Amino acid composition of the proteins from chylomicrons and human serum lipoproteins

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ABSTRACT By a combination of polyanion precipitation and ultracentrifugation, chylomicrons, very low density, low density, and high density lipoproteins have been isolated from human serum as discrete classes free from contamination with any other major class of lipoprotein or protein. After removal of the lipid, the proteins from each class were hydrolyzed and their amino acid compositions were determined by use of the amino acid analyzer. Application of the "t" test to the concentrations of amino acid residues showed that the amino acid composition of the proteins from each of these lipoprotein classes differs significantly from class to class. However, when the logarithms of the moles of amino acid residues are plotted, there are similarities in the amino acid "profiles" between the chylomicrons and high density lipoproteins on the one hand, and between the very low density and low density lipoproteins on the other.

The differences in amino acid composition between the lipoproteins suggest that any metabolic interconversions between them probably do not occur by simple lipolysis.

KEY WORDS lipoproteins · high density · low density · very low density · chylomicrons · human serum · lipids · protein composition · amino acid "profiles" · amylopectin sulfate · ultracentrifugation · electrophoresis · delipidation · amino acid analysis

KNOWLEDGE of the amino acid composition of the four major classes of human serum lipoproteins might help to throw more light on the possibility of interconversions between classes. Earlier workers were of the opinion that there was a stepwise conversion of the lower density to the higher density classes after the administration of a high-fat meal (1), the injection of heparin (2), or the injection of ^{131}I-labeled lower density lipoproteins (3). However, if nothing but lipid is removed during the metabolism of the lower density lipoproteins (including chylomicrons), then the amino acid composition of the protein should be the same in all of the classes. Investigations of the N-terminal amino acids that have been reported to date (4–8) indicate that each class has its own typical N-terminal amino acid pattern and apparently its own typical protein residues. The studies of the amino acid composition of the various classes reported here provide further evidence for clear-cut differences between the proteins of the major lipoprotein classes.

Preliminary accounts of this work were reported to the 46th Annual Meeting of the Federation of American Societies for Experimental Biology (Atlantic City, 1962) and the 35th Scientific Session of the American Heart Association (Cleveland, 1962).

Abbreviations: HDL, high density lipoprotein(s); LDL, low density lipoprotein(s); VLDL, very low density lipoprotein(s).

EXPERIMENTAL PROCEDURES

Preparation of Sulfated Amylopectin

Sulfated amylopectin was prepared by a considerable modification of the method suggested by Bernfeld, Donahue, and Berkowitz (9) and used as the dried potassium salt. Anhydrous pyridine was added to a reaction vessel and chilled to 0°C. Chlorosulfonic acid then was added dropwise with stirring. The pasty product which resulted was heated to 70°C and stirred. Amylopectin suspended in pyridine was added to the sulfation mixture and stirred for 5 hr at 70°C. The entire reaction mixture was then poured into distilled water where the unsulfated polysaccharide formed a white precipitate. This entire mixture was transferred to dialysis tubing and dialyzed for 4 days against running tap water. A precipitate was removed from the dialyzed material by centrifugation. The supernatant fraction was heated to 70°C and a solution of barium chloride was added dropwise with stirring to precipitate the sulfated polysaccharide. This precipi-

tate was dissolved in distilled water by adjusting the solution to pH 10 with ammonium hydroxide. The barium was removed by precipitation with ethylenediamine tetraacetate, sodium salt (EDTA). The supernatant solution, which contained the sulfated polysaccharide, was dialyzed against running tap water, then distilled water, and finally potassium acetate solution. The sulfated polysaccharide was precipitated by the potassium acetate and was recovered as the potassium salt, washed with methanol and ether, and then air-dried.

Isolation of Chylomicrons

Blood was obtained by venipuncture from fasting male donors under 40 yr.[1] The blood was allowed to clot on standing at 37°C for 30 min. The clot and cells were removed by centrifuging the blood at 9,500 rpm (9,800 *g*) for 30 min in a Beckman-Spinco type 21 rotor.[2] At this time the chylomicrons, which floated, were removed by aspiration. They were then layered under an equal volume of 0.15 M NaCl (d = 1.003) and centrifuged at 11,250 rpm (9,800 *g*) for 30 min in a Beckman-Spinco type 30 rotor. The chylomicrons, which floated, were removed by aspiration and emulsified with 0.15 M NaCl by repeated extrusion through a 20 gauge needle. This solution was centrifuged at 11,250 rpm for 30 min in a type 30 rotor to float the chylomicrons. This washing procedure was carried out 5–10 times until the infranatant solution was free from turbidity. The chylomicrons were then layered under distilled water and centrifuged at 22,500 rpm (44,110*g*) for 70 min in a type 30 rotor in order to tightly pack the chylomicrons into a buttery layer which was removed with a tube slicer.

Isolation of Low Density and Very Low Density Lipoproteins

Sulfated amylopectin at a concentration of 10 mg/ml in distilled water was added dropwise with stirring to the chylomicron-free serum at 4°C until a final concentration of polysaccharide in the serum of 0.5 mg/ml was obtained. During this time an orange-colored precipitate formed which was allowed to stand overnight at 4°C.

The precipitate was recovered by centrifugation at 9,500 rpm for 90 min at 4°C. The supernatant serum was retained for isolation of high density lipoproteins. To wash the precipitate, 180 ml of 0.1 M calcium acetate, pH 7, was added to each aliquot of precipitate obtained from 1 liter of serum. The mixture was mixed well by repeated extrusion through an 18 gauge needle until a finely divided suspension was obtained. The precipitate was again recovered by centrifugation and washed as before with the calcium acetate solution. Finally, the precipitate was recovered by centrifugation in a Beckman-Spinco type 30 rotor at 25,000 rpm (54,450 *g*) for 2 hr in order to pack the precipitate and allow the removal of as much calcium acetate as possible. The precipitate was then brought to a volume of 105 ml with 3.2 M NaCl containing 5% $BaCl_2$. When allowed to stand overnight at 4°C, the precipitate went into solution. This solution, which had a density[3] of 1.1539, was centrifuged for 30 min at 25,000 rpm in the type 30 rotor in order to remove the uncomplexed polysaccharide as the barium salt as well as any blood cells trapped in the complex. It was then layered under an equal volume of 0.15 M NaCl (d = 1.003)[4] in Beckman-Spinco type 30 rotor tubes and centrifuged for 34 hr at 27,500 rpm (65,900 *g*) in order to establish a density gradient. The very low density lipoproteins (VLDL) which rise to the top of the tube under these conditons were removed by a tube slicer. The low density lipoproteins (LDL) which rise only to the middle of the centrifuge tube were also removed. The VLDL fraction was then layered under an equal volume of 0.15 M NaCl (d = 1.003) and centrifuged in the type 40 rotor at 11,900 rpm (9,800 *g*) for 30 min. Any remaining chylomicrons that may have complexed with the polysaccharide floated to the top of the tube and were removed with the tube slicer. The infranatant solution was then adjusted to a density of 1.019 with crystalline NaCl and centrifuged at 37,500 rpm (92,660 *g*) for 22 hr in the type 40 rotor in order to float the VLDL, which then appeared at the top of the tube as an intense yellow band. Any LDL present under these conditions have sedimented.

The LDL fraction that had been recovered from the middle of the type 40 rotor tube had a mean density of 1.0511. This fraction was layered under an equal volume of NaCl (d = 1.019) and centrifuged at 35,000 rpm (80,700 *g*) for 25 hr. Any VLDL which remained floated to the top of the tube and were removed with a tube slicer. The infranatant solution was then adjusted to d = 1.063 and centrifuged at 35,000 rpm for 25 hr. The LDL were collected as a bright orange band at the top of the tube. To insure complete removal of albumin or any other serum proteins, we refloated the LDL in this fraction by adjusting it to d = 1.063 with crystalline NaCl and centrifuging at 35,000 rpm for 25 hr in the type 40 rotor. We studied the infranatant solution by removing 1 ml aliquots and analyzing each aliquot by paper electro-

[1] Blood was collected in sterile vacuum bottles by the Louisville Regional Blood Center of the American Red Cross. Blood from 22 units was pooled for the isolation procedures.

[2] All isolation procedures which made use of the Beckman-Spinco type 21, 30, and 40 rotors were carried out at 4°C.

[3] All density determinations were carried out by pycnometry with a deviation of ±0.0003 g/ml. The symbol d is used to represent density in g/ml at 26°C.

[4] All solutions used in the flotation of lipoproteins contained 0.01% EDTA, pH 7.

phoresis. It was noted that albumin and any other serum proteins had sedimented to the bottom of the tube.

Isolation of High Density Lipoproteins

The supernatant serum remaining after the VLDL and LDL had been removed by precipitation was adjusted to $d = 1.200$ with crystalline NaBr and centrifuged in a type 30 rotor at 20,000 rpm (34,850 *g*) for 70 hr. Any residual lipoproteins, principally the high density lipoproteins (HDL), floated to the top of the tube and were recovered. This fraction was then layered under an equal volume of NaCl ($d = 1.063$) in a type 40 rotor tube and centrifuged at 37,500 rpm for 40 hr. Under these conditions any VLDL or LDL float to the top of the tube. The HDL appeared as a pale yellow band in the middle of the tube and were removed with a tube slicer. Again, to insure the complete removal of albumin or other serum proteins, the HDL fraction was adjusted to $d = 1.200$ with NaBr and centrifuged in a type 30 rotor at 20,000 rpm (34,850 *g*) for 70 hr. The HDL appeared as a pale yellow band at the top of the tube. As before, we examined the infranatant solution by paper electrophoresis in order to demonstrate that albumin and any other serum proteins had sedimented to the bottom of the tube.

Prior to delipidation, the chylomicrons and each class of lipoproteins were examined by paper electrophoresis and analytical ultracentrifugation and were found to be free from contamination with any other major class of lipoprotein or protein.

Delipidation of the Lipoproteins

Each lipoprotein fraction was dialyzed against 0.05 M NaCl for 18 hr at 4°C. The lipoprotein solutions were then added dropwise with stirring to 15 times their volume of methylal–methanol 4:1. A finely divided precipitate formed which was allowed to stand at 4°C overnight. The precipitate was recovered by centrifuging in a Beckman-Spinco type 21 rotor at 11,000 rpm (12,700 *g*) for 45 min. The precipitate was then washed three times with the methylal–methanol solution, washed repeatedly with distilled water until there was a negative reaction for chloride ion when tested with a silver nitrate solution, and finally washed three times each with 80% methanol, absolute methanol, and diethyl ether. The protein residue was allowed to dry in the air at room temperature. About 90% of the protein from the intact lipoprotein was recovered in the residue. To insure the completeness of delipidation, all protein residues were examined for lipid phosphorus (10). None was detected. In addition, we examined all protein residues for the presence of albumin by suspending them in distilled water and carrying out immunoelectrophoresis against an antiserum to human albumin (11). Precipitation lines were formed with a crystalline human serum albumin that had been carried through all the delipidation procedures and with the protein residues when this "delipidated" albumin was added to them. No precipitation lines formed with the protein residues by themselves, which indicated that albumin was not present in the residues.

Hydrolysis and Analysis of the Proteins

Aliquots of the various proteins were dissolved in 200 times their weight of constant-boiling 6 N HCl, frozen in an ampule, thawed and degassed, refrozen and sealed while evacuated, and then hydrolyzed for 22 hr at 110 ± 0.5°C. The hydrolyzed protein was transferred from the ampule to a round bottom flask with distilled water. The water and HCl were removed on a rotary evaporator after the addition of an antifoaming agent such as octyl alcohol. The dried amino acids were dissolved in 0.2 N sodium citrate buffer, pH 2.2. Aliquots of this buffered mixture, containing the equivalent of approximately 2 mg of protein, based on the factor 6.25 times the Kjeldahl nitrogen content (12), were analyzed quantitatively for amino acid constituents on a Beckman-Spinco Model 120 amino acid analyzer. After corrections were applied for decomposition of serine (0.90), threonine (0.95), and tyrosine (0.95) during hydrolysis (13), and after tryptophan was determined independently (14), we found that about 94% of the hydrolyzed protein was recovered in the amino acids listed. All values were converted from μmoles of amino acid/mg of amino acids recovered to moles of amino acid/100,000 g of amino acid residues recovered with the use of residue molecular weights for each amino acid listed.

Chemical Analyses

Cholesterol and cholesteryl esters were determined by the method of Bloor (15), phospholipids by the method of Lowry, Roberts, Leiner, Wu, and Farr (10), triglycerides by the method of Van Handel and Zilversmit (16), tryptophan by the method of Spies and Chambers (14) protein nitrogen in the hydrolysates by the method of Lang (12), and total protein in the intact lipoproteins by the method of Lowry, Rosebrough, Farr, and Randall (17). In the last method crystalline human serum albumin that had been carried through the delipidation procedure was used as the standard protein and was compared with each lipoprotein protein in terms of the amount of color development. On the basis of color recovery as compared to albumin, chylomicrons = 100%, VLDL = 110%, LDL = 110%, and HDL = 100%.

Ultracentrifugal Analyses

As a test for homogeneity, chylomicrons, VLDL, and LDL were examined at 26°C in NaCl, $d = 1.063$, in a

Beckman-Spinco Model E analytical ultracentrifuge. HDL were examined at 26°C in NaBr, d = 1.200. The lipoprotein solutions were adjusted to d_{26} 1.063 or d_{25} 1.200 by addition of appropriate amounts of a higher density NaCl or NaBr solution, and the final density was checked by pycnometry. Flotation rate measurements for the VLDL and the LDL were made under the conditions described by DeLalla and Gofman (18) at 26°C in 1.748 M NaCl, d = 1.063. A rotor speed of 52,640 rpm was used in characterizing all classes of lipoproteins. Flotation patterns for the VLDL, LDL, and HDL indicated the presence of a single component. Flotation patterns for the chylomicrons indicated that no other lipoproteins or proteins were present.

Electrophoresis

The technique of Scheidegger (11) was used for conducting immunoelectrophoresis in an LKB (Stockholm, Sweden) immunoelectrophoresis apparatus. 2.5 ml of a 1% solution of special agar (Noble, Difco Labs., Detroit, Mich.) in 0.1 M Veronal buffer was applied in a coat of uniform thickness to standard microscope slides. To insure uniform water content we always used the plates shortly after preparation. The wells were filled with 1 μl of antigen and electrophoresis was carried out in 0.1 M Veronal buffer at pH 8.6 and constant voltage (250 v) for 1 hr. The antigen was allowed to diffuse against 40–60 μl of antiserum for 20 hr at room temperature in a moist chamber. Slides were then washed overnight in saline, rinsed in distilled water, and air-dried. The slides were stained for protein with Amidoschwartz 10B (0.6% in methanol–acetic acid–water, 45:10:45) for 10 min and then rinsed with the methanol–acetic acid–water solution.

Paper electrophoresis was carried out in a Beckman-Spinco Model R electrophoresis apparatus. Lipoprotein samples were dialyzed against 0.05 M Veronal buffer at pH 8.6 overnight. The samples were then applied to Schleicher & Schuell 2043A Mgl paper strips: 10 μl was applied to strips to be stained for protein, 40 μl to those to be stained for lipid. Prior to the application of the samples the strips were saturated with buffer and allowed to equilibrate for 1 hr. Electrophoresis was carried out in 0.05 M Veronal buffer at pH 8.6 with constant current (1.5 ma) for 18 hr. The strips were dried and stained for protein with bromophenol blue or for lipid with Oil Red O.

Materials

Chemicals were reagent grade. All organic solvents were redistilled before use. Amylopectin was purchased as Magnapol S from A. E. Staley Mfg. Co., Decatur, Ill. Antiserum to human albumin produced in rabbits was obtained from Hoechst Pharmaceuticals, Inc., Cincinnati, Ohio. Crystalline human serum albumin was purchased from Nutritional Biochemicals Corporation, Cleveland, Ohio. Amino acid calibration mixture, type 1, and custom research resins types AA-15 and AA-27 were obtained from Beckman Instruments, Inc., Palo Alto, Calif. Thiodiglycol and ninhydrin were purchased from Pierce Chemical Co., Rockford, Illinois. Brij 35 was obtained from Atlas Chemical Industries, Inc., Wilmington, Delaware.

RESULTS

Table 1 shows the chemical composition of the four major classes of lipoproteins as isolated in our laboratory prior to amino acid analysis of the protein. These values were calculated by determining the dry weight of an aliquot of lipoprotein and then determining in turn the free cholesterol and cholesteryl ester (15), phospholipid (10), triglyceride (16), and protein (17) concentrations of equal aliquots. These data are similar to those of others (19), particularly those of Bragdon, Havel, and Boyle (20). Of interest are the similarities between our VLDL and LDL composition data and those of these latter investigators, since they isolated these two classes by flotation while we isolated our fractions by polyanion precipitation. The protein composition of our HDL class resembles the HDL_3 subclass more than that of the HDL_2 subclass (21). This is not unexpected since HDL_3 is the most abundant in human serum (22) and a considerable amount of the HDL_2 present in serum apparently is converted to HDL_3 during the repeated ultracentrifugation required for the isolation of the HDL class (23). Flotation rate measurements in the analytical ultracentrifuge permitted the calculation of S_f 6.7 for the LDL and S_f 43.6 for the VLDL. The flotation rate system as devised by DeLalla and Gofman (18) does not include lipoproteins with densities greater than 1.063, thus the HDL are excluded from these calculations. The chylomicrons under these conditions have such a high flotation rate that no boundary can be observed in the centrifuge.

A single hydrolysate of the protein from chylomicrons, VLDL, LDL, and HDL, respectively, was analyzed in triplicate on the amino acid analyzer. The results were calculated in moles of amino acid/100,000 g of amino acid residues recovered (Table 2). Application of Student's "t" test to analyses in triplicate of these four hydrolysates[5] showed that at a 99% level of confi-

[5] It is assumed that each pair of hydrolysates is normally distributed with means μ_1 and μ_2 and equal variances. This assumption appears to be justified by a comparison of the standard deviations. The hypothesis to be tested by the t-test is $\mu_1 = \mu_2$ and the test statistic for triplicate samples is

$$t = 2.4(\bar{X}_1 - \bar{X}_2)/\{(X_{11} - \bar{X}_1)^2 + (X_{12} - \bar{X}_1)^2 + (X_{13} - \bar{X}_1)^2 + (X_{21} - \bar{X}_2)^2 + (X_{22} - \bar{X}_2)^2 + (X_{23} - \bar{X}_2)^2\}^{1/2}$$, where $f = n_1 + n_2 - 2 = 4$.

TABLE 1 CHEMICAL COMPOSITION OF THE VARIOUS LIPOPROTEIN CLASSES

Lipoprotein Class	Composition*					FC/TC Ratio	TC/PL Ratio	S_f
	FC	CE	TG	PL	Protein			
Chylomicrons	4.4	5.9	77.7	7.5	4.3	0.43	1.3	—
VLDL	7.4	11.1	54.2	19.2	8.3	0.39	0.96	43.6
LDL	8.5	28.8	10.5	27.9	22.7	0.23	1.3	6.7
HDL	2.9	9.2	5.9	24.7	58.1	0.24	0.49	—

FC, free cholesterol; CE, cholesteryl ester; TG, triglyceride; PL, phospholipid; TC, total cholesterol.
* Per cent of dry weight. Each value represents a determination in duplicate.

TABLE 2 AMINO ACID COMPOSITION OF THE PROTEINS FROM CHYLOMICRONS AND HUMAN SERUM LIPOPROTEINS*

Amino Acid	Chylomicrons	VLDL	LDL	HDL
	moles of amino acid per 100,000 g of amino acid residues recovered			
Alanine	80.86 ± 1.73	58.50 ± 0.61	54.35 ± 0.41	75.80 ± 2.14
Arginine	36.45 ± 0.62	35.06 ± 0.62	30.57 ± 1.26	39.30 ± 2.05
Aspartic acid	79.76 ± 0.98	93.31 ± 0.40	93.62 ± 0.17	71.72 ± 1.07
Half cystine	25.92 ± 7.27	2.13 ± 0.00	6.23 ± 0.00	6.67 ± 2.14
Glutamic acid	119.99 ± 1.59	106.14 ± 0.77	106.43 ± 0.57	152.57 ± 0.57
Glycine	40.61 ± 1.60	45.63 ± 1.18	43.13 ± 0.51	38.58 ± 0.30
Histidine	21.44 ± 0.28	18.45 ± 1.50	21.75 ± 0.44	15.36 ± 0.67
Isoleucine	19.66 ± 0.24	44.57 ± 0.79	50.13 ± 0.26	8.87 ± 0.10
Leucine	83.63 ± 0.10	93.95 ± 0.62	98.10 ± 0.14	110.12 ± 2.32
Lysine	81.09 ± 2.97	77.14 ± 0.66	72.49 ± 0.45	88.69 ± 2.73
Methionine	11.50 ± 0.17	12.40 ± 0.44	10.81 ± 0.36	8.14 ± 0.22
Phenylalanine	39.81 ± 1.45	46.80 ± 1.44	44.97 ± 0.58	32.67 ± 0.14
Proline	44.10 ± 2.95	30.87 ± 1.96	36.93 ± 0.32	38.83 ± 0.60
Serine†	58.02 ± 0.71	79.09 ± 0.53	75.81 ± 0.62	58.18 ± 0.76
Threonine†	51.07 ± 0.70	55.43 ± 0.52	57.23 ± 0.30	43.85 ± 1.40
Tryptophan‡	7.60 ± 0.24	9.33 ± 0.22	6.30 ± 0.00	8.89 ± 1.11
Tyrosine†	27.73 ± 0.20	27.04 ± 0.37	29.37 ± 0.17	28.57 ± 0.40
Valine	61.35 ± 4.04	48.87 ± 0.35	47.52 ± 0.96	53.22 ± 0.98

* Mean values and SD from triplicate analyses of a 22 hr hydrolysate.
† Corrected for decomposition during 22 hr hydrolysis (serine, 0.90; threonine, 0.95; and tyrosine, 0.95).
‡ Determined by the method of Spies and Chambers (14).

dence all four proteins were significantly different from each other (Table 3). However, if a plot is made of the logarithms of the moles of each amino acid in decreasing order of concentration in a manner similar to that suggested by Cook and Martin (24), there are similarities between the chylomicron and HDL proteins, and between the VLDL and the LDL proteins (Fig. 1). In this type of plot the slope of the line connecting the values for any two amino acids is a measure of the ratio in which these two amino acids occur in the protein. If the proteins in two samples are identical, the ratio of any two amino acids would be the same in each sample regardless of the absolute amount of protein analyzed. Consequently, identical proteins would have curves of exactly the same shape, regardless of their position on the plot (25). This is a useful procedure for establishing an amino acid "profile" for each protein.

DISCUSSION

Although the proteins from chylomicrons and HDL are not identical in terms of amino acid composition, their similarities are marked (Fig. 1). This finding supports the work of Rodbell and Fredrickson (7) who showed similarities between a Veronal buffer-soluble chyloprotein from human lymph and the HDL protein from human serum when compared by *N*-terminal analysis, paper electrophoresis, and "fingerprinting" of peptides after chymotryptic digestion. We have made no attempt in these experiments to fractionate the chyloprotein, although we have noted in the dog that the Veronal buffer-soluble chyloprotein from lymph and the HDL protein from serum do not have the same amino acid composition (26).

There apparently has been some doubt as to whether the VLDL exist as a class discrete from chylomicrons and LDL. On the one hand, Rodbell (6) found a small amount of *N*-terminal glutamic acid in this fraction which was not present in chylomicrons. Shore (5) found the principal *N*-terminal in VLDL to be serine while the major *N*-terminal in LDL was glutamic acid. The immunological studies of Briner, Riddle, and Cornwell (27), and the subsequent discussion by Aladjem (28), indicate that the VLDL and the LDL share some antigenic

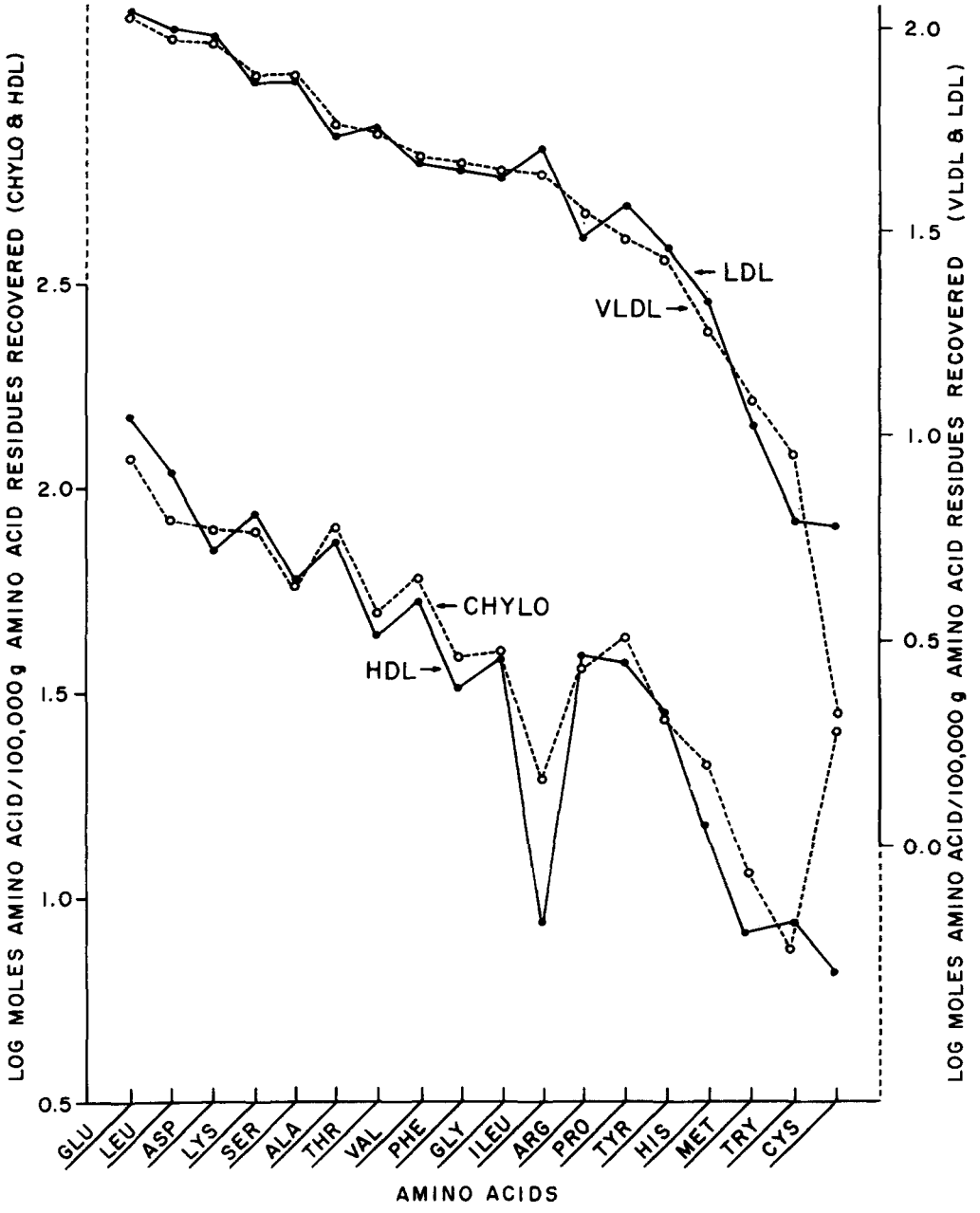

FIG. 1. Log plot of the concentrations of amino acids from proteins of chylomicrons, very low density, low density, and high density lipoproteins.

CHYLO, chylomicrons; GLU, glutamic acid; LEU, leucine; ASP, aspartic acid; LYS, lysine; SER, serine; ALA, alanine; THR, threonine; VAL, valine; PHE, phenylalanine; GLY, glycine; ILEU, isoleucine; ARG, arginine; PRO, proline; TYR, tyrosine; HIS, histidine; MET, methionine; TRY, tryptophan; CYS, half cystine.

components but not others, so that they are immunologically similar but not identical. In addition, when the distribution function, $g(S_f)$, is plotted against the flotation rate, (S_f), there is a marked difference in the flotation rate maxima between the VLDL and the LDL (29,30). On the other hand, Shore and Shore (31), Scanu and Hughes (32), and Granda and Scanu (33) reported that the amino acid composition of the VLDL protein was the same as the LDL protein. In an earlier report (34), we noted both similarities *and* differences between the VLDL and LDL proteins in terms of amino acid composition. As seen in Fig. 1, the proteins, while not identical, have similar "profiles" when the logarithms of the moles of their amino acid residues are plotted.

Margolis and Langdon (35) have compared the results of their analysis of the low density (β_1–) lipoprotein to ours (36) in some detail. Since they appparently used formula molecular weight rather than residue molecular

TABLE 3 *t* VALUES FOR THE COMPARISON OF AMINO ACID COMPOSITIONS OF PROTEINS FROM FOUR LIPOPROTEIN FRACTIONS

Amino Acid	HDL vs. Chylomicrons	VLDL vs. LDL	HDL vs. LDL	VLDL vs. Chylomicrons	HDL vs. VLDL	Chylomicrons vs. LDL
Alanine	3.16	*9.82*	*17.00*	*21.06*	*13.40*	*25.76*
Arginine	1.58	*5.54*	*6.00*	1.02	3.24	3.92
Aspartic acid	*9.52*	1.18	*34.92*	*21.76*	*32.32*	*23.98*
Half cystine	4.38	*10.00*	0.36	*5.64*	3.66	*4.66*
Glytamic acid	*34.42*	0.52	*92.28*	*14.08*	*114.22*	*13.24*
Glycine	2.38	3.24	*13.54*	4.56	*9.60*	4.56
Histidine	*14.54*	3.64	*13.78*	3.38	3.46	0.68
Isoleucine	*73.12*	*7.92*	*258.16*	*35.76*	*51.84*	*151.72*
Leucine	*18.18*	*9.92*	*8.84*	*28.62*	*11.64*	*64.18*
Lysine	1.86	*10.22*	*7.68*	2.24	*5.36*	*4.94*
Methionine	*21.02*	*4.92*	*11.06*	3.34	*15.08*	3.06
Phenylalanine	*8.54*	4.08	*35.74*	*5.92*	*16.90*	*5.72*
Proline	3.02	*4.92*	*4.88*	*6.30*	*6.26*	*4.16*
Serine	0.32	*7.28*	*37.40*	*42.48*	*50.50*	*32.62*
Threonine	*7.98*	*5.30*	*16.16*	*8.66*	*13.46*	*13.92*
Tryptophan	1.96	*24.66*	4.00	*9.38*	0.68	*9.64*
Tyrosine	3.22	*9.96*	3.44	2.80	*4.86*	*11.10*
Valine	3.38	2.30	*7.16*	*5.32*	*7.22*	*5.74*
No. of amino acids significantly different at the 99% level of confidence ($t_{0.99}$ = 4.604)	8	12	15	13	14	13

The values compared in this statistical analysis were the moles of each amino acid per 100,000 g of amino acid residues (Table 2).

weight, we have compared our data to their 22 hr hydrolysate by converting all of our values so that aspartic acid = 100 moles (Table 4). This procedure allows comparison regardless of the method of recovery of the amino acids since the ratio of each amino acid to the others will not change. Our greatest differences occur in tryptophan, methionine, lysine, and tyrosine, respectively. We have not noticed such a marked variation in tyrosine and lysine between hydrolysates as they report (26). In Table 4 we also have compared our data in a similar manner with those of Scanu and Hughes (32), Granda and Scanu (33), Wathen and Levy (26), and Levy and Fredrickson (23). We have noted the work of Shore and Shore (31) and Brown, Davis, Clark, and Van Vunakis (37), but

TABLE 4 AMINO ACID COMPOSITION OF THE PROTEIN FROM LOW DENSITY LIPOPROTEIN RELATIVE TO ASPARTIC ACID = 100 MOLES

Amino Acid	Scanu & Hughes (32)	Granda & Scanu (33)	Margolis & Langdon (35)	Wathen & Levy (26)	Levy & Fredrickson (23)	Present Work
Alanine	66	57	56	62	59	58
Arginine	33	27	30	34	—	33
Aspartic acid	100	100	100	100	100	100
Half cystine	0	3	6	—	—	7
Glutamic acid	113	107	114	113	118	114
Glycine	46	40	44	49	45	46
Histidine	17	19	23	24	—	23
Isoleucine	39	49	52	56	53	54
Leucine	110	103	106	112	108	105
Lysine	65	74	53	78	—	77
Methionine	1	13	16	12	13	12
Phenylalanine	53	45	49	50	49	48
Proline	41	30	37	41	40	39
Serine	92	80	75	88	74	81
Threonine	63	60	59	66	56	61
Tryptophan	—	13	4	—	—	7
Tyrosine	28	29	22	37	33	31
Valine	33	46	51	53	52	53
Average per cent difference	21	15	11	5	4	
Ratio of amino acids with greater than 5% difference	10/17	12/18	7/18	6/16	4/13	
Percentage of amino acids with greater than 5% difference	59	67	39	37	31	

we have confined our comparison to the data obtained by the Spackman, Stein, and Moore (38) method of automatic recording amino acid analysis. In order to gain some notion of similarities and differences between laboratories, we have calculated the per cent difference between each amino acid reported by each investigator and ourselves and have averaged these per cent differences. We also have noted the number of amino acids that differ by more than 5% and the percentage of the total that this represents. This gives us a primitive but simple way to compare our data. The comparison assumes that a reasonably good value for aspartic acid has been obtained. Under these conditions we notice the best agreement with Levy and Fredrickson (23), who isolated their material by flotation; next with Wathen and Levy (26), who isolated their material by precipitation with sulfated rice starch; and finally with Margolis and Langdon (35), who isolated their material by precipitation with dextran sulfate. One notices in comparing Table 4 to Table 5 that there is better agreement between laboratories on LDL analysis than HDL analysis, which would indicate that the LDL is being isolated as a more homogeneous fraction. It is disappointing that there is not better agreement between laboratories. Good replication within a given laboratory is possible: the analyzer is capable of distinguishing between two different proteins and of providing reproducible results when more than one hydrolysate is prepared from the same protein (26).

While the HDL can be fractionated by repeated ultracentrifugation into a number of separate components, most investigators have found little apparent difference in the amino acid composition between these components (21,23,32). We have compared our data to those obtained by these workers—using the data from Levy, Fredrickson, and Laster (39), which represented a more complete analysis than the data from Levy and Fredrickson (23)—as well as by Shore and Shore (40). We noted the work of Shore (5) but again confined our comparison to data obtained by the Spackman, Stein, and Moore method (38). As before we converted all data to aspartic acid = 100 moles (Table 5). Using the same procedure of calculating the per cent difference of each amino acid, we agree best with Levy, Fredrickson, and Laster (39). That the value for aspartic acid may be a reasonably true one is borne out by the fact that all laboratories report a low value for isoleucine which is generally very reproducible. The comparison of the data from the HDL analyses shows less agreement than the LDL analyses, even though all fractions were recovered by flotation, again indicating that the HDL is apparently not isolated in as homogeneous a form as the LDL.

The lack of good agreement between laboratories would indicate that we may not be analyzing the same lipoprotein components. This may be due to the fact that under differing conditions of lipid loading of the plasma donor, different lipoproteins may appear in the density spectrum where another normally appears as the

TABLE 5 AMINO ACID COMPOSITION OF THE PROTEIN FROM HIGH DENSITY LIPOPROTEIN RELATIVE TO ASPARTIC ACID = 100 MOLES

Amino Acid	Scanu & Hughes (32)	Scanu & Granda (21)	Shore & Shore (40)	Levy, Fredrickson, & Laster (39)	Present Work
Alanine	92	93	97	93	106
Arginine	81	56	67	66	55
Aspartic acid	100	100	100	100	100
Half cystine	—	6	8	7	9
Glutamic acid	238	233	227	242	213
Glycine	67	55	57	52	54
Histidine	16	19	25	22	21
Isoleucine	9	11	8	7	12
Leucine	166	176	162	161	154
Lysine	117	118	130	131	124
Methionine	7	12	17	11	11
Phenylalanine	55	46	40	41	46
Proline	91	62	75	58	54
Serine	99	82	82	82	81
Threonine	62	62	62	59	61
Tryptophan	—	39	—	—	12
Tyrosine	44	51	42	39	40
Valine	62	79	71	66	74
Average per cent difference	21	21	14	10	
Ratio of amino acids with greater than 5% difference	14/16	11/18	10/17	9/17	
Percentage of amino acids with greater than 5% difference	87	61	59	53	

major component. When VLDL from lipemic plasma are extracted exhaustively with cold ether and the resultant phospholipid–protein residues are examined in the analytical ultracentrifuge, we have noted a 13S component, typical of a residue from LDL, a 6S component, typical of a residue from chylomicrons, as well as a major 4–5S component, typical of the residue from the VLDL (8). These findings were corroborated to a large extent by the work of Gustafson, Alaupovic, and Furman (41) who examined phospholipid–protein residues of VLDL from hyperglyceridemic serum and found a 4S component (apolipoprotein A) typical of HDL residues, a 14S component (apolipoprotein B) typical of LDL residues, and a 7S component (apolipoprotein C) not typical of either residue but with *N*-terminal serine and threonine and typical, perhaps, of VLDL protein.

That lipemic VLDL represent a mixture of proteins is borne out by a comparison of the protein composition of VLDL (Table 6) from the fasting state (present work) and from lipemic donors (42) which indicates a substantial difference between fractions and which would be consistent with the presence of LDL, chylomicron, and even HDL proteins in the VLDL density range during lipemia. These would not be distinguished prior to delipidation since they have the same flotation rate as the VLDL and are recovered with it. It is likely that the same situation holds between laboratories, i.e., we are analyzing mixtures of different lipoproteins but with extremely similar flotation rates, particularly in the case of the HDL.

TABLE 6 Amino Acid Composition of the Protein from Very Low Density Lipoprotein Relative to Aspartic Acid = 100 Moles

Amino Acid	Bobbitt (42)	Granda & Scanu (33)	Present Work
Alanine	71	52	63
Arginine	48	30	38
Aspartic acid	100	100	100
Half cystine	1	4	2
Glutamic acid	135	109	114
Glycine	48	41	49
Histidine	17	14	20
Isoleucine	40	34	48
Leucine	94	100	101
Lysine	83	79	83
Methionine	16	14	13
Phenylalanine	43	44	50
Proline	38	25	33
Serine	68	69	85
Threonine	50	58	59
Tryptophan	—	17	10
Tyrosine	23	29	29
Valine	56	51	52
Average per cent difference	16	20	
Ratio of amino acids with greater than 5% difference	14/17	11/18	
Percentage of amino acids with greater than 5% difference	82	61	

The data for the amino acid analysis of VLDL by Granda and Scanu (33) are included in Table 6 as an example, perhaps, of variations resulting from differences in isolation techniques. These workers isolated their VLDL by flotation, while we isolated ours by complex formation with amylopectin sulfate. We have obtained preliminary evidence in our laboratory (43) that there are some slight differences in the phospholipid components of LDL isolated by complex formation as compared with LDL isolated by flotation. Since it appears that the polysaccharide may complex with the phospholipids, isolation by use of polyanions may select a more specific portion of the lipoprotein spectrum. Regardless of the method of isolation, the problem still remains of isolating a lipoprotein which contains a specific peptide or peptides and which is truly representative of its class.

Although the proteins from the plasma lipoproteins are rich in hydrophilic amino acids such as glutamic acid, aspartic acid, and lysine, respectively, and hydrophobic amino acids such as leucine, they are not different enough in this respect from other plasma proteins (44) to account for their great affinity for lipids. It is likely that this property is generated by their primary, secondary, tertiary, or quaternary structures which are essentially unknown at this time.

The comparison of the amino acid composition of the proteins from the four major classes of serum lipoproteins provides support for the view that their protein moieties are not identical and that interconversions generally cannot take place by a simple process of lipolysis. There are, however, some striking similarities between the proteins of the LDL and the VLDL on the one hand, and between chylomicrons and the HDL on the other. Certain of the variations between preparations and between results from different laboratories suggest that density may not be an entirely adequate means of distinguishing between true lipoprotein classes, a view which is quite clearly demonstrated by the finding of Levy, Fredrickson, and Laster (39) that an HDL protein can be found in the LDL density fraction in the serum from patients suffering from abetalipoproteinemia, and who have a complete deficiency of LDL protein.

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