

Heterogeneity of serum low density lipoproteins in normal human subjects

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Abstract Equilibrium density gradient ultracentrifugation of serum low density lipoprotein (LDL) from twelve healthy human subjects was used to separate six subfractions with mean density ranging from 1.0268 to 1.0597 g/ml. Mean corrected peak flotation rate (S_f^0) measured by analytic ultracentrifugation, and mean particle diameter determined by negative staining electron microscopy, both declined significantly with increasing density of the subfractions. Major differences in chemical composition of the subfractions were noted, including a significantly lower triglyceride content and higher ratio of cholesteryl ester to triglyceride in the middle fractions compared with those of highest and lowest density. Concentration of fraction 2 correlated positively with HDL ($P < 0.01$) and negatively with VLDL ($P < 0.001$); concentration of fraction 4 correlated negatively with HDL ($P < 0.05$) and positively with VLDL ($P < 0.001$) and IDL ($P < 0.01$). LDL may thus include subspecies of differing structure and composition which might also have different metabolic and atherogenic roles. — **Shen, M. M. S., R. M. Krauss, F. T. Lindgren, and T. M. Forte.** Heterogeneity of serum low density lipoproteins in normal human subjects. *J. Lipid Res.* 1981. **22**: 236–244.

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Lipoproteins in the density class of LDL (1.019–1.063 g/ml) have been found to represent a distribution of particles differing in size, hydrated density, and chemical compositions. Lindgren, Elliot, and Gofman (1) first showed that it is possible to fractionate the LDL into at least three subgroups by preparative ultracentrifugation in a fixed angle rotor. It was further indicated, in a later report, that molecular weights and peak flotation rates differ in LDL from normal men and women (2). Adams and Schumaker (3) reported LDL equilibrium banding on a density gradient under centrifugal force and found evidence for at least three density classes.

This and more recent evidence for the heterogeneity of LDL (4–9) led us to investigate the physical and chemical properties of LDL fractions separated by equilibrium density gradient ultracentrifugation

in normal subjects. Also, the concentrations of these subfractions in serum were tested for correlations with concentrations of the other lipoprotein classes (9) in order to determine any significant relationships between LDL and either VLDL or HDL that may not have been apparent when using measurement of total LDL.

EXPERIMENTAL PROCEDURES

Isolation of low density lipoproteins

Sera were prepared from blood samples obtained from twelve healthy normolipemic subjects who had fasted overnight. The donors were six males and six females between ages 25 and 56. Mean serum cholesterol concentration for these subjects was 194 ± 65 (SD) mg/dl and mean triglyceride concentration was 86 ± 61 mg/dl.

Blood was drawn into an evacuated tube containing merthiolate (1 mg/20 ml blood) and was allowed to clot for 1 hr at room temperature. Immediately after clotting, two low speed centrifugations (30 min at 1500 rpm in IEC) were performed to obtain the serum samples.

LDL were isolated from the serum of each subject by sequential preparative ultracentrifugation under standard conditions (2). Briefly, the serum was adjusted to $d = 1.019$ g/ml with solid sodium bromide and centrifuged at 40,000 rpm for 18 hr at 17°C in a Beckman 40.3 Ti rotor. The top 2-ml was decanted and the subnatant fluid was adjusted to $d 1.063$ g/ml with sodium bromide solution. After centrifugation of this mixture at 17°C for 24 hr at 40,000 rpm in a Beckman 40.3 rotor, the LDL were withdrawn in the top 1-ml and dialyzed in NaBr solution of $d 1.0400$ g/ml overnight with two changes of dialyzing solution.

Abbreviations: LDL, low density lipoproteins; IDL, intermediate density lipoproteins; VLDL, very low density lipoproteins; HDL, high density lipoproteins.

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Density gradient ultracentrifugation

The dialyzed LDL (2 ml) were layered carefully above an NaBr solution of d 1.0540 g/ml (2.5 ml) in a 1/2" by 3-1/2" Beckman cellulose nitrate centrifuge tube (holds 7 ml), and 2.5 ml of NaBr solution of d 1.0275 g/ml was layered above the LDL. The tubes were then centrifuged to equilibrium at 40,000 rpm for 40 hr in a Beckman SW 45 rotor at room temperature (22–24°C).

After the centrifugation of the LDL, from two to four bands of either yellowish or turbid appearance were observed in the tubes. Because it was not possible to separate and reproducibly withdraw these bands as discrete layers, the contents of each tube were withdrawn in an arbitrary sequence, beginning with the top 0.5-ml, then six 1-ml fractions, and the bottom 0.5-ml. As later proved by analytic ultracentrifugation, the top 0.5-ml contained intermediate density lipoproteins of $d < 1.019$ (IDL, S_f 12–20) and the bottom 0.5-ml contained high density lipoproteins. The bottom fraction was not studied further. The middle six fractions were numbered 1 to 6, beginning from the top. A background salt tube was included with each individual centrifugation, and the salt densities of the fractions in this tube were measured by refractometry.

Analytic ultracentrifugation

The LDL subfractions 1 through 6, as well as serum total lipoproteins and isolated LDL, were analyzed by analytic ultracentrifugation in a Spinco Model E instrument with schlieren optics as previously described (2). The runs were performed at 26°C and 52,640 rpm with salt density of 1.061 and 1.200 g/ml. The flotation coefficients were corrected for viscosity, temperature, and concentration. Hydrated densities were calculated from ηF° versus ρ -plot. Molecular weights were estimated using Stokes' frictional factor of 1.1 (5).

As part of the study, a computer program was designed to add the individual curves of the corrected analytic ultracentrifuge schlieren patterns of the fractions and normalize the sum to the total concentration of the unfractionated LDL that had been used as the starting material.

The VLDL, IDL, and HDL for each subject were also determined from the analytic ultracentrifugal profiles of the total serum lipoproteins. (VLDL, $S_f > 20$, IDL, S_f 12–20, HDL, $F_{1.20}^{\circ}$ 0–20).

Electron microscopy

Morphological characteristics of the subfractions were studied by electron microscopy as described by

Forte and Nichols (10). LDL fractions obtained from density gradient centrifugation were dialyzed against a 1% ammonium acetate buffer (pH 7.4) containing 5 mg/l EDTA. An equal volume of 2% sodium phosphotungstate (pH 7.4) was mixed with the dialyzed LDL. A drop of this mixture was applied to a Formvar/carbon-coated grid. Microscopy was performed in a JEM 100C (JEOLCO, Inc., Tokyo, Japan) operating at 100 kV. Mean particle size of the LDL fractions was calculated from measurements of 100–200 particles in each fraction in each of six subjects.

Chemical composition determination

Phospholipid was determined using the method of Bartlett (11). Total cholesterol, free cholesterol, and triglyceride concentrations were determined using enzymatic methods on a System 3500 Gilford Computer Directed Analyzer (Gilford Instruments, Oberlin, OH). Assay reagents used for the cholesterol assays were from Boehringer-Mannheim (Palo Alto, CA) and assay reagents for triglyceride were from Worthington Biochemical Corp. (Freehold, NJ). Cholesteryl ester concentrations were calculated as (total cholesterol – free cholesterol) \times 1.68. Protein concentrations were determined by the method of Lowry et al. (12).

The number of molecules of each component per LDL particle was estimated by using the particle molecular weight as calculated from analytic ultracentrifugation, and component molecular weights assumed to be as follows: phospholipid, 775; free cholesterol, 387; cholesteryl ester, 650; triglyceride, 850. Protein content was calculated as the number of amino acid residues per particle, assuming an average residue molecular weight of 100 (13). Significance of differences in mean values for composition of the six LDL subfractions ($P < 0.05$) was calculated by paired t -test.

RESULTS

Analytical ultracentrifugation

Fig. 1 shows analytic ultracentrifuge schlieren photographs of LDL taken from four of the subjects at 30 min (photograph 1) and 64 min (photograph 2) after final ultracentrifugal speed was attained. Single peaks are shown in cases A and C, while in cases B and D, multiple peaks are apparent, particularly at 64 min.

Fig. 2 shows the corrected schlieren curves for the S_f 0–20 lipoproteins of the twelve subjects. The computer program used for plotting these curves is based on the schlieren photographs taken

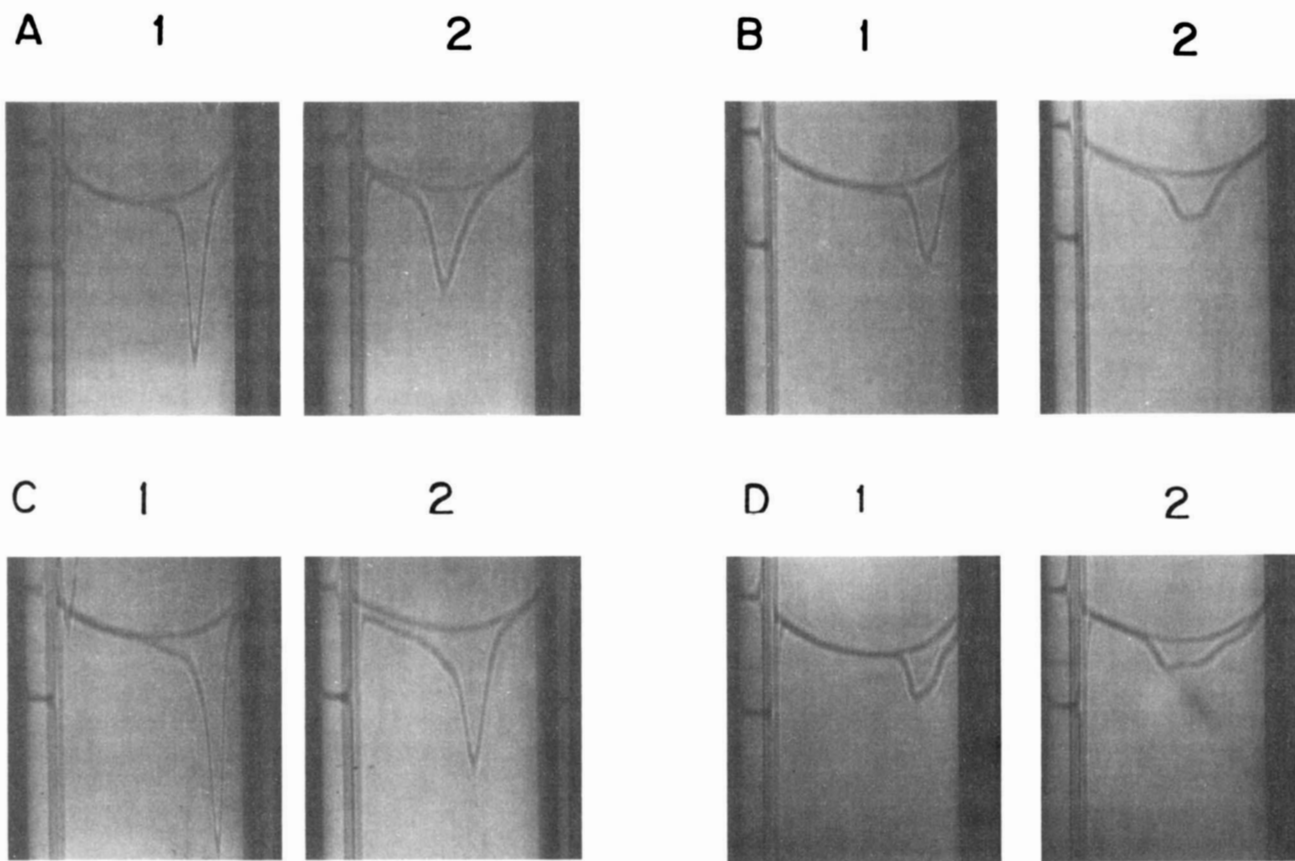


Fig. 1. Schlieren patterns during analytical ultracentrifugation of LDL from four normal subjects. Centrifugation speed was 52,640 rpm at 26°C with NaBr solution of density 1.061 g/ml. (A) and (B) are from men and (C) and (D) are from women. In each case, photograph 1 was taken at 30 min and photograph 2 at 64 min after the centrifuge reached the designated speed.

at 30 min, and thus multiple peaks, seen in four of the subjects (females 1 and 2, and males 1 and 2) at 64 min, are not clearly visible. A variety of S_f^0 distributions was present with peak S_f^0 rates ranging from 5.4 to 8.5 in the women, and from 5.4 to 7.1 in the men.

Shown in **Fig. 3a** are the corrected analytic ultracentrifuge schlieren patterns of the six fractions of LDL from one of the female subjects. While the curves for the individual fractions overlap, each fraction has a distinct peak S_f^0 rate.

The results of the computer program which added the individual curves and normalized the sum to the unfractionated LDL starting material can be seen in **Fig. 3b**. The schlieren pattern of the summed fractions is very similar to that of the original LDL. This suggests that there is uniform recovery and that there have been minimal changes in the flotation properties of the subfractions after density gradient ultracentrifugation.

The mean hydrated densities and peak flotation rates of the six LDL subfractions in the group of subjects are listed in **Table 1**. Peak S_f^0 ranges from

10.4 for men and 9.9 for women in fraction 1 to 1.7 for men and 2.0 for women in fraction 6. The mean values differed significantly for each subfraction and the differences in flotation rates of the individual fractions between the sexes were not statistically significant.

Electron microscopy

Fig. 4 shows representative electron micrographs of three of the six major LDL subfractions as seen in one of the subjects. The particles in all fractions appear to be round and relatively uniform in size (the standard deviation for the mean diameter of each subfraction was approximately 20Å). The particles as seen under electron microscopy are smaller in each succeeding subfraction as the density of the subfractions increases. This progression was analyzed further by plotting the mean particle diameter of each of the six fractions, as measured by electron microscopy in six subjects, against the mean flotation rate for each fraction. The plot (**Fig. 5**) displays a progressive decline in particle size with increasing density and decreasing flotation rate.

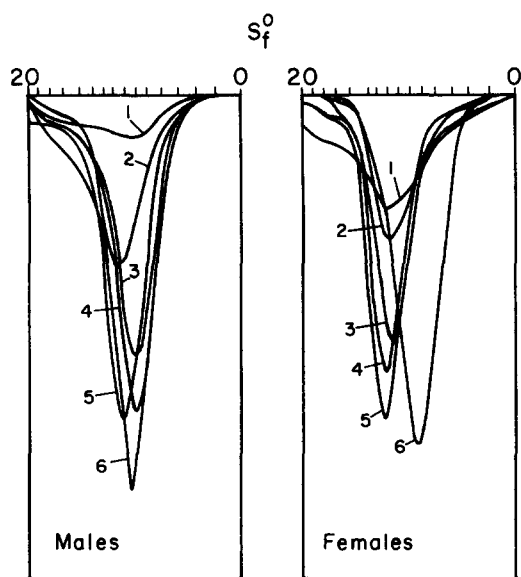


Fig. 2. Corrected computer-plotted schlieren curves of LDL (S_f 0–20 lipoproteins) from six male and six female subjects. See text for methods.

Concentration of chemical components

The concentration of protein in each of the LDL subfractions from the twelve subjects is shown in **Table 2**. Individual differences in the distribution of protein among the fractions were seen, and these paralleled the differences in total mass as determined by analytic ultracentrifugation (Fig. 2). Mean levels of protein in fractions 1 and 2 were slightly higher in women than men, and levels of fraction 3 and 4 slightly lower, but these differences did not approach statistical significance. Similarly, the absolute and relative concentrations of the major lipid classes in LDL did not differ between the sexes, and the composition data from all sub-

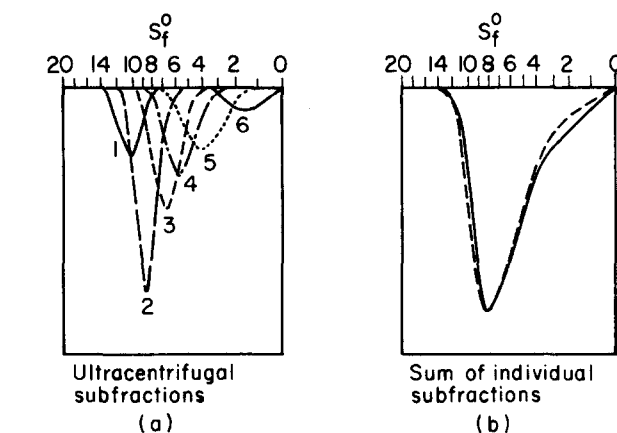


Fig. 3. (a) Schlieren patterns of 6 LDL subfractions from a representative subject. (b) LDL pattern of the same subject before subfractionation is indicated by the solid line (—) while the computer summation of patterns obtained in (a) is indicated by the dashed line (---).

jects were pooled. The results, expressed as weight percent, are shown in **Table 3**. The statistical significance of differences among the six fractions was tested using paired *t*-tests, and the significant differences ($P < 0.05$) can be summarized as follows: percent phospholipid was higher in fractions 1 and 3 than in fractions 5 and 6; free cholesterol was higher in fractions 1 and 2 than in fraction 3, lower in fraction 4 than 3, and higher in fraction 6 than in fractions 4 and 5; cholesteryl ester was lower in fraction 4 than in fraction 2, lower in fraction 5 than in fractions 2 and 3, and lower in fraction 6 than in fractions 1–4; triglyceride was higher in fractions 1 and 5 than in fractions 2–4, and higher in fraction 6 than in fractions 2–5; protein was higher in fraction 3 than in fraction 1, higher in fractions 4 and 5 than in fractions 1–3, and higher in fraction 6 than in fractions 1 and 2.

TABLE 1. The average densities and peak flotation rates of LDL subfractions in serum from twelve subjects

Fraction	Density ^a	σ -Intercept (n) ^b	Male	Female
			n = 6	n = 6
Top 0.5 ml	1.0244			
1	1.0268	1.0169 (9)	10.4 ± 0.6	9.9 ± 0.7
2	1.0308	1.0211 (9)	8.4 ± 0.3	8.3 ± 0.8
3	1.0357	1.0271 (9)	6.9 ± 0.5	6.7 ± 0.5
4	1.0414	1.0323 (8)	5.3 ± 0.5	5.3 ± 0.4
5	1.0492	1.0371 (8)	4.2 ± 0.3	4.1 ± 0.5
6	1.0597	1.0479 (3)	1.7 (n = 1)	2.0 ± 0.6 (n = 5)

^a The data are from identical gradients with no lipoprotein added and centrifuged simultaneously with the subfractionations.

^b These σ -intercept data and the corresponding S_f values to the right are consistent with reference (2). The substantial differences observed between such σ -intercept values and the corresponding 1-g gradient densities reflect both the substantial compression gradient at 39,000 rpm, as well as some difference in compressibility between the LDL subfractions and the solvent.

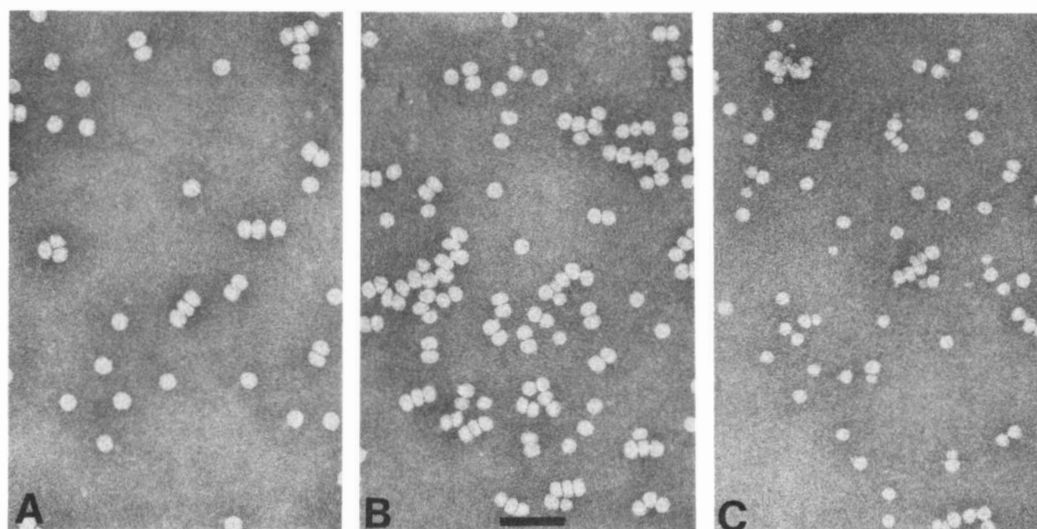


Fig. 4. Electron micrographs of negatively stained subfractions of LDL. (a) fraction 1, $271\text{Å} \pm 23\text{Å}$ SD diameter; (b) fraction 3, $247\text{Å} \pm 17\text{Å}$ SD diameter and (c) fraction 5, $203\text{Å} \pm 25\text{Å}$ SD diameter. Bar marker represents 1,000Å and applied to all micrographs.

In **Table 4** is shown the number of molecules of each component per LDL particle as estimated using particle molecular weights (see Materials and Methods). Again, using paired *t*-tests, statistically significant differences ($P < 0.05$) were identified among the fractions excluding fraction 6, for which insufficient data were available. From fraction 1 through fraction 5 there were significant ($P < 0.05$) progressive decreases in molecular weight, and in molecules of phospholipid (except in fraction 3 versus 2), free cholesterol, and cholesteryl ester

(except in fraction 2 versus 1). Triglyceride molecules decreased in number from fractions 1 through 3, then increased in fraction 5 versus 4. Amino acid residues were higher in fraction 1 than in fractions 2–5.

Table 5 shows the molar ratios of the components. Amino acid residues/phospholipid was higher in fractions 4 and 5 than in fractions 1 and 2; free cholesterol/phospholipid was lower in fraction 4 than in fractions 1–3; cholesteryl ester/free cholesterol was lower in fraction 1 than fractions 3 and 4, and cholesteryl ester/triglyceride was lower in fraction 1 than in fractions 2–4, and lower in fraction 5 than in fractions 3 and 4.

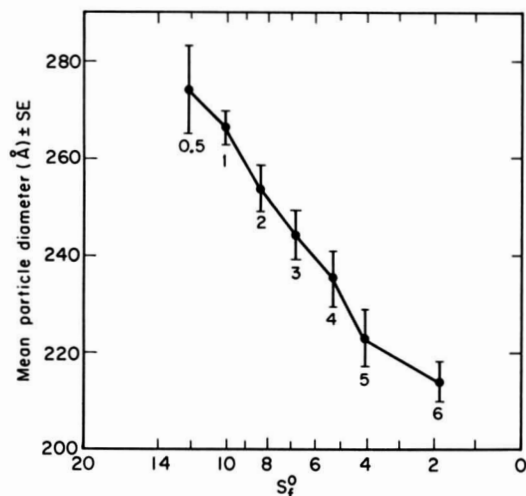


Fig. 5. Mean particle diameters of isolated LDL subfractions (numbers 1–6) determined by electron microscopy versus peak S_f^0 of these fractions determined by analytical ultracentrifugation. The top ½ ml fraction (containing LDL) is designated 0.5.

Statistical correlations and reproducibility of findings

Pearson correlations were calculated between serum concentrations of each of the six LDL subfractions and serum concentrations of VLDL, IDL, and HDL for each of the twelve subjects as measured by analytic ultracentrifugation (**Table 6**). VLDL and IDL concentrations were correlated strongly positively with fractions 4 and 5 and negatively with fraction 2, although the latter correlations did not achieve significance at $P = 0.05$. HDL levels were positively correlated with fraction 2 and negatively with fraction 4.

Four of these twelve subjects were recalled 6 to 12 months after the first study for a second study of LDL subfractions. No significant differences in the ultracentrifugal or chemical data were found be-

TABLE 2. Concentration of protein in LDL subfractions (mg/dl of serum^a)

Fraction	0-0.5 ml	1	2	3	4	5	6	Total
Males								
1	3.7	2.9	3.3	3.5	3.6	1.9	0.7	19.6
2	1.6	3.2	10.5	14.9	8.1	3.9	1.7	43.9
3	4.0	3.7	7.3	12.5	17.5	4.9	2.6	52.5
4	3.0	3.5	6.5	17.9	22.8	5.8	1.5	61.0
5	3.3	5.9	17.9	28.3	9.5	3.1	0.8	68.8
6	4.2	5.3	13.1	31.3	20.0	4.5	1.3	79.7
Mean ± SD	3.3 ± 0.9	4.1 ± 1.2	9.8 ± 5.2	18.1 ± 10.3	13.6 ± 7.6	4.0 ± 1.4	1.4 ± 0.7	54.3 ± 21.1
Females								
1	1.7	2.8	7.6	5.5	5.3	4.8	4.2	31.9
2	1.5	4.2	12.0	12.2	8.5	4.9	1.2	44.5
3	4.0	4.8	15.6	20.1	9.5	2.4	1.2	57.6
4	2.3	5.4	20.8	15.1	8.6	4.8	2.7	59.7
5	3.2	7.6	20.3	16.2	6.7	3.9	1.4	59.3
6	6.5	6.7	9.4	26.8	34.2	7.2	2.6	93.4
Mean ± SD	3.2 ± 1.9	5.3 ± 1.7	14.3 ± 5.6	16.0 ± 7.2	12.1 ± 10.9	4.7 ± 1.6	2.2 ± 1.2	57.7 ± 20.6

^a Unadjusted for dilution of LDL in dialysis (see Methods).

tween the first and second experiments. Therefore, the distribution and properties of the subfractions within an individual subject appear to be relatively stable.

DISCUSSION

LDL includes a spectrum of particles differing in molecular weight, ultracentrifugal flotation rate, and hydrated density (2). While discrete subclasses of LDL of S_f 0-12 have not been clearly identified in normal subjects, Nelson and Morris (4) studied 41 normal humans using analytic ultracentrifugation and found that 46% showed heterogeneous LDL. Hammond and Fisher (5) and Fisher (6) have reported that polydispersity of LDL of S_f 0-20 in hyperprebetalipoproteinemic subjects could be resolved into five major fractions separable by density gradient ultracentrifugation, three within the region S_f 0-12, and two within the S_f 12-20. Lee and Alaupovic

(7) prepared six density subfractions of LDL (d 1.006-1.063 g/ml) from normal subjects using sequential preparative ultracentrifugation and found constancy of size but variation in relative cholesteryl ester and triglyceride content in the three most dense fractions.

In the present study, an equilibrium density gradient ultracentrifugation procedure was used to separate six subfractions of LDL of S_f 0-12 from normal subjects. Analytic ultracentrifugation revealed that peak flotation rate declined progressively with increasing density of the fractions, and the mean values for neighboring fractions differed significantly. Mean particle size determined by electron microscopy also decreased substantially and progressively with increasing density of the fractions, paralleling the analytic ultracentrifuge data. The flotation rates, sizes, and relative chemical compositions of the fractions differed minimally among the subjects, despite wide individual variation in the distribution of mass among the fractions.

TABLE 3. Chemical composition of LDL subfractions^a

Fraction	Phospholipid	Free Cholesterol	Cholesteryl Ester	Triglyceride	Protein
<i>percent</i>					
1	22.3 ± 2.5	8.8 ± 1.9	40.2 ± 3.7	4.9 ± 1.3	23.8 ± 1.9
2	21.7 ± 2.4	8.5 ± 1.4	41.9 ± 2.9	3.2 ± 1.6	24.7 ± 1.5
3	22.2 ± 3.2	7.9 ± 1.3	40.8 ± 3.3	2.8 ± 1.0	26.2 ± 1.9
4	21.7 ± 3.1	7.4 ± 1.1	39.6 ± 3.5	2.9 ± 1.0	28.3 ± 1.8
5	20.7 ± 3.1	7.8 ± 1.9	37.9 ± 4.7	4.6 ± 1.6	29.0 ± 3.4
6	19.7 ± 3.9	8.9 ± 2.6	36.2 ± 6.6	5.6 ± 1.7	29.6 ± 6.3

^a In expressing percentage composition, the sum of the mass of all components in each fraction from a given subject was taken as 100%. This table shows the mean ± S.D. of the twelve subjects. Differences among fractions significant at *P* < 0.05 (paired *t*-test) are indicated in the text.

TABLE 4. Number of molecules of components per particle of LDL subfractions

Fraction	n	Mol. wt. $\times 10^{-6}$	Phospholipid	Free Cholesterol	Cholesteryl Ester	Triglyceride	Amino Acids
<i>Mean \pm S.D.</i>							
1	9	2.89 \pm 0.08	811 \pm 98	616 \pm 134	1790 \pm 208	178 \pm 46	7074 \pm 344
2	9	2.61 \pm 0.06	707 \pm 69	537 \pm 83	1715 \pm 132	108 \pm 49	6458 \pm 565
3	9	2.38 \pm 0.09	677 \pm 124	474 \pm 80	1488 \pm 145	82 \pm 39	6334 \pm 495
4	8	2.13 \pm 0.11	604 \pm 123	403 \pm 62	1281 \pm 108	74 \pm 24	6104 \pm 632
5	8	1.93 \pm 0.14	496 \pm 91	362 \pm 64	1139 \pm 214	110 \pm 38	5767 \pm 703
6	2	1.88	383	351	1077	123	6453

Differences among fractions significant at $P < 0.05$ (paired t -test) are indicated in the text.

The presence of up to four isopycnic bands after density gradient ultracentrifugation, and the appearance of up to three peaks in the schlieren curves of LDL in some subjects suggests that at least some of the differences observed among the six density subfractions are due to the presence of discrete subspecies of LDL. In the present study, it was not feasible to attempt isolation of the individual isopycnic bands since they were not clearly identifiable in all the subjects.

Recently, we have developed a procedure for densitometric scanning of the gradient tubes, and have analyzed in detail the isopycnic banding and analytic ultracentrifuge schlieren patterns in 12 additional subjects.² Preliminary results indicate that the least dense band comprises LDL of S_f^0 7.5–12, which is included primarily in subfractions 1 and 2 in the present procedure, while the most dense band represents LDL of S_f^0 0–4, which is contained in subfractions 5 and 6. These regions are also identifiable as distinct peaks in the analytic ultracentrifuge pattern in some subjects (cf. Fig. 1D). The other bands are centered between these two regions and overlap with them.

These ultracentrifugal findings do not exclude

² Krauss, R. M., and D. Burke. Unpublished data.

TABLE 5. Molar ratios of components per particle of LDL subfractions

Fraction	AA/PL	FC/PL	CE/FC	CE/TG
1	8.8 \pm 1.5	0.76 \pm 0.15	3.1 \pm 0.9	10.3 \pm 3.6
2	9.2 \pm 1.2	0.76 \pm 0.10	3.3 \pm 0.7	19.5 \pm 9.4
3	9.6 \pm 1.8	0.72 \pm 0.16	3.3 \pm 0.8	20.5 \pm 3.1
4	10.3 \pm 1.3	0.69 \pm 0.17	3.3 \pm 0.7	19.2 \pm 7.1
5	11.9 \pm 2.5	0.75 \pm 0.15	3.3 \pm 1.0	12.1 \pm 5.9
6	16.9	0.92	3.2	8.9

Abbreviations: AA, amino acids; PL, phospholipid; FC, free cholesterol; CE, cholesteryl ester; TG, triglyceride. All data are mean \pm S.D. based on the data in Table 4. Differences among fractions significant at $P < 0.05$ (paired t -test) are indicated in the text.

further heterogeneity on the basis of narrower isopycnic banding, nor do they exclude additional sources of heterogeneity within the density subfractions. In the case of fraction 6, a previous study by Albers, Chen, and Aladjem (8) of S_f^0 0–2 lipoproteins indicates that LP(a) and HDL are likely to be present in significant concentrations in some subjects.

Interpretation of differences among the density subfractions of LDL must also take into account the possibility that ultracentrifugation or other aspects of the preparation of these fractions may introduce changes in the structure or composition of LDL. Full assessment of this possibility awaits studies employing other techniques for LDL separation (chromatographic, electrophoretic). However the consistency of the differences in physical and chemical properties among the fractions described here merits attention and further study.

Major differences were found in chemical composition of the six LDL subfractions, particularly in relative content of nonpolar lipids, there being a significantly higher ratio of cholesteryl ester to triglyceride in fractions 3 and 4 than in the fractions of lesser or greater density. Such differences may contribute in part to variance in density among these fractions (7). Estimates of the number of molecules of the lipid and protein components per particle indicated a significantly lower number of triglyceride molecules in the middle fractions.

TABLE 6. Coefficients of correlation between concentrations of LDL fractions and other lipoproteins

	Fr 1	Fr 2	Fr 3	Fr 4	Fr 5	Fr 6
VLDL	-0.02	-0.43	+0.32	+0.84 ^a	+0.71 ^b	-0.05
IDL	+0.41	-0.09	+0.73 ^b	+0.79 ^b	+0.57	-0.28
HDL	-0.32	+0.78 ^b	-0.12	-0.62 ^c	-0.41	+0.16

^a $P < 0.001$,

^b $P < 0.01$, and

^c $P < 0.05$; significance of correlations.

The number of free cholesterol, phospholipid, and amino acid residues declined to varying extents between fractions 1 and 5 while the protein to phospholipid ratio increased progressively, consistent with the densities of the particles.

If, as is thought to be the case for larger triglyceride-rich lipoproteins (14), the nonpolar lipids are contained within the core of the LDL molecule, the compositions reported here are consistent with a progressive decrease in the core triglyceride content through fractions 1, 2, and 3. Possibly this decrease is due to lipolysis. The higher triglyceride content in fractions 5 and 6 suggests that these particles are either derived from another metabolic pathway or that there is a differential exchange of core lipids among these and other lipoprotein particles.

Comparison of the six LDL subfractions between the men and women in this study group revealed no significant differences in peak flotation rates or in composition of the subfractions between the sexes. The serum concentrations of LDL subfractions 1 and 2 were slightly higher in the women however, and levels of fractions 3 and 4 higher in the men, consistent with differences in LDL with faster and slower flotation rates reported on the basis of analytic ultracentrifuge measurements in normal populations (2).

Findings of much greater statistical significance were obtained when correlations were sought between concentrations of LDL subfractions and levels of VLDL and HDL in the present study population. Levels of fraction 4 were strongly positively correlated with VLDL and IDL concentrations and were negatively correlated with levels of HDL. On the other hand, concentrations of fraction 2 were negatively correlated with VLDL and positively with HDL, although the former correlation did not reach statistical significance at $P = 0.05$. These correlations are consistent with those recently reported by Krauss, Lindgren, and Ray (9) in which levels of smaller LDL of S_f 0–7 as measured by analytic ultracentrifugation in a normal population were positively correlated with VLDL levels and negatively with HDL in most age-sex categories. Levels of larger LDL of S_f 7–12 showed the opposite correlations in these groups. While these correlations cannot in themselves be taken to indicate differences in metabolic interrelationships of the larger (fraction 2) and smaller (fractions 4 and 5) LDL, this possibility is strongly suggested by the data. Possible differences in the pathways for production of larger and smaller LDL may also be reflected in the compositional differences, particularly differences in

cholesteryl ester: triglyceride ratio, among the LDL subfractions described here. While it has recently been reported that the apolipoprotein B in LDL may pass from S_f 10 to S_f 4 subspecies (6), it also has been shown that apolipoprotein B and triglyceride in LDL have different kinetics (15).

Since levels of HDL-cholesterol have been shown to correlate negatively with risk of coronary heart disease (16), and since HDL levels have been shown here to correlate negatively with levels of smaller LDL, the possibility is raised that a positive association may exist between levels of smaller LDL and coronary disease risk. Recently studies in cholesterol-fed rhesus monkeys (17) have suggested that unusually large LDL in the size range of human IDL may be primarily responsible for atherosclerosis. Camejo et al. (18) have reported that certain human LDL bind preferentially to a preparation of arterial wall mucopolysaccharide. Further studies in human populations will be required to resolve whether one or more portions of the LDL distribution might be preferentially involved in the atherosclerotic process. ■■

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