Charge properties of low density lipoprotein subclasses

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Abstract Measurement of electrophoretic mobility and particle size of low density lipoproteins (LDL) allowed use of standard electrokinetic theory to quantitate LDL charge characteristics from subjects with predominance of large LDL (pattern A, n = 9) or small LDL (pattern B, n = 8). Pattern A LDL was found to have significantly lower ($P \leq 0.001$) mobility (-0.22 ± 0.01 μm s^{-1} cm V^{-1}), surface potential (-4.2 ± 0.3 mV) and charge density (-500 ± 34 esu/cm^2) than pattern B LDL (-0.25 ± 0.01 μm s^{-1} cm V^{-1}, -4.9 ± 0.3 mV, and -580 ± 30 esu/cm^2), but no significant difference in particle valence (-22.0 ± 1.4 for pattern A vs. -21.8 ± 1.9 for pattern B). Thus, the greater mobility of pattern B LDL is due to similar net charge residing on a smaller particle. Comparison of subfractions in pattern B relative to pattern A LDL revealed greater surface potential in all pattern B subfractions and greater charge density in fractions of $d \geq 1.032$ g/ml. In a subset of subjects incubation with neuraminidase produced significant reductions in all LDL charge parameters for all subfractions, but did not abolish the differences between pattern A and B. Thus increased surface potential and charge density of unfractionated pattern B LDL is due both to charge properties of particles across the size and density spectrum as well as enrichment of pattern B LDL with smaller, denser particles that have higher surface charge density.—**La Belle, M., P. J. Blanche, and R. M. Krauss.** Charge properties of low density lipoprotein subclasses. *J. Lipid Res.* 1997. **38:** 690–700.

Supplementary key words agarose gel electrophoresis • LDL • charge • apoB • LDL subclass

LDL particles exhibit considerable heterogeneity in density, size, and chemical composition (1–7), and may also differ in charge (3, 8–10). LDL in the mid-density range (1.030–1.039 g/ml) have been found to have lower mobility on agarose gels than LDL of lesser or greater density (4, 11). This suggests that the most buoyant and dense LDL particles may have a relatively increased net negative electrical charge compared to mid-density LDL. Modifications that increase the net negative charge of LDL may have important metabolic consequences, such as reduced affinity for the LDL receptor (12, 13). Interestingly, mid-density LDL bind with higher affinity to the LDL receptor (5, 11, 14, 15) and undergo receptor-mediated uptake and degradation at greater rates than LDL of lesser or greater density (11, 15).

Non-denaturing gradient gel electrophoresis has shown that, in a majority of the population, the LDL particle spectrum may be characterized by a predominant peak of larger (pattern A) or smaller (pattern B) diameter LDL (14, 16, 17). We previously reported that the small, dense LDL predominating in pattern B subjects have decreased levels of glycosylation of apolipoprotein B (apoB) and decreased sialic acid content. Despite decreased sialic acid content, dense LDL have greater mobility on agarose gels (3) suggesting a greater net negative charge than the larger LDL that predominate in the pattern A phenotype. However, electrophoretic mobility of particles on agarose gels is determined by surface charge density, which in turn is a function of valence (net negative charge) and (inversely) of particle diameter. Thus, a smaller LDL particle with the same valence as a larger particle would have a greater surface charge density, resulting in greater mobility.

In the present study we used agarose gel electrophoresis to investigate differences in surface potential, charge density, and valence of LDL and LDL subfractions of differing size and density and to test whether there are altered charge characteristics of LDL in pattern B subjects.

MATERIALS AND METHODS

Materials

Bovine serum albumin (BSA), and neuraminidase (Type X) were from Sigma. Trolox (6-hydroxy-2,5,7,8-

Abbreviations: LDL, low density lipoprotein; apo, apolipoprotein; Lp[a], apolipoprotein[a]; TG, triglyceride; FC, free cholesterol; CE, cholesteryl ester; PL, phospholipid; esu, electrostatic unit; kD, kilodalton; LCP, lipoprotein-complexing proteoglycan; BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay; EDTA, ethylenediamine-tetraacetic acid; GGE, gradient gel electrophoresis; AnUC, analytic ultracentrifugation.

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tetramethylchroman-2-carboxylic acid) was from Hoffman La Roche. High molecular weight standards (BSA, lactate dehydrogenase, apoferritin, and thyroglobulin) and low molecular weight standards (phosphorylase B, BSA, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, α-lactalbumin) were from Pharmacia (Piscataway, NJ). Latex beads (0.038 μm diameter) were from Duke Scientific Corp. (Palo Alto, CA.).

Study subjects

Normolipidemic volunteers were recruited for this study based on previously determined LDL particle size as measured by non-denaturing gradient gel electrophoresis analysis of plasma followed by lipid staining. Subjects were categorized according to established criteria for LDL phenotype A or B, related to peak particle size, as described previously (17–21). Plasma samples were obtained after overnight fast from 10 men and 7 women, not on any medication known to alter lipid metabolism. None of the subjects were smokers. Blood was collected by venipuncture into vacutainers containing 1 mg of EDTA per ml and 10 μm Trolox, a water-soluble vitamin E analog. Blood cells were pelleted by centrifugation at 2000 *g* for 30 min at 4°C.

Isolation of LDL and LDL subfractions

LDL (d 1.019–1.063 g/ml) was isolated by sequential ultracentrifugation (22) and subfractionated by density gradient ultracentrifugation as previously described (6) except that 11 rather than 7 fractions were collected (3). The bottom 0.5-ml fraction was excluded from study because of the presence of HDL in some subjects (6). Whole LDL and LDL subfractions were dialyzed into 20 mM Tris-HCl (pH 7.5), 1 mM EDTA, 10 μM Trolox and stored at 4°C under nitrogen.

Determination of lipoprotein composition

Total and unesterified cholesterol and triglyceride were analyzed using enzymatic-endpoint reagent kits (Ciba-Corning Diagnostics Corp., Oberlin, OH), according to the manufacturers' instructions, on a Gilford Impact 500E auto analyzer (Ciba-Corning Diagnostics Corp). Phospholipids were measured using a standardized colorimetric method (23) and a phosphorus calibrator (Sigma Chemical Co., St. Louis, MO). LDL protein concentrations were determined by a modification of the method of Lowry et al. (24) using BSA as the standard. Apolipoproteins B and E were measured using standardized sandwich style ELISA. (25) Apoprotein B calibrators were standardized using CDC # 1883 serum reference material (Center for Disease Control, Atlanta, GA) and reference sera (Northwest Lipid Research Clinic, Seattle, WA). Apoprotein E was standardized using pooled reference sera (Northwest Lipid Research Clinic).

Determination of LDL particle size by gradient gel electrophoresis

LDL particle diameters were determined by nondenaturing 2–14% polyacrylamide gradient gel electrophoresis in 0.09 M Tris/0.08 M borate buffer (pH 8.3), 3 mM EDTA at 8–10°C. Samples were electrophoresed at 40 V for 15 min, then 80 V for 15 min, and then at 125 V for 24 h to allow all particles to run to their size exclusion limits (16, 18, 19). Gels were stained for protein with Coomassie Brilliant Blue R-250 and scanned at 555 nm with a Transidyne RFT densitometer. Particle sizes were calculated from a calibration curve using a high molecular weight reference protein mixture (Pharmacia Biotech., Piscataway, NJ), 380 Å latex beads (Duke Scientific Corp., Palo Alto, CA) and lipoprotein calibrators that are frozen at −80°C and included on each gel run. Plasma samples, stored at −80°C and used as controls for gradient gel analysis procedures, were run in duplicate on each gel. Particle size of LDL peaks in the controls were measured within ± 2 Å (coefficient of variation, ±1%).

Determination of LDL hydrated particle size by analytic ultracentrifugation

LDL subfractions (n = 12) were prepared from two individual subjects and from a pool of five plasma samples. Each LDL subfraction was divided into two equal volumes and dialyzed to NaBr-NaCl salt solutions, d 1.063 and 1.203 g/ml. Each pair of dialyzed samples and their respective salt background was measured by analytic ultracentrifugation. Peak flotation rate, molecular weight, and hydrated particle density (σ) were determined by established analytic methods (22). Minimum molecular weights were determined from flotation velocity measurements and corrected to bring results closer to results obtained by sedimentation equilibrium measurements, as described by Kahlon et al. (26). Particle size, assuming sphericity, was calculated from the following equation:

$$\text{particle diameter (Å)} = \sqrt[3]{\frac{\text{molecular weight}}{0.3153 \times \sigma}} \qquad \textit{Eq. 1)}$$

where hydrated particle density (σ) is closely approximated from the measurement of flotation velocity at background densities 1.063 and 1.203 gm/ml extrapolated to zero migration or rho-intercept, and the factor 0.3153 is the product of calculated molecular volume and Avogadro's number (22). In Eq. 2 the Stokes radius is calculated using the value of Kahlon et al. (26), to correct results obtained by flotation velocity for particle shape and/or hydration, and bring the estimated particle size closer to results obtained by sedimentation equilibrium.

$$\text{radius} = (f/f^0)\ (\text{diameter}/2) \qquad \textit{Eq. 2)}$$

$$\text{and: } f/f^0 = 1.215$$

Control samples were analyzed for Stokes radius of LDL in duplicate on three different analytic ultracentrifuges (Model E, Beckman Instruments, Fullerton, CA) and calculated LDL diameter was within ±1.0 Å (coefficient of variation, ±0.5%).

Particle diameter of the isolated LDL subfractions was also determined by GGE, and a regression equation representing the relationship of these two measurements was obtained and used to convert the GGE particle diameters of experimental samples to equivalent particle diameters based on Stokes radius (see Fig. 1, Results). These values were used for all electrokinetic determinations.

Agarose gel electrophoresis of LDL and LDL subfractions

LDL electrophoretic mobility was determined by electrophoresis of approximately 0.5–1 μg of LDL protein on Beckman Paragon Lipogels. Samples were applied to the gels and allowed to penetrate for 5–10 min before electrophoresis at 100 V for 30 min in the Beckman kit barbital buffer using the Beckman Paragon Electrophoresis System. To correct for gel to gel variations, two lanes of standards (Pharmacia low molecular weight standards) were run on each gel. The gels were then fixed in ethanol–acetic acid–water 4.5:1.4.5 (vol/vol) for 5 min, dried, stained for protein using Coomassie Brilliant Blue R-250, and destained in methanol–acetic acid–water 2:1:8. The gels were scanned using a Molecular Dynamics Personal Densitometer and the migration distance was determined as the distance from the sample application point to the center of the stained band. LDL charge characteristics (electrophoretic mobility, surface potential, valence, and charge density) were calculated from electrokinetic theory as described by Sparks and Phillips (20). Briefly, surface potential (S) was calculated according to equation 3

$$S = U6\pi n/Dc \qquad \textit{Eq. 3)}$$

where U is the corrected particle mobility expressed as (μm/s) × (cm/V), n is the coefficient of viscosity (0.0089 poise for barbital buffer), and Dc is the solvent dielectric constant (78.36 for barbital buffer). Converting from electrostatic volts to ordinary volts (1 statvolt = 300 volts) the equation becomes $S = U \times 9000 \times 6\pi n/Dc = U \times 19.27$. This number differs slightly from the value of 19.35 used by Sparks and Phillips (20) due to a small error in the original calculations.

Valence (V), the net number of negative charges, was calculated according to equation 4:

$$V = (1.049 \times 10^7)Ur(1 + Kr + Kr_i)/(f(1 + Kr_i)) \qquad \textit{Eq. 4)}$$

where U is the corrected particle mobility on the agarose gel, r is the Stokes radius in cm of the particle, r_i is the counterion hydrated radius (in this case Na, 2.5×10^{-8} cm [ref. 19]), f is a function of particle size and thickness of ionic double layer around the particle and is calculated from equation 5:

$$f = (3.66 \times 10^5 \times r) + (-1.74 \times 10^{11} \times r^2) + (3.54 \times 10^{16} \times r^3) + (-1.8 \times 10^{21} \times r^4) + 0.979, \qquad \textit{Eq. 5)}$$

and K is the Debye-Huekel constant which is calculated from equation 6

$$K = \sqrt{I}/(3.06 \times 10^{-8}) \times 10^{-11})/r^2. \qquad \textit{Eq. 6)}$$

where I is the electrolyte ionic strength (0.05 for barbital buffer). Charge density was calculated as a function of valence from equation 7:

$$Cd = (V \times 3.82) \qquad \textit{Eq. 7)}$$

Neuraminidase treatment of LDL

LDL samples from 13 subjects (6 pattern A and 7 pattern B) were dialyzed overnight at 4°C versus 80 ml of 40 mM sodium acetate buffer (pH 5.0), 1 mM EDTA, and 10 μM Trolox. The samples were then incubated in dialysis buffer containing 10 mM $CaCl_2$ and 50 mU of neuraminidase per 0.1 mg of LDL protein. Control samples were incubated in dialysis buffer with $CaCl_2$. After incubation for 24 h at 27°C under nitrogen, aliquots were removed for electrophoresis on agarose gels.

Statistical analysis

Student's paired *t*-test and correlation coefficients were determined using Statview (Abacus Concepts, Inc.). Multiple regression models including subject and subfraction density were used to assess relationships of LDL charge parameters with indices of density and composition (SuperANOVA, Abacus Concepts, Inc.). Post hoc analysis was performed using the Schiffe test. In all analyses $P < 0.05$ was considered significant.

RESULTS

Particle diameter and plasma lipid levels

Isolated LDL subfraction particle diameters measured by GGE correlated strongly ($r = 0.989$) with diameters measured by AnUC as shown in **Fig. 1.** LDL isolates were relatively homogeneous in size distribution as determined by gradient gel profiles (data not shown), and

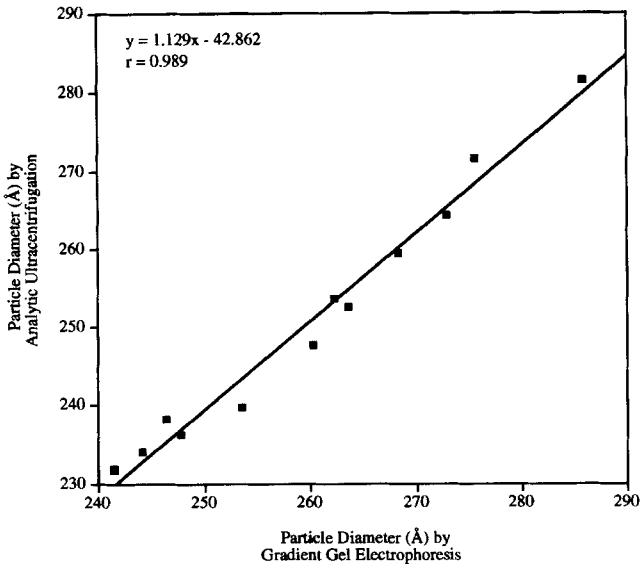

Fig. 1. Plot of isolated LDL subfraction particle diameters by GGE vs. AnUC (n = 12). The regression equation shown in the figure was used to adjust particle size of experimental samples measured by GGE to equivalent hydrated particle diameter as measured by AnUC.

the 12 fractions studied represented LDL species with peak particle sizes ranging from 286Å to 241Å by GGE and 281Å to 232Å by AnUC. Results for particle diameters using both methods are shown in **Table 1,** along with individual and mean values for plasma lipids. Subjects with LDL subclass pattern B did not differ significantly from the subjects with pattern A in age, total cholesterol, or LDL cholesterol, but as reported previously (16, 27) pattern B subjects had higher levels of plasma triglycerides and lower levels of HDL cholesterol.

Charge of LDL from pattern A and B subjects and effects of neuraminidase

Table 2 summarizes the charge characteristics of unfractionated LDL from all subjects. The charge values reported here differ from those reported previously by Sparks and Phillips (20) due to an error in the computer program originally used to calculate LDL charge (M. C. Phillips, personal communication). LDL from subjects with pattern A had significantly lower ($P \leq 0.001$) mobility, surface potential, and charge density than LDL from pattern B subjects. Notably, however, there was no difference in valence between pattern A and B LDL.

Treatment of LDL with neuraminidase (**Table 3**) resulted in significant ($P \leq 0.01$) reductions in LDL mobility, surface potential, charge density, and valence in both groups. Despite these changes, pattern B LDL retained significantly greater ($P < 0.03$) mobility, surface potential, and charge density than did pattern A LDL. The resulting negative valence was approximately 1 unit greater for pattern B than pattern A LDL, but this difference was not statistically significant. Thus, differences in degree of sialation do not appear to explain the charge differences found between pattern A and B LDL.

For all subjects combined, significant correlations were found between LDL diameter and both LDL valence ($r = -0.60$, $P = 0.01$) and charge density ($r = 0.62$, $P = 0.02$). When LDL pattern A and B phenotypes were analyzed separately, a significant correlation ($r = 0.89$, $P = 0.004$) was found between LDL diameter and valence in the pattern B group but not in the pattern A group. None of these correlations were significant in the subset of samples analyzed after neuraminidase treatment.

Charge differences among LDL subfractions

To determine whether the differences in charge of LDL from pattern A and B subjects were due to differences between the large LDL particles characteristic of pattern A and the smaller LDL prevalent in pattern B, or to differences throughout the LDL size and density spectrum, we examined subfractions generated by density gradient ultracentrifugation. **Table 4** shows the results from all subjects combined. Both surface potential and charge density were significantly higher in the most dense subfraction (d = 1.060 g/ml) whereas valence was greatest in the two most buoyant fractions (d $\leq$ 1.027 g/ml).

Comparison of the charge characteristics of the same LDL subfractions between pattern A and B subjects (**Fig. 2A–C**) showed that, for all the subfractions, pattern B LDL consistently had a significantly greater surface potential ($P \leq 0.03$) than did the corresponding pattern A subfractions. Similarly, the charge density of all LDL subfractions in pattern B was consistently greater than in the corresponding pattern A subfractions, although this difference reached statistical significance ($P \leq 0.04$) only in subfractions 4–10. The valence of pattern B LDL in subfractions 1–10 was also consistently greater than the corresponding pattern A LDL, reaching statistical significance ($P \leq 0.04$) in subfractions 1, 2, 5, and 10.

Incubation with neuraminidase significantly ($P < 0.001$) reduced surface potential and charge density in all LDL subfractions of both pattern A and B subjects (**Fig. 3**). After neuraminidase treatment, ANOVA revealed that the surface potential and charge density of subfraction 10 remained significantly greater ($P < 0.05$) than in subfractions 2 through 8. Treatment with neuraminidase reduced LDL valence significantly ($P < 0.001$) in all subfractions but in general did not alter the relative differences among the subfractions.

Further, after neuraminidase treatment, pattern B

TABLE 1. Comparison of age, sex, diameter of the main LDL, plasma triglyceride levels, and cholesterol levels of subjects in LDL subclass pattern A versus subjects in LDL subclass pattern B

Pattern	Age	Sex	Triglycerides	Cholesterol			Peak LDL Diameter from	
				Total	HDL	LDL	GGE	AnUC
			mg/dl		*mg/dl*		*Å*	
A	62	M	129	231	60	145	262	253
	42	F	67	169	63	93	266	257
	38	F	111	111	31	58	274	266
	63	M	62	199	60	127	278	271
	42	F	58	142	44	86	267	259
	42	F	41	166	63	95	274	266
	43	F	92	157	54	85	269	261
	49	M	103	189	66	102	264	255
	46	M	65	249	67	169	266	257
Average	47 ± 9		81 ± 29	179 ± 43	56 ± 12	107 ± 34	268 ± 6	261 ± 6
B	41	F	122	211	53	134	256	246
	42	M	89	176	54	104	256	246
	39	F	107	222	52	149	249	238
	48	M	112	266	45	199	258	248
	49	M	136	205	36	142	258	248
	47	M	194	114	32	43	237	225
	37	M	346	317	35	213	247	236
	69	M	228	264	44	174	246	235
Average	47 ± 10		167 ± 86	222 ± 62	44 ± 9	107 ± 34	251 ± 8	240 ± 8
Significant at P =	NS		0.01	NS	0.01	NS	0.0003	0.0003

LDL subfractions retained greater surface potential and charge density ($P < 0.05$) than did the corresponding pattern A LDL subfractions (Fig. 3), although this reached statistical significance only for surface potential (Fig. 3D) in subfractions 4–10, probably due to the smaller sample size, and for charge density in subfractions 3–6 and 8–10 (Fig. 3E). Valence of pattern B LDL after neuraminidase treatment remained greater in all pattern B subfractions and was significantly greater ($P < 0.05$) in subfractions 3, 5 and 8–10 (Fig. 3F).

Composition of LDL and LDL subfractions

Analyses of lipid and apoprotein content of unfractionated LDL and individual LDL subfractions were performed (**Table 5**) to assess whether variation in these parameters was related to the observed valence and charge density differences among the fractions.

Compared with pattern A, the percent by weight of protein and triglyceride in pattern B LDL was found to be significantly greater ($P = 0.04$ and 0.019, respectively) while the percent free cholesterol was significantly lower ($P = 0.008$) throughout. No significant differences were found between the percent cholesteryl ester or the percent phospholipid in pattern A and pattern B LDL. For the subfractions, pair-wise comparisons of LDL protein, cholesteryl ester, and phospholipid composition revealed no significant differences between pattern A and B. The triglyceride content of pattern B LDL subfractions 1 through 7 was significantly greater ($P < 0.04$) than in the corresponding pattern A LDL subfractions, while the free cholesterol content in pattern B LDL subfractions 3 through 9 was significantly lower ($P \leq 0.02$).

Multiple regression analysis (results not shown), ad-

TABLE 2. Charge characteristics of unfractionated LDL in pattern A or pattern B subjects

Variable	All Subjects	Pattern		P (A vs. B)
		A	B	
Mobility ($-\mu m\ s^{-1}\ cm\ V^{-1}$)	0.23 ± 0.02	0.22 ± 0.01	0.25 ± 0.01	0.001
Surface potential (−mV)	4.5 ± 0.4	4.2 ± 0.3	4.9 ± 0.3	0.001
Charge density ($-esu/cm^2$)	538 ± 52	500 ± 34	580 ± 30	0.0002
Valence (−e)	21.9 ± 1.6	22.0 ± 1.4	21.8 ± 1.9	NS

Values given are the mean ± SD.

TABLE 3. Charge characteristics of unfractionated LDL in pattern A and B subjects before and after neuraminidase treatment

Variable	± Neuraminidase	All Subjects	Pattern A	Pattern B	*P* (A vs. B)
Mobility (−μm s^{-1} cm V^{-1})	−	0.24 ± 0.02	0.23 ± 0.01	0.25 ± 0.01	0.003
	+	0.18 ± 0.03[a]	0.16 ± 0.01[a]	0.19 ± 0.03[a]	0.026
	+ Neur. loss	0.06 ± 0.02	0.07 ± 0.01	0.06 ± 0.02	NS
Surface potential (−mV)	−	4.7 ± 0.3	4.3 ± 0.4	4.9 ± 0.3	0.003
	+	3.5 ± 0.5[a]	3.1 ± 0.3[a]	3.7 ± 0.5*	0.026
	+ Neur. loss	1.2 ± 0.4	1.2 ± 0.3	1.2 ± 0.5	NS
Charge density (−esu/cm^2)	−	556 ± 39	521 ± 12	587 ± 24	0.001
	+	410 ± 61[a]	367 ± 27[a]	447 ± 59[a]	0.015
	+ Neur. loss	146 ± 46	153 ± 30	140 ± 58	NS
Valence (−e)	−	22.1 ± 1.6	22.4 ± 0.8	21.9 ± 2.1	NS
	+	16.3 ± 2.1[a]	15.8 ± 1.6[a]	16.7 ± 2.5[a]	NS
	+ Neur. loss	5.8 ± 1.8	6.6 ± 1.1	5.2 ± 2.2	NS

Values given are the mean ± SD.
[a]Significantly different from untreated LDL, $P < 0.01$.

justing for differences among individual subjects and subfractions, revealed no significant relationships between variation in subfraction valence or charge density and any of the measured lipids among the pattern A subfractions. However, there were strong negative relationships of relative protein content with valence and charge density ($P = 0.0001$; β coefficient = −0.992 and −18.6 for valence and charge density, respectively). In LDL subfractions from pattern B subjects, there were significant relationships between variation in valence and amount of protein ($P = 0.005$, $\beta = 0.022$), triglyceride ($P = 0.0001$, $\beta = -1.83$), free cholesterol ($P = 0.005$, $\beta = 0.725$), and cholesteryl ester ($P = 0.0001$, $\beta = 2.02$). Variation in subfraction charge density for pattern B also related significantly to content of triglyceride ($P = 0.005$, $\beta = 0.070$), free cholesterol ($P = 0.02$, $\beta = -0.004$), and cholesteryl ester ($P = 0.003$, $\beta = -0.072$).

DISCUSSION

In this study quantitative measurements of LDL charge parameters were used to identify the physical and chemical factors associated with the greater mobility of small dense LDL compared with large buoyant LDL on agarose gel electrophoresis, and to demonstrate differences in charge characteristics of LDL from subjects with a predominance of large (pattern A) versus small (pattern B) LDL. Among LDL density subfractions, charge density and surface potential were found to be greatest in the most dense LDL particles while LDL negative valence was greatest in the most buoyant LDL. This finding is consistent with results of an earlier study (4) although in that report the LDL subfractions at both extremes of the density range had greater mobility on agarose gels compared to the middle density fractions. These authors hypothesized that this was due

TABLE 4. Charge characteristics of LDL density subfractions

Subfraction	Volume (ml)	Density (g/ml)	Mobility (μmcm/Vs)	Surface Potential (−mV)	Charge Density (−esu/cm^2)	Valence (−e)
1	0.5	1.024	0.246 ± 0.033	4.7 ± 0.5	547 ± 48	27.6 ± 3.0[c]
2	1.0	1.027	0.241 ± 0.034	4.6 ± 0.5	536 ± 55	25.4 ± 2.8[d]
3	0.5	1.030	0.235 ± 0.026	4.5 ± 0.4	515 ± 45	23.5 ± 2.3
4	0.5	1.032	0.233 ± 0.024	4.5 ± 0.3	517 ± 37	22.8 ± 2.0
5	0.5	1.034	0.236 ± 0.023	4.5 ± 0.4	521 ± 44	22.1 ± 1.9
6	0.5	1.037	0.235 ± 0.027	4.5 ± 0.5	527 ± 58	21.4 ± 2.5
7	0.5	1.040	0.237 ± 0.028	4.5 ± 0.5	534 ± 50	20.5 ± 2.1
8	0.5	1.043	0.241 ± 0.020	4.6 ± 0.4	543 ± 44	20.2 ± 1.8
9	1.0	1.049	0.256 ± 0.029	4.9 ± 0.6	584 ± 70	20.3 ± 2.4
10	1.0	1.060	0.277 ± 0.034[a]	5.3 ± 0.7[a]	630 ± 75[b]	21.2 ± 3.0

Values given are the mean for all subjects ± SD.
[a]Indicates fractions significantly different ($P < 0.05$) from subfractions 3–7.
[b]Fractions significantly different from subfractions 1–8.
[c]Fractions significantly different from subfractions 3–10.
[d]Fractions significantly different from subfractions 6–9.

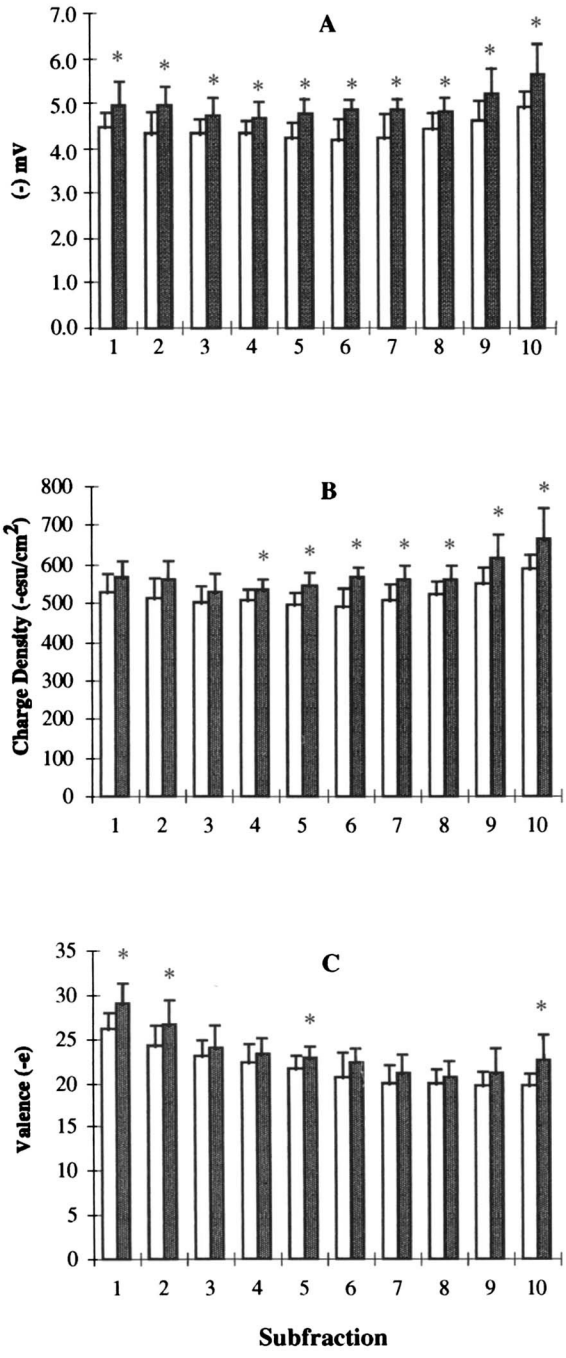

Fig. 2. Charge characteristics of LDL density subfractions from subjects with LDL subclass patterns A (n = 9) and B (n = 8). Charge characteristics were determined from the migration of 0.5–1.0 μg protein from each subfraction on agarose gels as described in Methods. Error bars show the standard deviation and an * indicates significant differences between pattern A (open bars) and B (shaded bars). (A) Surface potential of LDL subfractions, calculated from equation 3. (B) Charge density of LDL subfractions, calculated from equation 7. (C) Net negative electrical charge (valence) of LDL subfractions, calculated according to equation 4. In C, the horizontal line shows the average valence in unfractionated LDL for all subjects.

to increased net negative charge of the LDL at the extremes of the density spectrum. Our quantitative analysis shows that this hypothesis is correct only for the larger LDL particles found in the lower density subfractions. The increased mobility on agarose gels of the most dense LDL is primarily due to a smaller diameter relative to negative charge, resulting in an increased surface charge density.

Determination of particle valence and charge density is partially dependent on the hydrated particle diameter (equations 4 and 7). Measurements of LDL particle diameter by AnUC, GGE, electron microscopy, laser light scattering, and sedimentation equilibrium have all yielded particle diameters differing by as much as 68 Å (28) and each method has potential weaknesses in determining particle diameter GGE, laser light scattering, and AnUC have the advantage of high precision and reproducibility (±1 Å, CV = 0.5%). For GGE however, a correction factor(s) for any distortions from sphericity introduced by the gel matrix is not known, whereas for AnUC a correction factor for shape distortion due to particle compression by *g*-forces has been estimated (29). Because as shown in Fig. 1, LDL particle diameter measurements from AnUC and GGE are highly correlated, we used this linear relationship to convert the more easily obtained GGE results to AnUC particle diameters for calculating valence and charge density. As expected, based on the strong correlation between particle diameters estimated by GGE and AnUC, statistical significance of the differences in valence and charge density between LDL subfractions described here were similar when based on particle diameters measured by either technique (data not shown).

The increased valence of buoyant LDL could be related to higher sialic acid content of these subfractions. However, while all subfractions had significant decreases in all charge parameters after neuraminidase treatment, the valence of the most buoyant LDL fractions remained higher than in the denser fractions, although the differences did not reach statistical significance. In addition, denser LDL retained greater mobility and charge density. Thus differing sialic acid content does not appear to be responsible for differences in charge properties across the LDL density spectrum.

It is unlikely that variation in Lp[a] content contributed to the charge differences at any LDL subfractions as Lp[a] has pre-β mobility (30) and immunoblots for Lp[a] (not shown) demonstrated only trace amounts of Lp[a] in the denser LDL. Another possibility that might account for the increased mobility and charge density of the dense LDL is oxidative modification. Oxidation can increase LDL electrophoretic mobility (7) and small dense LDL is known to be more susceptible to oxidation than large buoyant LDL (7). In addition an LDL particle with increased electronegative charge

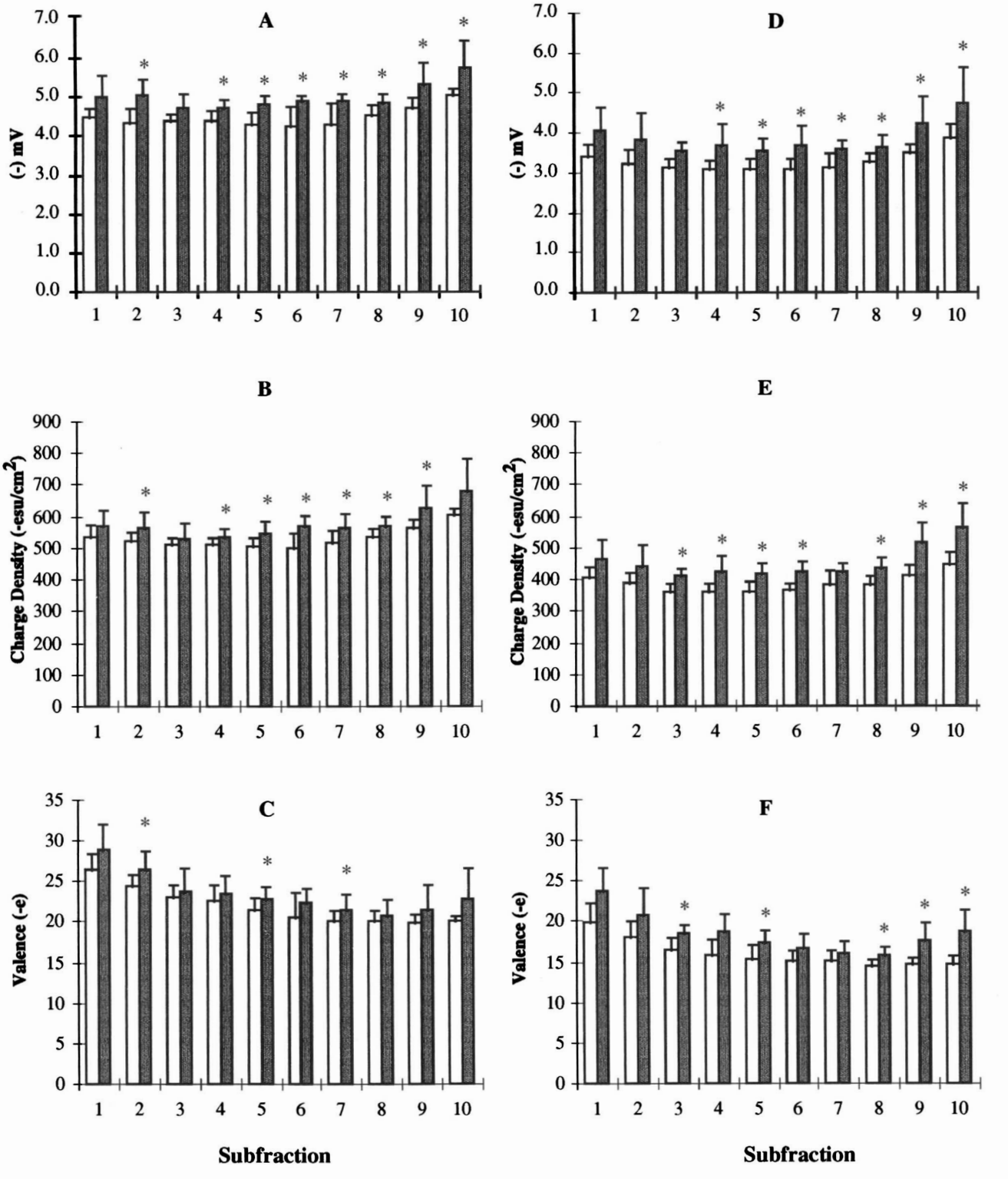

Fig. 3. Effect on LDL charge characteristics of neuraminidase treatment of LDL isolated from subjects with LDL subclass pattern A (n = 6) or pattern B (n = 7). LDL subfractions were incubated with or without neuraminidase as described in Methods. An * indicates significant differences between pattern A (open bars) and B (shaded bars). Error bars indicate the standard deviations. (A–C) Surface potential, charge density and valence, respectively, of untreated LDL subfractions. (D–F) Surface potential, charge density and valence, respectively, of neuraminidase-treated LDL subfractions.

TABLE 5. LDL composition and particle diameter in unfractionated LDL and LDL density subfractions from subjects with LDL subclass pattern A or LDL subclass pattern B

Subfraction	% of Total Protein	Particle Diameter (Å)	% Composition				
			Protein	TG	FC	CE	PL
Pattern A Subjects							
Unfractionated	—	261 ± 6	20.1 ± 0.8	4.4 ± 0.9	8.7 ± 0.8	44.8 ± 2.2	22.0 ± 1.5
1	4.4 ± 2.8	274 ± 8	16.9 ± 1.0	11.6 ± 4.3	8.8 ± 2.7	40.1 ± 4.1	22.5 ± 1.4
2	8.8 ± 3.5	268 ± 5	18.3 ± 1.5	7.3 ± 2.5	8.9 ± 1.6	43.2 ± 2.0	22.3 ± 1.0
3	8.4 ± 3.2	264 ± 5	18.2 ± 1.6	4.3 ± 1.3	9.3 ± 1.2	44.8 ± 1.7	23.3 ± 1.5
4	15.6 ± 2.6	261 ± 8	19.7 ± 1.2	3.5 ± 0.7	9.7 ± 1.0	44.9 ± 2.0	22.2 ± 1.1
5	19.7 ± 2.1	257 ± 8	21.1 ± 2.1	2.9 ± 0.6	9.7 ± 1.7	45.4 ± 2.4	20.8 ± 1.1
6	14.6 ± 2.1	254 ± 7	20.8 ± 1.1	2.8 ± 0.7	8.9 ± 0.6	45.4 ± 2.0	22.2 ± 1.8
7	10.6 ± 2.4	247 ± 6	22.3 ± 1.3	3.0 ± 1.0	7.6 ± 1.5	45.7 ± 2.6	21.4 ± 0.8
8	7.3 ± 2.2	241 ± 5	24.2 ± 3.0	3.3 ± 1.2	7.4 ± 0.9	44.4 ± 2.2	20.7 ± 2.1
9	7.6 ± 2.6	234 ± 3	26.2 ± 2.0	5.4 ± 1.4	7.1 ± 1.4	42.4 ± 5.1	19.0 ± 5.6
10	3.1 ± 2.4	226 ± 5	28.5 ± 4.7	5.5 ± 0.7	7.2 ± 4.0	40.4 ± 2.5	18.4 ± 6.8
Pattern B Subjects							
Unfractionated	—	240 ± 8	23.1 ± 1.5	7.8 ± 3.3	5.7 ± 0.6	42.8 ± 5.0	20.5 ± 2.9
1	4.5 ± 3.5	279 ± 10	15.0 ± 1.6	20.5 ± 6.5	7.8 ± 0.8	34.2 ± 6.2	22.5 ± 0.8
2	6.2 ± 3.9	269 ± 8	17.8 ± 2.2	12.4 ± 3.6	7.4 ± 0.7	39.4 ± 4.2	23.0 ± 1.8
3	4.0 ± 2.0	263 ± 5	19.8 ± 1.8	7.5 ± 0.9	7.6 ± 0.6	41.2 ± 4.4	23.9 ± 2.7
4	6.3 ± 4.5	260 ± 3	21.0 ± 1.9	7.0 ± 1.3	7.0 ± 0.8	41.4 ± 3.1	23.5 ± 1.7
5	9.0 ± 6.8	252 ± 6	21.3 ± 1.8	6.6 ± 1.3	6.7 ± 0.9	42.2 ± 3.2	23.2 ± 2.6
6	11.8 ± 4.7	246 ± 7	21.8 ± 1.9	5.9 ± 1.1	6.3 ± 0.8	42.9 ± 2.6	23.0 ± 2.3
7	15.3 ± 5.4	240 ± 7	23.5 ± 1.9	4.7 ± 1.0	5.5 ± 0.9	43.6 ± 3.0	22.7 ± 1.2
8	18.2 ± 7.8	236 ± 6	23.2 ± 4.6	4.0 ± 1.0	5.2 ± 0.8	49.1 ± 5.3	20.9 ± 2.0
9	20.0 ± 7.6	229 ± 6	25.6 ± 2.0	3.8 ± 1.4	4.7 ± 0.5	47.2 ± 5.8	21.0 ± 1.7
10	4.8 ± 4.0	224 ± 9	29.1 ± 1.7	5.8 ± 1.6	3.9 ± 1.5	43.1 ± 7.8	19.8 ± 3.6

Fractions have the same volume and density as for Table 4. Values given represent the mean ± SD, expressed as percent composition. The distribution of protein across the LDL subfractions, expressed as % of total protein, was calculated for each subject by taking the sum of the protein in all subfractions as 100%. For the compositional data, the sum of the mass of all LDL components for each subject was taken as 100%. Statistical analysis of the results is described in the text.

(LDL−) has recently been isolated from plasma (31–33). Although the density distribution of LDL− is shifted towards the more dense fractions (31, 33), the relative quantity measured in these fractions appears to be small and not sufficient to account for the increased charge density and mobility of the dense LDL fraction.

We have found that while pattern B LDL has significantly greater mobility, surface potential, and charge density than does pattern A LDL, valence of unfractionated LDL does not differ significantly between pattern A and B. Thus, a predominance of smaller particles with increased surface charge density accounts for the observed increased mobility on agarose gels.

Examination of the particle valence and distribution of protein in the density subfractions (Tables 4 and 5, respectively) explains why, in the unfractionated LDL, the smaller pattern B LDL possesses approximately the same valence as the larger pattern A LDL. In both pattern A and B subjects, density subfractions that account for a majority of the LDL protein (subfractions 4 through 6 for pattern A subjects and subfractions 7 through 9 for pattern B subjects) have essentially the same valence (Fig. 2C). Thus, while the pattern B LDL subfractions have increased valence compared to the same pattern A LDL subfractions, the subfractions comprising the majority of the LDL protein have very similar valence.

Electrophoretic mobility, surface potential, valence, and charge density of pattern B LDL were increased across the entire size and density range of LDL particles in pattern B subjects. A possible explanation for these differences would be greater sialation of pattern B LDL. While greater sialic acid content could result in increased negative charge, we have previously found that sialic acid content is lower in pattern B LDL than in pattern A LDL (3). Further, while incubation with neuraminidase produced significant reductions in all LDL charge parameters for both pattern A and B LDL and LDL subfractions (Table 3 and Fig. 3, respectively), this did not abolish the significant charge differences between pattern A and B LDL or result in pattern B LDL losing more net negative charge than did pattern A LDL. Moreover, although the difference did not reach statistical significance, neuraminidase-treated pattern A LDL showed a slightly greater loss in valence than did pattern B LDL, consistent with the increased sialic acid content in pattern A LDL reported previously (3). Thus differing LDL sialic acid content does not appear to

contribute to the charge differences between pattern A and B LDL.

Other components of LDL that can contribute to LDL charge are phospholipids, apoprotein components, and neutral lipid (e.g., by affecting protein conformation). Compositional analysis of unfractionated LDL and LDL density subfractions (Table 5) showed that pattern B LDL had significantly increased protein and triglyceride and decreased free cholesterol compared to pattern A LDL. As a number of studies have shown that alterations in LDL lipid composition can alter the expression of certain apoB epitopes (34–38), it is possible that such conformational differences result in altered exposure of charged amino acid residues. The finding of a significant relationship between relative protein content and valence in pattern A and B LDL subfractions and of valence with other lipid components in pattern B fractions is consistent with this hypothesis. It is of interest that total phospholipid content did not relate to LDL charge properties among the LDL subfractions. It remains possible, however, that differences in content of individual phospholipid species might contribute to charge differences among the subfractions.

Both more buoyant and dense LDL subfractions are apoE-enriched (11), and the distribution of the apoC lipoproteins among LDL density subfractions also varies (1). Thus, it is also possible that LDL subfractions with increased valence or charge density might be enriched in apoE and/or apoC.

The differences in valence and charge density that we have found among LDL particles in different density subfractions may affect the metabolic fate of these particles. Production of LDL with an increased net negative charge by modification of lysine residues by acetylation, carbamylation, glycation, or oxidation all lead to increased uptake by macrophages through the scavenger receptor system (39, 40). Increasing the net negative charge of LDL by acetylation or addition of various free fatty acids was also found to increase the rate of cholesterol transfer into LDL by cholesterol ester transfer protein (41, 42). Reducing the increased negative charge of acetylated LDL by addition of phospholipid significantly reduced its ligand activity for the macrophage scavenger receptor (43), suggesting that LDL surface charge density is a significant factor in interaction with the receptor. Additionally, LDL from subjects with hypercholesterolemia have been reported to show altered mobility on agarose gel electrophoresis suggesting an altered net particle charge which could result in (or perhaps be a consequence of) abnormal metabolism of these LDL particles (9, 10).

In conclusion, these results indicate that electrical charge differences across the LDL particle spectrum and between pattern A and pattern B LDL phenotypes are due to complex interactions among LDL particle diameter, valence, and chemical composition. The physical and chemical properties underlying these charge differences may have significant metabolic consequences.

We wish to thank Drs. Michael C. Phillips and Alex V. Nichols for valuable discussions, Joe Orr for analytic ultracentrifugation, and Laura Holl for gradient gel and biochemical measurements. This work was supported by the National Institutes of Health Program Project Grant HL 18574 from the National Heart, Lung, and Blood Institute, a grant from the National Dairy Promotion and Research Board and administered in cooperation with the National Dairy Council, and was conducted at the Lawrence Berkeley National Laboratory through the U.S. Department of Energy under Contract No. DE-AC03-76SF00098.

Manuscript received 7 August 1996 and in revised form 31 December 1996.

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