Isolation and Characterization of High Density Lipoprotein Apoproteins in the Non-human Primate (Vervet)*

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Six different apoproteins have been isolated and characterized from vervet high density lipoprotein (HDL). The apoproteins were isolated and purified by a combination of gel and ion exchange chromatography along with preparative isoelectric focusing. Measured properties of the apoproteins included: relative mobility on urea-polyacrylamide gel electrophoresis (PAGE), isoelectric point, molecular weight, amino acid composition, sialic acid content, ability to activate purified lipoprotein lipase, and relative content in HDL. Based on these characteristics, several analogies were seen between human and vervet HDL apoproteins. Vervet apoprotein A-I (apo-A-I) was polymorphic, had a molecular weight of 28,000, and was the major apoprotein of vervet HDL (69% of total HDL protein). Vervet apo-A-II, a monomeric protein with a molecular weight of 9,900, was the second most abundant HDL apoprotein (11% of total HDL protein). Vervet apo-C-III was a glycoprotein (2 mol of sialic acid/mol of protein) with a molecular weight of 9,500. Apo-C-III of the vervet was a potent activator of lipoprotein lipase, but it differed chemically (sialic acid and amino acid analysis) from human apo-C-II. The vervet threonine-poor apoproteins were small (Mᵦ = 13,900 and 11,500) monomeric proteins that were variable in amount among HDL of individual animals. Apo-C-I was not present in quantities sufficient for isolation and no arginine-rich apoprotein was detected in vervet HDL. It was concluded that: 1) vervet and human HDL apoproteins are similar based on chemical, physical, and functional characteristics, and 2) the relative amounts of the threonine-poor apoproteins in vervet HDL are greater and more variable than in normal human HDL.

HDL* concentrations are influenced by environmental conditions and have been reported to increase with exercise (1). * This work was supported by a National Heart, Lung, and Blood Institute Grant (SCOR) HL14164, by a North Carolina Heart Association grant, and by Institutional National Research Service Award HL07115 (to J. S. P.) and was carried out during completion of the requirements for the degree of Doctor of Philosophy in Comparative and Experimental Pathology from the Bowman Gray School of Medicine of Wake Forest University (by J. S. P.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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The abbreviations used are: HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; apo-A-I, -A-II, -C-I, etc., apoproteins A-I, A-II, C-I, etc.; apo-E, arginine-rich apoprotein; apo-HDL, protein portion of HDL; SDS, sodium dodecyl sulfate; RF, relative mobility; pI, isoelectric point; PAGE, polyacrylamide gel electrophoresis; IEF, isoelectric focusing.

and decrease with dietary modification such as a high cholesterol or high polyunsaturated fat diet, or both (2-4). HDL concentration also has been inversely correlated with the incidence of coronary heart disease (5) and increased HDL concentration is now thought to afford protection from atherosclerosis (6). These HDL responses have prompted the study of metabolism of the HDL apoproteins; presumably availability of at least some of the HDL apoproteins helps determine the plasma HDL concentration (7). However, studies of environmental influences on HDL metabolism are difficult to conduct in human beings since absolute control of the subjects' environment is hard to achieve.

We have sought to use nonhuman primates as animal models for study of HDL metabolism in atherosclerosis because of the close phylogenetic relationship of nonhuman and human primates. We were encouraged by the reported similarities in apo-A-I and apo-A-II of two nonhuman primate species compared to those of humans (8, 9). In order to find the most appropriate model we have examined the dietary-induced modifications of plasma lipoproteins of several nonhuman primate species. One of the species which has appeared promising is the African green monkey (Cercopithecus aethiops) of the vervet subspecies. In a study of male vervets, plasma LDL and HDL cholesterol were lowered in response to a diet rich in polyunsaturated fat, a finding similar to that found in humans by Shepherd et al. (3). Vervets ingesting a high fat diet have a lipoprotein composition and distribution similar to that of North American human beings (4). Earlier studies have shown that the African green monkey develops diet-induced atherosclerotic lesions with topography and morphology similar to those seen in human beings (10, 11).

The present study was undertaken to characterize the apoproteins of HDL in vervet monkeys in preparation for studies on diet-induced modifications of HDL metabolism. We report the isolation and some chemical, physical, and functional characteristics of HDL apoproteins in male vervet monkeys fed diets containing 40% of calories from fat and cholesterol levels covering the range of those in the diet of the American human primate population.

EXPERIMENTAL PROCEDURES

Animals—Adult male vervets available from ongoing experiments were studied. All were fed a diet with 40% of calories supplied as fat. Dietary fat was either lard, safflower oil, or butter and the cholesterol content varied from 0.034 to 0.79 mg/kcal. Blood samples from animals within a dietary group were pooled for the isolation of HDL apoproteins so that diet effects could be observed if present. Blood collection and plasma isolation methods have been detailed previously (12).

HDL and Apo-HDL Isolation—Plasma HDL was isolated by the combined ultracentrifugation and agarose column method described

L. L. Rudel, unpublished observations.
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by Rude1 et al. (13). Only HDL samples which exhibited a single migrating band on agarose electrophoresis (14) were used.

HDL samples were dialyzed exhaustively against 0.01% EDTA in glass-distilled water and lyophilized prior to delipidation. Delipidation of HDL was accomplished with freshly redistilled ethanol and ether according to the procedure of Strnad and Aurelian (15). Lipid-free apoproteins were stored dry at 20°C under a N2 atmosphere until ready for use.

Apo-HDL (50 to 60 mg) was fractionated by gel chromatography on Ultragel AcA34 (LKB Instruments, Inc., Rockville, Md.). The column (90 x 2.5 cm) was equilibrated and run with 0.05 M Tris-HCl, 6 M urea, pH 8.4, at 4°C. Urea (Fisher Scientific Co., Fairlawn, N. J.) solutions (10 M) were deionized just prior to use with Rexyn I-300 (Fisher Scientific Co.). Fractions (2.5 ml) with apoproteins were pooled and dialyzed exhaustively against 0.01% EDTA shortly after elution. The column flow rate was 10 ml/h.

Ion Exchange Chromatography of Smaller Molecular Weight Apoproteins—DEAE-cellulose (Whatman DE52, Whatman Ltd., Maidstone, England) was equilibrated with 0.01 M Tris-HCl, 6 M urea, pH 8.4, at a column flow rate of 3 cm/h. Small proteins of apo-HDL (30 to 40 mg) from region III of the AcA34 column were loaded onto the column in 6 to 8 ml of equilibrating buffer and were eluted with a 1000-ml linear buffer gradient from 0.025 to 0.07 M Tris-HCl, 6 M urea, pH 8.6. Equivalent results were obtained using smaller amounts of protein (10 to 20 mg) and a column flow rate of 1 cm/h. The flow rate of both columns was 10 ml/h.

Preparative isoelectric focusing—Flat bed isoelectric focusing in Ultragel granulated gel (LKB Instruments, Inc.) was done according to the method of Winter et al. (16), with slight modification. The gel slurry was 3 g of Ultradex, 39 ml of 10 M M urea, 5% glycerol solution. The cathode chamber was filled with 0.02 M NaOH followed by addition of the fluorescent reagent and sample was reduced to 2.2 ml. Fifty-microgram protein samples (20 to 50 mg) were applied to wells in 40 11 tubes with a Barnsted conductivity bridge PM-70 C B (The Barnsted Endend) was equilibrated with 0.01 M Tris-HCl buffer, pH 8.6. Equivalent results were obtained using smaller amounts of protein (10 to 20 mg) and a column flow rate of 1 cm/h. The flow rate of both columns was 10 ml/h.

Analytical isoelectric focusing—Analytical isoelectric focusing was done according to the method of Gidez et al. (17) with modification. Gels were 7.5% acrylamide (10 x 0.5 cm) containing 2% Ampholine (pH range, 4 to 7), 6.3 M urea, and 10% glycerol. Delipidated apoprotein samples (20 to 100 mg) were applied to the cathodic end of the gel in 100- to 200-ml of 0.01 M Tris-HCl buffer, 8 M urea, pH 8.6, and overlaid with 100 M of 1% Ampholine (pH range, 3 to 10), and 5% glycerol solution. The cathode chamber was filled with 0.02 M NaOH and the anode chamber, with 0.01 M H2PO4. Tubes were precooled at 100 V for 30 min prior to sample application and then at 0.1 watt/tube for 20 h at 4°C. After the run was stained with Coomassie blue according to the procedure of Malik and Berrie (18). The pl value of the proteins was determined by running a blank gel as described by Gidez et al. (17), with slight modification. The gel slurry was 3 g of Ultradex, 39 ml of 10 M M urea, 5% glycerol solution. The cathode chamber was filled with 0.02 M NaOH followed by addition of the fluorescent reagent and sample was reduced to 2.2 ml. Fifty-microgram protein samples (20 to 50 mg) were applied to wells in 40 11 tubes with a Barnsted conductivity bridge PM-70 C B (The Barnsted Endend) was equilibrated with 0.01 M Tris-HCl buffer, pH 8.6. Equivalent results were obtained using smaller amounts of protein (10 to 20 mg) and a column flow rate of 1 cm/h. The flow rate of both columns was 10 ml/h.

Amino Acid Analysis—Aldolase of individual apoproteins (200 to 400 mg) were lyophilized in 5-ml ampules and gaseous with nitrogen before addition of 0.5 ml of constant boiling HCl (6 N). The ampules were sealed under vacuum and hydrolysis was carried out for 24 h at 120°C. The hydrolysate was dried under nitrogen and 200- to 250-ml citrate buffer, pH 2.0, was added to the vial along with 40 ml of n-propanol (Calbiochem, San Diego, Calif.) as an internal standard in 40 ml of citrate buffer, pH 2.0. Amino acid analysis was done on a Technicon semimicrosample amino acid analyzer (Technicon Instruments, Tarrytown, N. Y.) after applying 100 ml of sample to both the acid and basic column. Tryptophan content was determined by the fluorimetric method of Pajot (23).

Lipid and Protein Assays—Methods used were described in detail by Winter et al. (16) and modified when necessary. Acetyl neuraminic acid (Sigma Chemical Co., St. Louis, Mo.) was used as the standard.

The assay mixture consisted of 25 ml of substrate (0.54 mg of triglyceride; 52,900 cmpg/mg), 60 mg of crystalline bovine serum albumin (Sigma Chemical Co.), 1 M of LPL, and 22 to 24 mg of an individual HDL apoprotein or 0.07 mg of protein added as intact HDL. The total volume of the incubation mixture was made up to 1 ml with 0.1 M Tris-HCl, pH 8.6. Reactions were stopped and the fatty acids were extracted by the method of Schotz et al. (24). Radioactive fatty acids released were counted in scintilux-2 (New England Nuclear). All radioactivity determinations were done on a model DPM 100 liquid scintillation counter. Sodium acetylation was done on a Technicon semimicrosample amino acid analyzer (Technicon Instruments, Tarrytown, N. Y.) after applying 100 ml of sample to both the acid and basic column. Tryptophan content was determined by the fluorimetric method of Pajot (23).

The cationic acid content of individual apoproteins was determined by the method of Murayama et al. (24) with slight modification. The fluorescent reagent was prepared with redistilled 90% ethanol instead of methanol and the total volume of the reagent and sample was reduced to 2.2 ml. Fifty-microgram aliquots of proteins were lyophilized in 12-ml conical centrifuge tubes and sialic acid was released from the proteins by incubation with 0.05 M H2SO4 at 37°C for 2 h as described by Codea et al. (25). The incubation was stopped by addition of an equal volume (100 ml) of 0.05 M NaOH followed by addition of 2 ml of fluorescent reagent. N-Acetyl neuraminic acid (Sigma Chemical Co., St. Louis, Mo.) was used as the standard.

Lipoprotein Lipase Assay—Radiolabeled [9,10-3H]triolein (New England Nuclear, Boston, Mass.), purified by thin layer chromatography as described by Catlett (26), was dissolved in an ethanol solution (87%) and used as substrate. A 2 ml of intralipid (Cutter Medical, Berkeley, Calif.). Substrate was prepared by sonicating 2 ml of intralipid, 3 ml of 0.1 M Tris-HCl, pH 8.6, and 20 ml of [9,10-3H]triolein (dissolved in ethanol) for 20 min in a Uitrafilter II sonice water bath (Buehler Ltd., Evanston, Ill.). Lipoprotein lipase was purified from bovine milk using heparin affinity chromatography as described by Egeberg and Ohrenberg (26).

The assay mixture consisted of 250 ml of substrate (0.54 mg of triglyceride; 52,900 cmpg/mg), 60 mg of crystalline bovine serum albumin (Sigma Chemical Co.), 1 M of LPL, and 22 to 24 mg of an individual HDL apoprotein or 0.07 mg of protein added as intact HDL. The total volume of the incubation mixture was made up to 1 ml with 0.1 M Tris-HCl, pH 8.6. Reactions were stopped and the fatty acids were extracted by the method of Schotz et al. (24). Radioactive fatty acids released were counted in scintilux-2 (New England Nuclear). All radioactivity determinations were done on a model DPM 100 liquid scintillation counter.
scintillation counter (Beckman Instruments, Inc.); samples were counted to a 2-s error of 2%.

Other Analyses—Protein determinations were performed by the method of Lowry et al. (28) using bovine serum albumin, Fraction V (Sigma Chemical Co.), as the standard. When ampholines were present, the Bio-Rad protein assay procedure (Bio-Rad Laboratories, Richmond, Calif.) using rabbit v-globulin (Bio-Rad Laboratories) as the standard. Cholesterol determinations were done according to the procedure of Rudel and Morris (29). Phospholipids were quantitated by the method of Fiske and Subbarow (30), while triglycerides were done according to the method of Sadesai and Manning (31). Free and ester cholesterol and triglycerides were quantitated after Folch extraction (32) and thin layer chromatography of the HDL lipids was done as detailed previously (33).

RESULTS

The percentage composition of vervet HDL is shown in Table I. The HDL was 45% protein and 55% lipid; over half of the lipid mass was phospholipid. There was no significant difference in the average composition of HDL from monkeys consuming diets containing low or moderate cholesterol levels.

Size fractionation of vervet monkey apo-HDL by gel chromatography on Ultragel AcA34 results in three distinct regions (Fig. 1, top). Region I was not analyzed in detail but sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) showed that this material behaved as large molecular weight aggregates, probably due to irreversible self-association of the region II material (34). Rechromatography of Peak I material (Fig. 1, bottom) was performed and a single peak was found which eluted in the same position as the original Peak II. SDS-PAGE of the Peak II material showed that a single band was present with an apparent molecular weight of 27,800 ± 1020 (mean ± standard error). This protein co-migrated with and appeared to be analogous to human apo-A-I (see below). It subsequently will be referred to as apo-A-I.

TABLE I

| Values given are mean ± standard error. |
|----------------------------------------|
| **Composition** | **Control (n = 19)** | **Test (n = 20)** |
|-----------------|----------------------|------------------|
| Protein         | 44.5 ± 0.5           | 44.7 ± 0.8       |
| Lipid           |                      |                  |
| Cholesterol ester | 19.4 ± 0.4           | 21.4 ± 0.4       |
| Free cholesterol | 3.6 ± 0.1            | 3.6 ± 0.1        |
| Phospholipid    | 30.6 ± 0.5           | 28.7 ± 0.6       |
| Triglyceride    | 1.9 ± 0.2            | 1.6 ± 0.3        |

* Animals were fed a diet containing 0.034 (control) or 0.79 mg of cholesterol/kcal (test) with 40% of the calories as fat (mainly lard).

Vervet apo-A-I was polymorphic and had at least four major bands as determined by urea-PAGE and IEF (Figs. 3 and 4, respectively). The *R* values for vervet apo-A-I ranged from 0.32 to 0.39 and the range of protein was 5.9 to 6.3. Vervet apo-A-I was found to contain relatively large amounts of glutamic acid, leucine, lysine, and alanine, while it was missing the amino acid isoleucine (Table II). This protein did not contain sialic acid and did not activate purified lipoprotein lipase (Table III). The content of apo-A-I in HDL from individual animal samples was relatively constant, i.e. 69 ± 4% (mean ± standard error) of the total HDL protein, as determined by densitometric scanning of IEF gels (n = 9).

Region III from the AcA34 column separation of apo-HDL (Fig. 1, top) was found to contain several proteins on SDS and urea-PAGE and further fractionation was attempted with ion exchange chromatography on DEAE-cellulose. Three elution peaks were consistently obtained as shown in the pattern of Fig. 2. Each peak was heterogeneous as seen on urea-PAGE and IEF gels (Figs 3 and 4) and contained more than one

**TABLE II**

Amino acid composition of apo-HDL proteins

| Amino acid | A-I | DI-1 | DI-2 | DI-1 | DI-2 | DII |
|-----------|-----|------|------|------|------|-----|
| Asp       | 16 (18)^b | 15 (14)^b | 5 (3)^b | 13 (13)^b | 3 (6)^d | 7 (7)^* |
| Thr       | 8 (10) | 1 (5) | 5 (6) | 2 (1) | 4 (10) | 8 (5) |
| Ser       | 12 (12) | 4 (8) | 4 (6) | 6 (9) | 5 (11) | 7 (10) |
| Glu       | 38 (35) | 11 (10) | 16 (16) | 10 (11) | 9 (18) | 13 (11) |
| Gly       | 11 (11) | 14 (11) | 2 (3) | 14 (10) | 2 (3) | 8 (3) |
| Ala       | 23 (23) | 21 (15) | 7 (5) | 16 (14) | 7 (9) | 12 (10) |
| Val       | 15 (12) | 1 (1) | 7 (6) | 2 (2) | 4 (5) | 5 (6) |
| Met       | 1 (3) | 1 (1) | 1 (1) | 0 (2) | 0 (2) | 1 (2) |
| Ile       | 0 (0) | 2 (2) | 0 (2) | 1 (2) | 1 (1) | 0 (0) |
| Leu       | 34 (35) | 5 (4) | 8 (8) | 4 (4) | 7 (10) | 7 (5) |
| Tyr       | 6 (6) | 3 (4) | 3 (4) | 1 (4) | 9 (6) | 1 (3) |
| Phe       | 5 (6) | 4 (7) | 4 (4) | 3 (8) | 1 (3) | 3 (4) |
| Lys       | 24 (20) | 4 (4) | 8 (9) | 5 (4) | 6 (5) | 6 (8) |
| His       | 7 (6) | 3 (2) | 0 (0) | 3 (2) | 0 (0) | 1 (1) |
| Arg       | 14 (17) | 9 (8) | 1 (0) | 7 (8) | 2 (1) | 3 (2) |
| Pro       | 12 (10) | 5 (4) | 4 (4) | 2 (4) | 4 (5) | 3 (2) |
| Trp       | 5 (7) | 0 (0) | 0 (0) | 0 (3) | 0 (3) | 0 (N. D.)^* |

^a Human A-I apoprotein composition (39).
^b Human threonine-poor apoprotein composition (37).
^c Human A-II apoprotein composition (41).
^d Human C-II apoprotein composition (43).
^e Human C-III apoprotein composition (43).
^f Determined by the fluorescence method of Pajot (23).
^* N. D., not determined.

**TABLE III**

Activation of purified lipoprotein lipase by isolated HDL apoproteins

| Protein | Amount of protein | Triglyceride released/ml incubation medium |
|---------|------------------|------------------------------------------|
|         | *μg* | cpm |                |
| DI-1    | 22   | 3753 |
| DI-2    | 23   | 3519 |
| DI-1    | 24   | 3494 |
| DI-2    | 24   | 2966 |
| DII     | 23   | 8570 |
| A-I     | 23   | 5266 |
| Enzyme blank | 67 | 729 |
| Activator blank | 67 | 3155 |
| Positive control | 67 | 35087 |
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**FIG. 2.** Ion exchange (DEAE-cellulose) chromatography of AcA34 Peak III (Fig. 1) material from apo-HDL. Fractions within the regions designated by roman numerals were pooled for further analysis.

**FIG. 3.** Urea-PAGE of apo-A-I and small molecular weight apoproteins isolated by DEAE-cellulose column chromatography as shown in Fig. 2. The roman numerals correspond to the elution peaks indicated in Fig. 2. Where peaks were asymmetrical, the front and back regions were analyzed separately.

**FIG. 4.** Analytical isoelectric focusing of apo-A-I and of the smaller molecular weight apoproteins isolated by DEAE-column chromatography as shown in Fig. 2. Isoelectric points are indicated for the bands. Fractions are identified as in Fig. 3.

**FIG. 5.** Urea-PAGE gels of the isolated and purified HDL apoproteins. Apo-HDL, apo-A-I, and AcA34-III, the pooled material Peak III from the AcA34 column (Fig. 1), were included for reference. Designations are based on the DEAE-column elution and urea-PAGE $R_F$, as described under "Experimental Procedures."

A nomenclature system for the small apoproteins was adopted based on the elution position from the ion exchange column (DEAE-cellulose) and $R_F$ on urea-PAGE as described under "Experimental Procedures." Since the isoelectric points were quite different for individual proteins within each DEAE peak (Fig. 4), preparative isoelectric focusing was used to purify the small proteins.

From Peak I (Fig. 2), DI-1 had a mobility on urea-PAGE of $R_F = 0.38$ and accounted for about half of the protein of the DI peak (Fig. 3). The position of DI-1 on urea-PAGE overlapped with that of apo-A-I (Figs. 3 and 5). The DI-1 protein had a $pI = 6.94$. After final purification by IEF, the DI-1 protein migrated as a single band on urea-PAGE (Fig. 5) and IEF (Fig. 6); its mobility in these systems was not modified by $\beta$-mercaptoethanol. A molecular weight of 13,900 ± 376 was found for DI-1 by SDS-PAGE on 20% acrylamide gels (Fig. 7). A low content of threonine, valine, and leucine, an absence of tryptophan, and a high content of aspartic acid, glycine, and alanine was found by amino acid analysis of the protein (Table II). DI-1 did not contain detectable sialic acid and did not activate purified lipoprotein lipase (Table III). The amount of DI-1 in HDL was much more variable than that for the other apoproteins of HDL. The relative content of DI-1 in vervet HDL ranged from 1 to 81% of the apo-A-I content in individual HDL samples (Table IV). Shown in Figs. 5 and 6 are representative gels of apo-HDL that illustrate the variability in DI-1 content; the sample on the right contained very little DI-1, while that on the left had substantial amounts. The characteristics of this apoprotein were similar to those of one of the threonine-poor apoproteins of human HDL (Table V).
The second protein (DI-2) eluting from the DEAE column overlapped with that of apo-A-II (DI-2) and the two proteins appeared as a single wide band on urea-PAGE in all of the individual apo-HDL samples (Fig. 5). The molecular weight of DI-2 was found to be 11,500 ± 351 (Fig. 7). The protein was low in threonine, valine, and leucine, while high in aspartic acid, glycine, and alanine (Table II). Methionine and tryptophan were not present in DI-1. The protein did not contain sialic acid and did not activate purified lipoprotein lipase (Table III). The amount of DI-1 protein in individual HDL samples ranged from 4 to 12% of that amount of protein present as apo-A-I (Table IV). This protein had many similarities to the threonine-poor apoproteins of human HDL (Table V).

On urea-PAGE DI-1 was the slower migrating of the two proteins of DEAE Peak II (Rf = 0.44). Most of the DII-1 protein eluted in the back region of Peak II of the DEAE column (Figs. 3 and 4). DII-1 had a pI = 6.44 (Fig. 4). After IEF purification, this protein migrated as a single band on urea-PAGE (Fig. 5) and IEF (Fig. 6); its mobility was not altered in either system when run in the presence of β-mercaptoethanol. The position of DI-1 on urea-PAGE gels overlapped with that of apo-A-II (DI-2) and the two proteins appeared as a single wide band on urea-PAGE in all of the individual apo-HDL samples (Fig. 5). The molecular weight of DI-1 was found to be 9,900 ± 308 (Fig. 7). DI-2 did not contain the amino acids isoleucine, histidine, and tryptophan, while the amino acid in highest content was glutamic acid (Table II). The protein had no detectable sialic acid and did not activate purified lipoprotein lipase (Table III). DI-2 was the second most abundant protein in vervet HDL and represented 8 to 13% of the total apo-HDL. The characteristics of this apoprotein indicated it represented the vervet equivalent to human apo-A-II, and it will subsequently be termed apo-A-II (Table V). The range of individual animal variability of apo-A-II content in vervet HDL relative to apo-A-I was 0.189 to 0.121 (Table IV), with an apparent molar ratio of 2:1 (A-I: A-II), a ratio similar to that reported for humans (35).

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![Fig. 6. Analytical isoelectric focusing of the isolated apo-HDL proteins. Designations are as described for Fig. 5.](image)

![Fig. 7. Standard curve for determination of protein molecular weight using SDS-PAGE on a 20% polyacrylamide slab gel. Rf values were determined as described under "Experimental Procedures." Five reference standards were run and their positions were plotted (O) and the linear least squares best fit line was drawn. The Rf for each of the vervet HDL apoproteins then was calculated and plotted on the line (O), and the molecular weight was calculated. Mean ± standard error values from triplicate determinations of molecular weights for individual apoproteins are indicated. cyt. c, cytochrome c.](image)

### Table IV

Range of protein to A-I content in apo-HDL of vervet monkeys

Ratios were obtained by densitometric scanning at 635 nm of analytical isoelectric focusing gels. Apo-HDL samples were obtained from individual animals (n = 51) consuming a variety of dietary regimens. The content of small proteins was normalized to A-I content by using a protein/A-I ratio, as the amount of apo-A-I in HDL varied little from sample to sample.

| Protein | Protein/A-I ratio |
|---------|------------------|
| DI-1    | 0.811-0.010      |
| DI-2    | 0.189-0.121      |
| DII-1   | 0.123-0.037      |
| DII-2   | 0.058-0.039      |
| DIII    | 0.053-0.025      |

### Table V

Characteristics of vervet high density lipoprotein apoproteins

Characteristics summarized are relative mobility on urea polyacrylamide gel electrophoresis (Rf), isoelectric point (pI), molecular weight (M), moles of sialic acid/mol in protein (SA), ability to activate purified lipoprotein lipase (LPL), and protein/A-I ratio.

| Protein | Rf  | pI  | M   | SA | LPL | Protein/A-I ratio | Apparent apoprotein analog in humans |
|---------|-----|-----|-----|----|-----|-------------------|-------------------------------------|
| A-I     | 0.32-0.39 | 5.9-6.3 | 27,800 | 0  | 0   | 1.0               | A-I"                              |
| DI-1    | 0.38 | 6.94 | 13,900 | 0  | 0   | 0.811-0.010      | Threonine-Poor"                    |
| DI-2    | 0.46 | 5.17 | 9,900  | 0  | 0   | 0.189-0.121      | A-II"                             |
| DII-1   | 0.44 | 6.44 | 11,500 | 0  | 0   | 0.123-0.037      | Threonine-Poor"                    |
| DII-2   | 0.51 | 5.20 | 8,000  | 1  | +  | 0.058-0.039      | C-II"                             |
| DIII    | 0.29 | 5.06 | 9,500  | 2  | +  | 0.053-0.025      | C-III"                            |

* Edelstein et al. (39).
* Small apoproteins are labeled according to their elution peak from DEAE-cellulose and relative mobility (Rf) on urea-polyacrylamide gel electrophoresis.
* Shore et al. (37).
* Scanu et al. (41).
* Brown et al. (45).
The second major protein (DII-2) which eluted from DEAE Peak II (Fig. 2) had a migration on urea-PAGE of \( R_F = 0.51 \) (Fig. 3). It was more acidic (\( p_I = 5.20 \); Fig. 4) than DII-1. DII-2 was present in equal amounts in the front and back regions of DEAE column Peak II (Figs. 2 and 3). Complete purification of DII-2 was not accomplished even after repeated ion exchange chromatography and preparative isoelectric focusing; DII-2 contained a minor faster migrating contaminant band which was approximately 7% of the stained protein as determined by densitometric scanning (Fig. 5). However, a single band was obtained for “purified” DII-2 on IEF gels (Fig. 6). The mobility of DII-2 on urea-PAGE gels was not affected by \( \beta \)-mercaptoethanol. The molecular weight of DII-2 was estimated to be 8,000 ± 335 by SDS-PAGE (Fig. 7). The amino acids methionine, histidine, and tryptophan were not present in DII-2, while glutamic acid was present in the highest amount. DII-2 was found to be a glycoprotein containing 31.2 ± 2.6 \( \mu \)g of sialic acid/mg of protein (five determinations) or 1 mol of sialic acid/mol of protein. The activity of purified lipoprotein lipase was stimulated 7-fold above control levels by DII-2 (Table III). Of the isolated and purified HDL proteins, DII-2 was found to be the least abundant among individual HDL samples, and the amount of DII-2 was proportional to the amount of apo-A-I and relatively constant (Table IV). Vervet DIII appeared analogous to human apo-C-III.

**DISCUSSION**

We have reported the isolation and characterization of six different apoproteins from vervet HDL. Comparison of our purified vervet HDL apoproteins with known human HDL apoproteins has indicated that similarities exist in some of the chemical, physical, and functional characteristics. Vervet apo-A-I and -A-II closely parallel their human counterparts in all measured characteristics (amino acid analysis, mobility on urea-PAGE gels, sialic acid content, molar ratio, lipoprotein lipase activation, and molecular weight) except one; human apo-A-II (36) can exist as a dimer (two identical monomers linked by a disulfide bond), while vervet apo-A-II does not. Remarkable similarity between vervet DI-1, DII-1, and the two human threonine-poor apoproteins and vervet DIII and human apo-C-III also was found for all measured characteristics. However, vervet DIII was not as heterogeneous as human C-III; DIII was present only as one glycoprotein species (2 mol of sialic acid/mol of protein). DII-2 was the only vervet apoprotein which differed from its presumed human counterpart, apo-C-II, based on chemical and physical characteristics. However, functional similarity was indicated for vervet DII-2 and human apo-C-II by their mutual ability to activate lipoprotein lipase. The many similarities between human and vervet HDL apoproteins indicate that the vervet is a useful animal model for the study of HDL apoprotein metabolism. For example, vervet HDL contains higher amounts of the threonine-poor apoproteins than human HDL (37), and vervet HDL concentrations on the average are twice the concentration of the human population, 560 ± 279 mg/dl (38). Study of vervet threonine-poor apoprotein metabolism may afford the opportunity to determine if these apoproteins have a role in the control of plasma HDL concentrations.

The most abundant protein of vervet HDL (69% of total HDL protein) had chemical and physical characteristics similar to human apo-A-I. The similarities between vervet and human apo-A-I included molecular weight, amino acid composition, elution profile from gel chromatography column, percentage of total HDL protein, and heterogeneity on urea-PAGE and IEF gels (39). Both vervet and human apo-A-I had an apparent molecular weight of 28,000 and neither contained sialic acid. Minor differences were seen in the content of aspartic acid, glutamic acid, alanine, valine, isoleucine, and arginine (Table II). There was also close agreement in the amino acid composition of apo-A-I from the vervet and that from the baboon and rhesus monkey (8, 9). Charge heterogeneity on urea-PAGE and IEF gels has been described for human apo-A-I (39) and was observed for vervet apo-A-I samples (Figs. 5 and 6). Immunological microheterogeneity of vervet apo-A-I using an antiserum prepared from purified apo-A-I also was detected. However, the immunological microheterogeneity was not due to different antigenic sites on each polymorphic form of apo-A-I (as visualized on IEF gels) but, rather, was due to different antigenic regions within the primary sequence (NH\(_2\) versus COOH-terminal), similar to that noted for human apo-A-I (40). IEF gels of purified apo-A-I appeared more heterogeneous than the apo-A-I region from gels of HDL samples (Fig. 6). Osborne and Brewer have suggested that apo-A-I heterogeneity might be due to protein concentration steps which utilize lyophilization or dialysis (34). Our results would support their findings as the apo-I HDL samples were concentrated only once by lyophilization, while the purified apo-A-I required several concentration steps during isolation. We have ruled out the possibility that the apo-A-I charge heterogeneity was due to carbamylation by the fact that human carbamylized was not detected in apo-A-I samples by amino acid analysis.

DII-2, a small monomeric protein (M\(_r\) = 9,900), was the second most abundant HDL protein for most vervet monkeys. Its chemical characteristics, including amino acid analysis, molecular weight, percentage of total HDL protein, and mobility on urea-PAGE gels, were similar to those of apo-A-II from human, baboon, and rhesus monkeys (8, 9, 41). The amino acid analysis of apo-A-II for all four species (human, rhesus, baboon, vervet) is remarkably similar. Vervet, rhesus, and baboon apo-A-II were all missing the amino acids isoleucine, histidine, tryptophan, and arginine. Apo-A-II was slightly smaller (M\(_r\) = 8,500) in the human, baboon, and rhesus monkey than we have found using SDS-PAGE for vervet DI-2 (M\(_r\) = 9900). Vervet apo-A-II was found to be a monomeric protein by SDS-PAGE, human apo-A-II, however, can exist as two identical subunits (M\(_r\) = 8,500) connected by an interchain disulfide bond (36, 41). The monomeric nature of nonhuman primate apo-A-II also has been described in the baboon and rhesus monkey (8, 9). Although apo-A-II was the second most abundant HDL protein in most animals, there were some animals which had greater amounts of DI-1 relative to apo-A-

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II. This resulted primarily from the variability in the DI-1 level because the variability in the relative amount of apo-A-II was minimal (Table IV).

Vervet DII-2 was compared with human apo-C-II on the basis of chemical, physical, and functional characteristics. Both DII-2 and human apo-C-II have similar mobility on urea-PAGE gels and the molecular weight estimate for DII-2 on SDS-PAGE gels was 8,000 compared to 8,837 for human apo-C-II determined from the primary structure (42). Major differences exist for the amino acid composition of DII-2 and human apo-C-II (Table II); the content of threonine, serine, and glutamic acid was the most different of the amino acids. DII-2 was found to be a glycoprotein (Table V), although human apo-C-II does not contain sialic acid (43, 44). DII-2 and human apo-C-II have functional similarity in that they both are activators of lipoprotein lipase (Table III); Refs. 45 and 46). Perhaps DII-2 is more effective than human apo-C-II in the activation of lipoprotein lipase due to the apo-C-II versus DII-2 chemical differences (amino and sialic acid). This could be a possible explanation for the low plasma triglyceride and VLDL concentrations in the vervet monkey (23 mg/dl)3 consuming a variety of dietary regimens (4). Based on the functional similarities, we have concluded that DII-2 is the vervet’s equivalent of human apo-C-II.

DIII was compared with human apo-C-III on the basis of chemical, physical, and functional characteristics. DIII and apo-C-III were similar in mobility on urea-PAGE gels in which both were the fastest migrating proteins (Fig 5; Ref. 47) and amino acid analysis (Table II). Both DIII and human apo-C-III were glycoproteins; however, apo-C-III contained 0, 1, or 2 mol of sialic acid/mol of protein (47), while DIII contained 2 mol of sialic acid/mol of protein (Table V). Similar heterogeneity due to different numbers of sialic acid residues apparently did not exist for vervet DIII. DIII had some potential for activating lipoprotein lipase (Table III); a similar finding has been reported for human apo-C-III (45, 46, 48). Based on our findings we would conclude that DIII is analogous to human apo-C-III.

Recently, Shore et al. have described two “new” threonine-poor apoproteins that appear to be minor protein components in HDL of normal human beings (37). Many similarities exist between the two human threonine-poor apoproteins and vervet DI-1 and DII-1. Their appearance on urea-PAGE and IEF gels was remarkably similar; pl values were 6.94 and 6.44 for DI-1 and DII-1, respectively, while the values for the human threonine-poor apoproteins were 6.5 and 6.0. DI-1 and one of the human threonine-poor apoproteins were found to migrate in the approximate position of apo-A-I on urea-PAGE, while DII-1 was found to overlap the migration of DI-2 (vervet apo-A-II). The overlapping migration on urea-PAGE gels of the threonine-poor apoproteins with apo-A-I and A-II may explain why the threonine-poor apoproteins were not detected in human HDL until amphotericin B treatment increased their content relative to other HDL apoproteins (37). The amino acid composition of DI-1 and DII-1 agreed reasonably well with that of the human threonine-poor apoproteins (Table II). These similarities suggest that vervet DI-1 and DII-1 are analogous to the human threonine-poor apoproteins (Table V). However, there was disparity in the molecular weight data between human threonine-poor apoproteins and vervet DI-1 and DII-1. Shore et al. reported that the less acidic threonine-poor apoprotein (pl = 6.5) existed mainly as dimeric protein (Mr = 40,000) linked by a disulfide bond (37). Upon reduction of the disulfide bond a Mr = 25,000 monomer was found. The more acidic threonine-poor apoprotein (pl = 6.0) had a molecular weight of 10,000. The vervet DI-1 and DII-1 were found to be monomeric and had molecular weight values of 13,900 and 11,500, respectively (Table V).

The amount of the threonine-poor apoproteins in normal human HDL is low. Treatment of humans with amphotericin B resulted in HDL that contained 25% or more of the total protein as the threonine-poor apoproteins (37). Normal vervet HDL contained 1 to 56% of the total HDL protein as DI-1 and 8 to 13% as DII-1 (Table IV). When this higher percentage is considered together with the higher concentration of total HDL protein in vervets, one concludes that much higher concentrations of these apoproteins are normally present in these monkeys compared to threonine-poor apoprotein levels in human beings. Shore et al. reported that the threonine-poor apoproteins were found in higher amounts in monkeys hyporesponsive to dietary cholesterol (37). In preliminary studies, we have not found any correlation between the amount of DI-1 or DII-1 in HDL and total serum cholesterol or α-lipoprotein cholesterol. We have not seen a consistent relationship of the levels of these apoproteins with the type of dietary fat or the level of dietary cholesterol. No apparent difference in fasted versus fed plasma samples was found. Further study is needed to define the metabolic role of these HDL apoproteins and to learn the significance of higher plasma levels of these apoproteins.

Two apoproteins which have been detected in human HDL, apo-C-1 and apo-E, were not present in vervet HDL in amounts sufficient for isolation. HDL samples from individual animals had a protein whose pl was more basic than DI-1 (see HDL gels in Fig. 6); apo-C-1 from human HDL samples has a similar appearance on IEF gels (37). However, the very low content of this protein in HDL would have made its isolation extremely difficult. We can only speculate that this protein may be a vervet analog for human apo-C-1. We have found apo-E in the size region between LDL and HDL peaks of the agarose column fractionation of plasma lipoproteins, but apo-E was not present in the HDL peak which was used for apoprotein isolation. It appears that the vervet has a larger sized HDL subfraction present in minor amounts that contain apo-E. This may be analogous to the situation in human beings’ (49).

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