Hereditary and dietary effects on apolipoprotein[a] isoforms and Lp[a] in baboons

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Abstract Baboons possess Lp[a] that is similar to human Lp[a], including the presence of the unique protein, apo[a]. Baboon apo[a] occurred in at least nine isoforms distinguishable by size. Isoforms were resolved by 3–12% polyacrylamide gradient gel electrophoretic separation of serum proteins, and were detected with baboon apo[a]-specific antibodies. Thirty one different apo[a] isoform phenotypes were detected in a population of 165 unrelated baboons. Identical isoform phenotypes were observed in different samples from individual baboons, and isoform phenotypes were unaffected by changes in diet. In one experiment, 16 baboons were fed a series of five diets differing in amounts of cholesterol and saturated or unsaturated fats. There was no significant effect of diet on serum Lp[a] levels. In another group of baboons (n = 70) controlled for age and dietary history, enrichment of the diet with cholesterol and saturated fat caused a small, but significant ($P < 0.005$), increase ($\bar{X}$ = 0.6 mg/dl) in serum Lp[a] concentration. Analysis of two large sire families suggested that apo[a] isoform patterns and serum Lp[a] concentrations were inherited. Putative parental alleles responsible for specific isoform bands appeared to segregate randomly. Heritability (h^2) of serum Lp[a] concentration was estimated to be 0.95 ± 0.04. We conclude that apo[a] isoform phenotypes and serum Lp[a] concentrations are inherited, and that Lp[a] concentrations are only slightly influenced by diet.—**Rainwater, D. L., G. S. Manis, and J. L. VandeBerg.** Hereditary and dietary effects on apolipoprotein[a] isoforms and Lp[a] in baboons. *J. Lipid Res.* 1989. **30:** 549–558.

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High levels of Lp[a] have been associated with human cardiovascular disease (1, 2). The especially close association of Lp[a] and disease may be due to the presence of the unique apolipoprotein, apo[a]. Human apo[a] has been reported to occur in several isoforms discriminated on the basis of electrophoretic mobility (3–5). Previous studies have suggested that human serum Lp[a] concentrations are highly heritable (6), and a recent report has described Mendelian patterns of inheritance of apo[a] isoforms in two families (7). Baboons possess Lp[a] that is similar to the human lipoprotein in many physicochemical properties, including possession of the unique apolipoprotein composition of Lp[a] (8, 9). This report describes the influence of diet and heredity on baboon Lp[a] concentrations and apo[a] isoforms.

EXPERIMENTAL PROCEDURES

Baboon serum samples

Baboons (genus *Papio*) were housed outdoors in gang cages and corrals, and were fed a chow diet (monkey chow, Ralston Purina Co., St. Louis, MO) except where stated otherwise. Prior to bleeding from the femoral vein, baboons were fasted overnight and immobilized with ketamine (10 mg/kg). Blood was permitted to clot, and the serum was prepared by low-speed centrifugation. Serum was treated with 0.3 mM phenylmethylsulfonyl fluoride to inhibit proteolysis. Samples were stored at −70°C as individual aliquots in plastic tubing segments which permitted the analysis of multiple aliquots that had been treated identically, i.e., subjected to a single freeze–thaw cycle (10).

Antibodies

Rabbit antibodies against baboon apoB and apoLp[a] (anti-apoB and anti-apoLp[a]) were prepared by injection of the purified baboon lipoproteins (9). Anti-apo[a] was isolated from anti-Lp[a] by removing antibodies that bound apoB. Immunoglobulins G were prepared by a combination of ammonium sulfate precipitation and gel filtration. Specificity was tested by serum immunoblotting after SDS-electrophoresis. When detected with avidin-peroxidase, the biotinylated anti-apo[a] and anti-apoB did not react with apoB and apo[a], respectively (9). However, anti-apo[a] did react faintly with at least two proteins smaller than myosin

Abbreviations: apo[a], apolipoprotein[a]; Lp[a], lipoprotein[a]; ELISA, enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate; LDL, low density lipoproteins; HDL, high density lipoproteins; VLDL, very low density lipoproteins.

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(either cross-reaction or minor contamination). Affinity-purified goat antibodies directed against rabbit immunoglobulin G and conjugated with horseradish peroxidase were obtained from Vector Laboratories (Burlingame, CA).

Lp[a] assay

Lp[a] was assayed using a sandwich-style ELISA as previously described (9). Estimates of Lp[a] concentrations were not altered in samples stored at −70°C, nor was free apo[a] detected in such samples (9). Freshly thawed sera were diluted and added to wells previously coated with the capture antibody (anti-baboon apo[a]). Bound Lp[a] was detected with biotinylated anti-apoB followed by avidin/biotinylated alkaline phosphatase (ABC-AP kit, Vector Laboratories). Alkaline phosphatase was assayed using *p*-nitrophenyl phosphate, and change in absorbance at 405 nm was monitored using a Dynatech MR 580 Microelisa reader. Each plate was standardized with purified baboon Lp[a] which was stored in 50 g/l nonfat dry milk in PBS as aliquots in plastic tubing (10) at −70°C; Lp[a] values were expressed as mg of Lp[a] protein per dl of serum. Lp[a] levels were comparable for all phenotypes because the detection system was directed against apoB (i.e., anti-apo[a] was used only to capture the Lp[a] particles).

Electrophoresis and immunoblotting

Serum samples were routinely diluted with five volumes of 0.125 M Tris-HCl, pH 6.8, containing 160 ml/l glycerol, 20 ml/l 2-mercaptoethanol, and 20 g/l SDS, and placed in boiling water for 5 min. Six microliters (containing 1 μl serum) were loaded into each well. Prestained high molecular weight standards were from BRL (Bethesda, MD). Concave gradient polyacrylamide gels (3–12% with 3% stacking gels) were cast from the solutions of Laemmli (11) and used in a Mini-Protean II apparatus (Bio-Rad Laboratories, Richmond, CA). Proteins were subjected to electrophoresis until the dye front (bromophenol blue) was at the bottom of the gel. Following electrophoresis, proteins in the gels were transferred to nitrocellulose paper (Schleicher and Schuell, Keene, NH) by holding 150 V constant for 3 hr (12). After transfer, the nitrocellulose was soaked 1 hr in the block buffer (50 g/l nonfat dry milk in PBS with 1.5 g/l Tween-20 and 0.1 g/l Antifoam A, Sigma). Anti-apo[a] was dissolved in the block buffer (13 mg IgG proteins/l) and exposed to the immobilized proteins for 1 hr. The detecting antibody was the horseradish peroxidase-coupled goat anti-rabbit IgG diluted 1000-fold in the block buffer; peroxidase activity was measured using H_2O_2 and 3-amino-9-ethylcarbazole (9).

Determination of apo[a] isoform phenotypes

Baboon apo[a] from serum was undetectable in gels or on blots by protein staining techniques. However, when stained using anti-baboon apo[a] in a highly sensitive detection system, isoform bands with different mobilities were observed. A high background often occurred under these conditions, and some minor staining of non-apo[a] proteins was observed. The additional color may have derived from nonspecific staining, but it is possible that some of the background was due to the presence of degraded apo[a] in the samples. On the basis of our experience with isolated lipoproteins, it was assumed that intact apo[a] bands had mobilities slower than that of myosin. Faintly staining bands with mobilities slower than myosin were interpreted to be nonspecific staining when they occurred in all lanes across the blot, and at relatively similar intensities. In contrast, apo[a] isoforms exhibited a diversity of size and intensity. The chief criterion for the identification of an apo[a] band was that it be larger than myosin, and that it stand out from the relatively constant background.

Some baboons had no detectable isoform bands (see Fig. 1, lanes 2 and 5), and this was termed the null, or N, phenotype. A standard that contained five different isoforms was prepared from the lipoproteins floating at d 1.1 g/ml from three selected baboons. In phenotyping, bands whose midpoint fell within the width of the largest standard band were designated B phenotype (see Fig. 2), and larger bands were designated A phenotype. The remaining bands of the standard were designated D, G, I, and K in order of increasing mobility; and the intervening regions were designated C, E and F, H, and J. The interval between standards D and G was wide, with apoB located between them. Therefore, bands occurring near or below apoB were designated F, and E was the region between D and F. The region below K was designated L.

Molecular weights of the five standard isoform bands were estimated from five different immunoblots by comparison in semi-log plots with the mobilities of the high molecular weight standards (BRL) of albumin (68,000), phosphorylase B (97,400), and myosin (200,000), and with baboon apoB (same mobility as human apoB, 538,000 with carbohydrate (13)). The apparent molecular weights were: isoform B, 692,000 ± 47,000 ($\bar{X}$ ± standard deviation); isoform D, 586,000 ± 25,000; isoform G, 463,000 ± 16,000; isoform I, 410,000 ± 9,000; and isoform K, 350,000 ± 7,000.

Density gradient ultracentrifugation

Fresh plasma (200 μl) was diluted with 0.5 ml of a KBr solution in 1 mM EDTA (d 1.385 g/ml). The density gradient was formed from a sequence of KBr solutions with the following densities (g/ml): 3.0 ml of d 1.21, 2.0 ml of d 1.125, 2.0 ml of d 1.07, 2.0 ml of d 1.05, 1.0 ml of d 1.02, and 1.0 ml of d 1.006 saline (14). After centrifugation in an SW41 rotor (Beckman Instruments, Palo Alto, CA) at 39,000 rpm for 24 hr, the gradient was displaced using Fluorinert FC-40 (Sigma Chemical Company, St. Louis, MO) through an ISCO UA-5 absorbance monitor (280 nm)

and collected in 0.45-ml fractions as described (8). Lp[a] in the fractions was assayed using the ELISA after a 50-fold dilution, and apo[a] in fractions was analyzed by immunoblotting after precipitation in 10 volumes of acetone–ethanol 1:1 at −20°C.

Diet experiments

The effects of diet on Lp[a] levels and apo[a] isoforms were investigated in two experiments. In the first experiment, each of 16 adult male baboons (6 to 12 years old) was fed sequentially five different diets for 6 weeks prior to sampling. The monkey chow diet was low in fat (10% of calories) and cholesterol (0.03 mg/kcal). The other four diets were formulated from a special chow mixture with no cholesterol or fat by supplementation with saturated fat from lard or unsaturated fat from corn oil (40% of calories) and with or without cholesterol (1 mg/kcal) (15).

In the second experiment, 70 baboons, both males and females, were weaned onto the monkey chow diet, and bled at 27 months of age (27.6 ± 1.0; $\bar{X}$ ± standard deviation). The baboons were then fed a challenge diet in which the special chow diet was enriched in cholesterol (1.7 mg/kcal) and saturated fat from lard (40% of calories), as above. After 7 weeks, the baboons were bled again for the challenge sample.

Lp[a] levels were determined in frozen serum samples using the ELISA. All samples from each baboon were determined at the same time. For the first diet experiment, the effects of different diets on Lp[a] levels were assessed by analysis of variance (16). For the second diet experiment, the effects of changing from chow to the challenge diets were analyzed using a paired *t* test (16).

Genetic studies

Serum samples were taken from baboons of both sexes and at different ages; samples were stored at −70°C for various lengths of time (from 0–8 years) prior to analysis. Population distributions of apo[a] phenotypes were assessed in 165 putatively unrelated baboons (i.e., acquired from outside the institutional colony; most were captured in Kenya). In pedigree analyses, the isoforms of family members (both progeny and parents) were compared on the same blot to assess the inheritance of parental isoforms, and then apo[a] isoforms in the family were identified by comparison with the isoform standard. Heritability (h^2), the proportion of the phenotypic variation that is attributable to additive genetic factors, was estimated using Lp[a] concentration data from 94 members of two sire families (17).

Determinations of protein, apoB, and cholesterol

Protein concentrations were measured relative to the bovine serum albumin standard (18). ApoB concentrations were determined by electroimmunoassay, and cholesterol by an enzymatic assay (19). HDL cholesterol was determined following heparin-Mn^{2+} precipitation (20), and VLDL + LDL cholesterol was estimated as the difference between total cholesterol and HDL cholesterol.

RESULTS

Baboon apo[a] isoforms

Serum samples from about 400 baboons were examined for apo[a] isoforms. **Fig. 1** illustrates apo[a] isoform sizes detected by anti-apo[a] in sera from eight baboons. Baboons possessed zero, one, or two isoform bands; in no case were more than two isoforms identified in an individual. Isoform phenotypes were identical when serum and plasma samples were compared. Immunoblotting sensitivity was optimized by using a large serum protein load, a relatively high concentration of primary antibody, and a sensitive secondary antibody. A consequence of enhanced sensitivity was that many other protein bands, including apoB, were usually stained, although not as intensely as the apo[a] isoforms. Most of the other proteins were much smaller in size, and did not interfere with apo[a] isoform identification. Apo[a] isoforms were detected as intensely staining bands readily distinguished from the background of the blot.

A standard that contained five separate isoforms aided the identification of apo[a] isoform phenotypes (**Fig. 2**). The standards and the location of apoB permitted the identification of 12 potential apo[a] isoform phenotype classes. Some bands with the same phenotypic designations were observed to differ slightly in mobility. For example, the two A bands in lanes 1 and 6 were apparently different, as were the two C bands in lanes 2 and 3 of Fig. 2. In the course of these studies, baboons possessing 9 of the possible 12 isoform size classes were identified (Fig. 2). The H, J, and L phenotypic designations were included in case they should be detected in future.

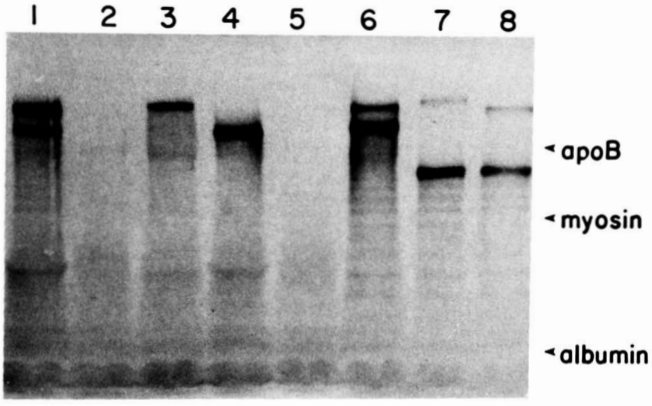

Fig. 1. Immunoblots of apo[a] isoforms resolved by polyacrylamide gradient electrophoresis and stained with anti-apo[a]. Locations of apoB, myosin, and albumin are indicated. Serum Lp[a] concentrations (mg/dl) measured for the eight different baboons were: lane 1, 9.1; lane 2, 0; lane 3, 4.0; lane 4, 11.2; lane 5, 0; lane 6, 17.2; lane 7, 16.9; and lane 8, 15.0.

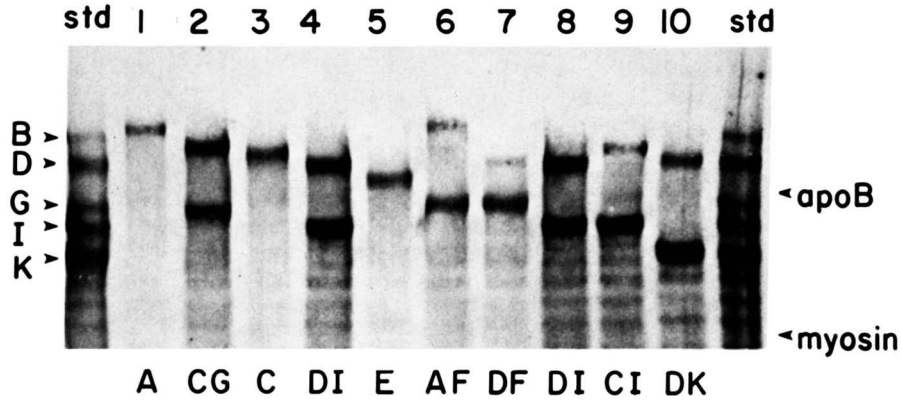

Fig. 2. Immunoblots of apo[a] isoform phenotypes and standards. Apo[a] isoforms were resolved by polyacrylamide gradient electrophoresis and stained with anti-apo[a]. Standards (std), with the five isoforms indicated, were placed in outside lanes. Apo[a] isoform phenotypes of baboons are indicated at the bottom.

Apo[a] isoform phenotypes in unrelated baboons

Table 1 shows that 31 different isoform phenotypes were observed in 165 unrelated baboons. Eight of the baboons (5%) had no detectable isoforms (N phenotype) which suggested the frequency of undetectable alleles to be 22% in the baboon population. Eighty two baboons (50%) had single isoform phenotypes, and they are underlined in Table 1 to indicate that the individuals could be homozygous (as tabulated) or heterozygous for an undetectable allele. The remaining baboons (45%) had two isoforms.

Relationship of apo[a] isoforms and Lp[a] density

The density of the lipoproteins bearing two different isoforms was assessed using density gradient ultracentrifugation. Baboon 1X1672 possesses high concentrations of two different-sized isoforms (D and I), and **Fig. 3** shows the lipoprotein density profile. Lp[a] was present in fractions 10 (d 1.048 g/ml) through 16 (d 1.079 g/ml). The smaller isoform, I, was concentrated in fractions 10 through 14, while the larger isoform, D, was concentrated in fractions 12 through 15. A similar pattern of isoform distribution in density fractions (i.e., the larger isoform was present in the denser fractions of the Lp[a] peak) was observed in experiments with two other baboons that exhibited phenotypes AI and FI (data not shown).

Effects of diet on Lp[a] levels and apo[a] isoforms

The effects of cholesterol and type of fat were assessed in 16 adult baboons; 8 were progeny of sire 1X1672, and 8 were progeny of sire 1X0102 (none were full sibs). **Table 2** shows the effects of the five different diets on Lp[a] levels. Regardless of diet, the progeny of 1X1672 had consistently higher ($P < 0.001$, two-way analysis of variance) serum Lp[a] levels than did the progeny of 1X0102. However, no significant effect of diet ($P = 0.573$), or interaction between sire and diet ($P = 0.680$), was detected.

Lp[a] levels were analyzed in sera from 70 young baboons controlled for age and dietary history. Baboons were fed a chow diet low in cholesterol and fat, followed by a challenge diet enriched in cholesterol and saturated fat. Serum Lp[a] levels increased significantly ($P < 0.005$, paired t test) from 5.2 ± 0.4 to 5.8 ± 0.5 mg/dl ($\bar{X}$ ± SEM) when animals were fed the challenge diet (**Fig. 4**). Lp[a] levels increased significantly in both males ($P < 0.025$, n = 38, paired t test), and in females ($P < 0.001$, n = 32); there was no significant difference ($P = 0.846$, two-way analysis of variance) between the increases in the males (0.550 ± 0.297 mg/dl; $\bar{X}$ ± SEM) and females (0.625 ± 0.218). Con-

TABLE 1. Apo[a] isoform phenotypes in 165 unrelated baboons

	Band 2												
Band 1	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*K*	*L*	*N*
A	14	3	4	6	3	·	5	·	1	·	·	·	·
B		26	4	8	5	6	7	·	·	·	1	·	·
C			15	2	4	3	3	·	1	·	·	·	·
D				11	2	·	·	·	1	·	·	·	·
E					9	2	3	·	·	·	·	·	·
F						·	1	·	·	·	·	·	·
G							5	·	·	·	·	·	·
H								·	·	·	·	·	·
I									1	·	·	·	·
J										·	·	·	·
K											1	·	·
L												·	·
N													8

Actual counts of baboons with indicated phenotypes are presented. Underlined numbers indicate baboons with single isoform phenotypes that could be homozygous or heterozygous for the *n* allele.

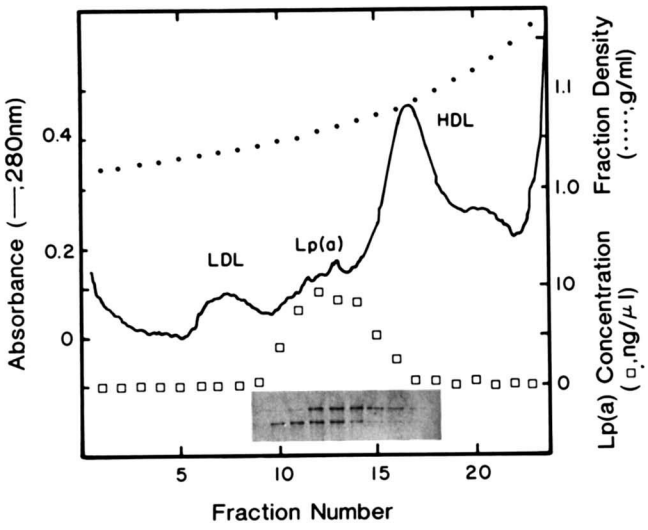

Fig. 3. Lp[a] resolved using density gradient ultracentrifugation. Plasma (200 μl) from baboon 1X1672 was subjected to density gradient ultracentrifugation. Density in fractions was determined by refractometry. One microliter of each fraction was diluted 50-fold and assayed for Lp[a]. Aliquots representing 5 μl of each fraction were subjected to SDS-electrophoresis and immunoblotted using anti-apo[a]. The primary antibody was biotinylated anti-apo[a] which was detected with peroxidase-labeled avidin.

centrations of total serum cholesterol, VLDL + LDL cholesterol, and HDL cholesterol increased by 77, 97, and 60%, respectively, in the baboons; in an unselected subset of 40 animals, serum apoB concentrations increased by 28%.

There was no effect of dietary cholesterol and fat on apo[a] isoform phenotypes in any of the 86 baboons studied in the two diet experiments. **Fig. 5** illustrates the lack of a dietary effect on isoform patterns in 4 different baboons used in the diet studies.

Inheritance of apo[a] isoforms in the pedigrees of two sires

Two large sire families (1X0102 and 1X1672) were selected for their contrasting serum Lp[a] levels. Included in **Fig. 6** are families from which samples from at least three progeny were analyzed. Apo[a] isoform phenotypes were assigned relative to the standards. Isoform bands within families (i.e., common by descent) had identical mobilities as detected by immunoblotting procedures. Presented in **Fig. 7A** are the isoforms observed in members of the second family (numbered from the left) of sire 1X1672 in which all four possible combinations of parental isoform bands were observed in the progeny. Occasionally, such as in the fourth family of 1X1672, two parental isoforms with the same designation (D) had slightly different mobilities when electrophoresed side by side, but could not be distinguished when both were present in an individual. In the two pedigrees, all progeny isoforms were traceable to their parents, except the very faint D isoform observed in the first two progeny of the fourth family of sire 1X0102 shown in Fig. 7B. Lp[a] levels in these two progeny were 0.41 and 0.49 mg/dl (rounded off to 0 mg/dl in Fig. 6). Analysis of one blot where the isoform bands were sharper suggested the presence of a very faint D isoform band in the dam. However, this putative D band did not stand out sufficiently from the background on other blots (n = 5) to justify altering the designation of the maternal phenotype. Thus, we report two apparent discrepancies in one family where progeny had nonparental apo[a] isoforms.

Table 3 summarizes the segregation patterns for apo[a] isoforms in the families illustrated in Fig. 6. This summary is based on the assumptions that: *1*) any individual that exhibited only one isoform and did not transmit it to all progeny was heterozygous for a null allele, *n* (gene product not detected), and *2*) individuals exhibiting no isoforms were homozygous for *n*. Families in which the mother exhibited one isoform, but could not be distinguished as heterozygous for a null allele on the basis of progeny phenotypes were excluded from the tabulation. The fourth family of sire 1X0102 also was excluded. By χ^2 analysis, the results do not differ significantly from Mendelian expectations (one to one segregation ratio).

Inheritance of Lp[a] levels in the pedigrees of two sires

Heritability of Lp[a] levels was estimated from data on two sire families (1X0102 and 1X1672) in Fig. 6. The two sires were selected for their extremes in Lp[a] levels, while their mates had Lp[a] levels closer to the population mean. The heritability (h^2) was estimated to be 0.95 ± 0.04.

TABLE 2. Average Lp[a] concentrations in two half-sibships of baboons fed five diets

	Supplement Added to Chow Diet				
Sire	Nothing	Unsaturated Fat	Unsaturated Fat + Cholesterol	Saturated Fat	Saturated Fat + Cholesterol
1X0102	2.5 ± 0.5	2.4 ± 0.5	2.8 ± 0.6	2.9 ± 0.6	2.7 ± 0.6
1X1672	14.1 ± 1.3	16.2 ± 1.3	17.3 ± 1.0	16.1 ± 2.2	16.9 ± 1.1

Numbers represent mean ± standard error of the mean (n = 8) of the serum Lp[a] concentrations (mg/dl). Diets are described in the Experimental Procedures section.

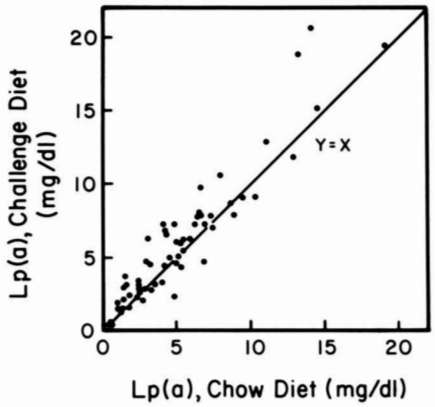

Fig. 4. Serum Lp[a] levels in baboons fed chow and the challenge diets. The theoretical line representing no difference due to diet (y = x) is plotted. Least squares fit of the data to a line gave a slope of 1.07 and an intercept of 0.19 mg/dl, with a correlation coefficient of 0.932 (n = 70).

Relationship of Lp[a] levels and apo[a] isoforms

Of 165 unrelated baboons presented in Table 1, 8 with no detectable isoforms averaged 0.3 ± 0.3 mg/dl ($\bar{X}$ ± standard deviation) of serum Lp[a]; 82 with one isoform averaged 4.8 ± 4.2 mg/dl; and 75 with two isoforms averaged 6.4 ± 3.7 mg/dl. Thus, there was a positive relationship between number of isoforms and serum Lp[a] levels.

The pedigrees of sires 1X0102 and 1X1672 were analyzed for the relationship between specific apo[a] isoforms and Lp[a] levels in **Fig. 8**. Only individuals with one isoform and deduced to be heterozygous for the *n* allele by inspection of the pedigree were utilized. Baboons possessing isoforms B and E had serum Lp[a] levels averaging 5.7 and 2.6 mg/dl, respectively, with each isoform observed in three different families. Other apo[a] isoforms were not as broadly represented, except in the case of isoform C which was present in four different families. However, in these families, Lp[a] levels appeared to occur in two clusters averaging 2.5 (three families) and 11.6 (one family) mg/dl. Lp[a] levels in baboons with isoform D also appeared to occur in two clusters of 0.5 (two families) and 14.5 (one family) mg/dl. Four members of one family possessed the I isoform, and they averaged 10.6 mg/dl.

DISCUSSION

In this report, we describe the isoforms of baboon apo[a] that are detected by differences in mobility in serum immunoblots. Baboon apo[a] isoforms, like the human proteins (2), are large, with mobilities similar to apoB. We observed heterogeneity in apo[a] isoform mobilities. If isoforms differ in numbers of Kringle IV-like repeats as hypothesized for human apo[a] (21), then addition of one repeat unit of about 13,000 molecular weight to a 500,000 molecular weight apo[a] would represent a change of less than 3%. For comparison, the average distance between myosin and apoB (molecular weight difference of about 300,000) was 12.1 ± 0.7 mm in 10 blots, and we calculate 0.5 mm difference in mobility per repeat unit increment. To distinguish between isoforms differing by a single repeat on different blots would be impossible with the present technique. A standard with five isoform bands helped define 12 size regions of apo[a] phenotypes, with the understanding that several allelic products should fall within each phenotypic region. Confirming this expectation was the observation of apparently different-sized isoforms occurring within several phenotypic regions (Fig. 2).

A total of 31 different apo[a] phenotypes was observed in 165 unrelated baboons; 50% of the baboons had single isoform phenotypes, and 45% had two isoform phenotypes (Table 1). Some baboons had no detectable isoform bands, and this was termed the null, or N, phenotype. It is likely that the N phenotype resulted from apo[a] alleles that produced very little or no protein occurring as Lp[a] particles in the serum. Eight (5%) of the baboons exhibited the N phenotype, enabling the frequency of alleles encoding undetectable apo[a] isoforms to be calculated as 22%. Utermann et al. (7) and Kraft et al. (22) have reported that 60–70% of human apo[a] alleles encode undetected iso-

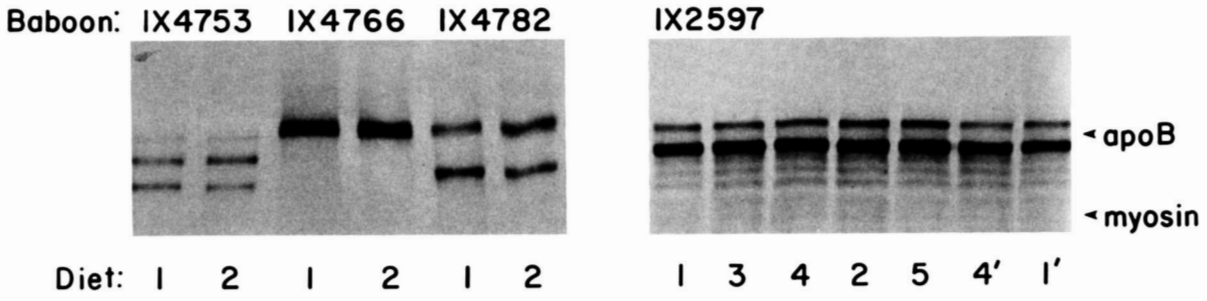

Fig. 5. Effect of diet on apo[a] isoform patterns in four baboons. Diet 1 was the chow diet; the other diets were: 2, saturated fat and cholesterol; 3, saturated fat; 4, unsaturated fat; and 5, unsaturated fat and cholesterol. The serum samples are organized in the same diet sequence as the baboons were fed; the primes mark repeats of the indicated diets.

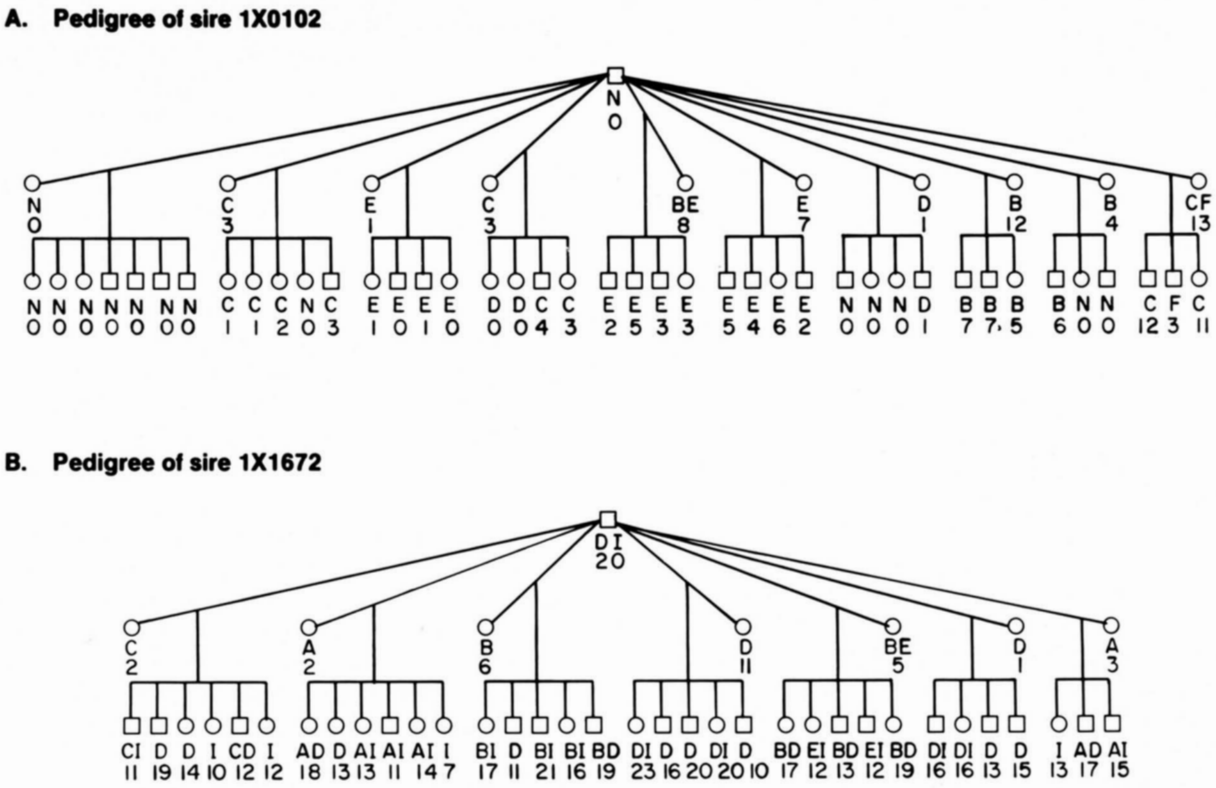

Fig. 6. Pedigrees of sires 1X0102 (A) and 1X1672 (B). The apo[a] isoform phenotype and serum Lp[a] level (mg/dl) are indicated under the symbol for each individual. Symbols for males are squares, and for females are circles. In the text, families are numbered from left to right.

forms. The higher frequency may be due to lower sensitivity of detection; 73 null phenotype humans averaged 4.4 mg/dl of serum Lp[a] (22). By comparison, the 8 null phenotype baboons in this study averaged 0.3 mg/dl of Lp[a] protein, or about 1 mg/dl of total Lp[a] (8). Of the baboons described in Table 1, the 10 with two isoforms and the lowest serum Lp[a] levels averaged 2.0 mg/dl, suggesting that about 1 mg/dl (protein) was sufficient to detect an apo[a] isoform. Therefore, the sensitivity of the present detection method required the electrophoresis of about 5 ng of an isoform (from 10 ng of apoLp[a]). In some cases, as little as 0.4 mg/dl was sufficient to detect an apo[a] isoform band (Fig. 7B).

Different isoforms had different distributions in lipoprotein density fractions (Fig. 3.), similar to previous observations of human apo[a] size forms (3). The larger isoform (D) was observed in the denser fractions, while the smaller isoform (I) was concentrated in relatively less dense fractions. It is

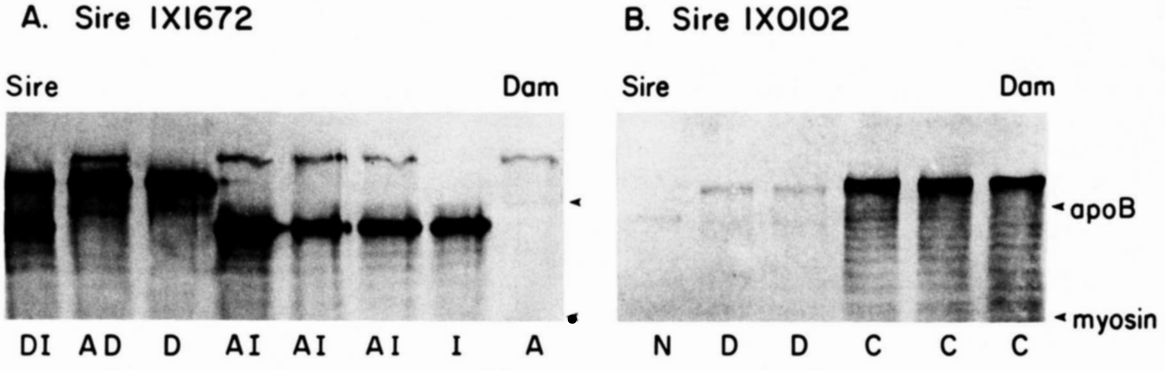

Fig. 7. Apo[a] immunoblots showing inheritance of apo[a] isoform phenotypes. Samples from the sire and dam occupy the outside lanes, and their progeny are in order of age. Apo[a] isoform phenotypes are indicated at the bottom of each lane. Presented are: family 2 of sire 1X1672 (A) and family 4 of sire 1X0102 (B).

TABLE 3. Segregation patterns for apo[a] isoforms

Putative Parental Genotype	Number of Progeny	Segregation Ratio
ax	9	6*a* : 3*x*
bx	17	8*b* : 9*x*
cx	14	8*c* : 6*x*
dx	29	12*d* : 17*x*
ex	9	6*e* : 3*x*
fx	3	1*f* : 2*x*
ix	34	18*i* : 16*x*
nx	32	14*n* : 18*x*
Σ	147	73 : 74

Segregation of apo[a] isoforms in the progeny of heterozygous parents. Heterozygosity was assigned from either isoform pattern or, in the case of a null (*n*) allele, by the incomplete transmission of isoforms. The putative alleles encoding the isoforms are designated by lower-case italicized letters. The symbol *x* refers to any other allele including the null allele. Data were taken from the two families presented in Fig. 6.

possible that the Lp[a] particles resolved on the basis of density and differing in apo[a] isoform composition, may also differ in other properties, including relative atherogenicity.

The effects of diet on Lp[a] characteristics were determined in 70 young baboons while fed a chow diet and then fed an atherogenic challenge diet supplemented with cholesterol and saturated fat. Lp[a] levels increased significantly ($P < 0.005$) in the second diet; however, the average increase was just 11% (5.2 to 5.8 mg/dl), which was small compared with average increases in total cholesterol (77%), VLDL + LDL cholesterol (97%), and apoB (28%) concentrations. Thus, baboon serum Lp[a] concentrations changed little in response to alterations in dietary fat as has been observed in humans (23–25) and rhesus monkeys (26). The modest increase in Lp[a] levels did not appear to result from increases in LDL level because purified LDL is not detected in the assay (9 and Rainwater, D. L., et al., unpublished data). Furthermore, consistent with observations in humans (27–29), Lp[a] levels did not appear to be related to LDL (apoB) levels; changes in both levels were not correlated by least squares analysis ($r = 0.110$, n = 40) in this experiment.

In another experiment, serum Lp[a] was characterized in 16 adult baboons fed sequentially five different diets. Alterations in amounts of dietary cholesterol and fat from corn oil and lard had no significant effect on Lp[a] levels. It seems likely that lack of statistical significance in this experiment, in contrast to the previous experiment, may have resulted from smaller sample size and greater heterogeneity of age and dietary history of the baboons.

No change in apo[a] isoform pattern was observed as a consequence of any of the diets.

Analysis of the two pedigrees presented in Fig. 6 suggested the inheritance and relatedness of apo[a] isoforms and Lp[a] levels. First, all apo[a] isoforms in 73 of 75 progeny could be traced back to parental phenotypes. Because isoforms must stand out from their background to be identified, apparent exceptions to parental derivation might be expected. In the case of the fourth family of 1X0102, it is likely that the mother had a heterozygous phenotype, CD, but that the faint D band was not detected because of the high background resulting from the nearby C band (Fig. 7B). In the two progeny not receiving the C band, the faint D band was detectable because of the lack of background contributed by the N phenotype sire. Second, analysis of segregation revealed that all alleles appeared to segregate independently (Table 3). Third, serum Lp[a] levels appeared to be inherited, with 95% of the phenotypic variation attributable to genetic effects in these two pedigrees. Finally, Fig. 8 shows that there appeared to be a relationship between serum Lp[a] concentrations and apo[a] isoform phenotypes in individuals presenting a single isoform phenotype and deduced to be heterozygous for the *n* allele. Among individuals with similar phenotypes, there was less variation within families than between families. In both the C and D phenotype family groups, there appeared to be two distinct clusters of Lp[a] levels. Thus, these observations suggest that within families, Lp[a] levels and apo[a] isoform phenotypes are transmitted together in baboons.

The baboon provides an important model of human Lp[a] that may be utilized to further understand the regulation of Lp[a] and apo[a] phenotypes. The baboon model of Lp[a] closely resembles what is known about human Lp[a] in terms of physicochemical properties (8, 9). We now

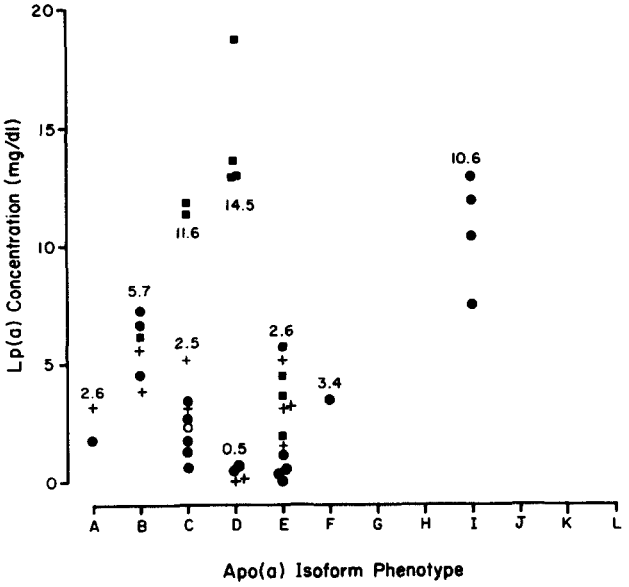

Fig. 8. Relationship of Lp[a] levels and apo[a] isoforms. Baboons deduced to possess the indicated isoform and an *n* allele were selected from the pedigrees in Fig. 6 by inspection. Each symbol within a column represents isoforms that are identical by descent. The mean Lp[a] levels of baboons with each apo[a] isoform phenotype are indicated; means for the upper and lower clusters of isoforms C and D are indicated separately.

report that in baboons, as in humans, Lp[a] levels (6) and apo[a] isoforms (7) are highly heritable. However, contrary to observations in humans (7, 22), we did not observe an inverse relationship between apo[a] isoform size and Lp[a] level in baboons. Furthermore, it appears, at least in the case of the C and D phenotypes, that we have observed more than one Lp[a] level associated with a single isoform phenotype (Fig. 8). If Lp[a] level is regulated at the apo[a] structural locus, then we have detected in both cases two alleles dictating the same isoform phenotype. We have detected more variability of apo[a] isoforms and isoform phenotypes than was detected in humans (7, 22), perhaps due to increased sensitivity of our methods. Preliminary observations (Rainwater, D. L., et al., unpublished results) with the present method have suggested greater heterogeneity of human apo[a] isoforms than has been reported; further research is in progress to extend these observations.

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