Low density lipoprotein heterogeneity in the cebus monkey

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Abstract In studies of cebus monkey plasma lipoproteins, we have used an ultracentrifugally generated density gradient to isolate two distinct species of low density lipoproteins (LDL). Compositional analyses revealed that each of the ultracentrifugally isolated fractions was enriched in cholesteryl esters and contained a single apolipoprotein which in terms of its mobility on SDS gels corresponded to apolipoprotein B-100, the major apolipoprotein of human LDL. Hydrodynamic measurements carried out in the analytical ultracentrifuge showed that $F_{1.20}$ values were 30.0 for LDL_1 and 23.5 for LDL_2. In a solution of density 1.0069 g/ml, the sedimentation rates were 5.9 and 7.2 S for LDL_1 and LDL_2, respectively. In addition to sedimentation velocity data, we describe a new approach for using these same data to obtain calculated values for molecular weight. The hydrated densities calculated for the two fractions were 1.033 and 1.045 g/ml and calculated molecular weights were 3.08 million for LDL_1 and 2.42 million for LDL_2. Hydrated density values were in excellent agreement with those calculated from compositional data. Electron microscopy data showed that LDL_1 had a larger mean diameter of 26.7 nm than LDL_2 which had a diameter of 19.3 nm. Native gel electrophoretic analyses of the two LDL fractions in 3.5% acrylamide showed that, consistent with its size, LDL_1 had slower mobility than LDL_2.—**Puppione, D. L., R. J. Nicolosi, M. C. Kowala, and V. N. Schumaker.** Low density lipoprotein heterogeneity in the cebus monkey. *J. Lipid Res.* 1989. **30:** 641–650.

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Although frequently considered as being a metabolically homogeneous entity, human LDL has been known to consist of subfractions for over 30 years (1). Having demonstrated the existence of LDL subclasses through the use of the analytical ultracentrifuge, Gofman and coworkers (2–4) proposed that differences in the levels of LDL subclasses might be related to the degree of human susceptibility to atherosclerosis. In addition to these variations in subclass concentrations, the heterogeneity of human LDL since has been extended to include measurable differences both in the shapes of schlieren patterns and in the flotation rates of their major peaks which in certain cases can be as many as two or more (5–13). Moreover, recent studies strongly indicate that LDL which are both less massive and less buoyant than normal are associated with increased risk of atherosclerosis (14–17).

Schlieren analyses also have revealed heterogeneous LDL in certain species of nonhuman primates (18–26). Hill, Martin, and Douglas (18) reported the presence of two components in the schlieren patterns of both rhesus and cebus LDL. The LDL density class of rhesus monkeys has since been shown by Fless et al. (19, 20, 24–26) to contain three different lipoproteins, each containing apoB and having molecular weights of 3.32, 2.75, and 3.47 million. The LDL with the largest molecular weight of 3.47 million have been demonstrated to be Lp[a] (25, 26).

Because of our ongoing studies of lipoproteins in New World monkeys, we were interested in determining the nature of the LDL heterogeneity in the cebus. Hill et al. (18) found that the two components of cebus LDL had flotation rates of 22 and 19 negative Svedbergs in a salt solution of density 1.20 g/ml. Consistent with their observations, we also were able to detect two components in the schlieren patterns of cebus LDL when analyses were done at densities of 1.059 and 1.213 g/ml. In this communication, we report on our procedures for isolating in an ultracentrifugal gradient the lipoproteins that comprise the two LDL components observed in the schlieren pat-

Abbreviations: VLDL, very low density lipoproteins, d < 1.006 g/ml; IDL, intermediate density lipoproteins, d 1.006–1.019 g/ml; LDL, low density lipoproteins, d 1.019–1.063 g/ml; HDL, high density lipoproteins, d 1.063–1.21 g/ml; Lp[a], large polydisperse lipoproteins having densities primarily between 1.04 and 1.09 g/ml and containing two large molecular weight apolipoproteins, apoB-100 and Lp[a]; coco, coconut oil; apo, apolipoprotein; SDS, sodium dodecyl sulfate. $F_{1.20}$ denotes a flotation rate measured in negative Svedbergs at a density of 1.20 g/ml. S_f denotes a flotation rate measured in negative Svedbergs at a density of 1.063 g/ml and at 26°C.

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terns. In characterizing the physicochemical properties of each component, we carried out hydrodynamic studies, agarose and polyacrylamide gel electrophoresis, electron microscopic measurements, and detailed chemical compositional analyses. In contrast to the studies of rhesus LDL (25, 26), we found that neither of the cebus LDL were Lp[a]. Instead, the two LDL contained apolipoprotein B as the only demonstrable protein, and they differed from each other in both size and density.

MATERIALS AND METHODS

Animals and diets

Six adult male cebus monkeys (*Cebus albifrons*), 8½ years of age were fed essentially from birth a diet previously described (27). The caloric distribution was 21% from protein, 49% from carbohydrate, and 30% from fat. Edible coconut oil was selected as the fat instead of butter or animal fat because it does not contain cholesterol.

Blood specimens

After an overnight fast, the monkeys were tranquilized with Ketamine (Bristol Laboratories, Veterinary Products, Syracuse, NY) and blood samples were drawn from the femoral vein into EDTA-containing tubes to which a final concentration of 1.125% N-ethylmaleimide (Sigma, St. Louis, MO) and phenyl methyl sulfonylfluoride (2×10^{-4} M) was added to inhibit lecithin:cholesterol acyltransferase (LCAT) and proteolytic activity. Plasma was separated by low speed centrifugation at 4°C.

Lipoprotein isolation

Lipoproteins were isolated by a sequence of steps or one-step ultracentrifugation in a fixed-angle rotor 70.1 Ti rotor (Beckman Instruments Inc., Palo Alto, CA) as described by Schumaker and Puppione (28). The electrophoretic mobility of the lipoprotein fractions was determined using agarose gels.

Schlieren analyses

The isolated lipoprotein fractions with densities less than 1.063 g/ml were sent refrigerated to the Molecular Biology Institute at UCLA by express mail. Fractions were dialyzed overnight at 4°C against a 1.57 molal NaCl solution containing 0.04% $Na_2 \cdot$ EDTA and 0.05% NaN_3, d 1.059 g/ml. Analytical ultracentrifugation was carried out with an An-D Beckman rotor using double sector cells. Flotation velocity measurements were done in duplicate at a speed of 52,640 rpm. After fractions had been dialyzed against a 3.02 molal NaBr solution containing 0.04% $Na_2 \cdot$ EDTA and 0.05% NaN_3, d 1.213 g/ml, a second series of flotation velocity measurements were made in duplicate at 35,600 rpm. The Beckman model E analytical ultracentrifuge used in this phase of the study was equipped with schlieren optics. The temperature for each run did not vary by more than 1°C. Salt solution densities were measured with a Mettler/Paar Densitometer at a temperature of 26°C. Moving boundary flotation rates of the major LDL components were calculated from a ln $\times$ versus t plot using a computer program. Flotation rates were corrected for temperature. From these data, it was possible to estimate the hydrated density of the two cebus LDL components, seen in Fig. 1, from a plot of ηs versus ϱ as has been described by Lindgren et al. (8).

Density gradient separation of lipoproteins

Having obtained information on their hydrated densities, the following gradient was established to isolate the two components of cebus LDL in a Beckman SW 41 rotor. Samples of plasma obtained from cebus monkeys were pooled. After the plasma had been adjusted to a density of 1.070 g/ml with a 5.75 molal NaCl solution (1:0.5 plasma to NaCl solution), aliquots of 2 ml were transferred to the bottoms of six cellulose nitrate tubes. Each was overlaid with 4 ml of a NaCl solution of density 1.050 g/ml and then 4 ml of a NaCl solution of density 1.035 g/ml. The tubes were filled with 1.5 ml of a NaCl solution of density 1.019 g/ml. Following 43 h of ultracentrifugation at 40,000 rpm and at 20°C, twenty 0.6-ml fractions were collected from the top with an ISCO Density Gradient Fractionator (Lincoln, NE). Corresponding fractions obtained from each tube were pooled for the measurements described below.

Sedimentation and flotation measurements

The pools of the major fraction of the two components were sent refrigerated to UCLA. Following dialysis against a 0.21 molal NaCl solution containing 0.04% $Na_2 \cdot$ EDTA and 0.05% NaN_3, d 1.0060 g/ml, sedimentation runs were done simultaneously for both pools at 52,000 and then 40,000 rpm. Pools then were dialyzed against a 2.75 molal NaBr solution having a density of 1.1954 g/ml and flotation runs of both pools were done at 18,000 and 22,000 rpm. Analyses were done using a Beckman Model E ultracentrifuge equipped with a scanner optical system set at a wavelength of 280 nm. Sedimentation and flotation coefficients were measured using double sector cells and a Beckman An-F Titanium rotor. In these runs, the signal taken directly from the chart recorder was sent to the A/D converter of a DEC PDP 11/03 computer, and stored on flexible disks. Using programs written for a PDP 11/45 computer, derivative plots of individual scans were obtained. Sedimentation and flotation coefficients were calculated utilizing the maximum ordinate from the derivative plots obtained from the computer output. Reported values have been corrected for temperature.

Electron microscopy

Samples of LDL_1 and LDL_2 were negatively stained with 2% (v/v) phosphotungstate as we have described recently (27). Preparations generally were made at least in triplicate, viewed on a JEOL 100S electron microscope (JEOL Co., Japan) at 80 KV and photographed at a magnification of 50,000 ×. Final magnification was 125,000 ×. Particle diameters were measured with a micro dissecting scope equipped with an eye-piece micrometer and a photograph scale marker; approximately 150–200 free-standing lipoprotein particles were measured on multiple photographs taken from different areas of the grid.

Polyacrylamide gel electrophoresis

The apolipoprotein distribution of each LDL fraction was determined by SDS polyacrylamide gel electrophoresis according to the method of Weber and Osborne (29). Aliquots of each lipoprotein were dialyzed against 0.15 M NaCl containing 0.04% $Na_2 \cdot EDTA$ and 0.01% NaN_3 (pH 7.0), and delipidated with methanol–ether 3:1 and washed twice in the same solvent. Separation of the different molecular weight species of apoB using 3.5% SDS-PAGE was performed essentially according to the method of Kane, Hardman, and Paulus (30). β-Mercaptoethanol was added as a reducing agent to some samples to compare patterns between reduced and nonreduced apolipoproteins. After electrophoresis, gels were fixed for 48 h in 20% (w/v) trichloroacetic acid (TCA) with several changes of TCA and stained overnight in 50% (v/v) methanol, 5% (v/v) acetic acid containing 0.19% (w/v) Coomassie Blue R-250. Destaining was carried out in 5% (v/v) methanol, 7.5% (v/v) acetic acid. Native electrophoresis of LDL subclasses was carried out as we have previously described on 3.5% polyacrylamide gels (31).

Analytical measurements

Protein values determined by the method of Lowry et al. (32) were corrected for differences in chromogenicity between the standard, bovine serum albumin (fraction V; Sigma Chemical Co., St. Louis, MO) and apoB-100 (33). Cholesterol (34) and triacylglycerol (35) were quantified using enzymatic methods. Unesterified cholesterol was measured by omitting the cholesterol esterase from the enzymatic reagent (34), and phospholipids were quantitated by the method of Eibl and Lands (36). Because dense beta lipoproteins, similar to what we describe in this study, have been reported in human subjects with hyperthyroidism (37), measurements of the levels of the thyroid hormones, T_3 and T_4, in cebus plasma were made. Values were found to be normal.

RESULTS

Schlieren analysis

Flotation rate analyses of cebus LDL in the analytical ultracentrifuge revealed a schlieren pattern with two components. Experiments were performed in two different solutions (NaCl, d 1.0585 g/ml and NaBr, d 1.2134 g/ml). **Fig. 1** shows the two cebus components in the NaCl solution at 35 min after the rotor had reached speed. The component with the higher flotation rate is referred to as component 1 or LDL_1. Hill et al. (18) had reported only a single component for squirrel monkey LDL. Having been isolated and treated in an identical fashion to cebus LDL, squirrel monkey LDL served as a control. In both salt solutions, cebus LDL_1 had flotation rates comparable to the single component of squirrel monkey LDL. The single component seen for squirrel monkey LDL suggests that the two cebus monkey components are not artifactually produced during preparation and isolation. In the NaCl solution, cebus LDL_1 had a flotation rate of 4.90 ± 0.16 versus 4.56 ± 0.06 for the squirrel LDL. In the NaBr solution, the flotation rates were 28.71 ± 0.22 for cebus LDL_1 and 38.23 ± 0.25 for squirrel LDL. The other cebus component, LDL_2, had flotation rates of 2.32 ± 0.13 in the NaCl solution and 22.98 ± 0.10 in the NaBr solution. The flotation rate data, uncorrected for concentration, were used to obtain estimates for the

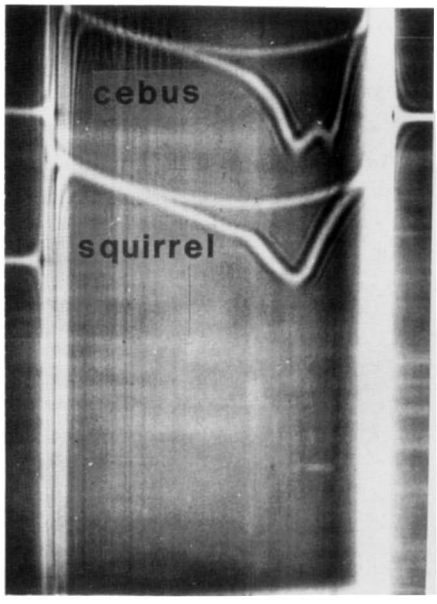

Fig. 1. Schlieren patterns of cebus and squirrel monkey LDL (solution density 1.059 g/ml) spun in an analytical ultracentrifuge for 35 min. Note the two components for cebus monkey LDL compared to the single component seen for the LDL of the squirrel monkey.

buoyant densities of cebus LDL_1 and LDL_2. By plotting the viscosity-corrected flotation coefficients as a function of the density (8), the intercepts with the abscissa yielded density values of 1.030 g/ml for LDL_1 and 1.045 g/ml for LDL_2.

Swinging bucket density gradient fractionation

Knowledge of the estimated densities of the two groups of cebus LDL allowed us to develop the swinging bucket gradient isolation procedure described in the Materials and Methods. The distribution of the lipoproteins within the resulting gradient was obtained by monitoring the absorbance of each fraction at 280 nm. The extent of the separation can be seen in **Fig. 2.** The relative amounts of these two cebus LDL varied among animals although the amount of LDL_1 was, in most cases, greater than that of LDL_2.

Sedimentation and flotation velocity studies

The major fractions containing only one of the components were combined to obtain separate pools of LDL_1 and LDL_2. Measurements were made of the sedimentation and flotation coefficients using each of these pools. **Fig. 3** shows superimposed scanner tracings for LDL_1 and LDL_2 obtained during a single ultracentrifugal run using a multiple cell rotor. In both cases, the flat plateau regions leading the respective boundaries indicated that there was no demonstrable aggregation. Values corrected to 20°C are listed in **Table 1.** Because the concentrations used were all quite low, these measured values were within 0.2% of the infinite dilution values. Plotting the corrected sedimentation velocity measurement (ηs) versus densities (ϱ) yielded the ϱ-intercept, and the slope, $d\ \eta s/d\varrho$.

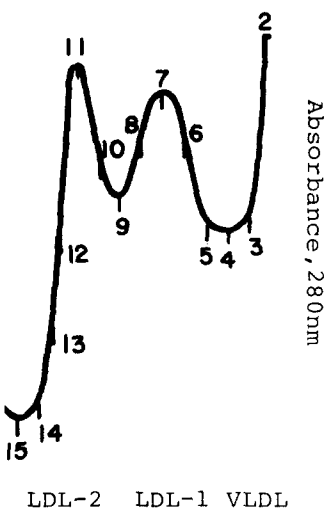

Fig. 2. Profiles of absorbance (280 nm) versus fraction number of cebus monkey plasma lipoproteins separated by density gradient ultracentrifugation. Note the extent of separation of the two LDL components.

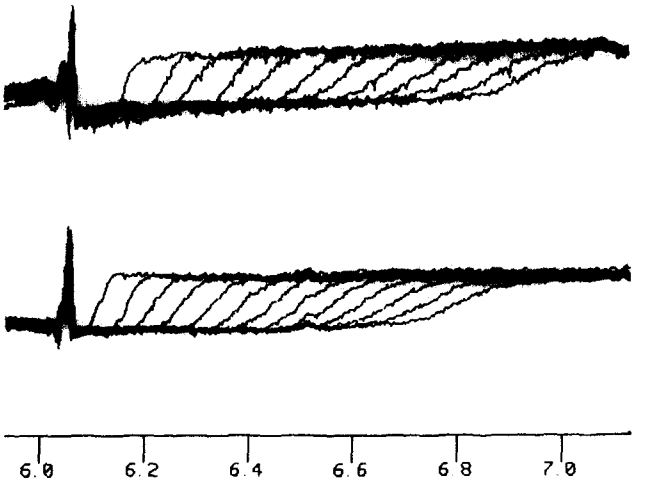

Fig. 3. A series of integral scans, taken at 8-min intervals and showing the movement of the two LDL components (solution density 1.0069 g/ml; rotational speed, 52,000 rpm). The scale at the bottom indicates the radial distances (cm) of the boundary from the axis of rotation. LDL_1 (upper series); LDL_2 (lower series).

The value of the ϱ-intercept equals the buoyant density, ϱ_b. With these values, the molecular weights of the two LDL components were calculated using the following expression which is derived in Appendix A:

$$M = \left[6\pi N \left(\frac{0.75}{\pi N \varrho_b} \right)^{1/3} \varrho_b \, (f/f_o) \, (-d\eta s/d\varrho) \right]^{3/2}$$

where f/f_o is the translational frictional ratio. A zero value for the preferential hydrations in these NaBr solutions was assumed, since this value was observed by Fisher, Granade, and Mauldin (38) for KBr solutions. The molecular weights were calculated assuming the value for the translation frictional coefficient to be 1.1, as previously determined for human LDL by Fisher et al. (38). Resulting values for molecular weight are listed in Table 1.

Electrophoretic analyses

On agarose, both LDL exhibited beta electrophoretic mobility, with LDL_2 having a slightly greater mobility than LDL_1 (figure not shown). On 3.5% native acrylamide gels, the influence of size as well as charge resulted in a clear separation between the two LDL as shown in **Fig. 4.** Consistent with a smaller size, LDL_2 migrated more rapidly than LDL_1.

Fig. 5 shows for each density fraction the apolipoprotein distribution determined on a 3.5% SDS polyacrylamide gel. Because the same volume was applied from each fraction, the relative concentrations across the gradient may be visualized. In each fraction, the apoprotein was found to consist of a single band which, based on its mobility, corresponded in molecular weight to apolipoprotein B, the

TABLE 1. Hydrodynamic properties of cebus LDL, hydrated densities and molecular weights

Component	$S_{20,\ 1.0069}$	$F_{20,\ 1.1954}$	Density[a]	Molecular Weight[b]
			g/ml	
1	5.73	30.18		
1	5.87	29.77		
	5.80 ± 0.12	29.98 ± 0.29	1.033	3.08×10^6
2	7.02	23.17		
2	7.20	23.74		
	7.11 ± 0.13	23.46 ± 0.40	1.045	2.42×10^6

[a]Densities were determined from the intercept of the ηs versus ϱ plot.
[b]Molecular weights were determined from the slope of the ηs versus ϱ plot.

major apolipoprotein of human LDL. This also was observed in a 12.5% SDS gel, as well as the absence of any bands with molecular weights less than 200,000 (figure not shown). Reduction with β-mercaptoethanol had no effect on the apolipoprotein pattern. These observations, together with absence of pre-β bands on agarose gels, indicate that neither of the two cebus LDL is Lp[a].

Electron microscopic studies

A size difference between the LDL_1 and LDL_2 was seen when the lipoproteins were examined in the electron microscope following negative staining. Representative electron micrographs of both LDL are shown in **Fig. 6.** Free-standing particles were round, and no other morphological characteristics were noted. Consistent with the above data, LDL of component 1 were larger than those of component 2 with mean diameters of 27.7 ± 0.5 nm versus 19.3 ± 0.3 nm.

Compositional studies

Percent compositions of individual components of the two lipoproteins are given in **Table 2.** In both cases, the major lipid class consisted of cholesteryl esters. In agreement with their molecular weight difference, the lipid to protein ratio was larger for component 1 (4.7) as compared with component 2 (3.6).

The partial specific volume of a lipoprotein can be determined from the weighted sum of its constituent protein and lipid moieties by assuming additivity of volumes. A value of 0.716 ml/g (39) was used for the partial specific volume of the protein moiety of LDL. The partial specific volume of the lipid moiety was calculated using published values for the partial specific volumes for each of the component lipid classes: 0.984 ml/g for phospholipids (40), 1.097 ml/g for triacylglycerol (41), 1.042 ml/g for cholesteryl esters (42), and 0.957 ml/g for unesterified cholesterol (43). The density values calculated from these partial specific volumes and the compositional values listed in Table 2 are 1.032 and 1.044 g/ml for LDL_1 and LDL_2,

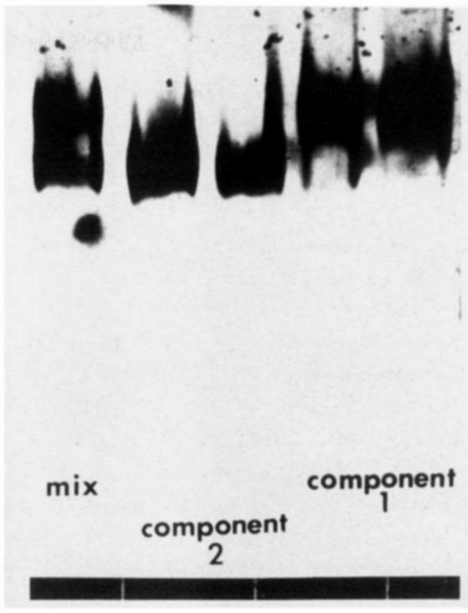

Fig. 4. Native gel (3.5%) electrophoretic separation of components 1 and 2 of cebus monkey LDL showing differences in migration. Mixture of the two shown for comparison.

respectively. These calculated values agree quite well with the observed hydrated density values listed in Table 1. Moreover, consistent with the evidence that each β-lipoprotein contains a single apoB-100 as well as the published values for the molecular weight of this apolipoprotein in humans (44–46), the molecular weights of the pro-

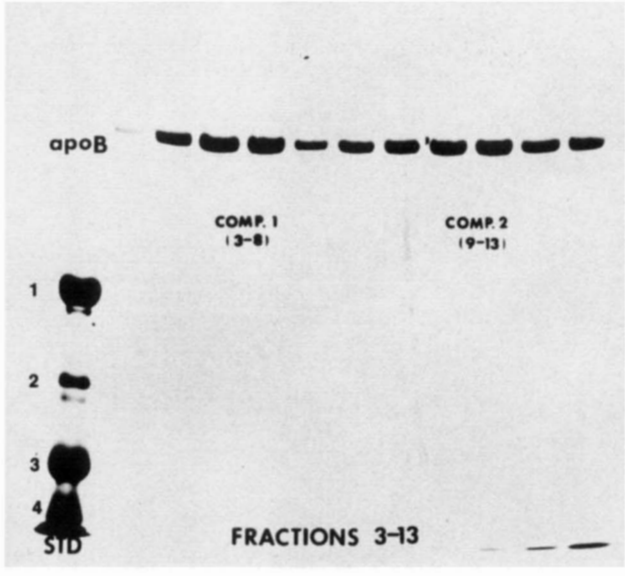

Fig. 5. SDS polyacrylamide gel electrophoresis (3.5%) of cebus monkey LDL fractions (numbers indicated in parentheses) collected from density gradient ultracentrifugation. Molecular weight standards: 1, myosin, 200,000; 2, beta-galactosidase, 116,500; 3, phosphorylase B, 92,500; and 4, bovine serum albumin, 66,200. Note fractions contain only large molecular weight apoB.

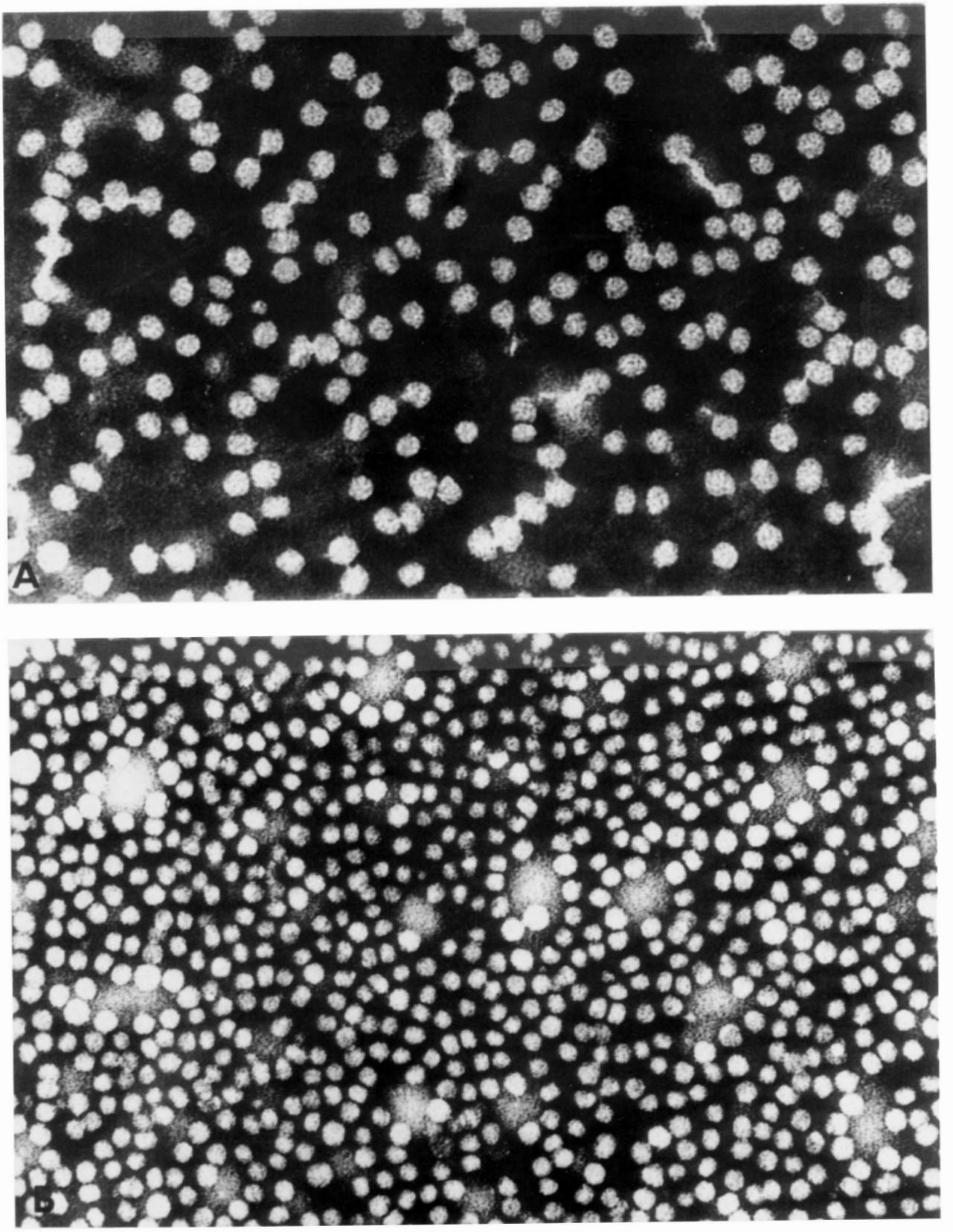

Fig. 6. Electron micrographs of the two cebus monkey LDL components showing the larger size of LDL_1 (6A) versus LDL_2 (6B). Magnification factor, 125,000. Approximately 150 to 200 free-standing lipoprotein particles were measured.

TABLE 2. Composition[a] of cebus monkey LDL subclasses

Subclass	Unesterified Cholesterol	Cholesteryl Ester	Triacylglycerol	Phospholipid	Protein[b]
LDL_1	0.36 ± 0.02	1.44 ± 0.02	0.22 ± 0.03	0.53 ± 0.02	0.54 ± 0.03
	(11.8 ± 0.5)	(46.6 ± 0.8)	(7.0 ± 1.1)	(17.1 ± 0.6)	(17.5 ± 0.9)
LDL_2	0.24 ± 0.03	0.96 ± 0.05	0.25 ± 0.07	0.44 ± 0.01	0.53 ± 0.07
	(9.9 ± 1.1)	(39.8 ± 2.2)	(10.3 ± 3.0)	(18.3 ± 0.5)	(21.7 ± 2.9)

[a]Values shown in this table were determined from the compositional data obtained on LDL_1 and LDL_2 fractions isolated from the plasmas of three different monkeys. Duplicate measurements were made to determine each individual component. The first values are composition data ± standard deviation expressed as grams ($\times 10^6$) of each component per gram-mole of lipoprotein. Values in parentheses are the percent compositions.

[b]Values for the protein content were corrected for chromogeneity in the Lowry assay according to the formula: grams of apoB = 0.8 grams of BSA equivalent.

tein moiety of LDL_1 and LDL_2 calculated from data in Tables 1 and 2 as percent protein × lipoprotein molecular weight were 539,000 ± 28,000 and 525,000 ± 70,000 respectively. Also listed in Table 2 are the compositions in terms of g ($\times 10^6$) of each component per gram-mole of lipoprotein. This presentation of data permits one to envision how a specific LDL might be modified to form another.

Finally, the molecular weights, M, and the molecular volumes (M/ϱ) of components 1 and 2 fall precisely on the previously published plots (10, 47) of these quantities of human LDL and IDL and our data are in agreement with the proposed model for LDL "in which the amount of protein is consistent with a molecular weight of about 510,000 daltons" (47).

DISCUSSION

Our studies have demonstrated that two components observed in the schlieren patterns of cebus LDL can be isolated and characterized. However, our flotation rates in solutions of comparable density were larger than previously reported values (18) by as much as 8 and 4 Svedbergs for components 1 and 2, respectively. In their study, Hill et al. (18) analyzed a concentrated lipoprotein solution containing a mixture of all the density classes, and probably a major part of the differences is due to the concentration dependence of the sedimentation coefficient.

In studying LDL heterogeneity in rhesus plasma, Fless et al. (24–26) characterized two cholesteryl ester-rich lipoproteins that differed in molecular weight and in density but which in both cases contained apoB-100 as the major apolipoprotein. One of these LDL, having a molecular weight of 3.32 million and a density of 1.027 g/ml was more massive and less dense than either of the cebus LDL in our study. The size (2.75 million) and density (1.036 g/ml) of the second rhesus LDL were comparable to cebus LDL_1. A second type of rhesus LDL, exhibiting pre-β electrophoretic mobility, was more massive (molecular weight, 3.47 million) than the other two LDL and was demonstrated to be Lp[a] (24–26). The Lp[a] lipoproteins, which have been reported in the plasmas of humans, certain other Old World primates and hedgehogs, range in density between 1.04 and 1.09 g/ml (25, 48–50). The anomalously high density range for a lipoprotein of this size is a result of a low lipid to protein ratio due to the presence of both apo Lp[a] and apoB-100 (51). Although the density of cebus LDL_2 was within this range, a pre-β electrophoretic band was not observed and our reported value for the molecular weight of LDL_2 is considerably lower than those for Lp[a] lipoproteins. Moreover, our compositional analyses together with the SDS electrophoretograms indicate that cebus LDL contain only apoB-100.

LDL heterogeneity can be induced in nonhuman primates by diets high in cholesterol and saturated fats (19, 21, 25, 52–57). In some of these cases, the LDL are enriched in either apoE (52) or apoA-I (57) and both types exhibit α electrophoretic mobility. In our studies, the animals were fed no cholesterol and only 30% of the calories was derived from coconut oil. Parallel studies in which the dietary fat is corn oil indicate that the levels of LDL are lower than values found in this reported study but LDL_1 and LDL_2 still could be detected. Hill et al. (18) made their observations of cebus LDL heterogeneity on animals being fed Purina chow supplemented with butter, applesauce, and nutrients. Independent of the nature of the dietary fat, both cebus LDL have the physicochemical properties of human LDL. They are enriched in cholesteryl esters, exhibit β electrophoretic mobility and have apoB-100 as the major apolipoprotein. In addition, our values for hydrated densities and molecular weights are within the range defined for human LDL (8, 12, 47). The hydrodynamic and compositional data plus the absence of pre-β and α electrophoretic bands eliminate the possibility of two cebus LDL being one of the other types of lipoproteins that can be isolated within this density interval in other species of nonhuman primates.

In a recent review, Rudel et al. (58) discussed the relevance or applicability of the heterogeneity seen among nonhuman primate β lipoproteins to their counterparts in human plasmas. They noted that in several species of nonhuman primates cholesterol feeding will result in a marked increase in the concentration of "large cholesteryl ester-enriched apoB-100"-containing lipoproteins, having densities in the IDL range. The formation of these IDL was thought to be due to a limited exchange of cholesteryl esters with triacylglycerol molecules because of the low levels of TG-rich lipoproteins in nonhuman primate plasmas (59). In the case of humans with elevated levels of VLDL, Rudel et al. (58) proposed that the increased exchange of cholesteryl esters for triacylglycerol would lead to formation of small LDL with size and density comparable to values that we report for cebus LDL_2. However, in the cebus monkey the small LDL are present in spite of this species having low levels of VLDL. As in other species of monkeys, the TG levels in cebus plasma are quite low (60). Preliminary turnover data, which indicate that LDL_1 and LDL_2 intraconvert in cebus plasma, have made it difficult to deduce the metabolic origins of these two types of LDL. One type could be derived stepwise from IDL precursors and the other could be nascent LDL (61) secreted directly from the liver. LDL which are lighter in mass and higher in density than those isolated from the majority of human plasma are reportedly atherogenic (11–17). We intend to utilize the cebus monkey model to obtain insight into the formation and metabolism of these small LDL.

APPENDIX A

Molecular weight analysis. To determine molecular weights, the data were analyzed by a new approach having the advantage of eliminating a term which is very sensitive to density heterogeneity, viz. $(1-\bar{v}\,\varrho)$. The sedimentation equation, written for the hydrated macromolecules, may be multiplied by the solution viscosity to yield:

$$\eta s = \frac{M_h (1 - \bar{v}_h \varrho)}{Nf'} \qquad Eq.\ 1$$

where η is the solvent viscosity, s is the observed sedimentation coefficient at infinite dilution, M_h is the hydrated molecular weight, $\bar{v}_h$ is the hydrated partial specific volume, which is determined as the reciprocal of the buoyant density, ϱ is the solution density, N is Avogadro's number, and f' is the translational frictional coefficient divided by the solution viscosity. Taking the derivative of this equation with respect to ϱ yields the slope of the η s vs ϱ plot. Thus:

$$\frac{d(\eta s)}{d\varrho} = -\frac{M_h \bar{v}_h}{Nf'} \qquad Eq.\ 2$$

The hydrated molecular weight may be written in terms of the preferential hydration parameter as $M_h = M(1 + \Gamma')$. The hydrated apparent specific volume is given by the reciprocal of the observed buoyant density, ϱ_b. Thus:

$$\frac{d(\eta s)}{d\varrho} = -\frac{M(1 + \Gamma')}{Nf'\,\varrho_b} \qquad Eq.\ 3$$

The translational frictional coefficient divided by viscosity may be related to the frictional ratio, using the expression

$$f' = 6\pi \left(\frac{f}{f_o}\right)\left(\frac{3/4\ \bar{v}\ M}{\pi\ N}\right)^{1/3} \qquad Eq.\ 4$$

where f/f_o is the frictional ratio and $\bar{v} = \bar{v}_h + \Gamma'(\bar{v}_h - \bar{v}_w)$, where $\bar{v}_w$ is the partial specific volume of water. Combining equations 3 and 4 yields an expression relating the molecular weight to the slope and the intercept of the plot of ηs vs ϱ:

$$M = \left[6\pi N \left(\frac{f}{f_o}\right)\left(\frac{3/4\ \bar{v}}{\pi N}\right)^{1/3} \frac{\varrho_b}{(1 + \Gamma')}\left(\frac{-d\eta s}{d\varrho}\right)\right]^{3/2} \qquad Eq.\ 5$$

Animals used in this study were maintained in accordance with the guidelines of the Committee on Animals of the University of Lowell Research Foundation and those prepared by the Committee on Care and Use of Laboratory of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 85-23, revised 1985). The authors would like to acknowledge the secretarial assistance of Nancy Kenon and the technical assistance of Dorothy Arrigo. Corn oil was generously provided by Best Foods, CPC North America, Englewood Cliffs, NJ. This work was supported by grants from the National Institutes of Health HL36101, HL36200, and HL38567 awarded to Dr. Nicolosi and GM13914 awarded to Dr. Schumaker.

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