

papers and notes on methodology

Lipoprotein separation and low density lipoprotein molecular weight determination using high performance gel-filtration chromatography

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Abstract Plasma lipoproteins were isolated at $d < 1.225$ g/ml from nonhuman primates of three species, cynomolgus, rhesus, and African green (vervet) monkeys. Individual lipoprotein classes were separated by high performance gel-filtration chromatography and low density lipoprotein (LDL) molecular weight was determined. A comparison was made using column configurations including TSK 3000 SW, 4000 SW, and 5000 PW columns. Due to its relative simplicity, stability, and economy, a single 5000 PW column was selected for most of the work. The recovery of lipoprotein cholesterol from the column averaged $91 \pm 2.5\%$. A comparison of the immunologic, chemical, and electrophoretic properties of high density lipoproteins (HDL) and LDL isolated by this technique with those of HDL and LDL isolated by conventional agarose column chromatography indicated that lipoproteins isolated by high performance gel-filtration chromatography were intact and reasonably free of cross contamination. A standard preparation of ^{125}I -labeled LDL was added to the $d < 1.225$ g/ml lipoprotein fraction just prior to separation and a relative size index, r_1 , was determined. When r_1 values for a large number of samples were compared with the log of the LDL molecular weight (determined by agarose column chromatography) a linear relationship was found with a correlation coefficient, $r = 0.85$. The regression equation for this relationship could be used to calculate LDL molecular weights from the r_1 value. These values agreed with LDL molecular weight determined by flotation equilibrium analysis in the analytical ultracentrifuge. We conclude that high performance gel-filtration chromatography using the TSK 5000 PW column provides an analytical and preparative technique for simultaneous separation of individual lipoproteins and determination of LDL molecular weight.—Carroll, R. M., and L. L. Rudel. Lipoprotein separation and low density lipoprotein molecular weight determination using high performance gel-filtration chromatography. *J. Lipid Res.* 1983. **24**: 200–207.

Supplementary key words agarose gel • agarose column chromatography • apolipoprotein • cholesterol • high density lipoproteins • non-human primates.

Classically, plasma lipoprotein fractions have been defined by their hydrated density differences (1) and are isolated by sequential preparative ultracentrifugation (2). An alternative method for preparative isolation of lipoproteins uses a separation by size during agarose column chromatography (3–6). This technique has the advantage that it is simultaneously preparative and analytical. A visual profile of the relative distribution by size is obtained during preparation that permits an evaluation of heterogeneity among and within individual samples. A method has been developed that permits the determination of LDL molecular weight during preparative isolation of the lipoproteins (7). This has been important because LDL size has been shown to be a good predictor of the degree of coronary artery atherosclerosis in nonhuman primates (8, 9). The primary disadvantages of agarose column chromatography are the limited availability of agarose that will give a satisfactory resolution of lipoprotein classes and the time required for the separation (~24 hr).

Recent advances in development of column packings for high performance gel-filtration chromatography (HPGC) of large molecular weight proteins provide an alternative method for rapid chromatographic isolation

Abbreviations: HDL, high density lipoproteins; HPGC, high performance gel-filtration chromatography; ILDL, intermediate-sized low density lipoproteins; LDL, low density lipoproteins; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; VLDL, very low density lipoproteins.

of lipoprotein classes. Several reports have recently appeared describing the use of HPGC for analysis of lipoproteins (10–16). In this report we have extended the observations of these investigators by directly comparing agarose column chromatography and HPGC for lipoprotein isolation and molecular weight determination of LDL. We have demonstrated that lipoproteins elute essentially quantitatively from the HPGC columns and remain intact with respect to electrophoretic mobility, apolipoprotein composition, and chemical composition.

MATERIALS AND METHODS

Animals

Fasting blood samples for lipoprotein isolation were obtained as previously described (7). Three species of nonhuman primates. African green (vervets), cynomolgus, and rhesus monkeys were utilized in this study. The feeding of control (0.15 mg cholesterol/Kcal) and test (0.75 mg cholesterol/Kcal) diets containing 40% of calories as fat allowed us to maximize the observed range of LDL molecular weights.

Lipoprotein isolation and analyses

The density of plasma was raised to 1.225 g/ml with solid KBr and lipoproteins were isolated from individual plasma samples by centrifugation in a SW40 rotor (Beckman Instruments, Fullerton, CA) at 40,000 rpm for 40 hr at 15°C in a Beckman L5-50 ultracentrifuge (16). An aliquot of the isolated total plasma lipoprotein mixture was applied to an agarose column (Bio-Gel A-15m, 200-400 mesh, Bio-Rad Labs, Richmond, CA) and lipoproteins were eluted as previously described (6). A second aliquot of the total plasma lipoprotein mixture was chromatographed on Toyo Soda high performance gel-filtration columns. The HPGC was carried out using a DuPont Model 850 liquid chromatograph (DuPont Instruments, Wilmington, DE) equipped with a DuPont Model 837 variable wavelength spectrophotometer and a Hewlett Packard Model 3380A integrating recorder (Hewlett Packard Instruments, Avondale, PA). The chromatography columns, TSK 3000 SW (7.5 mm × 600 mm), TSK 4000 SW (7.5 mm × 600 mm), TSK 5000 PW (7.5 mm × 600 mm), and TSK 5000 PW pre-column (7.5 mm × 100 mm) were obtained from Beckman Instruments, Altex Division, Berkeley, CA. Unless stated otherwise, HPGC was carried out at room temperature after applying a 250- μ l aliquot of the plasma lipoprotein mixture to the column and eluting it in a buffer consisting of 0.25 M Tris-PO₄, pH 7.6.

Material eluted from the HPGC columns was moni-

tored at 280 nm and was collected in 0.5-ml fractions. The contents of the tubes containing individual lipoprotein peaks were pooled for further analysis. Agarose electrophoresis was performed according to the method of Noble (17). Immunoreactivity to anti-apoB, anti-apoA-I, anti-apoA-II, and anti-albumin was checked by immunodiffusion (18). Antisera were prepared in goats to monkey apoproteins as previously described (19). The lipid composition of individual fractions was determined as previously described after measurement of total, free, and ester cholesterol (20), phospholipid (21), and triglycerides (22). Protein was measured by the method of Lowry et al. (23) using bovine serum albumin, fraction V (Sigma Chemical Co., St. Louis, MO) as the standard.

Apoprotein analysis was carried out on isolated lipoprotein fractions using polyacrylamide gel electrophoresis in the presence of SDS. Samples were dialyzed against 0.01% EDTA, lyophilized, and then they were delipidated and resolubilized by heating to 80°C for 10 min in 0.025 M Tris-0.2 M glycine buffer, pH 8.3, containing 0.02 M sodium dodecyl sulfate and 0.3 M mercaptoethanol. Final protein concentrations were 1 μ g/ μ l. Apoprotein separation was carried out in a horizontal slab gel containing a 5% polyacrylamide stacking gel and a 12% running gel, as described previously (24). After electrophoresis, gels were fixed in methanol-water-acetic acid 45:45:10 (v/v/v) and were stained with Coomassie blue (25).

Column calibration

The HPGC columns were calibrated for molecular weight determination by a method similar to that described previously for the agarose column (7). Tracer amounts of a reference ¹²⁵I-labeled LDL of known molecular weight was added to each lipoprotein sample. Radioactivity eluted from the column was monitored by placing each tube from the fraction collector in a Beckman Gamma 4000 Counting System (Beckman Instruments, Fullerton, CA). A relative size index (r_1) was determined for each sample by dividing the elution volume at the center of the ¹²⁵I-labeled LDL peak by the elution volume at the center of the OD₂₈₀ peak of the sample LDL. The r_1 values for each sample were then plotted against the log of the LDL molecular weight that had been determined on separate aliquots of the same lipoprotein sample by agarose column chromatography. The relationship was described by linear regression analysis and the regression equation was used to determine the LDL molecular weight from HPGC. Aliquots of several of the agarose column purified LDL samples were sent on wet ice to the laboratory of Dr. Charles Nelson, and their molecular weights were measured in the analytical ultracentrifuge by the flotation

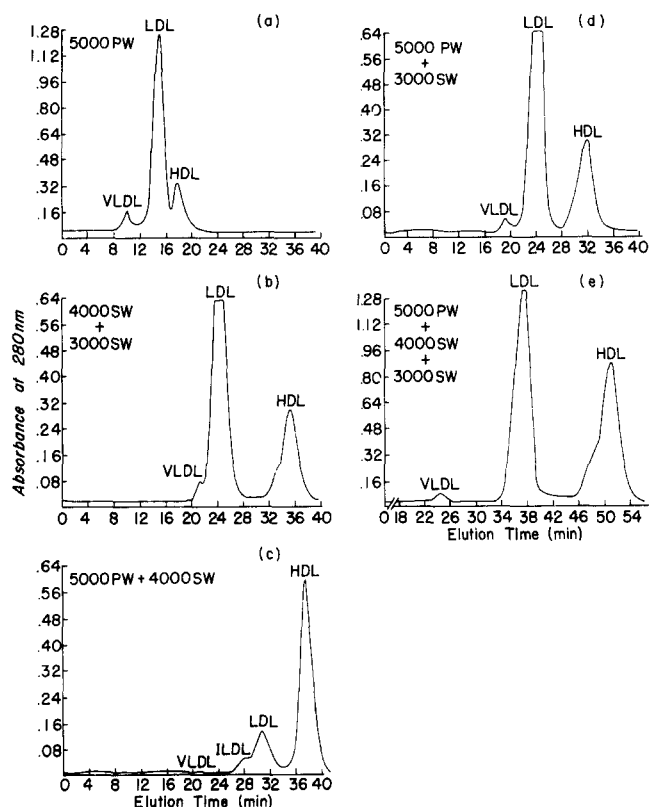


Fig. 1. Effect of column configuration on the elution profiles of vervet monkey lipoproteins. For each panel, 250- μ l aliquots of $d < 1.225$ g/ml lipoprotein samples (equivalent to lipoproteins from 2.5 ml of plasma) were chromatographed on (a) a TSK 5000 PW, (b) a TSK 4000 SW + TSK 3000 SW, (c) a TSK 5000 PW + TSK 4000 SW, (d) a TSK 5000 PW + TSK 3000 SW, and (e) a TSK 5000 PW + TSK 4000 SW + TSK 3000 SW column. The dimensions of each column were 75 mm \times 600 mm. The column buffer consisted of 0.25 M Tris- PO_4 , pH 7.6. Chromatography was carried out at a flow rate of 1.0 ml/min at ambient temperature. The sample in (c) was from an animal fed a control diet; other samples were from test diet-fed animals.

equilibrium method of Nelson et al. (26), and this molecular weight value was compared to the HPGC value.

Statistical analysis

Statistical evaluations of the data were carried out as indicated throughout based on statistical methods described by Snedecor and Cochran (27).

RESULTS

High performance gel-filtration chromatographic separation of $d < 1.225$ g/ml lipoproteins isolated from vervet monkey plasma samples was carried out using a number of different column configurations (**Fig. 1a–e**). The 5000 PW, 5000 PW + 3000 SW, 5000 PW + 4000 SW, and the 5000 PW + 4000 SW + 3000 SW all provided adequate resolution of the major lipoprotein classes. The position of each of the lipoprotein classes

indicated was verified using purified lipoproteins isolated by agarose column chromatography. The best resolution of individual lipoproteins was obtained with the 5000 PW + 4000 SW + 3000 SW. However, due to a shorter analysis time, longer column stability, and the decreased cost of using a single column system, the 5000 PW column was selected for use during most of the remaining studies. The lack of stability of the 3000 SW and 4000 SW was evidenced by absorption of lipoproteins to the columns after 1–3 months of use. This binding resulted in an almost complete retention by the column of the applied LDL. The bound material could be subsequently eluted by a detergent-containing buffer. We have not observed this type of column deterioration using the 5000 PW column.

The flow rate of the buffer through the 5000 PW column affects lipoprotein resolution as illustrated in **Fig. 2**. The separation among the lipoprotein classes was improved by decreasing the flow rate from 1.0 ml/min to 0.5 ml/min. Further decreases in flow rate improved resolution only slightly and were not advantageous because of the increase in time required for sample analysis. The results in **Fig. 3** show that varying the Tris- PO_4 buffer concentrations from 0.05 M to 1.0 M markedly altered the elution profile of $d 1.225$ g/ml lipoproteins when a single 5000 PW column was used.

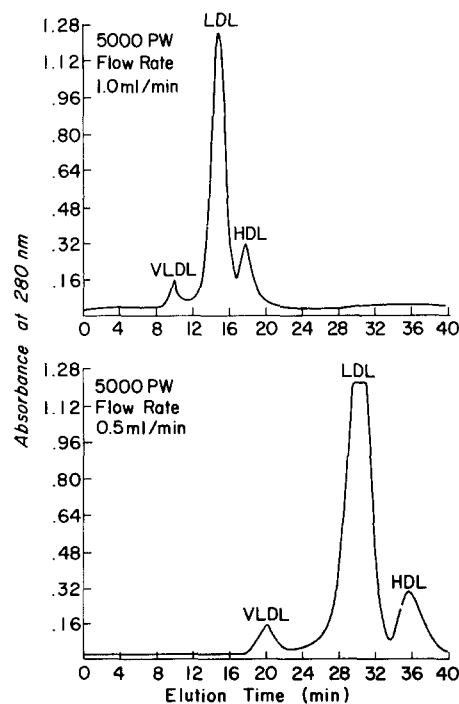


Fig. 2. Effect of flow rate on lipoprotein separation. Separate 250- μ l aliquots of a single vervet monkey $d < 1.225$ g/ml lipoprotein sample were chromatographed on a 5000 PW column at a flow rate of 1.0 ml/min (top) or 0.5 ml/min (bottom). The column buffer consisted of 0.25 M Tris- PO_4 , pH 7.6.

The VLDL, which are excluded from the column packing, were eluted at the same position regardless of the Tris concentration while the LDL and HDL elution time increased with increasing buffer concentration. This resulted in peak broadening and, in the case of HDL, appeared to result in resolution of HDL into multiple subfractions. The r_1 for the LDL fraction increased from 0.9745 at 0.05 M Tris-PO₄ to 1.0145 at 1.0 M Tris-PO₄. A Tris-PO₄ concentration of 0.25 M appeared to give maximum resolution of individual lipoprotein classes and was used as the elution buffer in the remaining studies.

The HPGC method was then compared to agarose column chromatography for lipoprotein separation using lipoproteins isolated from plasma by ultracentrifugation at 1.225 g/ml. A representative comparison is shown in Fig. 4. The lipoprotein separation obtained by the two techniques was similar although the intermediate-sized low density lipoprotein (IDL) fraction was less well defined in the separation on the HPGC column. The agarose column separation required 26 hr for completion while that of the HPGC method required only 40 min. We have chromatographed up to 5 mg of lipoprotein protein (in a total volume of 500 μ l) on the HPGC column without loss of resolution of individual lipoprotein classes.

As a further indication of the performance of the 5000 PW column, three LDL and three HDL samples isolated by agarose column chromatography were rechromatographed on HPGC either individually or as a 1:1 mixture of LDL plus HDL. Representative chro-

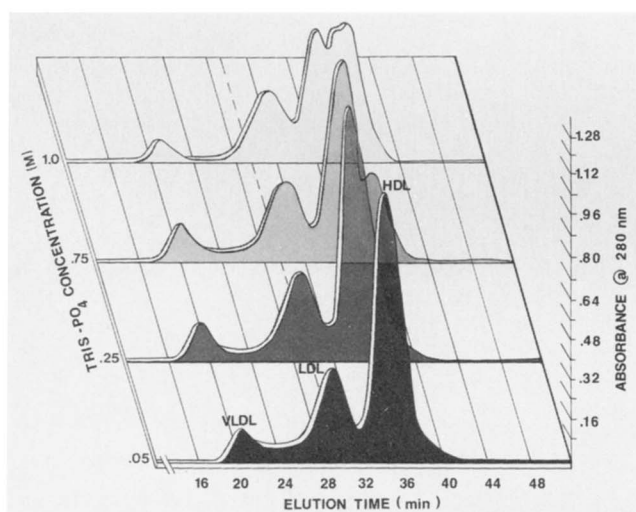


Fig. 3. Effect of buffer concentration on the elution of plasma lipoproteins. In each case, a 250- μ l aliquot of a single vervet monkey < 1.225 g/ml lipoprotein sample was chromatographed at a flow rate of 0.5 ml/min. Four buffer concentrations were compared as indicated. The dotted line in the figure represents the elution time at the center of the ¹²⁵I-labeled LDL internal standard peak.

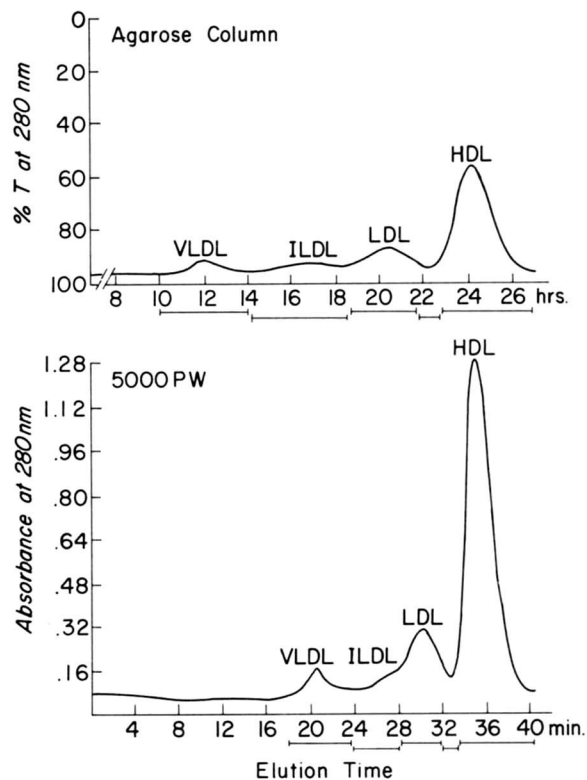


Fig. 4. Comparison of lipoprotein separation by HPGC and by agarose column chromatography. Aliquots of a single vervet monkey < 1.225 g/ml lipoprotein sample were chromatographed on a 1.5 cm \times 90 cm Bio-gel A-15m, 200–400 mesh, agarose column (top) or a 75 mm \times 6000 mm TSK 5000 PW column (bottom). A 1.0-ml aliquot of lipoprotein sample was applied to the agarose column and the sample was eluted at 7 ml/hr with 0.9% NaCl, 0.01% EDTA, 0.02% NaN₃, pH 7.4, at 4°C. A 250- μ l aliquot of lipoprotein sample was applied to the HPGC column and was eluted at 0.5 ml/min in 0.25 M Tris-PO₄ buffer, pH 7.6, at ambient temperature. The elution profile was used as the basis for pooling fractions into VLDL, ILDL, LDL, and HDL, as indicated by the brackets under each profile.

matograms are shown in Fig. 5. The LDL and HDL samples showed single symmetrical peaks when rechromatographed and the LDL:HDL mixture gave two symmetrical peaks proportional in size to the individual LDL and HDL peaks. The cholesterol recovery of the isolated lipoproteins in this instance was 100% \pm 3.6%, mean \pm SD, and recovery of lipoprotein cholesterol of LDL and HDL from the mixture was 93% \pm 1.73% and 114% \pm 3.21%, respectively.

To further characterize the HPGC procedure, fractions containing individual lipoprotein classes were pooled after elution from the HPGC column and were further analyzed. Agarose electrophoresis indicated that LDL and HDL from HPGC had mobilities similar to those of the lipoproteins in unfractionated plasma (data not shown). Analysis of the apoproteins of HPGC-isolated LDL from four animals and HPGC-isolated HDL from three animals (Fig. 6) indicated that their

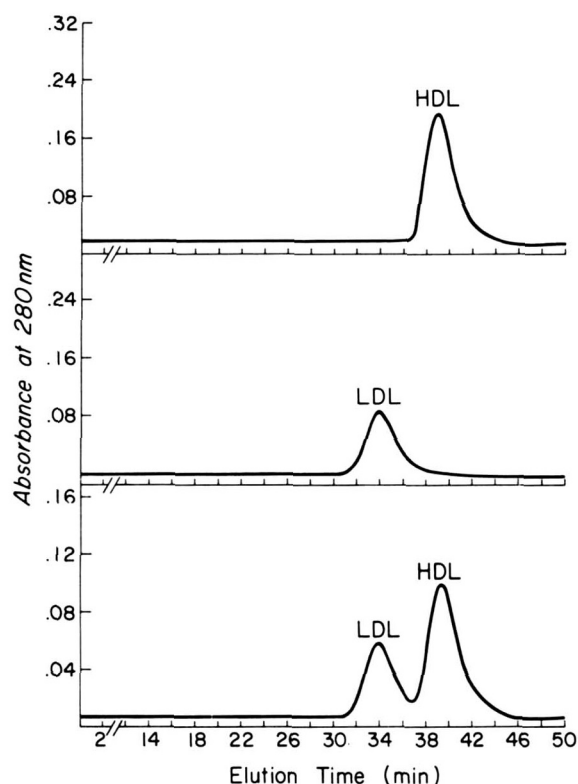


Fig. 5. Comparison of HPGC of isolated LDL, HDL, and an LDL:HDL mixture. Aliquots of a single $d < 1.225$ g/ml lipoprotein sample were chromatographed on a 1.5 cm \times 90 cm Bio-gel A-15m, 200–400 mesh, agarose column. The fractions containing LDL and HDL were pooled and the HDL (top), LDL (middle), and a 1:1 mixture of LDL and HDL (bottom) were chromatographed on a 7.5 mm \times 60 cm 5000 PW column. HPGC was carried out at a 0.25 M flow rate of 0.5 ml/min at ambient temperature with Tris- PO_4 , pH 7.6, as the column buffer.

apoprotein profiles were similar to those of LDL and HDL isolated by other techniques (7). The LDL contained primarily apoB and were free of apoA-I. The HDL contained primarily apoA-I and apoA-II and did not contain apoB. These findings were confirmed by immunodiffusion using goat anti-monkey apoB, anti-monkey apoA-I, and anti-monkey apoA-II. The HPGC isolated lipoproteins also were shown by immunodiffusion to be free of albumin.

Table 1 shows a comparison of cholesterol distribution among lipoprotein classes separated by agarose column chromatography and HPGC. The recovery of lipoprotein cholesterol from the HPGC column averaged $91 \pm 2.5\%$ (SEM), a value similar to the 94% recovery reported for the agarose columns (6). The mean values for the percentage of plasma cholesterol in individual lipoprotein classes were not significantly different when the two methods were compared statistically although the distributions were not identical for each sample. A comparison of the two methods by repeated chromatography of a pooled lipoprotein sample on HPGC and

agarose columns indicated that the percentage values for the VLDL-ILDL fraction were lower when HPGC was used. However, no differences in the percentages of cholesterol in HDL or LDL were observed when HPGC and agarose column techniques were compared. The variability between methods is presumably related to the incomplete resolution of the ILDL and LDL fractions by HPGC. The data for the chemical compositions of LDL and HDL isolated by the two column chromatographic techniques are shown in **Table 2**. The percentages obtained on samples from each of the two isolation techniques were similar. The ratios of total cholesterol to protein and total cholesterol to phospholipid were higher in the HPGC-isolated HDL samples. This difference, although small, was statistically significant ($P < 0.05$, paired *t*-test).

A tracer amount of reference standard ^{125}I -labeled LDL was added to each of several samples to permit LDL molecular weight determination (7). The standard curve for the relative size index from HPGC versus the log of LDL molecular weight obtained from agarose column chromatography is shown in **Fig. 7**. The data in the figure represent determinations made on 12 rhesus, 15 African green, and 38 cynomolgus monkey LDL samples including material isolated from animals from both control and test diet groups. The correlation coefficient for these data was $r = 0.85$. The coefficient of variation for the retention volume of the ^{125}I -labeled

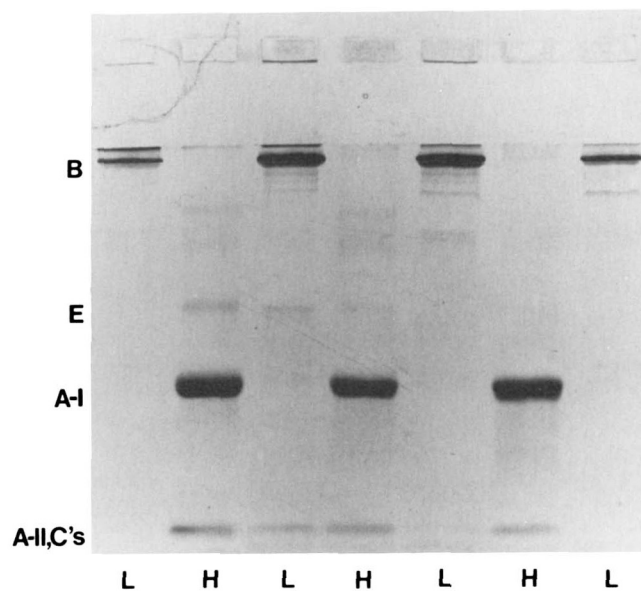


Fig. 6. SDS-PAGE profiles of apoproteins obtained from LDL and HDL isolated by high performance gel-filtration chromatography. The apoproteins are designated on the left by: B, apoprotein B; E, apoprotein E; A-I, apoprotein A-I; A-II, apoprotein A-II; C's, C apoproteins. LDL are designated at the bottom by L, HDL by H. LDL from four different vervet monkeys fed the test diet are shown, and HDL from three of the same animals are shown. Approximately 40 μg of protein was applied to the gel for each sample.

TABLE 1. Comparison of cholesterol distribution obtained by agarose column and high performance gel-filtration chromatography

Animal #	% Cholesterol (w/w)					
	VLDL + ILDL		LDL		HDL	
	Agarose	HPGC	Agarose	HPGC	Agarose	HPGC
2	10.75	6.04	32.47	29.85	56.78	63.80
3	8.07	8.98	30.63	35.33	61.30	55.24
4	6.54	11.31	26.59	34.41	66.87	53.89
98	12.60	7.07	15.67	17.41	71.72	75.51
101	3.61	3.55	7.11	8.61	89.28	87.85
102	5.25	4.73	15.36	21.58	79.38	73.43
103	7.40	10.43	13.75	22.66	78.90	67.31
233	5.43	8.43	22.64	30.78	71.94	60.29
235	6.69	14.24	33.89	33.09	59.41	51.62
236	8.01	5.32	32.30	39.61	59.69	54.72
Mean	7.08	6.88	21.24	26.11	71.68	66.61
Std. Dev.	2.61	3.93	9.83	9.87	11.23	12.66
Pool mean ^a	7.39	3.73	58.55	57.85	34.06	38.41
Std. Dev.	1.08	1.31	2.90	4.05	2.06	4.15

^a The values in this portion of the table represent the mean and standard deviation for five agarose and eight HPGC separations of a pooled plasma lipoprotein sample.

LDL internal standard was 0.46%. The recovery of the ¹²⁵I-labeled LDL radioactivity applied to the column was 84 ± 2%. We also compared several LDL molecular weight values obtained from HPGC with those determined by flotation equilibrium analysis on the analytical ultracentrifuge. For eight samples, good agreement was found. The column-derived value was 2.96 ± 0.06 g/μmol (mean ± SEM) compared to 2.97 ± 0.9 g/μmol when measured in the analytical ultracentrifuge. Although most of the LDL molecular weight evaluations were done using the 5000 PW column, we also validated the use of two combinations of columns for this measurement: i.e., 5000 PW + 3000 SW and the 5000 PW + 4000 SW. Twenty samples were run on each of these

combinations, the equations for the regression lines obtained were $y = 8.33 r_1 - 7.88$ and $y = 7.50 r_1 - 7.14$ for the 5000 PW + 3000 SW and the 5000 PW + 4000 SW, respectively. The slope of the 5000 PW + 3000 SW combination was the largest, indicating that this combination may offer the most sensitivity for molecular weight determination of the column arrangements evaluated.

DISCUSSION

The present findings indicate that HPGC is a non-destructive analytical and preparative technique for an-

TABLE 2. Chemical compositions for LDL and HDL isolated by HPGC and agarose column chromatography

Sample	N		Percent by Weight							
			PL	FC	CE	TG	Pro	EC/TC	TC/Pro	TC/PL
HPGC	6	LDL	20.72 ^a ± 2.41	8.98 ± 1.17	46.36 ± 3.43	1.07 ± 0.93	19.21 ± 2.06	0.74 ^c ± 0.01	1.85 ^c ± 0.25	1.70 ^c ± 0.21
Agarose	6	LDL	19.04 ± 2.06	9.36 ± 0.67	48.49 ± 2.71	0.82 ± 0.41	19.16 ± 1.16	0.74 ± 0.01	1.88 ± 0.25	1.84 ± 0.28
HPGC	5	HDL	17.38 ± 4.80	4.44 ± 0.98	20.06 ± 2.80	5.02 ± 3.12	43.45 ± 10.03	0.73 ^c ± 0.04	0.28 ^b ± 0.02	0.69 ^b ± 0.08
Agarose	5	HDL	17.70 ± 5.09	3.34 ± 0.74	17.99 ± 5.21	3.60 ± 1.45	47.68 ± 8.00	0.75 ± 0.06	0.23 ± 0.03	0.56 ± 0.09

^a Values represent the mean ± SD for samples from six animals.

^b $P < 0.05$, Students' *t*-test. Ratios for HDL and LDL isolated by HPGC and agarose column chromatography were compared.

^c Not significantly different at $P = 0.05$.

Abbreviations: PL, phospholipid; FC, free cholesterol; CE, cholesteryl ester; TG, triglyceride; Pro, protein; EC esterified cholesterol; TC = EC + FC.

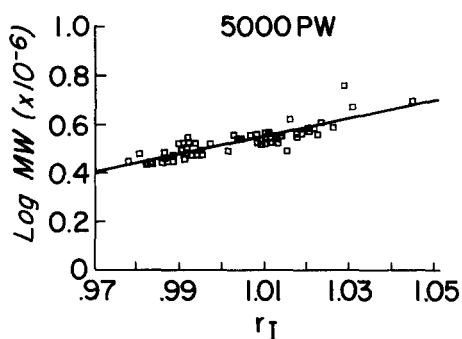


Fig. 7. Comparison of the relationship between log molecular weight (MW) of LDL, as determined by agarose column chromatography, versus the relative size index (r_1), as determined by HPGC. Aliquots for 65 individual $d < 1.225$ g/ml lipoprotein samples were chromatographed on a 75 mm \times 600 mm TSK 5000 PW column; separate aliquots were chromatographed on agarose (7). The line drawn is the least squares best fit regression line. The equation of the line determined by linear regression analysis is $y = 3.76055 r_1 - 3.25308$. The correlation coefficient for these data is 0.85.

alyzing lipoprotein spectra of individual plasma samples. Our findings confirm the data of others showing that the Toyo Soda gel filtration columns will separate lipoprotein classes (10–16), and they extend these observations in several ways. These data are the first to show that essentially quantitative recovery of lipoprotein cholesterol and ^{125}I -labeled LDL can be obtained from the column. We have documented that the individual lipoproteins eluted from the column are intact, based on chemical composition, and are relatively free of contamination from other lipoproteins, based on electrophoretic mobility, apoprotein patterns, and immunological criteria. The average distribution of cholesterol among lipoprotein fractions obtained after HPGC was found to be comparable to that obtained by agarose column chromatography. Finally, we have shown that the HPGC columns work reproducibly by documenting their ability to accurately predict LDL molecular weight, and by the small coefficient of variation in the elution volume of the ^{125}I -labeled LDL reference standard, $14.87 \text{ ml} \pm 0.46\%$. We conclude that HPGC can be used as an accurate and reproducible method for the separation and quantitation of plasma lipoproteins. Nonhuman primate plasma lipoproteins were used for most of our validation work because of the availability of large numbers of samples. Our work using lipoproteins isolated from human beings has been much less extensive. However we have found that lipoproteins from human beings are separated equally well. Our data suggest that HPGC can be applied to the human situation with equal likelihood of success.

The question of which particular column or column combination to select does not have a single solution. Clearly, the best separation of lipoprotein classes was obtained using a three-column set-up (Fig. 1). However,

this is an expensive set-up, the analysis time is longer, and the stability of the 3000 SW and 4000 SW columns is suspect. For this reason, we chose to do most of our work with the 5000 PW column. The resolution of individual lipoprotein classes is acceptable although not as good as other configurations. The advantages were that the column was stable during constant usage for over a year (the 3000 SW and 4000 SW columns had useful lives of about 1 and 3 months, respectively) while the cost and analysis time were minimized.

The buffer concentration clearly modified the lipoprotein separation on the 5000 PW column. It is possible that the heterogeneity seen in the HDL region at higher buffer concentrations is related to the heterogeneity in the HDL that has been defined by other procedures (28) although we have no data to confirm this possibility. However, all of the peaks became broader and the separation between LDL and HDL was partially compromised at high concentrations of buffer so we decided to use the 0.25 M Tris concentration for the majority of our studies. Our evidence suggests that at low buffer concentrations, ion exclusion effects in the column may prevent lipoprotein particles from entering the pores in the column packing. The increasing buffer concentration may reduce this effect and increase the sieving properties of the column (29).

The chemical composition data (Table 2) demonstrate that the HDL and LDL eluted from the column are basically intact. However, the small but statistically significant difference in the ratios of total cholesterol to protein and total cholesterol to phospholipid in HDL suggest that some of the surface constituents of the lipoproteins may have been lost to the column. We monitored the recovery of cholesterol from the column and it was excellent (91%). The recovery of ^{125}I -radioactivity was good (84%) but not as complete. In some samples a small, irregularly shaped OD_{280} peak was found that eluted near 55 min. On one occasion, the material in this peak was concentrated and the presence of a protein with the size of apoA-I was demonstrated by SDS PAGE. In the future, it will be important to monitor the recovery of specific apolipoproteins and phospholipids depending on the application for which the technique is to be used. We have used lipoproteins for HPGC separation that were first exposed to high salt concentrations and ultracentrifugation. It may be that these procedures have partially modified the lipoproteins, although one would not expect equivalent results from HPGC and agarose column isolated lipoproteins in this case.

In summary, although lipoprotein-column interactions may not be totally nil using the HPGC procedures described here, the data obtained demonstrate that this is a promising technique for lipoprotein evaluation that

will likely become increasingly important in the future. ■

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