Identification of multiple subclasses of plasma low density lipoproteins in normal humans

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Abstract Density gradient ultracentrifugation of low density lipoproteins (LDL) from 12 normal subjects showed multiple, distinct isopycnic bands. Densitometric scanning of the gradient tubes revealed that each band could be assigned to one of four density intervals and that the boundaries of these intervals were consistent among all the subjects. Analytic ultracentrifuge flotation (S_f^0) rates were assigned to the four density intervals, and there was a strong correlation between peak S_f^0 rate and peak isopycnic banding position (R_f) of the LDL in the 12 subjects. The S_f^0 value corresponding to the boundary between the two most buoyant LDL density subgroups was 7.5. This value is close to that previously demonstrated to define two LDL subdivisions (S_f^0 0–7 and S_f^0 7–12) that were discriminated by differing concentrations in men and women, and differing statistical relationships with levels of HDL and VLDL in a normal population. Further delineation of distinct subspecies of LDL was afforded by electrophoresis in 2–16% gradient polyacrylamide gels. Densitometric scans of protein-stained gels revealed multiple peaks, and particle diameters were assigned to these peaks using calibration markers. Particles of diameter ≥ 280 Å included both IDL and Lp(a), the latter defined by pre-β mobility on agarose electrophoresis and density > 1.050 g/ml. LDL particles with diameters 220–272 Å could be grouped into seven size intervals defined by modes in the distribution of gradient gel electrophoretic peaks in LDL from a group of 68 healthy men and women. Particle diameters of the major peaks in each of seven density subfractions decreased with increasing density of the fractions. However, particles within each of the size groups were distributed across a range of densities. Use of a lipid-staining procedure allowed identification of electrophoretic bands in whole plasma which corresponded to those seen in isolated LDL, eliminating the possibility that ultracentrifugation was responsible for formation of the subspecies detected by the gradient gel procedure. The application of density gradient ultracentrifugation and gradient gel electrophoresis provides a means of characterizing LDL from normal humans in terms of multiple distinct subpopulations which may also prove to have differing metabolic and pathologic properties.—**Krauss, R. M., and D. J. Burke.** Identification of multiple subclasses of plasma low density lipoproteins in normal humans. *J. Lipid Res.* 1982. **23:** 97–104.

Supplementary key words serum lipoproteins • very low density lipoproteins • high density lipoproteins • density gradient ultracentrifugation • analytic ultracentrifugation • polyacrylamide gel electrophoresis

Human plasma low density lipoproteins (LDL) are a heterogeneous collection of particles which vary in buoyant density, size, and lipid and protein composition (1–9). Recently, we have described a technique for equilibrium density ultracentrifugation of low density lipoproteins (d 1.019–1.063 g/ml) from normal human serum (6). Major differences were observed in analytic ultracentrifuge properties, particle diameters, and chemical composition of LDL among six sequential 1-ml density subfractions isolated by this procedure in 12 subjects. With increasing density, peak flotation rates (S_f^0) and mean particle diameters decreased progressively, while protein/phospholipid ratio increased. Cholesteryl ester/triglyceride ratio increased from the first (least dense) to third fractions, and then declined significantly in the two most dense fractions. It was also observed that concentrations of fraction 2 (mean peak S_f^0 8.4) were correlated inversely with levels of VLDL and positively with levels of HDL, while concentrations of fraction 4 (mean peak S_f^0 5.3) were correlated positively with levels of VLDL and inversely with HDL.

In the course of performing these studies, we observed the presence of up to four turbid or yellow bands in the LDL density range after the density gradient ultracentrifugation procedure. We wished to characterize the isopycnic banding further in order to determine if the presence of a set of discrete LDL subclasses could account for the variations among the density subfractions. Identification of these subclasses was made possible by the application of analytic procedures described in the present report.

MATERIALS AND METHODS

Subjects were healthy volunteers (42 men, 26 women), aged 27–58 years, who were within 20% of ideal body weight, were moderate or non-consumers of alcohol, and were not taking hormones or drugs known to affect plasma lipid levels. Plasma cholesterol and triglyceride concentrations were below the 90th percentile for age and sex (10) in all subjects. Blood samples were drawn from subjects after they had fasted 12–14 hr overnight. The blood was collected in tubes containing K_2EDTA

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

or Na_2EDTA, 1 mg/ml, centrifuged at 4°C, and plasma was separated. The d < 1.063 g/ml fraction of plasma was prepared for analytic ultracentrifugation and gradient gel electrophoresis (see below) as described previously (1).

Detailed studies of LDL were performed in 12 of the subjects (6 male, 6 female). LDL was isolated by ultracentrifugation between d 1.019 and 1.063 g/ml (11), and equilibrium density ultracentrifugation of the LDL was performed as described recently (6). Following ultracentrifugation, the tubes were scanned at 455 nm using a Transidyne RFT Scanning Densitometer mounted vertically and then the contents of the tube were withdrawn by pipette in eight fractions, starting with 0.5 ml at the top, then six 1-ml fractions (Fractions 1–6), and 0.5 ml at the bottom (6). A tube containing the salt solutions only was included in each individual centrifugation, and the salt densities of the fractions in this tube were measured by refractometry.

Analytic ultracentrifugation of LDL was carried out at d = 1.063 in a Spinco Model E instrument with schlieren optics as previously described (1). Flotation coefficients were corrected for viscosity, temperature, and concentration, and concentration of S_f^0 0–20 lipoproteins was computed as mg total mass per dl plasma (1).

Electrophoresis of LDL and LDL density subfractions was carried out at 12–14°C in 2–16% polyacrylamide gradient gels (Pharmacia PAA 2/16) at 125V for 24 hr using Tris (0.09 M)–boric acid (0.08 M)–Na_2EDTA (0.003 M) buffer, pH 8.3. Gels were pre-run at 125V for 15 min prior to loading samples. Samples were adjusted to 20% sucrose and 3–10 μl of sample was applied to each lane. The voltage was first adjusted to 20V for 15 min, then to 70V for 15 min, and finally to 125V for 24 hr. The gels were fixed in 50% methanol plus 10% acetic acid containing Coomassie Brilliant blue R-250. Gels were destained with 20% methanol plus 9% acetic acid and were scanned at 555 nm using a Transidyne RFT densitometer. Migration distances for each of the absorbance maxima were determined, and the molecular diameter corresponding to each of these peaks was calculated from a calibration curve using standards of known diameters. The standards included apoferritin and thyroglobulin (Pharmacia), with molecular diameters of 122 Å and 170 Å, respectively, and carboxylated latex beads of diameter 380 ± 7.5 Å (Dow Chemical). The diameter of the latex beads was confirmed by negative staining electron microscopy. For lipid staining, gradient gels were stained with 0.04% Oil Red O (Sigma) in 60% ethanol at 55°C and destained with 10% acetic acid (9).

SDS-polyacrylamide gel electrophoresis was performed using the methods of Kane, Hardman, and Paulus (8) and Laemmli (12). Agarose electrophoresis was carried out as described by Hatch et al. (13).

RESULTS

Ultracentrifugal analyses of LDL

The corrected analytic schlieren patterns of the S_f^0 0–20 lipoproteins in 12 normal subjects are shown in **Fig. 1.** The LDL (S_f^0 0–12 lipoproteins) of most of the subjects showed single, relatively symmetrical peaks. The schlieren pattern of LDL from subject 10 showed two broad peaks. In addition, subjects 5 and 11 showed distinct IDL (S_f^0 12–20) bands.

Inspection of the centrifuge tubes following equilibrium density gradient ultracentrifugation of the LDL (see Materials and Methods) revealed single major bands in some subjects and multiple bands in others. Scanning of the tubes at 455 nm produced patterns depicted in **Fig. 2.** Distinct peaks and shoulders are visible in the scans from each of the subjects. The peaks can be grouped

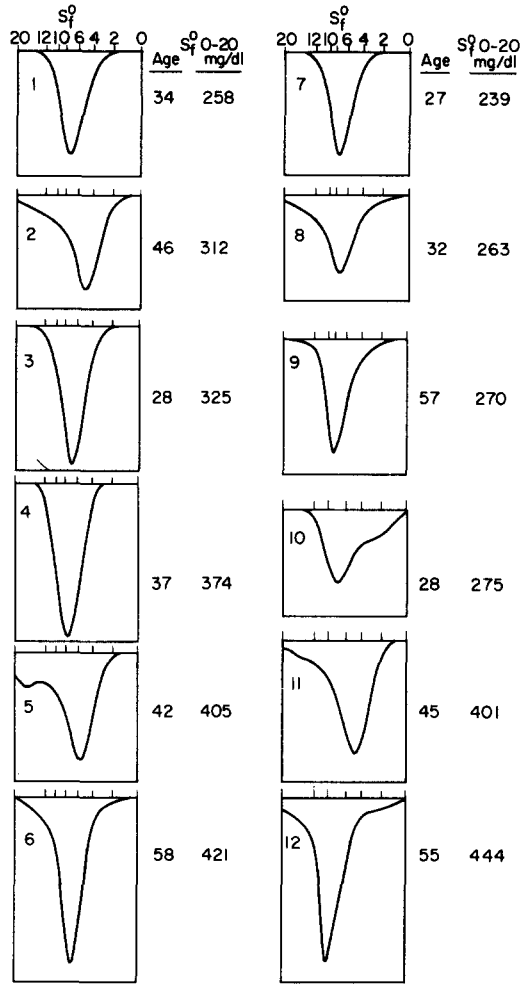

Fig. 1. Analytic ultracentrifuge schlieren patterns of S_f^0 0–20 lipoproteins from 12 normal fasting subjects. Subjects 1–6 are men, 7–12 are women. Subject ages and plasma concentrations of the S_f^0 0–20 lipoproteins (LDL plus IDL) are shown to the right of each pattern.

into four categories according to their isopycnic banding positions, and these groups have been designated I–IV from least to most dense (Fig. 2). Major peaks occur in density group I in subjects 9, 10, and 12; in group II in subjects 3, 4 and 6–10; in group III in subjects 2, 5, and 11, and in group IV in subject 10. Shoulders on the major peaks are seen in most of the subjects. These shoulders are aligned within one or more of the four density groups, e.g., in groups I and III in subjects 3, 4 and 8; and in group II in subjects 2, 5, and 11. The LDL of subjects 1 and 12 banded near the boundary of groups I and II and were not clearly contained within either group. The LDL of subject 10 banded in four distinct peaks. This pattern was reproduced on four separate occasions over a two-year period.

Further characterization of the LDL in each of the four density groups in subject 10 was achieved by analytic ultracentrifuge measurements of peak flotation rates (S_f^0) of seven fractions withdrawn serially after the density gradient ultracentrifugation procedure. These S_f^0 values are plotted in **Fig. 3** on the same scale as the densitometric tracing of the LDL banding pattern. Also plotted are the densities of the background salt solutions measured at

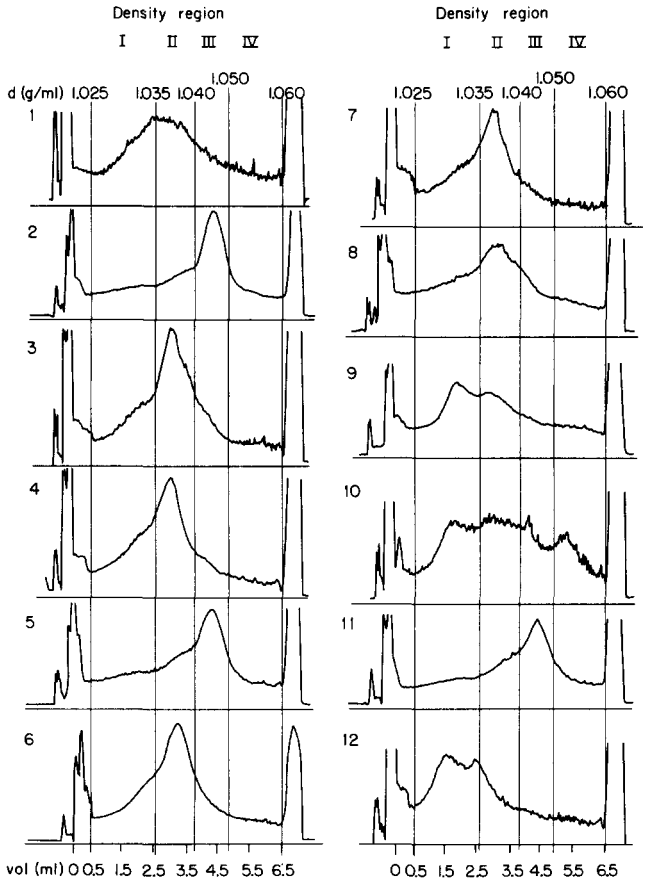

Fig. 2. Densitometric scans (at 455 nm) of equilibrium density gradients of LDL from 12 normal subjects. Subjects are numbered in the same sequence as in Fig. 1.

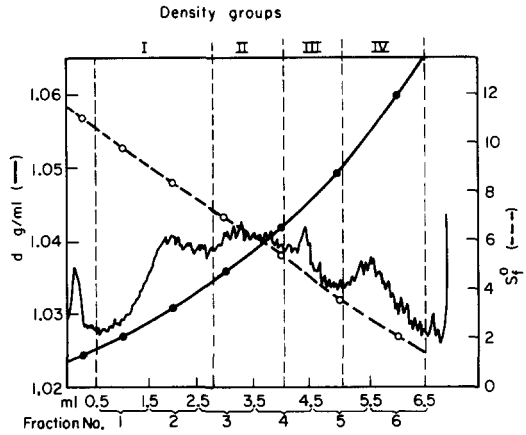

Fig. 3. Densitometric scan (at 455 nm) of the equilibrium density gradient from Subject 10, showing the corrected peak flotation rates (S_f, O – – – O) measured in each of the fractions indicated along the bottom of the figure. Also shown are the densities (g/ml, ● —— ●) of analogous fractions from a gradient containing containing only the salt solutions and centrifuged under the same conditions.

equilibrium in the same fractions. The values obtained matched closely the mean values for these two measurements in LDL subfractions obtained from a group of 12 normal subjects published previously (6). From Fig. 3, it is possible to designate the salt densities and S_f^0 values that encompass the mid-range of each of the four LDL density subgroups: group I, S_f^0 7.5–10, d 1.025–1.035 g/ml; group II, S_f^0 5.7–7.5, d 1.035–1.040 g/ml; group III, S_f^0 4.2–5.7, d 1.040–1.050 g/ml; group IV, S_f^0 1–4.2, d 1.050–1.060 g/ml. These S_f^0 ranges are consistent with the peak S_f^0 and peak density of the major isopycnic bands of the LDL in each of the other subjects. The correspondence between peak S_f^0 and peak band density for the 12 subjects is shown in **Fig. 4.** The strong correlation between these parameters is consistent with the known dependence of peak flotation rate on the buoyant density of LDL particles (1).

Gradient gel electrophoresis of LDL

We next sought to determine whether the LDL particles within each banding region could be discriminated by size as well as by buoyant density. Previous measurements of diameters of LDL subfractions using negative staining electron microscopy established that mean particle diameter decreased with increasing density (6). The diameters, however, appeared to decline progressively and the data did not indicate the presence of discrete size groups which might correspond with the density bands described in the present report. Electrophoresis in polyacrylamide gradient gels (2–16%) provided another means for detecting possible size differences among LDL of differing densities. Application of this technique revealed multiple bands within the total LDL fraction of each of the 12 subjects (**Fig. 5**). Increasing the time of electrophoresis from 24 to 30 hr did not significantly

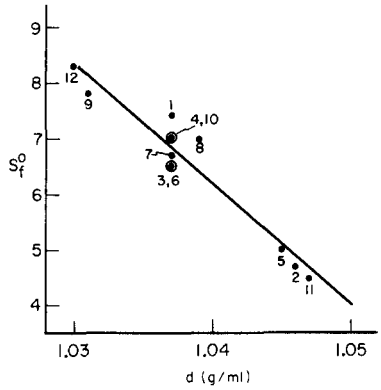

Fig. 4. Correspondence between S_f^0 of the major peak in the analytic ultracentrifuge schlieren pattern and the density at which the predominant LDL species bands in the equilibrium density gradient for each of the 12 subjects presented in Figs. 1 and 2. The line represents a linear regression analysis for the 12 points (r = 0.968).

affect the relative mobility of the LDL peaks. Based on the assumption that there are no major deviations from sphericity among the LDL, migration distance under these conditions is inversely related to particle diameter. The standard deviation of results from eight measurements performed on individual samples (duplicate samples in four different gel runs) ranged from 2.0–2.8 Å (coefficient of variation 0.8–1.0%).

To assess the size heterogeneity of the LDL in relation to the LDL density subgroups, seven LDL subfractions prepared using the density gradient ultracentrifugation procedure described above were analyzed by the gradient gel electrophoresis procedure. Examples of the gradient gel scans of the LDL and LDL subfractions from two of the subjects are shown in **Fig. 6.** Particle diameters of the identifiable LDL peaks were then calculated from a calibration plot using standards of known size (see Methods). These values are shown for each of the cases in Fig. 6. Peaks with particle diameters similar (±3 Å) to those of each of the major peaks identified in the total LDL are discernible in one or more of the density subfractions. In some fractions peaks are seen which are not resolved in the total LDL preparation. The range of particle diameters comprised by the LDL (218–278 Å) corresponds closely to the ranges previously determined by negative staining electron microscopy in a similar group of 12 normal subjects recently studied in this laboratory (6). SDS polyacrylamide gel electrophoresis of the above fractions revealed only B-apolipoprotein.

Particles classified as intermediate density lipoproteins (IDL) by density (<1.020 g/ml) and flotation rate ($S_f^0 \geq 12$) formed a turbid band in the top 0.5 cc (Fraction 0) of the density gradients (Figs. 2 and 3). Diameters of the major gradient gel electrophoretic bands in this fraction generally exceeded 280 Å, although some overlap with LDL-sized particles was also seen (Fig. 6). In some cases, particles with diameters 280–340 Å were also detected in the fractions with highest density (Fractions 5 and 6, d > 1.050 g/ml). This is illustrated in the case of Subject 10 (Fig. 6A). Agarose gel electrophoresis of these fractions stained with Fat Red 7B showed a major band with pre-β mobility in addition to a band with typical β mobility. The particle diameters, banding densities, and electrophoretic mobilities of the lipoproteins in these fractions are consistent with the presence of Lp(a) lipoprotein (14). Additional studies including radioimmunoassay of the Lp(a) antigen in these fractions are in progress to further characterize these particles. In subsequent analyses in this report, only LDL with particle diameters less than 280 Å are included.

Distribution of LDL species according to size and density

Fig. 7 depicts the distribution of particle diameters in the six LDL density fractions for all 12 subjects. The

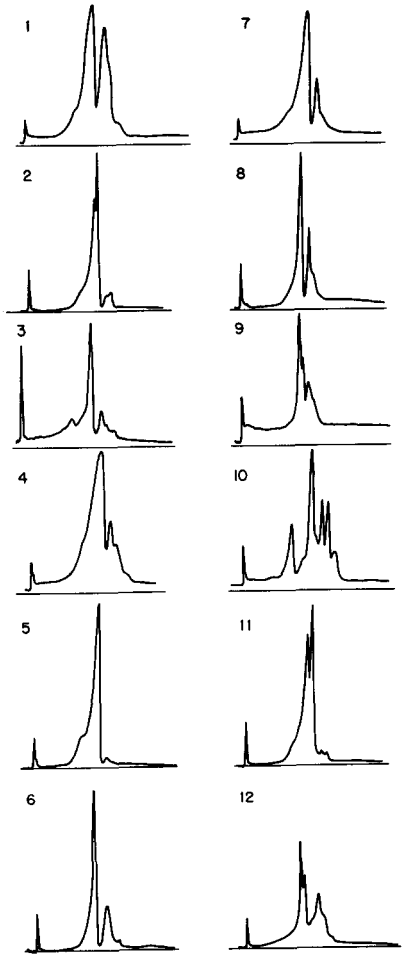

Fig. 5. Gradient gel electrophoretograms of total LDL from 12 normal subjects. Details are described in Materials and Methods. The sharp peak at the left end of each scan represents the top of the gel. Subjects numbered as in Fig. 1.

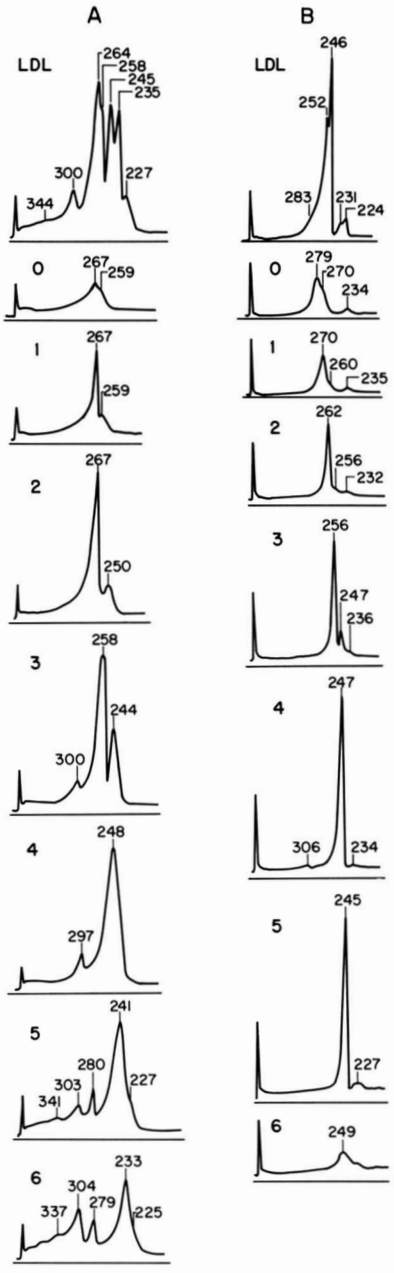

Fig. 6. Gradient gel scans of LDL and LDL density gradient fractions from two normal subjects. Column A shows the scan of the LDL and the LDL fractions from Subject 10. Fraction 0 is the top 0.5-ml fraction from the equilibrium density gradient, while fractions 1–6 are the subsequent 1.0-ml fractions pipetted from the top of the tube. In column B are scans of analogous fractions from Subject 2.

data show that particle diameters of the major LDL species tend to decline with increasing density of the fractions. In each fraction, however, species of smaller and sometimes larger size are also detectable as separate peaks. Thus, while mean diameters of the major species in fractions 1 and 2 are 270 and 267 Å, respectively, minor species are found with peak diameters ranging from 265 to 238 Å. Fractions 5 and 6 include minor species with diameters as small as 219 to 221 Å.

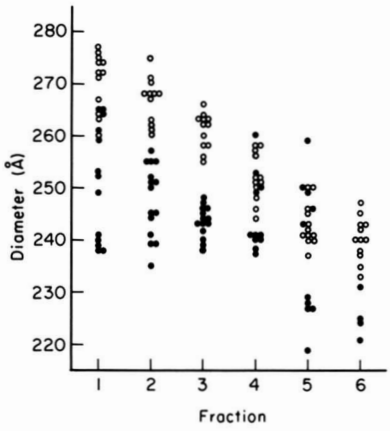

Fig. 7. Particle diameters of the major (○) peaks and minor (●) peaks in density gradient fractions 1–6 from 12 normal subjects. A major peak was identified as the single peak of greatest amplitude in each of the density fractions.

In Fig. 7, there appears to be clustering of values for particle diameters in several size regions. To define possible modes in the particle size distribution of LDL subspecies, the gradient gel electrophoresis procedure was carried out on the d < 1.063 g/ml lipoproteins in a group of 68 normal subjects, including the 12 whose LDL subfractions had been studied in detail. Particle diameters of each distinct electrophoretic peak in LDL from each subject were tabulated and plotted on a histogram within 2-Å size intervals between 220 and 278 Å (**Fig. 8**). The resulting distribution reveals seven discrete modes between 223 and 272 Å. The size groups corresponding to these modes have been designated A–G from largest to smallest diameter. Support for the concept that these groups represent distinct subpopulations of LDL derives from the additional observation that LDL from each individual subject contained at least two, and up to five, peaks with diameters corresponding to different size groups. Furthermore, the multiple peaks in individual LDL represented all possible combinations of pairs of size groups.

It should be noted that the distribution in Fig. 8 does

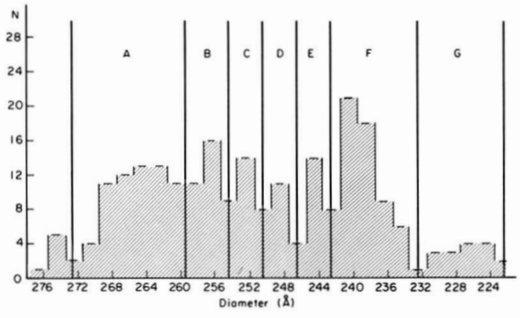

Fig. 8. Distribution of particle diameters of identifiable gradient gel electrophoretic peaks between 220 and 278 Å from the d < 1.063 g/ml lipoprotein fraction from 68 normal subjects.

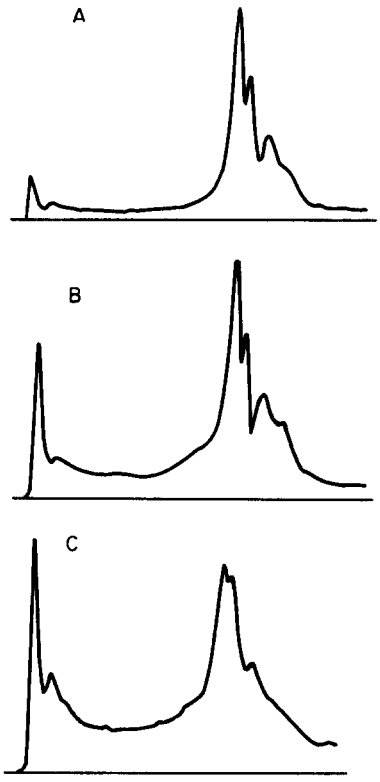

Fig. 9. Gradient gel scans of ultracentrifugally isolated LDL and of whole plasma from Subject 9. Panel A shows Coomassie Brilliant blue staining of isolated LDL; panel B shows lipid stain of isolated LDL; panel C shows lipid staining of whole plasma.

not indicate the absolute incidence of LDL size groups in this population of subjects, but rather the frequency with which individual peaks may be discriminated. For instance, peaks in regions C–E might be underrepresented because of overlap from neighboring peaks. Also, the distribution does not indicate the relative concentrations of the individual species. For example, peaks in size region F are observed frequently, but almost always as minor species in these normal subjects (see Fig. 7). Finally, the groupings represent a minimum number of distinct species, as indicated by the fact that in seven cases more than one peak was observed in size group A.

From Fig. 7, it is apparent that particles in any one of the size groups, and particularly those in groups B–F, may be found across a range of densities after the density gradient ultracentrifugation procedure. The basis for this density heterogeneity cannot be inferred from the present data, but it is possible that the phenomenon represents variations in lipid:protein ratio without detectable change in size or shape of the parent macromolecular structure. The possibility must be considered that the ultracentrifugal procedure may alter LDL structure in such a way as to result in this density heterogeneity, or even in the formation of one or more of the different size species detected by gradient gel electrophoresis. This possibility was examined by comparison of the results of gradient gel electrophoresis of fresh, unfractionated plasma with that of LDL isolated by ultracentrifugation between d 1.019–1.063 g/ml. Staining for protein resulted in the appearance of many bands in whole plasma not detected in the ultracentrifugal fraction. A lipid stain was ultilized to visualize only the lipoproteins. Scans of the lipid-stained gels (**Fig. 9B** and **C**) revealed comparable peaks in whole plasma and isolated LDL, although there was a small difference in the relative amplitudes of the peaks in the two samples. The peaks in LDL visualized by lipid staining also coincided with those observed in the protein-stained electrophoretogram of isolated LDL (Fig. 9A). These findings have been reproduced in 16 additional comparative studies. The results do not rule out the possibility that ultracentrifugation might alter lipid:protein ratios of the LDL subspecies and thus contribute to their density heterogeneity, but it is apparent that ultracentrifugal artefact is not responsible for the formation of the LDL subspecies detected by the gradient gel electrophoresis procedure.

DISCUSSION

Although generally isolated and measured as a single class of lipoproteins within the density range 1.019–1.063 g/ml, human plasma LDL are known to exhibit heterogeneity using several different criteria. These include ultracentrifugal flotation rate and buoyant density (1–6, 15–17), lipid (4–6, 16, 18), and apolipoprotein (7, 8) constituents, particle diameter (6, 9), and isoelectric point (19). In the present report, we have provided evidence for the existence of discrete subpopulations of LDL in normal subjects that can be discriminated on the basis of characteristic hydrated densities and particle sizes.

Previous studies of hydrodynamic properties of LDL have described polydispersity manifest as multiple peaks in the analytic ultracentrifuge schlieren pattern (2, 3, 16, 17). Such polydisperse patterns were found more commonly in patients with hyperprebetalipoproteinemia than in the normal population (3, 20), where the incidence was estimated to be about 9% (20). Most normal subjects were shown to have a major single peak which appeared to be monodisperse (17, 20). The molecular weight of this major species ranged from 2.4–3.9 $\times$ 10^6 daltons in individual subjects and had a strong genetic determinant (20).

Of the 12 normolipemic subjects whose LDL was studied in detail in the present report, only one (Subject 10) had more than one discernible peak in the LDL schlieren pattern. While the LDL in the other subjects appeared to be monodisperse in the analytic ultracentrifuge, further analyses by density gradient ultracentrifugation and gradient gel electrophoresis revealed poly-

dispersity of LDL in all the subjects. Furthermore, the LDL could be characterized in the entire group of subjects by a set of four modes in the density distribution (Figs. 3 and 4), and at least seven modes in the distribution of particle diameters (Fig. 8). This indicates that LDL is more aptly described as paucidisperse (containing a set of discrete components) in all subjects, rather than polydisperse in some and monodisperse in others.

The range of molecular weights of the seven size subgroups of LDL, calculated from increments in particle size and according to the formula Δmolecular weight = Δvolume $\times$ N $\times$ ρ, where ρ is 0.97 g/cc, (2), is approximately 1×10^6 daltons. This figure is comparable to the range of molecular weights of major LDL peaks found among normal individuals as described by Fisher et al. (20). The present data do not permit quantitation of concentrations of individual subspecies, but a qualitative appraisal of the gradient gel electrophoretic peaks in individual subjects (Fig. 5) indicates a great variety of distributions across the entire range of particle size shown in Fig. 8. Thus, the set of components described here represents a major form of LDL heterogeneity present in LDL from all normal subjects that, in most cases, is not resolved by analytic ultracentrifugation.

Within each of the major LDL subgroups defined by gradient gel electrophoresis, there is further heterogeneity of both particle size and density (Fig. 7). The size variation involves a much narrower range than that encompassed by the major LDL species (Fig. 8) and is in part due to the limits of resolution of the electrophoresis procedure (2–3 Å). The density heterogeneity involving particles of similar size (Fig. 7) may have several possible explanations. Some mixing of the isopycnic bands may have occurred as a result of diffusion or disturbance of the density gradient during withdrawal of fractions. It is unlikely, however, that this mixing would account for the entire spectrum of density distribution of the LDL subclasses. Furthermore, mixing would not explain the fact that the range of this density distribution varied considerably, even for particles of similar size and density. It is more likely, as has been suggested previously (5), that certain or perhaps all of the LDL species exhibit differences in buoyant density as a result of variations in lipid content and these variations may not detectably alter overall macromolecular diameter. This may occur as a result of in vivo processes such as lipid or lipid-protein transfers. It is possible that lipid-protein redistributions may occur in vitro, perhaps as a result of ultracentrifugation or other aspects of sample preparation and storage.

However, it is unlikely that the major LDL size groups are formed as a result of preparative artefact, since the number of electrophoretic bands and their relative mobility are the same in whole plasma as in isolated LDL. Similarly, the diameters of gradient gel peaks of the density subfractions agree, within experimental error, with diameters of major peaks present in the total LDL (Fig. 6). These results suggest that the centrifugation procedures do not result in major alterations in LDL particle size, although minor alterations might have gone undetected.

Irrespective of the possible effects of ultracentrifugation, the reproducibility of the size and density groups, and the consistency of the physical and chemical characteristics of the isolated density fractions in subjects with differing LDL distributions (6) support the conclusion that these distributions are determined by variations in concentration of a limited set of distinct LDL components. The gradient gel electrophoresis procedure has been shown here to provide a reproducible method for identifying these components in ultracentrifugal fractions or in whole plasma. In separate studies we have shown that the calculated particle diameters of purified LDL subspecies agreed well (±10%) with those measured by electron microscopy, and were highly correlated with peak analytic ultracentrifuge rates.[1] The latter relationship indicates that the heterogeneity revealed by gradient gel electrophoresis does not differ from that observed by hydrodynamic techniques.

The identification of distinct LDL subspecies with defined size, density, and flotation characteristics provides a framework for investigating physiologic and pathologic determinants of the LDL distribution. It is noteworthy that the boundary between density bands I and II was found to correspond to S_f^0 7.5, a value close to that (S_f^0 7) previously shown (21) to define two major LDL subdivisions that differed in concentration in men versus women. These subdivisions had differing and, in most cases, opposite correlations with serum levels of VLDL, IDL, and HDL (18). It is reasonable to suppose that the triglyceride-enriched LDL found in the larger and less dense subfractions of $S_f^0 > 7$ (6), and represented here primarily in size group A and density region I, may give rise to smaller, dense LDL subspecies via lipolysis and loss of core triglyceride and other lipids. This supposition is supported by studies of heparin-induced lipolysis (22, 23) and by studies of turnover of isotopically labeled LDL subspecies (24). However, in the smallest and most dense LDL subspecies, triglyceride content is increased (6), suggesting that another metabolic pathway is involved in their formation. Some of the smaller, denser subspecies may also conceivably be precursors of larger, less dense LDL subspecies formed as a result of transfers of lipid and protein components to and from these particles.

From a clinical and epidemiologic standpoint, it is apparent from the present findings that measurements of total LDL or LDL cholesterol will fail to distinguish

[1] Burke, D. J., R. M. Krauss, and T. M. Forte. Unpublished results.

LDL distributions which vary widely with regard to relative concentrations of individual subspecies. It has been shown that serum levels of the smaller, slower floating subspecies of $S_f^0 < 7$ are generally higher in men than in women, and are correlated inversely with levels of HDL (21). With further development and application of the techniques described here, it may be possible to identify specific LDL components that are primarily responsible for these relationships and to determine whether measurement of these components can improve the prediction of atherosclerotic risk.

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