Plasma lipoproteins in the Golden Syrian hamster (*Mesocricetus auratus*): heterogeneity of apoB- and apoA-I-containing particles

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Abstract We present the quantitative and qualitative characteristics of the apolipoprotein (apo) B- and apoA-I-containing lipoprotein subspecies in the plasma of male Golden Syrian hamsters. The spectrum of hamster lipoproteins of d < 1.172 g/ml was subfractionated by isopycnic density gradient ultracentrifugation. ApoB-containing subspecies were distributed up to a density of 1.074 g/ml. Hamster very low density lipoproteins (VLDL, d < 1.018 g/ml; ~120 mg/dl plasma) were triglyceride (TG)-rich, deficient in cholesteryl ester (CE), and highly heterogeneous in size, containing chylomicron-like particles. ApoVLDL contained proteins analogous to human apoB-100, apoB-48, and apoE. ApoB-containing subspecies with physicochemical properties typical of low density lipoproteins (LDL) were identified as a single, major size species in the density interval from 1.019 to 1.074 g/ml, particle diameter decreasing progressively with increase in density. Hamster LDL-like subspecies were distinguished from their human counterparts by a relative deficiency in core CE (<30% by wt) and by enrichment in triglyceride. The high M_r form of apoB was the major apolipoprotein of all LDL-like subfractions, in which apoE was detected as a minor component. Total plasma levels of LDL (d 1.019–1.074 g/ml) amounted to ~140 mg/dl (~25% of d < 1.172 g/ml lipoproteins). The distribution of dense apoB-containing subspecies overlapped that of apoA-I-containing, high density lipoprotein-1 (HDL_1)-like particles in the density interval ~1.039–1.074 g/ml. ApoA-I-containing subspecies with the physical and chemical characteristics of HDL were exclusively present over the density interval 1.074–1.172 g/ml. Quantitatively, these subspecies predominated in hamster plasma (~270 mg/dl). Light, HDL_2-like particles of d 1.065–1.103 g/ml (HDL_L) were preponderant, (~66% of total HDL). Marked size heterogeneity was evident, and was associated with distinct particle contents of minor apolipoproteins. Both HDL_L and heavy HDL (HDL_H, d 1.103–1.172 g/ml) were enriched in CE as evidenced by elevated weight ratios of CE:FC (7–9:1) and of CE:TG (up to ~50:1). Considered together, the core lipid contents of apoB- and apoA-I-containing lipoproteins are consistent with the hypothesis that the hamster is partially deficient in neutral lipid (CE, TG) transfer activity.—**Goulinet, S., and M. J. Chapman.** Plasma lipoproteins in the Golden Syrian hamster (*Mesocricetus auratus*): heterogeneity of apoB- and apoA-I-containing particles. *J. Lipid Res.* 1993. **34:** 943–959.

Supplementary key words density gradient ultracentrifugation • lipoprotein subspecies profile • physicochemical properties • apolipoprotein polymorphism

Among the rodents, the hamster (*Mesocricetus auratus*) has proven of singular importance as an animal model for comparative studies of lipid metabolism. Indeed, the pioneering studies of Spady, Dietschy, and colleagues (1, 2) revealed that the hamster is distinct from the rat and guinea pig in displaying rates of hepatic cholesterol synthesis that closely resemble those typical of humans. Subsequently, it has become evident that hamster and humans are alike in transporting a significant proportion of plasma cholesterol in the form of low density lipoproteins (LDL) and moreover, that similar mechanisms are involved in the regulation of plasma LDL-cholesterol levels in response to dietary lipids (1–6). As in humans, the hamster LDL receptor plays a key role in such control mechanisms, a finding that prompted the recent structural analysis of the corresponding cDNA (7). Furthermore, considerable information is now available on production rates of both VLDL and LDL, and of the kinetics of receptor-dependent and receptor-independent LDL degradation in the major tissues of *M. auratus* (8–11). Finally, similarities in bile acid composition and in biliary sterol secretion have been noted in hamster and humans (12, 13).

The hamster has also attracted interest as a potential model for atherosclerosis research, particularly because this species develops arterial lesions in association with a marked hyperlipidemia when fed a diet supplemented with cholesterol and saturated fat (14–16). More recently, *M. auratus* has been exploited as an animal model in which to screen and evaluate the hypolipidemic activity of new pharmacological agents (17, 18).

Despite the extensive use of the hamster model, there

Abbreviations: apo, apolipoprotein; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; EDTA, ethylenediamine tetraacetic acid; PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis.

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is a paucity of data on the lipid transport system in this species, a situation reflected in its absence from a comprehensive review of mammalian plasma lipoproteins in 1986 (19). Several investigators have, however, reported plasma lipid or lipoprotein lipid levels, or both, in Golden Syrian hamsters fed a chow diet (1, 5, 8, 9, 20, 21), and comparison reveals marked variation among them (e.g., fasting plasma triglycerides, range 80–149; cholesterol, 66–132; VLDL-triglycerides, 32–73; LDL-cholesterol, 23–34; and HDL-cholesterol, 41–71 mg/dl). Indeed, elevated concentrations of VLDL have been detected under fasting conditions (8, 9, 21). High density lipoproteins appear to dominate the lipoprotein profile however (1, 9), with apoA-I representing the most abundant apolipoprotein of hamster plasma (126 mg/dl) (22); levels of hamster apoB-100, of hepatic origin, are substantially lower (~30 mg/dl) (22, 23).

As a prelude to investigation of the mechanisms underlying the perturbation of lipid transport and metabolism in inbred stains of Golden Syrian hamster by hypolipidemic agents, we judged it essential to establish the principal features of plasma lipoprotein and apolipoprotein profile in this species. Using a single-step, density gradient methodology of high resolution, we describe herein the qualitative and quantitative characteristics of the plasma profile of apoB- and apoA-I-containing lipoprotein subspecies in *M. auratus*. In particular, lipoprotein particle heterogeneity has been evaluated around the classical 1.063 g/ml density limit for separation of LDL from HDL (24, 25), as earlier studies in the rat and mouse have documented extensive overlap of apoB- and apoA-I-containing particles in this region. Equally, the polymorphism of the major non-B apolipoproteins of the VLDL and HDL subclasses has been assessed by two-dimensional gel electrophoresis.

MATERIALS AND METHODS

Animals and diets

Sexually mature, male Golden Syrian hamsters, weighing 100–120 g and aged 8 and 10 weeks, were used throughout our studies; these animals were weaned at 3 weeks. The animals were raised in an established colony at the Pharmaceuticals Division of ICI PLC (Macclesfield, U.K.); this colony was originally derived from the Lake View strain as supplied by Charles River. Animals were maintained in collective cages in an air-conditioned room at ambient temperature (23 ± 1°C) with a 14-h light cycle. Hamsters had free access to water and food until the day before killing. The diet consisted of pellets composed (as % wt) of 4.3% lipid, 22.7% protein, 3.9% fibre, and 51.6% of carbohydrate and a vitamin and mineral salt mixture (Special Diet Services Rat and Mouse No 3, PB Nutrition, Witham, Essex, U.K.).

Blood samples

After overnight fasting, blood samples were withdrawn by heart puncture under halothane anesthesia, and collected individually. Blood (2–3 ml/animal) was drawn into tubes containing EDTA and gentamycin (final concentrations 1 mg/ml and 0.005%, respectively). Plasmas were rapidly separated by low speed centrifugation and then transported at 4°C by air freight to Paris. Upon arrival, plasma lipoprotein fractionation was commenced rapidly after addition of a cocktail of reagents to preserve native lipoprotein structure. This cocktail consisted of: *i)* antioxidants, EDTA (final concentration 0.26 mM), reduced glutathione (1.6 mM) and BHT (0.02 mM); *ii)* inhibitors of bacterial growth, sodium azide (1.5 mM) and gentamycin sulfate (0.005%); and *iii)* protease inhibitors, Pefablock SC (1 mM) and ϵ-aminocaproic acid (10 mM). Equally, EDTA, sodium azide, and gentamycin sulfate were added at final concentrations of 0.26 mM, 1.5 mM, and 0.005%, respectively, to all density solutions used in lipoprotein fractionation.

For comparative purposes, blood was drawn from healthy, fasting, normolipidemic male subjects (age 35–45 years), into tubes containing Na_2-EDTA (final conc. 1 mg/dl). Plasma was then isolated by low speed centrifugation (1200 *g* for 20 min) at 4°C, and treated as above.

Lipoprotein isolation

Hamster and human plasma lipoproteins were subfractionated on the basis of their hydrated density by the isopycnic ultracentrifugal density gradient procedure described for human serum lipoproteins by Chapman et al. (26), with the modification that 2.5 ml (instead of 3 ml) of the d 1.006 g/ml NaCl solution was added to the top of each gradient (a final volume of 12.0 ml). Gradients were constructed in Ultraclear (Beckman) tubes of the Beckman SW41-Ti rotor as described earlier (26). Ultracentrifugation was performed in a Beckman L8-55 or a Sorvall OTD-50 ultracentrifugation at 40,000 rpm (56×10^7 *g*-min) for 48 h at 15°C, using slow acceleration and deceleration modes to minimize gradient perturbation as the buckets passed from the vertical to the horizontal position and vice versa. On completion of ultracentrifugation, fractions of 0.4 ml were collected successively from the meniscus of each tube with a Gilson precision pipette; however, the first fraction (VLDL, d < 1.018 g/ml) was removed in a volume of 0.5 ml with a narrow-bore Pasteur pipette in order to permit a more satisfactory separation of VLDL, which tended to adhere to the tube walls. Corresponding fractions from different gradient tubes were pooled only when derived from the plasma of the same animal, or from the same plasma pool. Lipoprotein fractions were exhaustively dialyzed in Spectrapor tubing (Spectrum Medical Industries, Los Angeles, CA; molecular weight cut-off 12,000–14,000) for 24 h at 4°C against

a solution containing 0.15 M NaCl, 10 mM HEPES, 0.01% NaN_3, 0.01% EDTA, and 0.005% gentamycin, pH 7.4.

For apolipoprotein analysis by isoelectric focusing, the major lipoprotein classes were separated by sequential ultracentrifugal flotation (27) as follows: VLDL, d < 1.019 g/ml and HDL d 1.074–1.103 g/ml.

Chemical analysis of plasma lipids and lipoproteins

The lipid (total cholesterol, free cholesterol, triglycerides, and phospholipids) contents of whole plasma and of lipoprotein subfractions were assayed by use of enzymatic kits supplied by Biomérieux (Charbonnières-les-Bains, France). The amount of cholesteryl ester (as mass in mg/ml) was calculated using the formula: cholesteryl ester = (total cholesterol − free cholesterol) × 1.67. These enzymatic procedures were based on the following methods: *i)* free and total cholesterol, Richmond (28); *ii)* phospholipids, Takayama et al. (29) (this method measures only choline-containing phospholipids, i.e., sphingomyelin, lysolecithin, and phosphatidyl choline); *iii)* triglycerides, Fossati and Prencipe (30), involving hydrolysis to glycerol. The precision of these analyses was estimated by calculation of the technical error, the latter being defined as $\sqrt{d^2/2N}$, where d is the difference between duplicate estimations and N is the number of duplicates. The technical errors were: triglyceride, 2.3%, cholesterol, 0.8%, and phospholipid, 1.4%.

Lipoprotein protein content was estimated by the bicinchoninic acid (BCA) procedure of Smith et al. (31); bovine serum albumin (Sigma) was used as the working standard. This assay was linear over the range from 0 to 2000 μg protein/ml. Like the procedure of Lowry et al. (32), distinct differences may occur between the chromogenicity of the apolipoproteins on the one hand, and on the other, of the protein (i.e., albumin) upon which their quantitation is based (33). The technical error for the BCA protein assay was 5.7%.

Electrophoretic analyses of the protein moieties of lipoprotein subfractions (see below) suggested the presence of albumin. The albumin content in such subfractions was therefore quantitated by a specific colorimetric assay based on the formation of a complex between bromocresol green and albumin with peak absorbance at 628 nm. This assay was carried out by use of a commercial kit (Biomérieux) based on the method of Doumas and Biggs (34).

Morphological analysis

Samples of hamster VLDL (d < 1.018 g/ml) were negatively stained with 2% potassium phosphotungstate (pH 7.4) on Formvar carbon-coated grids, and examined at 60 KV with a Philips CM10 microscope (35). The procedures used for microscope calibration and the calculation of particle sizes were as outlined earlier (35).

Electrophoretic analyses of native lipoproteins

Agarose gel. One μl of plasma or 1 μl of each lipoprotein subfraction (diluted to a final conc. of 0.2 mg protein/ml) were electrophoresed on agarose gel slabs (Corning, Palo Alto, CA) using the Corning ACI system, essentially as described by Noble (36). Lipoprotein bands were stained for lipids with Fat Red 7B.

Nondenaturing polyacrylamide gradient gels. Lipoprotein fractions were electrophoresed in polyacrylamide gradient gels made up to contain continuous gradients from 2–16% or 4–30% (PAA 2/16 and 4/30; Pharmacia Fine Chemicals, Uppsala, Sweden); the GE-2/4 LS gel electrophoresis apparatus was used in accordance with the procedure of Nichols, Krauss, and Musliner (37) and Anderson et al. (38). The 2–16% gels were used for analysis of VLDL, IDL, and LDL subfractions, whereas 4–30% gels were used for analysis of particles in the HDL size range. Samples (10 μg protein) were applied in a maximal volume of 20 μl; dilute samples (<0.25 mg protein/ml) were concentrated by centrifugal ultrafiltration in Centrisart I tubes (Sartorius; size cut-off 10,000); tubes were centrifuged for 5 min at 2500 *g*-max and 4°C. After electrophoresis, gels were stained with a solution containing 0.7% (w/v) Coomassie Brilliant Blue R250, 22.5% (v/v) methanol, 2.2% (w/v) sulfosalycilic acid, 7.5% (w/v) trichloroacetic acid, and 67.8% (v/v) water. The migration distance of each stained lipoprotein band was determined and the corresponding Stokes diameter was calculated from a calibration curve obtained by simultaneous electrophoresis of a series of protein standards of known hydrated diameter (Pharmacia). For the 2–16% and 4–30% gels, standards included thyroglobulin (Stokes diameter 170 Å), ferritin (120 Å), catalase (104 Å), lactic dehydrogenase (81 Å), and albumin (71 Å); latex beads (380 Å) were used as an additional standard on 2–16% gels.

Electrophoretic analyses of apolipoprotein moieties

The constituent apolipoproteins of the protein moieties of lipoprotein subfractions were defined according to their molecular weight and isoelectric point.

Sample preparation. Lipoprotein fractions (15 μg protein) were first lyophilized and then delipidated with a mixture of ethanol–diethyl ether (peroxide-free, Merck) 3:1 (v/v), as described by Brown, Levy, and Fredrickson (39). After centrifugation (1200 *g*-max, 20 min at 4°C), the pellet was washed with diethyl ether alone; after sedimentation by centrifugation as before, the washed protein extracts were dried under a stream of N_2 and solubilized in a solution containing 0.5% SDS, 10 mM Tris-HCl, and 10 mM DTT at pH 6.8.

Molecular weight estimation: one-dimensional analysis. In order to separate and determine the molecular weights of non-B-apolipoproteins with mass in the range of 10^4–10^5

daltons, the protein moiety of each lipoprotein gradient subfraction was examined in an SDS-polyacrylamide gradient gel system (SDS-PAGE) constructed essentially according to the procedure of Irwin et al. (40), using a dual vertical slab electrophoresis cell (Hoeffer Instruments, San Francisco, CA). Slabs with a thickness of 1.5 mm and a length of 13 cm were constructed with a peristaltic pump (Gilson Minipuls II) and a gradient former (Bio-Rad, Richmond, CA) to give a continuous linear gradient from 5 to 19% acrylamide. After the running gel was formed, a stacking gel was added in 3% acrylamide.

For size estimation of the B apolipoproteins, a similar minigel system (Mini-Protean II System, Bio-Rad, Richmond, CA) made up to contain 3.5% polyacrylamide, was used. Conditions of electrophoresis were again as outlined by Irwin et al. (40) (30 mA/gel, 4 h at 5°C for 5–19% gels; 20 mA/gel, ~1.5 h at 5°C for 3.5% gels). On completion of electrophoresis, gels were stained with Coomassie Brilliant Blue R250.

A calibration curve was constructed from protein standards electrophoresed in parallel; for 5–19% gels, molecular weight markers were myosin (205 kD), β-galactosidase (116 kD), phosphorylase B (97.4 kD), bovine albumin (66 kD), ovalbumin (45 kD), carbonic anhydrase (29 kD), and α-lactalbumin (14.2 kD). For 3.5% gels, a series of polymerized molecular weight standards derived from phosphorylase B were used (584.4, 487, 389.6, 292.2, 194.8, and 97.4 kD, respectively). In both cases, the correlation coefficients for these curves were typically greater than −0.98.

Two-dimensional analysis. Apolipoproteins with size in the range from 10^4 to 10^5 daltons were also analyzed by the two-dimensional procedure of O'Farrell (41). Analytical isoelectric focusing was performed in the first dimension by the method of Warnick et al. (42) for plasma apolipoproteins. After electrofocusing in the pH range 4–6 (Biolytes, Bio-Rad), the isoelectric points of individual apolipoprotein bands were determined from a calibration curve constructed from a series of standard markers (carbonic anhydrase, pI range 4.8–6.7; Carbamalyte, Pharmacia); the correlation coefficient for this curve was ~0.99.

For migration in the second dimension, focusing gels were placed along the upper surface of 5–19% SDS-polyacrylamide gradient gels (40), and electrophoresed initially at 10 mA/gel for 30 min, then subsequently at 20 mA/gel for ~1 h. Gradient gels were colored first with Coomassie R250 (see above), and color-intensified with silver nitrate ("Silver stain plus", Bio-Rad). Molecular weights corresponding to the positions of stained spots were then calculated as described above.

Electroimmunoblotting of apolipoproteins

In order to identify individual hamster apolipoproteins on the basis of their immunological cross-reactivity with antibodies to their human counterparts, electroimmunoblotting was performed according to the general principles of Towbin, Staehelin, and Gordon (43). After electrophoretic migration either in SDS-PAGE or in focusing gels, proteins were electroeluted from the gel into a polyvinylidene fluoride membrane (PVDF, Millipore, Bedford, MA) using a buffer containing 25 mM Tris, 192 mM glycine, 20% methanol, and 0.05% SDS; electrotransfer was performed for 2 h at 90V for SDS-PAGE and for 1 h at 4°C for isofocusing gels. Protein transferred to PVDF membranes was revealed as detailed below for dot immunobinding. The monospecific antisera used were: a sheep polyclonal to human apoB (44), a sheep polyclonal to human apoA-I (from Immuno AG, Vienna, Austria), a sheep polyclonal to human apoE (45), and a sheep polyclonal to human Lp[a] (Immuno AG, Vienna, Austria).

For determination of either the molecular weights or isoelectric points of protein bands detected on PVDF, protein standards were stained with aminoblack by the method of Hancock and Tsang (46).

Evaluation of apolipoprotein content by dot immunobinding

For the qualitative immunological detection of specific apolipoproteins in individual hamster lipoprotein subfractions, dot immunobinding was performed by the procedure of Hawkes, Niday, and Gordon (47) using monospecific polyclonal antibodies to human apoB, apoA-I, apoE, and Lp[a] as above. By use of a Bio-Dot Apparatus

TABLE 1. Total concentrations of the major lipids of hamster plasma

	Total Cholesterol[a]	Cholesteryl Ester[b]	Free Cholesterol	Triglyceride	Phospholipid
			mg/dl[c]		
Hamster	114.6 ± 8.4	151.2 ± 13.8	22.8 ± 1.3	148.7 ± 26.4	199.8 ± 18.3
Human	181.2 ± 7.4	225.7 ± 35.8	46.2 ± 15.9	75.0 ± 20.6	196.2 ± 8.4

[a]Values for total cholesterol represent the sum of free and esterified cholesterol.
[b]Values of cholesteryl ester correspond to the difference between those of total cholesterol and free cholesterol multiplied by a coefficient of 1.67.
[c]Values are the mean ± SEM of duplicate analyses of five pools of hamster plasma representing an average of 10–25 animals per pool, and of three normolipidemic subjects. The same pools were used for lipoprotein isolation by density gradient ultracentrifugation.

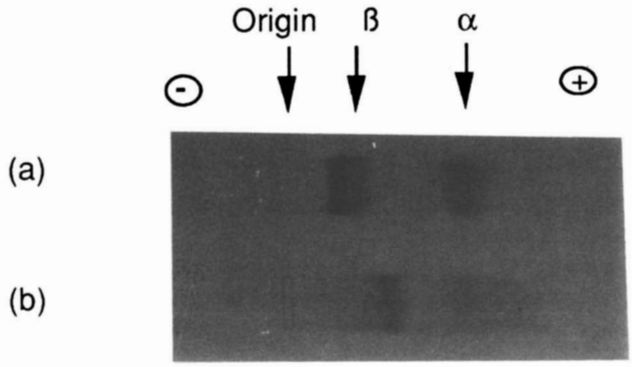

Fig. 1. Electrophoretic mobility of native lipoproteins in (a) human and (b) hamster plasmas (1 μl) on 1% agarose gel. Lipids were stained with Fat Red 7B; the positions of lipoprotein bands in human plasma are indicated by arrows.

(Bio-Rad, Richmond, CA), aliquots of lipoprotein fractions (1–2 μg protein/well) were deposed in wells and applied to a nitrocellulose membrane (Bio-Rad, Richmond, CA) by vacuum suction. The subsequent procedures for blocking, washing, antibody reaction, and detection were essentially as outlined earlier (44). Peroxidase-labeled rabbit antibodies to sheep immunoglobulins were obtained from Dako (Dakopatts a/s, Glostrup, Denmark) and diluted 100-fold.

RESULTS

Lipid and lipoprotein content of whole plasma

The concentrations of lipids in pooled hamster plasma are presented in **Table 1.** Despite fasting for ~16 h, triglyceride levels were almost twofold greater than those in humans. Total cholesterol levels in hamsters represented ~63% of those in normolipidemic subjects, and most of the cholesterol (~80%) was in esterified form. Phospholipid levels in humans and hamster were alike. In order to evaluate possible inter-animal variability in plasma lipid levels, total cholesterol was assayed in 19 male hamsters; values ranged from 87 to 120 mg/dl, with a mean of 108.7 ± 2.0 mg/dl. Such values are quite comparable to those already reported for male hamsters in the literature (1, 5, 8, 9, 20, 21).

The electrophoretic mobility of hamster lipoproteins in whole plasma varied to a minor degree between individuals. A representative pattern (**Fig. 1**) revealed two bands; the predominant band displayed α-mobility and migrated to a position similar to that of human HDL. A second band migrated with "prepre-β" or α-2 mobility. A third component(s) remained at the origin, and probably represented either chylomicrons, and their remnants, or large VLDL, or a mixture of both (see below). A distinct band of β-mobility was not discernible in the hamster pattern.

Density distribution, concentration, electrophoretic mobility, and chemical composition of native lipoprotein subfractions

The density distribution of the various lipoprotein classes of hamster plasma was evaluated over the density range up to 1.172 g/ml, and is compared to that of normolipidemic subjects in **Fig. 2.** Qualitatively, a marked resemblance was evident between the hamster and human profiles. Furthermore, the lipoprotein mass profile in our hamsters markedly resembled that reported earlier in overnight-fasted, adult Golden Syrian hamsters by Groot et al. (9). Thus, the hamster profile was characterized by the presence of three peaks that occurred at densities of less than 1.016, 1.029–1.039, and 1.083–1.103 g/ml, respectively, thereby closely resembling the human pattern. Significant quantitative differences were, however, in evidence. The quantitative distribution and mean weight % chemical compositions of individual lipoprotein subfractions from pooled hamster plasmas are summarized in **Table 2** and **Table 3.** In agarose gel, gradient fractions 1 and 2 ($d < 1.019$ g/ml) migrated as broad diffuse bands extending from the origin to the pre-β position, suggesting that they contained a highly heterogenous mixture of

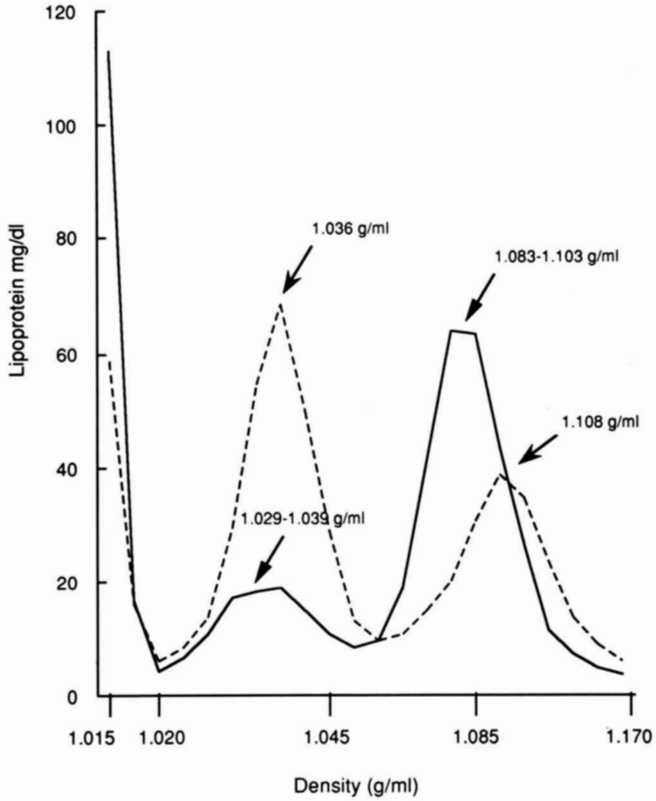

Fig. 2. Quantitative distribution of hamster and human plasma lipoproteins as a function of density after separation by gradient density ultracentrifugation. The data represent the mean of five pools of hamster plasma (—), and of three normolipidemic subjects (--).

TABLE 2. Profile of lipoprotein subfractions isolated from hamster plasma by density gradient ultracentrifugation

Variable	Density Gradient Fractions[a] 1	2	3	4	5	6	7	8	9	10	11
Density limits[c] (g/ml)	d<1.018	1.018–1.019	1.019–1.021	1.021–1.023	1.023–1.026	1.026–1.029	1.029–1.033	1.033–1.039	1.039–1.044	1.044–1.050	1.050–1.057
Electrophoretic mobility[d]	VLDL	VLDL	LDL	LDL	LDL	LDL	LDL	LDL	LDL HDL	LDL HDL	LDL HDL
Serum lipoprotein[e] (mg/dl)	120.6 ± 5.8	16.3 ± 3.3	4.2 ± 0.3	6.2 ± 0.4	10.7 ± 1.3	16.9 ± 1.5	18.3 ± 0.7	18.9 ± 1.4	14.6 ± 1.5	10.4 ± 0.9	8.1 ± 0.7
Serum lipoprotein protein content (mg/dl)	6.2 ± 2.8	1.5 ± 0.4	0.8 ± 0.3	1.5 ± 0.3	3.4 ± 1.8	4.6 ± 0.5	5.2 ± 1.0	5.3 ± 1.0	4.3 ± 1.1	3.1 ± 0.6	2.5 ± 0.5
Serum lipoprotein lipid content (mg/dl)	114.4 ± 19.6	14.8 ± 2.0	3.4 ± 0.3	4.9 ± 0.5	7.3 ± 1.1	12.3 ± 2.6	13.1 ± 0.8	13.6 ± 1.7	10.3 ± 2.1	7.3 ± 1.3	5.6 ± 1.0
Percent of total lipoprotein of d<1.172 g/ml	22.4	3.0	0.8	1.2	2.0	3.2	3.4	3.5	2.7	1.9	1.5

[a]Fractions were of ± 0.4 ml except fraction 1 which was removed in a volume of 0.5 ml.
[b]Lipids present in the bottom fraction, (no. 25, 1.200<d<1.283 g/ml) amounted to less than 5% of total plasma content.
[c]Density limits were taken from a standard curve of density vs. volume derived from control gradients containing only salt solutions.
[d]Electrophoretic mobility in agarose gel slabs was compared to that of corresponding lipoproteins of human serum.
[e]The values for each lipoprotein fraction represent the sum of the individual components determined chemically, and are the mean ± SEM of duplicate analyses of each fraction isolated from five density gradients. Each gradient was constructed from a pool of an average of 10–25 hamster plasmas.

TABLE 3. Chemical composition of lipoprotein subfractions isolated from hamster plasma by density gradient ultracentrifugation

Variable	Density Gradient Fractions[a] 1	2	3	4	5	6	7	8	9	10	11
Density limits[a] (g/ml)	d<1.018	1.018–1.019	1.019–1.021	1.021–1.023	1.023–1.026	1.026–1.029	1.029–1.033	1.033–1.039	1.039–1.044	1.044–1.050	1.050–1.057
Component (mean weight)[b]											
Protein[c]	4.8 ± 0.9	8.6 ± 1.1	16.3 ± 3.2	23.7 ± 1.3	25.9 ± 4.0	27.8 ± 1.7	28.2 ± 2.0	27.8 ± 1.2	29.3 ± 1.1	30.0 ± 1.0	30.8 ± 0.7
Free cholesterol	5.8 ± 0.3	6.5 ± 1.4	8.4 ± 1.7	8.8 ± 0.4	7.9 ± 0.7	7.8 ± 0.2	8.2 ± 0.3	7.6 ± 0.2	7.5 ± 0.3	7.6 ± 0.3	7.5 ± 0.4
Cholesteryl ester	3.1 ± 0.6	6.2 ± 1.1	23.1 ± 3.5	23.4 ± 2.2	24.2 ± 1.5	26.0 ± 1.8	27.1 ± 1.5	30.2 ± 0.9	29.9 ± 0.9	29.5 ± 0.9	27.7 ± 0.8
Triglyceride	72.5 ± 0.6	62.0 ± 4.8	30.3 ± 3.5	23.2 ± 2.4	21.4 ± 2.9	17.3 ± 1.9	14.6 ± 1.7	12.5 ± 1.5	11.2 ± 1.4	9.3 ± 1.3	6.8 ± 1.1
Phospholipid	13.8 ± 0.2	16.7 ± 2.0	21.9 ± 1.3	20.9 ± 0.9	20.6 ± 0.8	21.3 ± 0.9	21.9 ± 0.7	21.9 ± 0.6	22.1 ± 0.6	23.6 ± 0.4	27.2 ± 0.6

[a]See Table 2.
[b]Values are the means ± SEM of duplicate analyses of each fraction isolated from five separate pools of hamster plasma. Each gradient was constructed from a pool of an average of 10–25 hamster plasmas.
[c]Albumin was detected immunologically in fractions 1 to 22, and was quantified by a specific colorimetric assay (see Methods). Albumin content was deducted from the protein content of each fraction prior to calculation of % wt chemical composition.

VLDL (and possibly chylomicron) particles (data not shown), a finding subsequently confirmed by electron microscopic analysis (see below). Each hamster subfraction constituting the density interval from 1.019 to 1.074 g/ml (nos. 3–13) migrated as an intensely stained band to the same position as that of the corresponding human LDL subspecies. Alpha-migrating HDL were, however, detected in hamster over the range from d 1.039 to 1.172 g/ml (subfractions 9–22). Considered together, these electrophoretic analyses suggest that LDL- and HDL-like particles overlapped in the density range from 1.039 to 1.074 g/ml.

VLDL in fraction 1 (d < 1.018 g/ml) exhibited elevated triglyceride content (72.5%), and represented about 22%

Density Gradient Fractions[a]												
12	13	14	15	16	17	18	19	20	21	22	23	24[b]
1.057–1.065	1.065–1.074	1.074–1.083	1.083–1.092	1.092–1.103	1.103–1.114	1.114–1.126	1.126–1.138	1.138–1.150	1.150–1.164	1.164–1.172	1.172–1.186	1.186–1.200
LDL HDL	LDL HDL	HDL	HDL	HDL	HDL	HDL	HDL	HDL	HDL	HDL		
9.4 ± 1.8	18.5 ± 3.1	41.0 ± 6.2	63.6 ± 6.9	63.5 ± 5.7	43.6 ± 3.5	26.6 ± 3.8	11.4 ± 0.7	7.0 ± 0.5	4.5 ± 0.3	3.4 ± 0.4	5.1 ± 2.5	8.6 ± 2.7
2.9 ± 1.3	6.3 ± 2.4	14.1 ± 4.8	21.9 ± 6.6	22.5 ± 6.3	15.7 ± 3.6	15.3 ± 1.7	4.7 ± 0.4	3.2 ± 0.4	2.2 ± 0.2	1.9 ± 0.3	4.0 ± 5.2	7.1 ± 2.4
6.5 ± 2.3	12.2 ± 3.9	26.9 ± 7.9	41.7 ± 8.1	41.0 ± 5.4	27.9 ± 3.5	11.3 ± 1.7	6.7 ± 0.2	3.8 ± 0.6	2.3 ± 0.4	1.5 ± 0.5	1.4 ± 0.4	1.6 ± 0.4
1.8	3.4	7.6	11.8	11.8	8.1	5.0	2.1	1.3	0.9	0.6		

Density Gradient Fractions[a]													
12	13	14	15	16	17	18	19	20	21	22	23	24	25
1.057–1.065	1.065–1.074	1.074–1.083	1.083–1.092	1.092–1.103	1.103–1.114	1.114–1.126	1.126–1.138	1.138–1.150	1.150–1.164	1.164–1.172	1.172–1.186	1.186–1.200	1.200–1.283
30.5 ± 1.0	34.3 ± 1.9	34.3 ± 1.8	34.1 ± 2.3	37.8 ± 2.1	35.6 ± 1.5	42.5 ± 4.9	41.2 ± 0.6	45.9 ± 1.0	48.5 ± 2.3	58.3 ± 4.2	69.7 ± 9.3	85.3 ± 3.2	95.7 ± 4.6
5.7 ± 0.6	4.9 ± 0.3	3.8 ± 0.1	3.4 ± 0.2	2.5 ± 0.1	2.9 ± 0.1	2.6 ± 0.2	2.6 ± 0.2	2.5 ± 0.5	2.6 ± 0.7	1.5 ± 0.9	1.1 ± 1.1	0.4 ± 0.4	0.1 ± 0.1
24.6 ± 3.2	25.7 ± 0.7	25.6 ± 1.0	25.9 ± 1.0	25.6 ± 1.2	26.1 ± 1.0	23.5 ± 2.3	24.1 ± 0.6	22.3 ± 0.8	21.1 ± 1.3	17.9 ± 1.5	12.0 ± 3.4	2.7 ± 1.1	0.3 ± 0.3
3.6 ± 1.5	1.6 ± 0.6	0.7 ± 0.2	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.2	0.8 ± 0.5	1.2 ± 0.8	1.3 ± 1.3	1.5 ± 1.5	2.1 ± 2.1	0.8 ± 0.8	3.7 ± 4.1
35.6 ± 5.1	33.5 ± 1.9	35.6 ± 1.8	36.2 ± 2.0	36.5 ± 2.1	32.9 ± 1.4	31.1 ± 2.7	31.3 ± 0.8	28.1 ± 1.1	26.5 ± 1.4	20.8 ± 2.1	15.0 ± 4.1	10.8 ± 3.0	

of the total d < 1.172 g/ml lipoproteins of hamster plasma. Protein content was typically low and of the order of 5%; in addition, VLDL were atypical in that the proportion of cholesterol in the free form (5.8%) was greater than that in esterified form (3.1%). Intermediate density lipoproteins (IDL) were isolated in the density range 1.018–1.019 g/ml (fraction 2), and were again rich in triglyceride (62%), but were minor components (3% of total d < 1.172 g/ml substances). Some degree of contamination of IDL by the VLDL fraction during gradient fractionation cannot be excluded.

A transition in chemical composition was seen in fractions 3 and 4 (d 1.019–1.023 g/ml) in which marked decrease in triglyceride content was found. The propor-

tions of the core lipids, triglycerides, and cholesteryl esters were, however, similar in these subfractions, accounting for 53–46% of particle mass. Significant increase in protein content (to 16–24% from 5% in VLDL) paralleled increase in density, as did that of phospholipid (to ~21% from ~14% in VLDL). In their chemical compositions, certain features of fractions 5–11 (d 1.023–1.057 g/ml) resembled their counterparts in humans (26), with the exception that their triglyceride contents were elevated (range ~21–7%). In addition, a relative deficiency in cholesteryl esters was detected; nonetheless, cholesteryl esters were the dominant component of the lipid core (~24–30%). Total core lipids diminished with increase in density (from 45 to 34%). These LDL-like subfractions together accounted for a minor proportion (~18%) of the total hamster lipoproteins.

Fraction 12, of d 1.057–1.065 g/ml, was a minor component (~2% of total lipoproteins), but exhibited a chemical composition intermediate between those of human LDL and HDL (26). In its phospholipid content (~36%) it was akin to HDL, and more specifically to HDL_2, whereas the proportions of the remaining components were more typical of LDL (26), with the exception that cholesteryl ester content remained low (~25%).

In the hamster, total HDL (fractions 13–22, d 1.065–1.172 g/ml) accounted for ~53% of the d < 1.172 g/ml lipoproteins, and HDL_3-like fractions (nos. 17–22, d 1.103–1.172 g/ml), for some 34% of total HDL. Fractions 13–16 (d 1.065–1.103 g/ml), the major HDL subfractions, corresponded to human HDL_2 in hydrated density (26), and were characterized by low triglyceride contents (1.6–0.3%), elevated ratios of cholesteryl ester:free sterol (~5–10:1), protein contents in the range of ~34–38%, and high proportions of phospholipid (~35%).

Fractions 17–22 (d 1.103–1.172 g/ml), corresponding approximately to the hydrated density of HDL_3 in humans (d 1.100–1.150 g/ml), were characterized by high protein content (~36–58%) and cholesteryl ester:free cholesterol ratios in excess of 8 (range 8–11.9:1); it is noteworthy that proportions of phospholipid in these heavy HDL (HDL_H, range 20.8–32.9%) were typically less than in light HDL subfractions (HDL_L, range 33.5–36.5%).

Hamster subfractions of d 1.172–1.283 g/ml displayed chemical compositions in which protein was the principle constituent (70–96%); they accounted for only ~1% of total lipoprotein lipids, thereby corresponding to VHDL in humans (26).

Comparison of the chemical compositions of the major classes of hamster plasma lipoproteins with the corresponding human fractions is shown in **Table 4.** Hamster LDL, HDL_L, and HDL_H were calculated as the sum of the weights of the individual components in the constitutive subfractions as indicated. The differing amounts of core (apolar; triglyceride, cholesteryl ester) and polar surface components (apolipoproteins, phospholipids, and

TABLE 4. Comparison of the chemical composition of the major classes of hamster and human plasma lipoproteins isolated by density gradient ultracentrifugation

Variable	Hamster VLDL	Human VLDL	Hamster LDL	Human LDL	Hamster HDL			Human HDL			
					HDL_L	HDL_H	Total	HDL_2	HDL_3	Total
Fraction number[a]	1	1	5–11	5–11	12–16	17–22	12–22	12–16	17–20	12–20
Density limits[a] (g/ml)	d<1.018	d<1.018	1.023–1.057	1.023–1.057	1.057–1.103	1.103–1.172	1.057–1.172	1.057–1.103	1.103–1.150	1.057–1.150
Component[b] (mean weight %)										
Protein	4.8 ± 0.9	9.0 ± 6.9	30.0 ± 2.3	22.0 ± 1.6	34.2 ± 2.0	40.6 ± 1.9	36.3 ± 1.8	37.4 ± 2.5	45.8 ± 2.5	42.3 ± 2.3
Free cholesterol	5.8 ± 0.3	6.1 ± 1.4	8.1 ± 0.3	9.8 ± 0.3	3.7 ± 0.1	2.6 ± 0.2	3.3 ± 0.1	5.0 ± 0.1	2.7 ± 0.1	3.7 ± 0.1
Cholesteryl ester	3.1 ± 0.6	14.6 ± 5.8	24.3 ± 2.6	41.5 ± 1.7	25.5 ± 1.0	24.3 ± 1.2	25.2 ± 1.0	23.0 ± 1.1	19.1 ± 0.3	20.8 ± 0.2
Triglyceride	72.5 ± 0.6	52.2 ± 7.7	13.1 ± 1.5	5.4 ± 0.9	0.7 ± 0.2	0.5 ± 0.2	0.6 ± 0.2	4.6 ± 1.1	3.3 ± 0.9	3.8 ± 1.1
Phospholipid	13.8 ± 0.2	18.1 ± 12.9	24.5 ± 1.1	21.3 ± 0.5	35.9 ± 1.9	32.0 ± 1.2	34.6 ± 1.6	30.0 ± 1.6	29.1 ± 1.3	29.4 ± 1.3
Ratio CE/TG	0.04	0.3	1.8	7.7	36.4	48.6	42.0	5.0	5.8	5.5

[a]See Table 2.
[b]Values are the mean ± SEM of duplicate analyses of each fraction from five different pools of hamster plasmas and from three separate human plasmas.

some free cholesterol) are noteworthy. Thus total core lipids in hamster VLDL amounted to ~75% of the weight, but to only 67% in the human fraction, clearly suggesting that larger particles predominate in the former fraction (see Morphometric analysis, below). As in VLDL, hamster LDL was significantly poorer in cholesteryl ester and richer in triglyceride than its human counterpart. Both the light and heavy subfractions of hamster HDL showed an overall resemblance to the corresponding human particles in chemical composition, although triglyceride contributed less, and phospholipid and cholesteryl ester proportionately more, to particle mass in the hamster. Indeed, cholesteryl ester:triglyceride ratios (~36–49:1) were several-fold higher in both hamster HDL subfractions than in their human counterparts (~5–6:1).

Morphometric analysis

Negative stain electron microscopic examination of hamster VLDL (d < 1.018 g/ml) revealed particles that were essentially spherical when free standing, but tended to deform upon contact (**Fig. 3**). Marked heterogeneity was observed in particle size; the sizes of hamster VLDL were distributed asymmetrically, ranging from ~288 to 1218 Å in diameter with a mean of 703 Å.

Particle size and heterogeneity

The particle size and heterogeneity of hamster lipoprotein subfractions were evaluated by electrophoresis in nondenaturing polyacrylamide gel, and the corresponding electrophoretic patterns are shown in **Fig. 4;** these results are representative of three separate pools of hamster plasma. Data in hamster were compared to those obtained upon fractionation of normolipidemic plasmas from three healthy subjects (data not shown).

In 2–16% polyacrylamide gradient gels, lipoprotein particles with diameters greater than ~360 Å are incompletely resolved due to lack of penetration into the gel, thereby necessitating the electron microscopic analysis outlined above. It is nonetheless noteworthy that the minimal particle diameter detectable in fractions 1 and 2 was ~260 Å, a finding consistent with electron microscopic analysis.

Over the density interval from 1.019 to 1.065 g/ml (fractions 3–12), the diameter of the major particle species diminished with increase in density from 259 Å in fraction 3 to 254 Å in fraction 12. Over this same density range, the major subspecies of human LDL decreased in size from 274 to ~254 Å (data not shown). It is noteworthy that the human particles were slightly larger than their counterparts in the hamster over the density range involving light LDL (d 1.019–1.033 g/ml, fractions 3–7; hamster, 259–255 Å; human, 274–262 Å). These findings tend to corroborate those of our chemical analyses, in which the proportion of core lipids in fractions 4–7 was

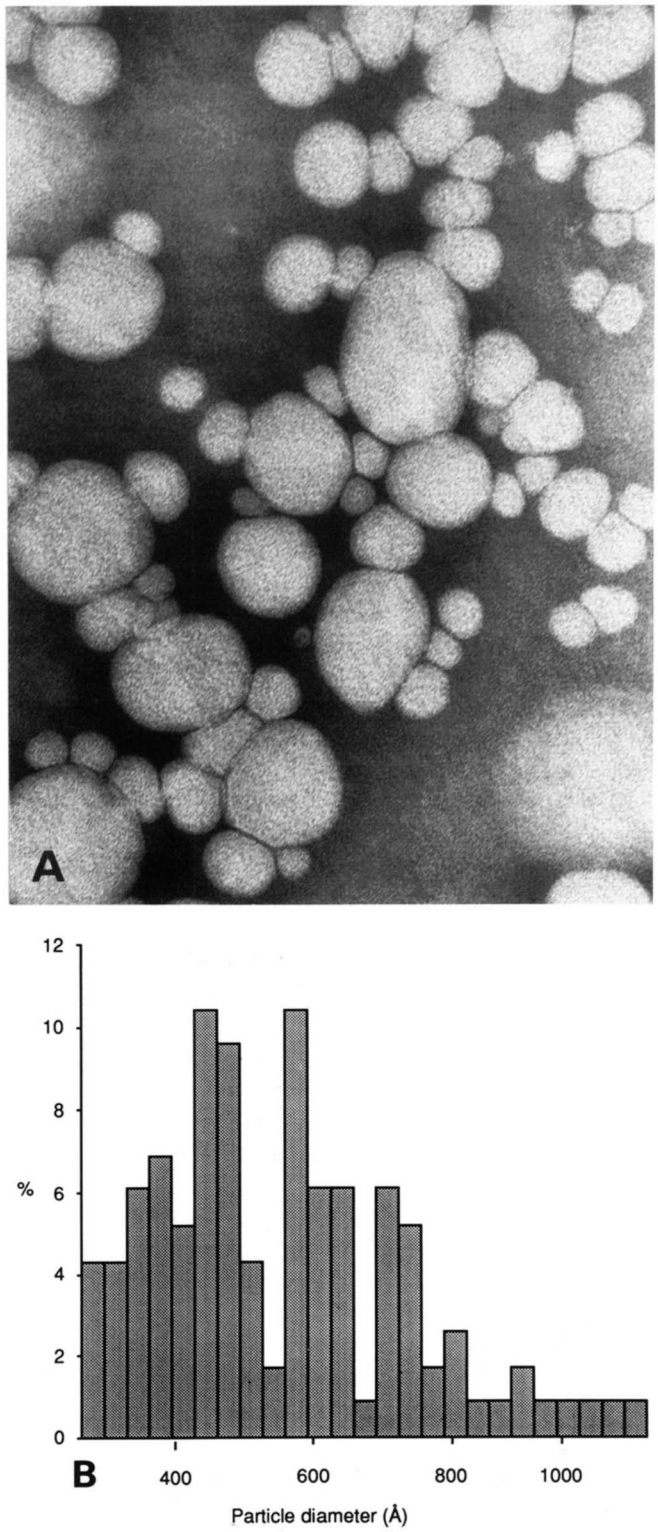

Fig. 3. (A) Electron photomicrographs of negatively stained hamster plasma lipoproteins of d < 1.018 g/ml, and (B) the frequency distribution of particle diameters.

lower in hamster LDL (~47–42%) than that of the corresponding human subfractions (51–48%). Equally, the proportion of hydrophilic surface components (mainly

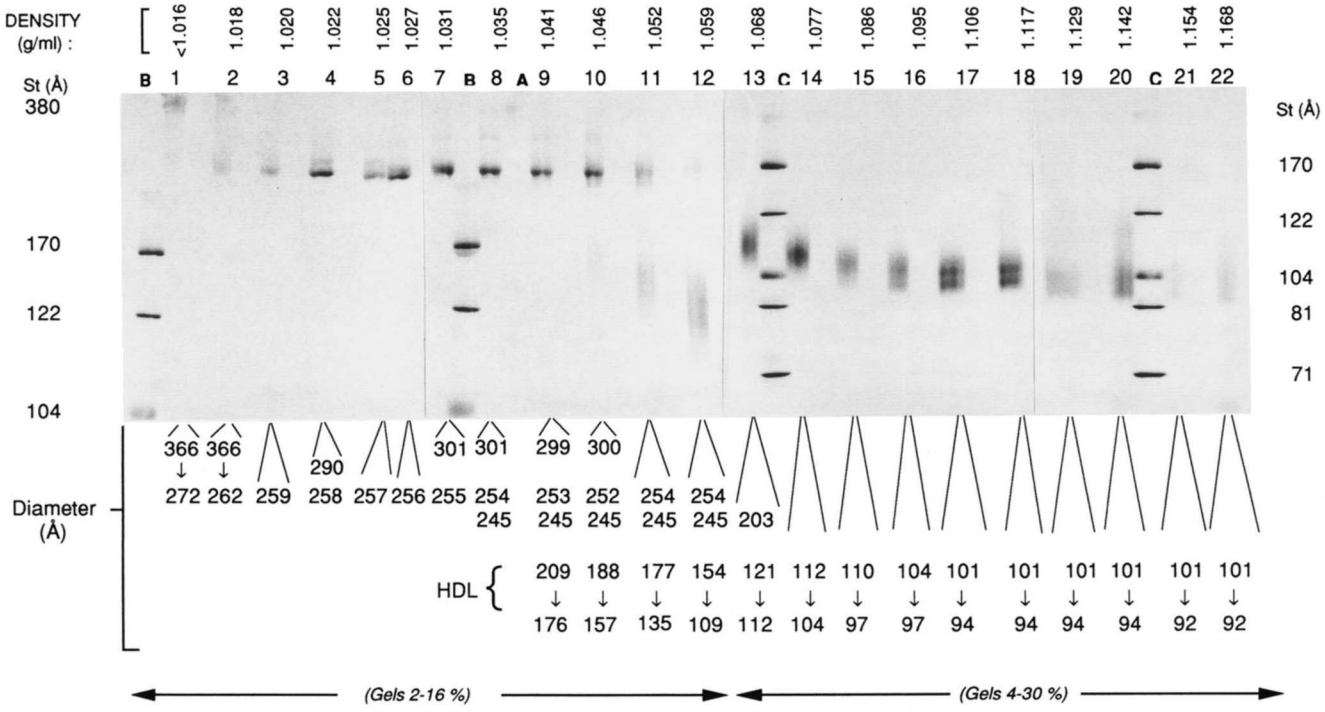

Fig. 4. Nondenaturing polyacrylamide gradient gel electrophoresis of native lipoprotein subfractions from hamster plasma. Electrophoresis was performed in 2–16% or 4–30% slabs on successive gradient fractions (10 μg protein/well) whose average densities (g/ml) are shown at top. The correspondence between lipoprotein particle diameters and the positions of individual stained gel bands was calculated as detailed in Methods; migration position and Stokes diameters of latex beads (380 Å) and each marker protein (St, Å) are indicated at left and right (standard protein markers were loaded in lanes A, B, and C). Gels were stained with Coomassie Brilliant Blue R-250. For diffuse bands such as those of HDL in fractions 9–22, the diameters corresponding to the leading and trailing edges, respectively, of the bands are indicated beneath the figure. Densities indicated above correspond to the mean density of each subfraction.

protein and phospholipid) was greater in the hamster subfractions over this same density range (fractions 4–7; hamster 44–50%; human 38–42%). These findings then suggest that light LDL-like particles in the hamster are smaller than their human counterparts. In addition to the major size subspecies, a second minor component with a diameter of ~300 Å was detected in hamster subfractions 7–10 (d 1.020–1.050 g/ml); the size of this subspecies was invariant with density.

Chemical analyses suggested that fraction 12 (d 1.059–1.065 g/ml) represented a transition between the LDL-like particles and those typical of HDL in hamster (Table 2). This observation was confirmed by the observation that particle species with sizes typical of both LDL and of HDL were detected together in fraction 12 (Fig. 4). Thus a faint band of diameter ~254 Å was detected, in addition to a diffuse component with size in the range ~109–154 Å, and whose proportion appeared to be similar as judged by the relative staining intensity of the respective bands. Traces of the latter HDL-like particles were also seen in fractions 9, 10, and 11 (d 1.039–1.057 g/ml); their size range diminished from 176–209 Å in fraction 9 to 135–177 Å in fraction 11. In sum, the hamster profile presents two particle size species corresponding to LDL and HDL that are present together over the density interval 1.039–1.074 g/ml. A similar overlap was seen in the human particle profile, but in this case, the overlap occurred over the range d 1.057–1.092 g/ml (data not shown).

Electrophoresis in 4–30% gradient gels of fractions 13–22 showed HDL particles as a single wide, diffuse band decreasing in size from a range of 112–121 Å to ~92–101 Å in the densest fractions. It is of interest that hamster HDL particle size appeared to remain constant in fractions 16–22 (d 1.092–1.172 g/ml), although the diffuse stained band was subdivided into two components of diameters ~94 Å and 100 Å, respectively (Fig. 4). Human HDL of similar density displayed slightly smaller particle sizes (82–97 Å) as compared to those of hamster.

Distribution and characterization of apolipoproteins

Dot immunobinding. A first approach to the characterization of the apolipoprotein content of hamster lipoprotein subspecies was performed by dot immunobinding, and in-

volved studies of subfractions isolated from three separate pools of hamster plasma with monospecific polyclonal antibodies to the human B, E, A-I and [a] apolipoproteins. The hamster counterpart to human apoB was detected in gradient subfractions 1–13 ($d < 1.074$ g/ml) as determined by positive dot blots (results not shown). A hamster protein cross-reacting with an antibody to human apoE was present along the entire length of the density gradient, but was detected as only a trace component in the interval 1.083–1.125 g/ml. The density distribution of a protein homologous to human apoA-I varied in fractions 8 or 9 (d 1.033 or 1.039 g/ml) according to the plasma pool examined, but was typically present up to fraction 22 (d 1.172 g/ml).

A protein cross-reacting with an antiserum to human apo[a] could not be detected in gradient fractions 1–22; nonetheless a positive reaction was obtained upon dot blotting of whole plasma, and similarly upon dot blotting of an aliquot of the densest fraction (no. 24, $1.186 < d < 1.200$ g/ml) containing plasma proteins. These results may be accounted for by a minor degree of cross-reactivity between the antibody to human Lp[a] and hamster plasminogen.

Molecular weight determination. A representative pattern obtained upon electrophoresis of the protein moieties of hamster lipoprotein subfractions in 5–19% SDS-polyacrylamide gel slabs under reducing conditions is shown in **Fig. 5.** For comparative purposes, gradient subfractions derived from human plasma were electrophoresed in parallel. The identity of the major hamster apolipoproteins resolved in such gels was estimated simultaneously by electroimmunotransfer or Western blotting (see Methods); the same series of antibodies as above was used in such studies, which were performed on four subfractions, i.e., 1, 6, 13, and 19, corresponding, respectively, to major components of hamster VLDL, LDL, HDL_L, and HDL_H.

Two bands of high M_r were detected (Fig. 5). The first, a prominent, intensely stained component of ~210,000 was present in fractions 1–13 ($d < 1.074$ g/ml), corresponding to the density intervals of VLDL, LDL, and a portion of that of HDL_L; only trace amounts of this protein were detected in the densest subfraction (d 1.068 g/ml). A second minor component of M_r ~168,000 was present in VLDL and IDL (fractions 1 and 2, respectively). The migration of the two high M_r components corresponded closely to that of human apoB-100 and apoB-48, respectively, in the same gel system; the identity of the hamster proteins was further confirmed by a positive reaction on Western blots with anti-human apoB

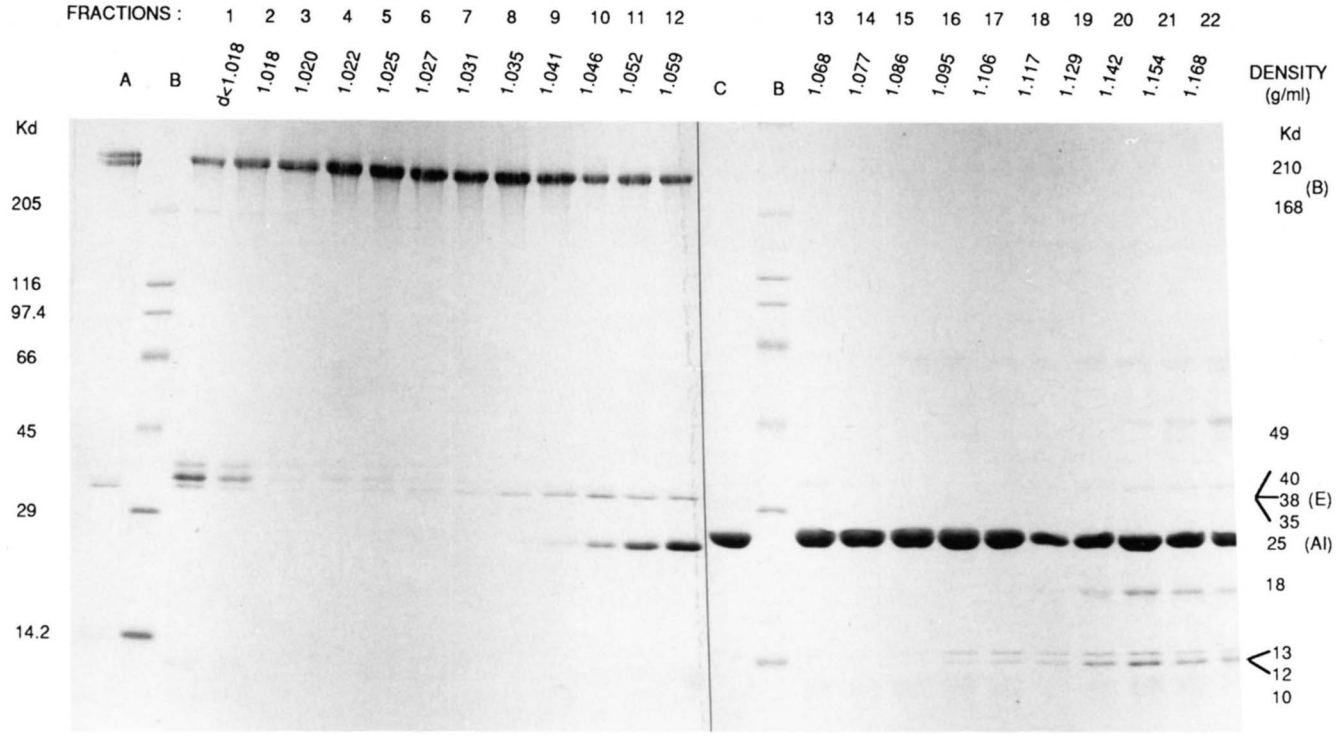

Fig. 5. Electrophoretic patterns in SDS-polyacrylamide gradient (5–19%) gel slabs of the apolipoproteins of hamster lipoprotein subfractions isolated by gradient density ultracentrifugation. Samples are: lane A, human apoVLDL (15 μg protein/well); lane B, purified standard molecular weight markers; lane C, human apoHDL (15 μg protein/well); lanes 1–22, hamster lipoprotein gradient subfractions (15 μg protein/well). Gels were stained with Coomassie Brilliant Blue R250. On the left hand side of the figure, the molecular weights of standard protein markers are indicated; at right, the M_r values and identities of the hamster proteins are noted. Densities indicated above correspond to the mean density of each subfraction.

(data not shown). It is relevant that our 5–19% polyacrylamide gel system is of insufficient resolution to allow accurate estimation of the sizes of proteins greater than ~100,000 daltons, and, indeed, significantly underestimates the M_r of the B apolipoproteins (human apoB-100 and apoB-48, 549 and 264 kD, respectively (48)).

Use of a lower acrylamide concentration (3.5%) allowed electrophoretic migration of the B proteins and, under such conditions, the sizes of the hamster B proteins were estimated as 534 and 334 kD, respectively. We have defined the larger major protein as hamster $apoB_H$ and the minor component as hamster $apoB_L$ (data not shown). The B proteins were not, however, the sole protein constituents of hamster VLDL and LDL. Indeed, three additional polypeptides of M_r 35,000, 38,000, and 40,000 were also present in fractions 1 to 13, the M_r 35,000 form predominating; their electrophoretic and immunological behavior suggested that they represent the hamster counterparts to human apoE. These components were most prominent in VLDL and IDL (fractions 1 and 2, respectively).

The hamster counterpart to human apoA-I was identified on the basis of its molecular weight (~26,000) and immunological reactivity (data not shown) in subfractions 8–22 (d 1.033–1.172 g/ml). Moreover, the hamster protein migrated to a position essentially identical to that of human apoA-I (Fig. 5, lane C), suggesting that the actual molecular weight of the hamster protein is ~27,000. In addition, it is noteworthy that the calculated size of hamster apoA-I based on amino acid analysis is 26,954 daltons (22). Hamster apoA-I was, however, a trace component of fractions 8–11, becoming the major apoprotein in HDL subfractions nos. 12–22. The protein moiety of light HDL (fraction nos. 13–17, d 1.065–1.114 g/ml) was constituted essentially exclusively of apoA-I, whereas additional apolipoproteins appeared in heavy HDL (fraction nos. 18–22, d 1.114–1.172 g/ml). With the exception of traces of apoE, we were unable to identify these components immunologically. A component with M_r 49,000 migrated similarly to human apoA-IV (M_r 46,000). A polypeptide of M_r 18,000, present mainly in heavy HDL, has no equivalent in humans. Finally, a group of small proteins equivalent to the human C proteins were detected in HDL subfractions 16–22. They consisted of two components of M_r 12,000 and 13,000, and a third diffuse band of M_r 8,000–10,000. Traces of such polypeptides were also detected in VLDL.

The density profile of hamster apolipoproteins showed only minor quantitative differences in studies of different plasma pools.

Isoelectric point determination and apolipoprotein polymorphism. The polymorphism of hamster apolipoproteins was evaluated by two-dimensional electrophoresis in denaturing gels (see Methods). The first dimension allowed determination of the isoelectric points (pI) of individual isoforms by analytical isoelectric focusing, and the second, determination of their molecular weights. Analytical isoelectric focusing studies were performed simultaneously on hamster and human VLDL (d < 1.019 g/ml) and HDL (1.074–1.103 g/ml) subfractions (**Fig. 6**). ApoE and apoA-I were identified in each species by electroimmunotransfer (see Methods).

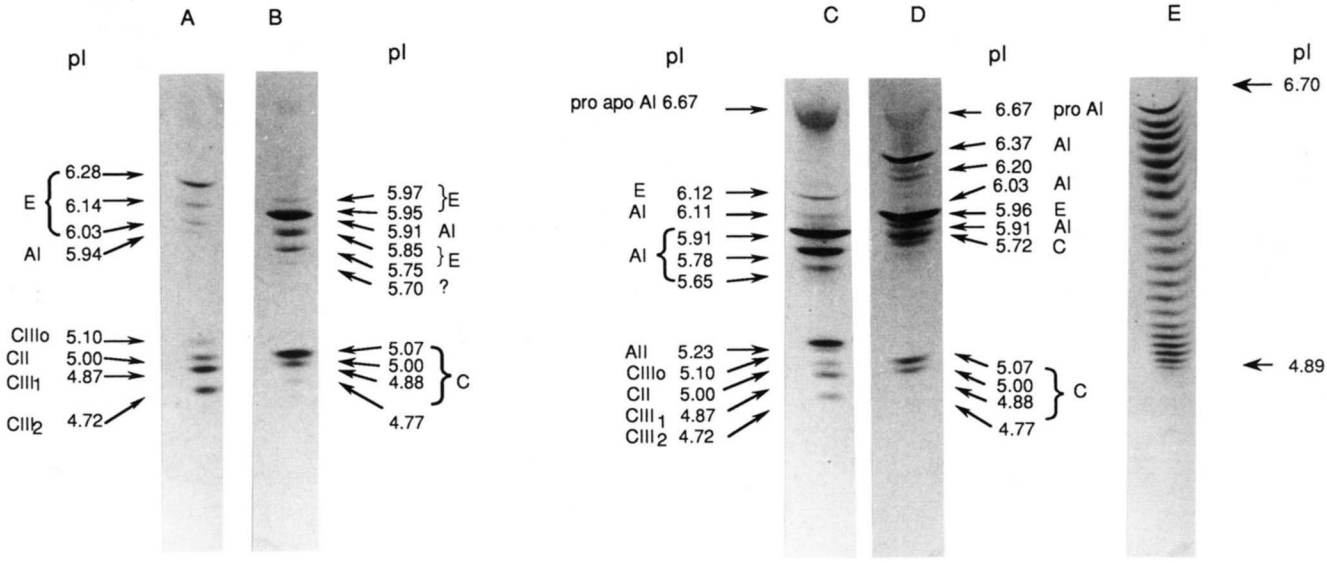

Fig. 6. Analytical isoelectric focusing pattern of hamster and human apolipoproteins in the pH range 4–6.5. Gels were stained with Coomassie Brilliant Blue R250. The pI values of the respective apolipoprotein bands were estimated from a calibration curve determined with carbamylated carbonic anhydrase standards (see Methods) of known pI. The identity of individual apolipoprotein bands was determined by immunoblotting (see Methods); samples are: (A) human apoVLDL (20 μg protein), (B) hamster apoVLDL (20 μg protein), (C) human apoHDL (15 μg protein), (D) hamster apoHDL (15 μg protein), and (E) standard.

In hamster apoVLDL, four isoforms (one major and three minor) of apoE were resolved (Fig. 6B). These isoproteins displayed more acidic pIs (5.95 for the major form; 5.97, 5.85, and 5.75 for the minor forms) than their human counterparts (6.28, 6.14, and 6.03) (Fig. 6A). In 2-D gels of apoVLDL (**Fig. 7A**), hamster apoE presented a complex series of spots, varying in both charge and size. The major form was of pI 5.95 and M_r 35,000; approx. six minor forms with slightly more acidic pI and higher M_r were also visualized. A minor component of pI 5.91 was also detected in apoVLDL, and was identified immunologically as apoA-I; on 2-D gels, this protein exhibited an M_r of ~26,000. On analytical isofocusing, a series of four bands (two major, two minor) with acidic pI in the pH interval 4.77–5.07 were also detected, thereby resembling the pI values of the human C peptides (pI 4.72–5.10). This group of proteins displayed M_r values in the range of 12,000 to 13,000 in 2-D gels. The major isoform of pI 5.07 and M_r 13,000 and the minor bands of pI 5.70 and 4.88 exhibited the same size. The second major isoprotein of pI 5.00 was slightly less than M_r 12,000.

Upon analytical isoelectric focusing of hamster apoHDL, apoA-I was resolved as three major isoproteins of pI 5.91, 6.03, and 6.37 (Fig. 6D); a minor, basic component of pI 6.67 was also identified as apoA-I on the basis of both its immunological reactivity and size. The identity of these four isoproteins as apoA-I was confirmed by their reactivity upon electroimmunoblotting with a polyclonal antibody to human apoA-I (data not shown). The hamster apoA-I isoproteins thus appear more basic than their human counterparts (5.65–5.78–5.91) (Fig. 6C); it is relevant that the pI 6.67 component displayed an electrophoretic behavior identical to human pro-apoA-I. Equally, an apoE isoform of pI 5.96 was identified in both 2-D gels (Fig. 7B) and by immunoblotting. As in the protein moiety of hamster VLDL, a series of polypeptides of low M_r and pI in the range of 4.77–5.07 was resolved. The pI 5.70 protein of M_r 13,000 was again present. In contrast, an isoprotein equivalent to human apoA-II with pI 5.23 was absent in hamster; we were also unable to identify an apoA-II counterpart on 2-D gels (Fig. 7B). It is noteworthy that values for the various isoforms of the human A-I, E, and C apolipoproteins obtained in the present studies are comparable to those reported in the literature (for a review, see ref. 19), with minor variation.

DISCUSSION

By application of a methodology involving lipoprotein fractionation by density gradient ultracentrifugation, coupled to immunological and electrophoretic analyses of apolipoprotein components, we have determined the principle features of the density profile of apoB- and apoA-I-containing lipoprotein subspecies in the plasma of chow-fed Golden Syrian hamsters. Prominent among these features, discussed in detail below, is the transport of a significant proportion of plasma cholesterol by apoB-containing lipoproteins (~43% of total), a finding largely consistent with the density distribution of cholesterol in hamster plasma originally described by Spady and Dietschy (1) and confirmed by others (5, 9, 20, 21).

ApoB-containing lipoproteins were distributed in hamster up to the density of ~1.074 g/ml (Fig. 5). As in other mammalian species (19), two major forms of this apolipoprotein were detected; the largest, denoted apoB_H and a counterpart to human apoB-100, was present over the density spectrum up to ~1.074 g/ml, while the smaller

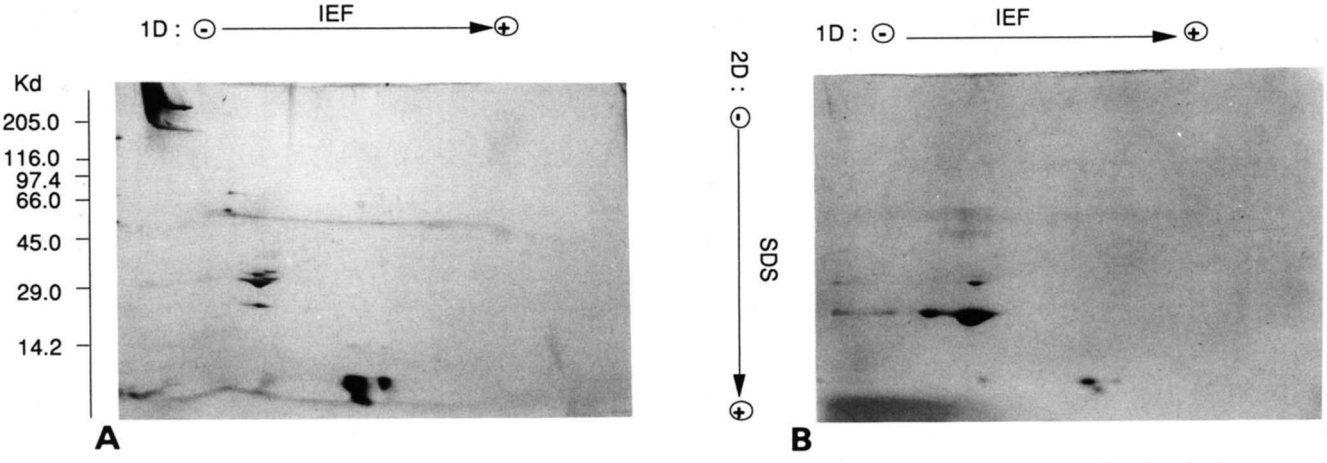

Fig. 7. Electrophoretic patterns in 2-D polyacrylamide gradient (5–19%) gel slabs of the apolipoproteins of hamster lipoproteins. Samples are: A, apoVLDL, d < 1.019 g/ml; B, apoHDL, d 1.074–1.103 g/ml. Gels were initially stained with Coomassie Brilliant Blue R250; coloration was subsequently intensified with silver nitrate. Isoelectric focussing was performed in the first dimension (1D); in the second dimension (2D), electrophoresis was performed under denaturing conditions in the presence of SDS (see Methods). The positions of molecular weight markers, electrophoresed in parallel in the second dimension, are indicated at left.

minor species, apoB_L, a counterpart to human apoB-48, was restricted to the triglyceride-rich lipoproteins, i.e., VLDL (fraction 1, d < 1.018 g/ml) and IDL (fraction 2, d 1.018–1.019 g/ml). Like the rat, VLDL in hamster were distinguished by their enrichment in apoE (Fig. 5), which presented as three molecular weight forms, that of M_r 35,000 predominating. Furthermore, the triglyceride-rich lipoproteins were highly heterogenous in their physicochemical properties (net electrical charge and particle size); their elevated triglyceride contents (~62–73%), considered together with the presence of a particle population with diameters of ~300–1200 Å, suggested the presence of particles of both intestinal and hepatic origins, i.e., of a mixture of chylomicrons, VLDL, and their respective remnants. The exclusive hepatic and intestinal origins of apoB-100 and apoB-48, respectively, in hamster supports this contention (23). The presence of significant amounts of chylomicron-like particles in VLDL under fasting conditions is, however, exceptional. It may reflect the ability of this animal to store substantial amounts of food in its cheek pouches, which may be subsequently digested during the fasting period. Under such conditions, hamster VLDL represented ~22% of the total d < 1.172 g/ml lipoproteins (~140 mg/dl) and accounted for transport of ~29% of total lipoprotein lipids. Such elevated VLDL levels distinguish the hamster from other rodent species, and are entirely consistent with the findings of Ontko, Cheng, and Yamamoto (8), in which the major esterification pathway implicated in the hepatic metabolism of plasma-derived free fatty acids in hamster is that of triglyceride synthesis. In consequence, increased hepatic production of VLDL ensues.

ApoB-containing particle subspecies with certain physicochemical characteristics typical of LDL were detected over the density interval from 1.019 to 1.074 g/ml, their concentration (~140 mg/dl) representing ~25% of the total d < 1.172 g/ml lipoproteins and a similar proportion of total lipoprotein lipid. These lipoproteins were of preβ-mobility on agarose gel and displayed a single major particle size subspecies whose diameter decreased from ~274 to 254 Å with progressive increase in density, thereby resembling their human counterparts (data not shown); nonetheless, they were deficient in cholesteryl esters (range 23–30% by wt) and rich in triglycerides (up to 30% in fraction 3 of d 1.019–1.021 g/ml). Moreover, the proportion of core lipids was greater in human than in hamster LDL (Table 4; ~47 and 37%, respectively). Hamster LDL subspecies of 1.026–1.039 g/ml were most abundant and contained traces of the M_r 35,000 form of apoE, a second size form (M_r 38,000) being present in addition over the density range 1.019–1.033 g/ml. As in humans, the B-100 component of hamster LDL (d 1.026–1.039 g/ml) thus appears to play a key role in particle binding to the cellular LDL receptor (K_d ~23 nM in a homologous experimental system in vitro; S. Goulinet, unpublished studies). Interestingly however, and despite the high degree of structural homology between the hamster and human LDL receptors, the respective receptors appear to recognize different domains on the surface of the homologous B-100 protein (49).

Dot immunobinding analyses showed apoA-I to be present together with apoB-100 and apoE as a component of subfractions 9–13 (d 1.039–1.074 g/ml), a finding confirmed by the identification of apoA-I as a prominent constituent of the protein moieties of such subfractions upon SDS-PAGE and Western blotting. Indeed, apoA-I was a major protein component of subfractions 11 and 12 (d 1.050–1.065 g/ml), and predominated over apoB in subfraction 13 (1.065–1.074 g/ml). At least two possibilities could account for these observations. First, apoB and apoA-I could occur on separate particle subspecies within the same subfraction, or could occur on the same particles, or a combination of these two possibilities. Our analyses by electrophoresis in agarose gel and in nondenaturing polyacrylamide gradient gels are most readily compatible with the former hypothesis. Furthermore, unpublished studies in our laboratory (S. Goulinet and M. J. Chapman) involving use of a Concavalin A affinity column have shown that apoA-I and apoB occurred on distinct lipoprotein particles possessing physicochemical characteristics akin to those of HDL and LDL, respectively. By contrast, apoE was present in both apoA-I- and apoB-containing particles. Clearly then, the distribution of dense apoB-containing particle subspecies overlapped that of apoA-I-containing, HDL_1-like particles in the density interval from ~1.039–1.074 g/ml. Moreover, it is noteworthy that the size of HDL_1 particles decreased (from ~175–210 Å at d ~1.040 g/ml) to attain diameters (~112–120 Å at d ~1.074 g/ml) resembling those of light HDL (~100–112 Å). In this context then, the hamster resembles other rodent species such as rat and mouse, in which significant overlaps of apoB- and apoA-I-containing particles occur in the density range ~1.039–1.074 g/ml, but is distinct from the guinea pig (19, 25).

Nondenaturing polyacrylamide gel electrophoresis revealed a minor apoB-containing particle subspecies of ~300 Å diameter over the density interval 1.020–1.050 g/ml. Its invariable size despite increase in density suggested that this particle might represent the hamster counterpart of Lp[a]. On the contrary, results of dot blot and Western blots using a polyclonal antibody to human apo[a] proved negative.

With the exception of trace amounts of apoB-containing particles in fraction 13 of d 1.065–1.074 g/ml, apoA-I-containing particle subspecies with physicochemical properties typical of HDL were exclusively present over the density interval from 1.065 to 1.172 g/ml. Indeed, HDL were the major lipoprotein class of hamster plasma (283 mg/dl; ~53% of total d < 1.172 g/ml substances), situating this species as a member of the "HDL mammals"

(19). Application of the density limits of HDL_2 (d 1.065–1.103 g/ml) showed light HDL to represent the major subclass (66% of total HDL). Both light and heavy hamster HDL subclasses were, however, enriched in cholesteryl esters, exhibiting elevated ratios of both cholesteryl ester:free cholesterol (7–9:1) and of cholesteryl ester:triglyceride (up to ~50:1). It is noteworthy that marked particle size heterogeneity was revealed in apoA-I-containing subspecies, and more specifically, that two size species of diameter ~100 and 94 Å, respectively, occurred simultaneously over the range d 1.092–1.172 g/ml corresponding mainly to the heavy HDL subpopulation. Whether such size heterogeneity may in part be accounted for by distinct particle contents of the minor apolipoproteins of M_r 49,000, 38,000 (apoE), 18,000, and 12,000–13,000 (apoCs) remains indeterminate. The dense HDL subclass may also be distinct in containing a counterpart (M_r ~49,000) to the human A-IV protein (M_r 46,000). Moreover the M_r ~18,000 polypeptide that we detected, which apparently possesses no human counterpart to date, may correspond to the "PX" polypeptide of M_r 19,500 identified recently in the rat by Blatter et al. (50).

Considered overall then, the density distribution of hamster apoA-I appears analogous to that in humans, this apolipoprotein being essentially absent from the apoB-containing lipoproteins, with the exception of those rich in triglyceride (d < 1.018 g/ml). The intestinal origin of a B-48-containing subpopulation of these lipoproteins (23), considered together with the presence of apoA-I in human chylomicrons, provides at least a partial explanation for this finding.

On a comparative basis, the hamster lipoproteins are primarily distinguished from their human counterparts on the basis of hydrophobic core lipid content. Thus, both hamster VLDL and LDL are cholesteryl ester-deficient and triglyceride-rich as compared to the human particles (26), whereas hamster HDL subspecies are cholesteryl ester-rich and triglyceride-poor (Table 4). Moreover, the light, cholesteryl ester-rich HDL subpopulation (HDL_L, equivalent to human HDL_2) predominated. Indeed, the chemical compositions of the hamster HDL subclasses are similar to those seen in human subjects presenting a genetic deficiency of the cholesteryl ester transfer protein (CETP) (51). It is therefore no surprise that a substantially diminished level of CETP activity has been detected in *M. auratus* (~15% of that in humans) (52). The hamster thus forms part of a group of mammals including rat, sheep, cow, pig, and dog in which neutral lipid transfer activity is less than a quarter of that in humans (53). These findings raise the question as to the nature of the principal pathways by which the hamster effects reverse cholesterol transport, i.e., from peripheral tissues to the liver. In this context, it will be of interest to evaluate the role of selective hepatic uptake of cholesteryl ester from HDL in this species, particularly as this process represents the principal mechanism in the rat for hepatic cholesteryl ester uptake from HDL (54, 55).

In conclusion, the present studies reinforce earlier proposals that *M. auratus* may represent a useful model for study of the synthesis, intravascular metabolism, and tissue catabolism of plasma lipoprotein subspecies, and equally of both nutritional and pharmacological factors that may modulate these processes.

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