Density distribution and physicochemical properties of plasma lipoproteins and apolipoproteins in the goose, *Anser anser*, a potential model of liver steatosis

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Abstract The fractionation and physicochemical characterization of the complex molecular components composing the plasma lipoprotein spectrum in the goose, a potential model of liver steatosis, are described. Twenty lipoprotein subfractions (d < 1.222 g/ml) were separated by isopycnic density gradient ultracentrifugation, and characterized according to their chemical composition, particle size and particle heterogeneity, electrophoretic mobility, and apolipoprotein content. Analytical ultracentrifugal analyses showed high density lipoproteins (HDL) to predominate (~ 450 mg/dl plasma), the peak of its distribution occurring at d ~ 1.090 g/ml ($F_{1.21}$ ~ 2.5). The HDL class displayed marked density heterogeneity, HDL_1-like particles being detected up to a lower density limit of ~ 1.020 g/ml, particle size decreasing progressively from 17–19 nm at d 1.024–1.028 g/ml to 10.5–12 nm (d 1.055–1.065 g/ml), and then remaining constant (~ 9 nm) at densities greater than 1.065 g/ml. HDL subfractions displayed multiple size species; five subspecies were present over the range d 1.103–1.183 g/ml with diameters of 10.5, 9.9, 9.0, 8.2, and 7.5 nm, four in the range d 1.090–1.103 g/ml (diameters 10.5, 9.9, 9.0, and 8.2 nm) and three over the range d 1.076–1.090 g/ml (diameters 10.5, 9.9, and 9.0 nm). ApoA-I (M_r 25,000–27,000) was the major apolipoprotein in all goose HDL subfractions, while the minor components (apparent M_r 100,000, 91,000, 64,000, 58,000, ~ 42,000, 18,000 and apoC-like proteins) showed marked quantitative and qualitative variation across this density range (i.e., 1.055–1.165 g/ml). The d 1.063 g/ml boundary for separation of goose low density lipoproteins (LDL) from HDL was inappropriate, since HDL-like particles were present in the density interval 1.024–1.063 g/ml, while particles enriched in apoB (M_r ~ 540,000) and resembling LDL in size (~ 20.5 nm) were detected up to a density of ~ 1.076 g/ml. Goose LDL itself was a major component of the profile (90–172 mg/dl) with a single peak of high flotation rate (S_f ~ 10.5). The physicochemical properties and apolipoprotein content of intermediate density lipoproteins (IDL) and LDL varied but little over the range d 1.013–1.040 g/ml, presenting as two particle species (diameters 20.5 and 21 nm) of essentially constant chemical composition; LDL (d 1.019–1.040 g/ml) were separated from HDL_1 by gel filtration chromatography and appeared to contain primarily apoB with lesser amounts of apoA-I. Very low density lipoproteins (VLDL) (d < 1.013 g/ml), a minor component (< 30 mg/dl) in our fasted, immature male geese, were enriched in triglyceride (~ 44%) and apoB, and ranged in size from ~ 19 to 64 nm (mean diam. 37.9 nm). At least 4 minor apolipoproteins of M_r ~ 100,000 or less were detected in VLDL and LDL, and up to 12 in HDL; their identities remain to be elucidated. Indeed, the polymorphism of certain acidic, low M_r apolipoproteins which we have presently documented may be of particular relevance to the susceptibility of the goose to liver steatosis. — **Hermier, D., P. Forgez, P. M. Laplaud, and M. J. Chapman.** Density distribution and physicochemical properties of plasma lipoproteins and apolipoproteins in the goose, *Anser anser*, a potential model of liver steatosis. *J. Lipid Res.* 1988. **29:** 893–907.

Supplementary key words isopycnic density gradient ultracentrifugation • analytical ultracentrifugation • gradient gel electrophoresis • chemical composition • isoelectric point

Fatty liver is characterized by an elevated content of fat in the liver, fat exceeding 5% of liver wet weight (2). Fatty liver occurs widely in both man and animal species, representing a response of the organism to various forms of acquired or inherited metabolic disorders. When acquired, the etiology of fatty liver may be primarily toxic, nutritional, or infectious. Histologically, fatty liver may be associated with cell degenerative or inflammatory processes (2).

Fatty liver occurs commonly in avian species, and especially in the laying female, in which hepatic steatosis is relat-

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; IDL, intermediate density lipoproteins; HDL, high density lipoproteins; VHDL, very high density lipoproteins; S_f, flotation coefficient in an NaCl medium of density 1.063 g/ml; F, flotation coefficient in an NaCl–KBr medium of density 1.21 g/ml; EDTA, ethylenediamine tetraacetic acid, sodium salt; SDS, sodium dodecyl sulfate; M_r, apparent molecular weight. The centile nomenclature for the B proteins has been adopted (1).

ed to modifications in lipid metabolism associated with vitellogenesis during egg formation (3, 4). Another type of fatty liver is induced in certain strains of geese or ducks that have been force-fed for production of "foie gras." This "foie gras" represents a quasi-pure form of acquired liver steatosis of nutritional origin, since degenerative events such as necrosis or cirrhosis occur rarely. Force-feeding with a diet enriched in carbohydrate (as maize) for 2–3 weeks typically induces intense cellular metabolic activity (as indicated by total amounts of hepatic protein, phospholipid, and RNA) (5), together with increased lipemia and glycemia (6, 7), resulting in steatosis due to the specific accumulation of triglyceride by the intact parenchymal liver cell (8, 9). In geese, liver weight may increase tenfold to represent 10% of the animal's weight; there is no equivalent of this rapid and intense phenomenon in other animals, including man.

Interestingly, only specific strains of geese, such as the Landes goose, may be force-fed with resultant induction of liver steatosis. Thus, although fatty liver is an acquired disorder in the goose, there is a genetic basis for susceptibility to liver steatosis in this species, for which no metabolic explanation is presently available. Furthermore, there is a paucity of knowledge of the mechanisms leading to excessive triglyceride accumulation in the liver. One hypothesis suggests that normal lipogenesis is associated with a defect in synthesis or secretion, or both, of nascent hepatic lipoproteins. In contrast to mammals, the liver is the major site of lipogenesis in birds (10, 11); endogenous triglycerides synthesized from dietary carbohydrate are released into the circulation as VLDL. Clearly then, any defect in VLDL production will prevent hepatic triglyceride clearance and rapidly lead to steatosis.

Since only meagre data are available on the lipid transport system in the goose *Anser anser* (12), detailed study of the plasma lipoprotein profile is a prerequisite to further investigation of the biochemical mechanisms leading to steatosis in this model. We presently describe the density profile and physicochemical characteristics of the lipoproteins and their apolipoproteins in immature male geese of the Landes strain, following their subfractionation by a single step, gradient density ultracentrifugal procedure (13).

MATERIALS AND METHODS

Animals and diets

Young male geese of the Landes strain and destined for production of fatty liver were bred under natural conditions of light and temperature at the Experimental Station for Goose Breeding, I.N.R.A., Artiguères, France. The birds were fed ad libitum a standard diet consisting mainly of maize and wheat and containing 13% protein, 2.8% fat, and 2600 kcal of metabolizable energy per kg.

Blood samples

At 22 weeks of age, 12 animals were fasted overnight for 18 hr, with water provided ad libitum. Blood (20 ml) was then withdrawn by puncture of the occipital venous sinus, collected on 0.12% (w/v) EDTA in a vacuum tube and kept at 2–4°C during the subsequent procedures. Individual plasmas were separated by low speed centrifugation. Antibacterial agents (sodium azide and sodium merthiolate, at final concentrations of 0.01% and 0.001% (w/v), respectively) and a chelator of metal cations (EDTA, 0.04%, w/v) were then added to each plasma sample.

Lipoprotein isolation

Lipoproteins were fractionated on the basis of their hydrated density by the isopycnic ultracentrifugal density gradient procedure described for human serum lipoproteins by Chapman et al. (13) and modified by Hermier, Forgez, and Chapman (14). Ultracentrifugation was performed in a Beckman SW41 rotor of a Beckman L8-55 ultracentrifuge at 40,000 rpm (56×10^7 *g*-min) for 48 hr at 15°C, using no brake at the end of the run. On completion of centrifugation, 20 fractions of 0.5 ml were collected successively from the meniscus of each tube by aspiration with a narrow-bore Pasteur pipette; the bottom fraction, essentially devoid of lipoproteins, was removed in a volume of ~ 2 ml. Our gradient fractionation did not take into account the position of visible lipoprotein bands, but their positions were noted relative to the number of the various fractions. Lipoprotein fractions were exhaustively dialyzed in Spectrapor tubing (Spectrum Medical Industries, Los Angeles, CA; mol wt cut-off 12,000) for 48 hr at 4°C against either a solution containing 0.05 M NaCl, 0.005 M NH_4HCO_3, 0.04% EDTA, and 0.01% sodium azide at pH 7.4, or against a solution of 0.01 M NH_4HCO_3 when samples were destined for electrophoretic analysis of their protein moieties.

Analytical ultracentrifugation

These experiments were carried out in an MSE Centriscan 75 analytical ultracentrifuge, according to Laplaud, Beaubatie, and Maurel (15). Briefly, plasma lipoproteins were first isolated by flotation in the preparative ultracentrifuge, either at a solvent density of 1.063 g/ml or of 1.21 g/ml. The former samples (i.e., d < 1.063 g/ml lipoproteins) were used for ultracentrifugal analysis of the spectrum of VLDL (S_f 20–100 and S_f 100–400) and LDL (S_f 0–12 and S_f 12–20), while the HDL profile was determined on lipoproteins obtained at d 1.21 g/ml (i.e., d < 1.21 g/ml). Quantification of the different lipoprotein classes was based on preliminary calibration of the Centriscan 75 using appropriate solutions of bovine serum albumin.

Gel filtration chromatography

Low density lipoproteins were initially isolated by sequential flotational ultracentrifugation (16) in the density interval 1.019–1.040 g/ml (see Results for justification of these density limits) from pooled goose plasma (~ 160 ml). After dialysis, this fraction (15 mg of protein) was applied to an agarose column of Bio-Gel A-15m (Bio-Rad, Richmond, CA) (dimensions 150 × 1.5 cm) and eluted at a flow rate of ~ 10 ml/hr with a buffer whose composition was the same as that of the dialysis solution (see above). This chromatographic step was performed with the aim of separating the subpopulations of particles constituting the d 1.019–1.040 g/ml fraction (see Results of nondenaturing polyacrylamide gradient gel electrophoresis). The elution position of column fractions containing lipoproteins was determined by continuous monitoring of the eluate at 280 nm.

Characterization of plasma lipoprotein fractions

Whole plasma and lipoprotein fractions were characterized by electrophoretic, chemical, and morphological analyses. The protein moieties of lipoprotein subfractions were defined according to the charge, molecular weight, and isoelectric point of their constituent apolipoproteins.

Electrophoresis

Agarose gel. Samples (5 μl) of whole plasma and lipoprotein fractions were electrophoresed on agarose gel slabs (Corning, Palo Alto, CA) using the Corning ACI system, essentially as described by Noble (17). Lipoprotein bands were stained for lipid with Oil Red O.

Gradient gels. Lipoprotein fractions were electrophoresed in polyacrylamide gradient gels made up to contain continuous gradients from 4–30% (PAA 4/30; Pharmacia Fine Chemicals, Uppsala, Sweden); the GE-2/4 LS gel electrophoresis apparatus was used in accordance with the procedure of Nichols, Blanche, and Gong (18). Gels were stained with Coomassie Brilliant Blue R250. 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; high molecular weight calibration kit). These standards included thyroglobulin (Stokes diameter 17 nm), ferritin (12.2 nm), catalase (10.4 nm), and lactic dehydrogenase (8.1 nm). Stokes diameters were calculated using the Stokes-Einstein equation (19). The correlation coefficient for the regression line of the relationship between the logarithm of the diameter of the calibration proteins and their migration distance was typically > -0.96.

Morphological analysis

Samples of goose VLDL (d < 1.013 g/ml; gradient fraction 1) were negatively stained with 2% potassium phosphotungstate at pH 7.4 on Formvar carbon-coated grids, and examined at 60 KV with a Philips EM 300 electron microscope (20). The procedures employed for microscope calibration and the calculation of particle sizes were as outlined earlier (20).

Chemical analysis

The following components were quantified in each fraction and in whole plasma as follows: protein by the method of Lowry et al. (21) using bovine serum albumin (Sigma) as the working standard; free cholesterol, total cholesterol, and phospholipids by enzymatic methods (22, 23), using the kits provided by Bio-Merieux (Charbonnière-les-Bains, France), and triglyceride by the colorimetric method of Biggs, Erickson, and Moorehead (24) using triolein (Sigma) as a standard. The amount of cholesteryl ester was calculated using the formula:

$$\text{cholesteryl ester} = (\text{total cholesterol} - \text{free cholesterol}) \times 1.67.$$

In this calculation, it was assumed that the factor for the ratio of the average molecular weight of goose plasma cholesteryl esters to that of the molecular weight of free cholesterol (i.e., 1.67), was the same in man and the goose. 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 3.8%, cholesterol 1.2%, phospholipid 2.0%, and protein 5.7%.

Analysis of protein moieties

Delipidation. Lipoprotein fractions were first lyophilized and then delipidated at −20°C with a mixture of ethanol–diethyl ether (peroxide-free, Merck) 3:1 (v/v) as described by Brown, Levy, and Fredrickson (25).

Molecular weight estimation. In order to evaluate simultaneously the molecular weights of apolipoproteins with masses in the range of 10^4 to 10^6 daltons, the protein moiety of each lipoprotein gradient subfraction was examined in an SDS-polyacrylamide gradient gel system constructed essentially according to the procedure of Irwin et al. (26), using a dual vertical slab electrophoresis cell (Hoeffer Instruments, San Francisco, CA). Slabs with a thickness of 1.5 mm and a length of 28 cm were constructed with a peristaltic pump (Gilson Minipuls II) to give a continuous nonlinear gradient from 3 to 25% acrylamide; more precisely, one volume of light solution (3% acrylamide monomer) was pumped into the heavy solution (25% monomer) while one volume was delivered to the slab. After running gel construction, a stacking gel was added in 3% acrylamide and Tris–glycine buffer (pH 6.8) to a height of 3 cm.

Samples (100 μg of protein) of each subfraction were dissolved in a solution containing 0.5% SDS, 1% β-mercaptoethanol and 10 mM Tris-HCl, and incubated for 2 hr at 37°C and applied to individual sample wells. Electrophoresis was then performed at 15–17 mA/slab for 20 hr and at 15°C. On completion of electrophoresis, gels were stained with Coomassie Brilliant Blue R250 (27). Two calibration curves were constructed from a series of polymerized molecular weight markers ranging in mass from 56,000 to 280,000 daltons, and from 14,300 to 71,500 daltons (BDH Biochemicals, Poole, U.K.). Proteins with a molecular weight above 80,000 were compared to a standard curve constructed with the high molecular weight markers, and proteins with M_r below this limit were evaluated with the other standard curve. In both cases, the correlation coefficients for these curves were typically greater than −0.99.

Isoelectric point estimation and mobility in alkaline-urea gels. The urea-soluble apolipoproteins of gradient subfractions were examined by electrophoresis in the alkaline polyacrylamide disc gel system of Davis (28) as modified by Kane (29). Gels were fixed, stained, and destained as described elsewhere (30). Finally, analytical isoelectric focusing was performed on the urea-soluble apolipoproteins of each lipoprotein fraction according to the procedure of Pagnan et al. (31), using polyacrylamide gels (7.5% monomer) containing 6 M urea and 2% ampholine (LKB) to give a pH range of 4 to 6. Samples containing up to 200 μg of protein were loaded onto each gel, except for LDL subfractions 5–7, for which 300 μg were loaded. Fractions 2 & 3 and 8 & 9 were pooled inasmuch as insufficient material was available in the individual fractions. Details of the procedure have been outlined earlier (14); gels were stained by the same procedure as that described above for the SDS gels (27).

RESULTS

Lipid and lipoprotein content of whole plasma

The mean concentrations of the major lipids of goose plasma were: free cholesterol 29 ± 4, cholesteryl ester 177 ± 20, triglyceride 81 ± 22, and phospholipid 198 ± 21 mg/dl, respectively. Total plasma cholesterol levels were 134 ± 15 mg/dl. Electrophoresis of goose plasma on agarose gel slabs separated the constituent lipoproteins essentially on the basis of their net negative charge and particle diameter, and revealed a pattern of three bands closely resembling that typical of human plasma, i.e., a diffuse α-migrating band, a narrow, faintly stained band of pre-β-mobility, and a third diffuse band of β-mobility (data not shown). The identification of these bands as goose HDL, VLDL, and LDL, respectively, was confirmed upon electrophoresis of isolated density gradient fractions under the same conditions (see below and Table 2).

Quantification and characterization of plasma lipoproteins

Analytical ultracentrifugation. Despite the possibility that the density limits and flotation properties of goose lipoproteins may be quite distinct from those of their human counterparts, we initially evaluated the qualitative and quantitative aspects of the goose lipoprotein profile by analytical ultracentrifugation. This procedure necessarily implies the application of hydrodynamic criteria originally derived from human lipoproteins (32).

The lipoprotein profile in two geese was analyzed using this technique. The results are presented in **Table 1,** while **Fig. 1** is representative of the pattern obtained in two animals. With regard to profiles obtained at d 1.063 g/ml, our data show that VLDL of S_f 20–400 were present at only moderate levels, i.e., 20.7 and 47.9 mg/100 ml, respectively, in both geese. Larger VLDL (S_f 100–400) accounted for the difference observed between these two values, being present in trace amounts in one animal and at a concentration of 25.2 mg/dl in the other. In the two geese studied, the density distribution of LDL (S_f 0–20) was essentially composed of particles with S_f in the 4.5–5.0 to 20.0 range. This resulted in elevated peak S_f values, i.e., 10.3 and 10.6, respectively, and in relatively high proportions of IDL (S_f 12–20), these latter particles accounting for 34% and 20% of total LDL, respectively. The plasma concentration of LDL amounted to 90.1 mg/100 ml in one goose and 172.3 mg/100 ml in the other.

Analytical ultracentrifugal profiles obtained at a density of 1.21 g/ml allowed the determination of the HDL distribution in our birds. This distribution took the form of an almost symmetrical peak, with peak flotation rates of F 2.5 and 2.2, respectively; the lower density limit of the distribution was approximately F 9.0 in both geese. HDL concentrations were elevated, amounting to 480.9 and 553.4 mg/dl, respectively, while the value of the ratio of the concentrations of the lighter (F 3.5–9.0) to heavier (F 0–3.5) HDL was 0.26 in one animal and 0.43 in the other. This higher latter value was the consequence of both a higher peak F rate and a greater concentration of HDL within the F 5–9 range. HDL were clearly the major lipoproteins of goose plasma, representing 71.5–81.3% of the total d < 1.21 g/ml fractions in these two birds, and LDL substantially less (15.3–22.2%).

Gradient density ultracentrifugation. The density limits of the successive fractions of 0.5 ml collected over the density gradient (except for the bottom fraction of ~ 2 ml) are taken from the profile established by Hermier et al. (14) for control NaCl/KBr gradients, and are shown in **Table 2.** Chemical analyses (lipid and protein components; Table 2) of individual lipoprotein subfractions allowed construction of a profile of the quantitative distribution of goose lipoproteins over the density range from ~ 1.010 to 1.210 g/ml **(Fig. 2).** Only minor differences in profile were

TABLE 1. Concentrations of goose plasma lipoproteins determined by analytical ultracentrifugation[a]

	S_f 1.063 Range				$F_{1.21}$ Range		
	400–100	100–20	20–12	12–0	9–3.5	3.5–0	Total
				mg/dl			
Goose #961	traces	20.7	30.5	59.6	98.6	382.3	591.7
Goose #967	25.2	22.7	35.3	137.0	166.2	387.2	773.6

[a]Concentrations were determined by analytical ultracentrifugation at a solvent density of 1.063 g/ml for lipoproteins of S_f 0–400 and at 1.21 g/ml for lipoproteins of $F_{1.21}$ 0–9, according to the conditions of Laplaud, Beaubatie and Maurel (15).

observed in the 12 animals studied, and these primarily concerned a slight displacement in the peak density of the major class (corresponding to high density lipoproteins) and minor variations in the absolute concentrations and relative proportions of the various subfractions comprising both the low density and high density peaks (see Table 2).

Minima occurred in the profile at densities of ~ 1.015 and ~ 1.055 g/ml respectively (Fig. 2), and corresponded to zones at which no colored bands were visible in the density gradient tube. The first well-defined peak occurred in the low density range (~ 1.020–1.040 g/ml) with a maximal concentration at ~ 1.030 g/ml, while the second and major peak occurred in the high density range (~ d 1.055–1.165 g/ml) at ~ d 1.090 g/ml. The profiles of both the low density and high density peaks were essentially symmetric. No distinct peaks of VLDL or IDL were detectable in these fasted animals.

Chemical composition

The percentage weight chemical compositions of the successive lipoprotein subfractions from the density gradient are shown in Table 2, and revealed increasing proportions of protein with diminution in lipid content as density increased. Fraction 1, of d < 1.013 g/ml, was rich in triglyceride (40–45%) and of pre-β mobility, suggesting its correspondence to VLDL; present at low levels in fasted birds, VLDL represented only ~ 7% of the total d < 1.205 g/ml lipoproteins. Intermediate density lipoproteins (d 1.013–1.020 g/ml, fractions 2 and 3) were similarly minor components of the goose lipoprotein spectrum, amounting to ~ 5% of the total. Chemically, they were distinct from VLDL in their elevated contents of cholesteryl ester (30–33%) and protein (~ 24%), and diminished proportion of triglyceride (~ 14%). Indeed, subfractions with hydrated density in the range classically attributed to low density lipoproteins (1.020–1.040 g/ml), i.e., fractions 4–7, appeared to represent a continuum with IDL, differing but little in their chemical compositions from the latter; thus their major components were cholesteryl esters (30–33%). These particles exhibited β-mobility and represented ~ 15% of the total d < 1.205 g/ml lipoproteins. Subfractions of d 1.040–1.055 g/ml (no. 8 and 9) occurred in minor amounts (< 3% of total lipoproteins) in a region in which lipoproteins of β- and of α-mobility overlapped in distribution (Table 2); their compositions were intermediate between those of the low and high density components.

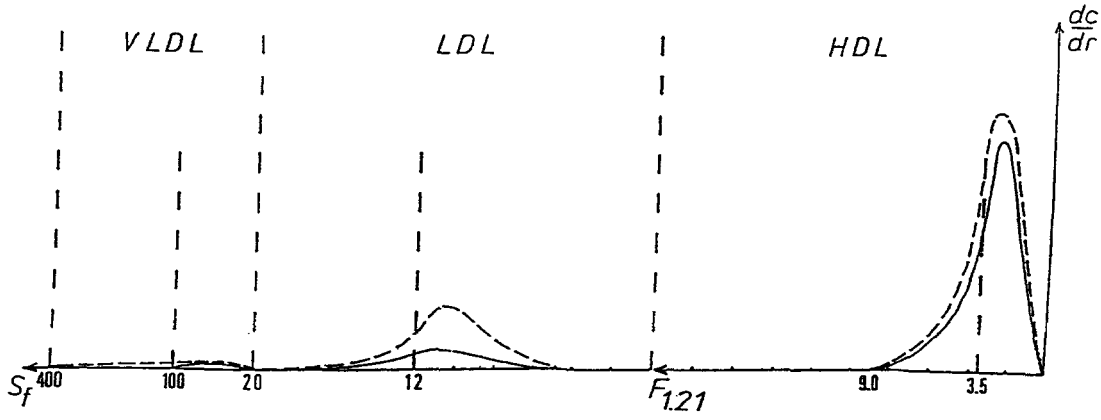

Fig. 1. Analytical ultracentrifugation pattern of goose plasma lipoproteins. VLDL and LDL were analyzed at a density of 1.063 g/ml and HDL at a density of 1.21 g/ml as described in Materials and Methods. Vertical dotted lines indicate the limits between the different density classes and subclasses as conventionally defined in human plasma. The profile represented by a solid line relates to lipoproteins from goose 961, while the dotted line refers to goose 967 (see Results, analytical ultracentrifugation).

TABLE 2. Density distribution, chemical composition and electrophoretic mobilities of

Component[b]	Density Gradient 1	2	3	4	5	6	7	8	9
		(Density Limits,							
	<1.013	1.013 -1.016	1.016 -1.020	1.020 -1.024	1.024 -1.028	1.028 -1.034	1.034 -1.040	1.040 -1.048	1.048 -1.055
									mean
Free cholesterol	7.0 ± 0.6	10.6 ± 0.2	10.4 ± 0.3	10.1 ± 0.4	9.7 ± 0.4	9.4 ± 0.6	9.9 ± 0.4	10.2 ± 0.6	10.2 ± 0.6
Cholesteryl ester	19.9 ± 0.9	35.2 ± 2.1	35.5 ± 1.3	35.5 ± 1.4	34.3 ± 1.7	34.1 ± 1.3	33.9 ± 1.6	29.8 ± 4.2	27.9 ± 2.6
Triglyceride	44.0 ± 2.3	20.9 ± 3.2	21.3 ± 2.5	13.9 ± 1.0	11.7 ± 1.2	11.2 ± 1.1	11.4 ± 1.2	12.0 ± 0.9	10.7 ± 1.0
Phospholipid	17.2 ± 0.8	15.6 ± 3.1	17.3 ± 1.1	17.1 ± 1.4	19.1 ± 0.6	19.6 ± 0.9	18.7 ± 1.4	20.0 ± 3.3	24.2 ± 2.6
Protein	12.0 ± 1.1	21.7 ± 1.1	20.6 ± 1.0	23.5 ± 0.7	25.3 ± 1.0	25.7 ± 1.4	26.2 ± 0.6	25.2 ± 0.7	27.0 ± 1.3
Electrophoretic mobility[d]	pre-β	β	β	β	β	β	(α) β	β α	(β) α
Plasma lipoprotein[c] (mg/dl)	30 ± 3	36 ± 2		92 ± 6				28 ± 1	

[a]Fractions were 0.5 ml.
[b]Values are the means ± SEM of duplicate analyses of each fraction isolated from plasma of 12 geese; n.d., not detectable.
[c]Density limits were taken from a standard curve of density versus volume derived from control gradient containing only salt solutions (14).
[d]Electrophoretic mobility in agarose gel slabs was compared to that of corresponding fractions of human serum. Trace amounts are indicated by parentheses.
[e]The values for the concentrations of each lipoprotein fraction or series of fractions represent the sum of the individual components determined chemically, and are the mean ± SEM of duplicate analyses of each fraction isolated from the individual plasmas of 12 geese.

Fractions 10 to 17 (d 1.055–1.165 g/ml) approximated the range of hydrated densities attributed to HDL in man (i.e., d 1.070–1.150 g/ml) (13, 33, 34), and indeed their chemical compositions and electrophoretic mobility confirmed their close correspondence. Thus, their protein contents ranged from ~ 34 to 50%, and their cholesteryl ester, triglyceride, and phospholipid contents from ~ 22 to 13%, 6 to 2%, and 32 to 22%, respectively; the proportion of free cholesterol varied little across this same range. All subfractions exhibited α-mobility. In confirmation of data obtained in analytical ultracentrifugal analyses, high density lipoproteins dominated the lipoprotein profile, accounting for up to 70% of the total substances of d < 1.205 g/ml.

Very high density lipoproteins (d 1.165–1.205 g/ml; fractions 18 and 19) were a trace component in the goose, amounting to only ~ 3% of the total plasma pool; they were characterized by elevated protein contents (~69–78%). These subfractions are intermediate in their physicochemical characteristics between those of human $VHDL_1$ (62–65% protein; hydrated density ~ 1.16 g/ml) and $VHDL_2$ (97–99% protein; isolated as lipoproteins of d > 1.250 g/ml) (33–35).

Particle size and particle heterogeneity

A representative pattern obtained in nondenaturing polyacrylamide gradient gels (4–30%) upon electrophoresis of a series of goose lipoprotein gradient subfractions is presented in **Fig. 3**; this pattern is typical of those obtained in four animals in which little qualitative variation was seen. Fraction 1 (VLDL, d < 1.013 g/ml) migrated into the gel to only a short distance (< 3 mm), and presented as a diffuse band with an apparent Stokes diameters of 22 nm. Such behavior raised some doubt as to the accuracy of size estimation under these conditions, and therefore two separate preparations of goose fraction 1 were examined by negative stain electron microscopy. By this methodology, the average particle diameter of goose VLDL was 37.9 nm, with a mode of 38.5 nm and an overall range of 19.2 to 64 nm. The frequency distribution of particle diameters gave a symmetrical profile; the morphology of the particles strongly resembled that typical of VLDL in other animal species (12), particles being essentially spherical in shape and deforming upon contact.

Fractions 2 to 10 (d 1.013–1.065 g/ml) contained a major particle species migrating as a single band with an apparently constant Stokes diameter of 20.5 nm; this same particle species was accompanied over this density range by a second, minor band of lower mobility and larger diameter (21 nm). A third component was visualized as a diffuse and faintly stained band whose diameter diminished (from ~ 19–17 nm in fraction 5 to ~ 12–10.5 nm in fraction 11) with increase in density. Indeed, the pattern of particle size species became complex in fraction 11 (d 1.065–1.076 g/ml) in which, in addition to the LDL band at 20.5 nm, three bands with HDL-like mobility were visible, with apparent diameters of ~ 10.5, 9.9, and 9.0 nm; the first of these was particularly diffuse and appeared to represent a continuation of the species of decreasing size seen in fractions 5 to 10 (Fig. 3). This transition continued into fraction 12 (d 1.076–1.090 g/ml), in which the band of diameter 9 nm

lipoprotein subfractions isolated from goose plasma by density gradient ultracentrifugation

Fraction Number[a]										
10	11	12	13	14	15	16	17	18	19	20
g/ml)[c]										
1.055 -1.065	1.065 -1.076	1.076 -1.090	1.090 -1.103	1.103 -1.120	1.120 -1.130	1.130 -1.146	1.146 -1.165	1.165 -1.183	1.183 -1.205	1.205 -1.222
weight %										
8.0 ± 0.8	6.5 ± 1.4	4.5 ± 0.5	3.7 ± 0.3	3.4 ± 0.2	3.2 ± 0.2	3.4 ± 0.3	2.8 ± 0.4	3.5 ± 0.6	2.6 ± 0.3	n.d.
25.0 ± 1.3	21.6 ± 1.3	21.0 ± 1.2	20.0 ± 1.3	19.4 ± 1.2	18.4 ± 1.3	17.2 ± 1.4	15.8 ± 1.5	10.9 ± 1.4	7.3 ± 1.0	n.d.
8.2 ± 0.7	7.4 ± 0.2	6.6 ± 0.4	6.0 ± 0.6	5.7 ± 0.5	5.9 ± 0.7	5.9 ± 0.8	4.2 ± 0.7	5.2 ± 0.5	2.1 ± 0.5	n.d.
27.7 ± 1.4	27.6 ± 0.5	27.7 ± 0.4	27.0 ± 0.2	25.9 ± 0.6	23.4 ± 0.7	19.3 ± 1.9	16.4 ± 3.3	13.8 ± 2.6	n.d.	n.d.
31.2 ± 1.2	37.5 ± 2.1	40.3 ± 1.0	43.4 ± 1.0	45.6 ± 1.1	49.1 ± 1.1	54.2 ± 2.0	60.5 ± 1.7	70.8 ± 2.7	92.7 ± 3.2	98.5 ± 2.2
α	α	α	α	α	α	α	α	α	α	α
447 ± 14								31 ± 1		

predominated over those of 9.9 and ~ 10.5 nm. Indeed, the major component of fractions 13–19 (d 1.090–1.205 g/ml) was the particle species of 9 nm diameter, accompanied by the 9.9 nm- and 10.5-nm species. A fourth band appeared as of fraction 13 (diameter 8.2 nm) and a fifth of diameter 7.5 nm as of fraction 14. Thus, the high density distribution was characterized by the presence of up to five particle size species, but whose individual diameters were constant over the density range of 1.090–1.205 g/ml.

Albumin was detected in fraction 19 (d 1.183–1.205 g/ml) in which a band was found with mobility identical to that of the bovine serum albumin used as a standard.

Characterization of the protein moieties

Molecular weight estimation. Electrophoresis of the total protein moieties of the gradient subfractions in extended SDS-polyacrylamide gradient gels **(Fig. 4)** revealed a high

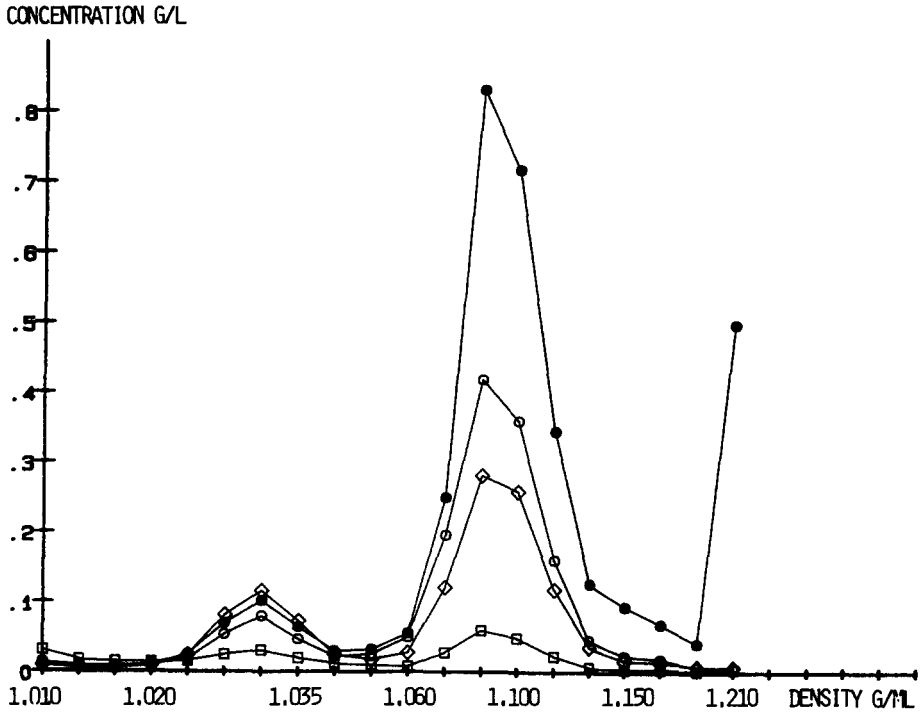

Fig. 2. Quantitative distribution of goose plasma lipoproteins as a function of density after separation by gradient density ultracentrifugation. Each curve shows the distribution of a single chemical component; (●) protein; (○) phospholipid; (◇) cholesteryl ester; (□) triglyceride. The data are taken from one goose representative of twelve.

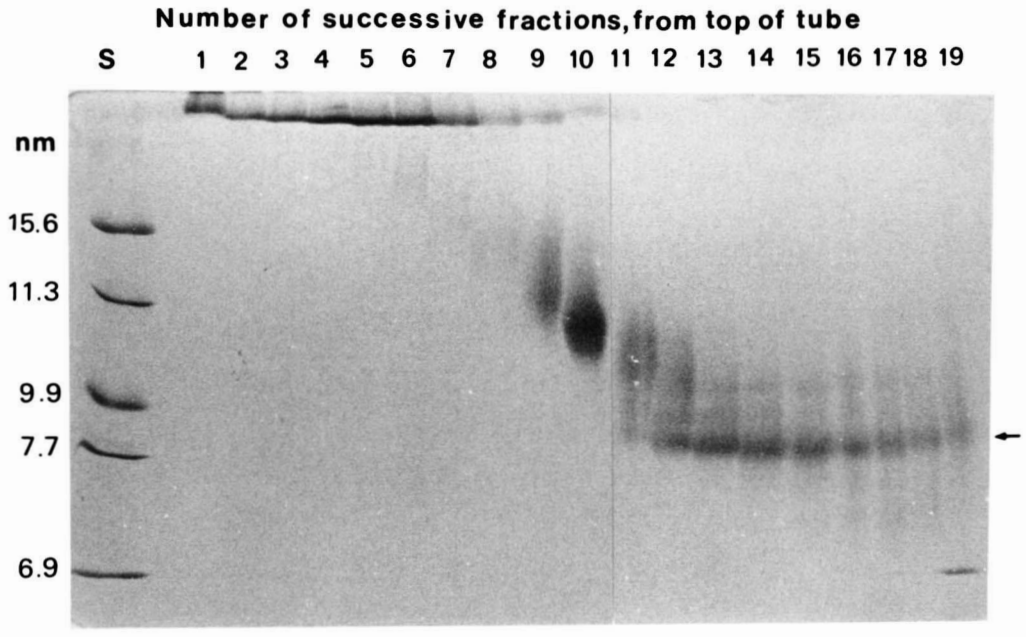

Fig. 3. Electrophoresis of native lipoprotein gradient subfractions (1-19, d < 1.205 g/ml) on polyacrylamide gel slabs containing a gradient of 4-30% acrylamide. Standard protein markers were loaded in lane S, and their respective Stokes diameters are indicated on the left. The arrow at right indicates the position of the major HDL particle size species.

molecular weight, apoB-like component (avg. M_r 540,000) to predominate over the density range from the lightest subfraction (fraction 1, d < 1.013 g/ml) to d 1.048 g/ml (fraction 8); at this latter density, substantial amounts of an apoA-I-like protein (avg. M_r ~ 26,000) appeared. Nonetheless, the apoB-like protein was strongly represented in subfractions 9 and 10 (d 1.048–1.065 g/ml), but was detectable in only trace amounts in fraction 11 (d 1.065–1.076 g/ml). Goose apoB-containing lipoproteins are therefore distributed up to a density of 1.076 g/ml in our isopycnic density gradient system.

Human apolipoprotein B-100 displayed the same electrophoretic mobility in our gel system as the goose protein of high molecular weight described above, and thus a similar apparent M_r (~ 540,000) (Fig. 4, lane A). We were, however, unable to detect a goose counterpart to the human B-48 protein.

All goose lipoprotein subfractions over the density range up to 1.189 g/ml (fraction 18) displayed the apoA-I-like protein (avg. M_r ~26,000). However, whereas this component was a minor constituent of the protein moieties of fractions 1 to 8 (i.e., d < 1.048 g/ml), it was the major apolipoprotein of fractions 9 to 18 (d 1.048–1.189 g/ml). Coelectrophoresis of the human A-I protein with goose HDL subfractions showed that it comigrated with the goose protein and thus possessed the same apparent molecular weight (data not shown).

Four components of intermediate size (apparent M_r 100,000, 91,000, 64,000, and 58,000, respectively) (Fig. 4) were barely visible in subfractions 1 to 8 (d < 1.048 g/ml), but appeared as strongly stained bands over the same density range as that over which the A-I component predominated, i.e., fractions 9 to 18 of d 1.048–1.189 g/ml. An additional apoprotein with M_r in the range of 41,000–43,000 was detected in several subfractions, and notably in fractions 1–5, 9–11, and 16–18 corresponding to density intervals of d < 1.028, 1.048–1.076, and 1.130-1.183 g/ml, respectively.

Subfractions with densities in the range of very low and low density lipoproteins (no. 1–8, d < 1.048 g/ml) were distinguished by the presence of an apoprotein (M_r 25,000) migrating immediately in front of the A-I component (Fig. 4); the proportion of this polypeptide decreased with increase in density, to become a trace component in fractions 7 and 8. Furthermore, subfractions occurring within the density ranges of very low and low density lipoproteins (i.e., 1–9, d < 1.055 g/ml) were also distinct in possessing a single size component of low molecular weight (M_r 18,000); this same component was present in trace amounts in fractions 10–13 (d 1.055–1.103 g/ml), and appeared to increase in fractions 14–18 (d 1.103–1.183 g/ml). Equally, a diffusely stained band with M_r ~ 15,000 appeared in fraction 10 (d 1.055–1.065 g/ml), becoming a prominent component in fractions 13–18 (d 1.090–1.183 g/ml); an additional band with M_r intermediate between those of 15,000 and 18,000 appeared in fractions 15–18. It is noteworthy that the

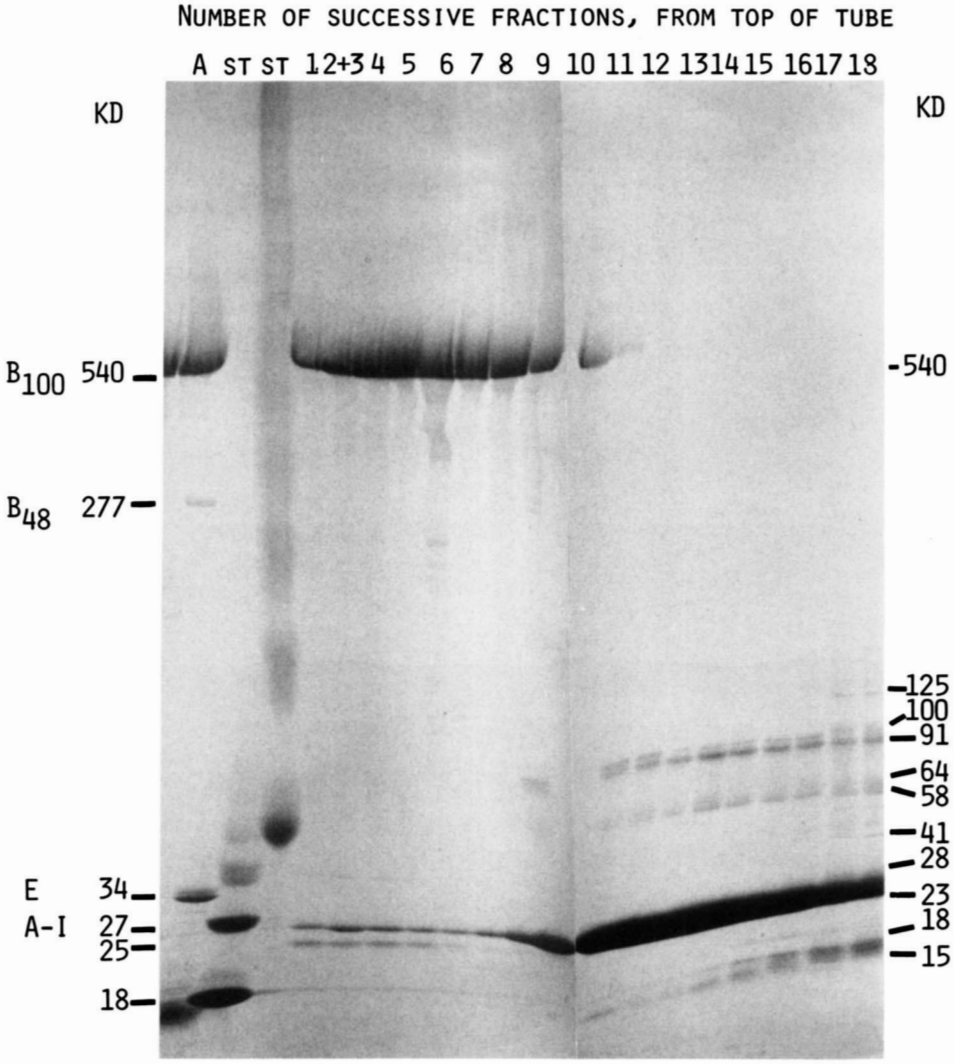

Fig. 4. Electrophoretic patterns in SDS-polyacrylamide gradient (3–25%) gel slabs of the apolipoproteins of goose lipoprotein subfractions isolated by gradient density ultracentrifugation. Samples are: lane A, human apoVLDL (100 μg of protein); lanes ST, purified standard molecular weight markers; lanes 1–18, goose lipoprotein gradient subfractions (100 μg of protein). Note that subfractions 2 and 3 were pooled in view of their low protein contents. Gels were stained with Coomassie Brilliant Blue R250. On the left hand side of the figure are indicated the molecular weights and identities of human apoproteins; at right are indicated the M_r values for the goose proteins. The density ranges of individual lipoprotein subfractions are provided in Table 2.

molecular size of the latter components may be overestimated, since the C apolipoproteins in a sample of human apo-VLDL (Fig. 4, lane A) migrated to a similar region (~ 25% acrylamide) of the gradient gel.

Isoelectric point estimation. The electrophoretic patterns obtained upon isoelectric focusing of the urea-soluble apoproteins of the goose lipoprotein gradient subfractions are shown in **Fig. 5** and compared to that of human apoHDL (Fig. 5, gel A). The major component in all gradient fractions focused as a sharp band of pI 5.66, and cofocused with the principal isoform of human apoA-I. The second major isoform of the human protein also appeared to possess a counterpart in the goose, with similar pI of 5.60. This latter isoform presented as a faintly stained band in fractions 1 to 6 (d < 1.034 g/ml), but became progressively more intense with increase in density; the band with pI ~ 5.55 displayed a distribution similar to that of pI 5.60, suggesting that it also might be a form of goose A-I.

In fractions 1–7 of d < 1.040 g/ml, only a single component more acidic than the apparent A-I isoforms was detectable, displaying a pI (~ 5.42) slightly more basic than that of human apoA-II (pI ~ 5.16) (36). Satellite bands of both the pI 5.55 and 5.42 components were present in the densest subfractions (no. 14–18, d 1.103–1.183 g/ml). Two prominent basic apoproteins (pI 5.83 and 5.77) also characterized the denser gradient subfractions (no. 8–18, d 1.040–1.183 g/ml), being present only as minor bands in those of d < 1.040 g/ml.

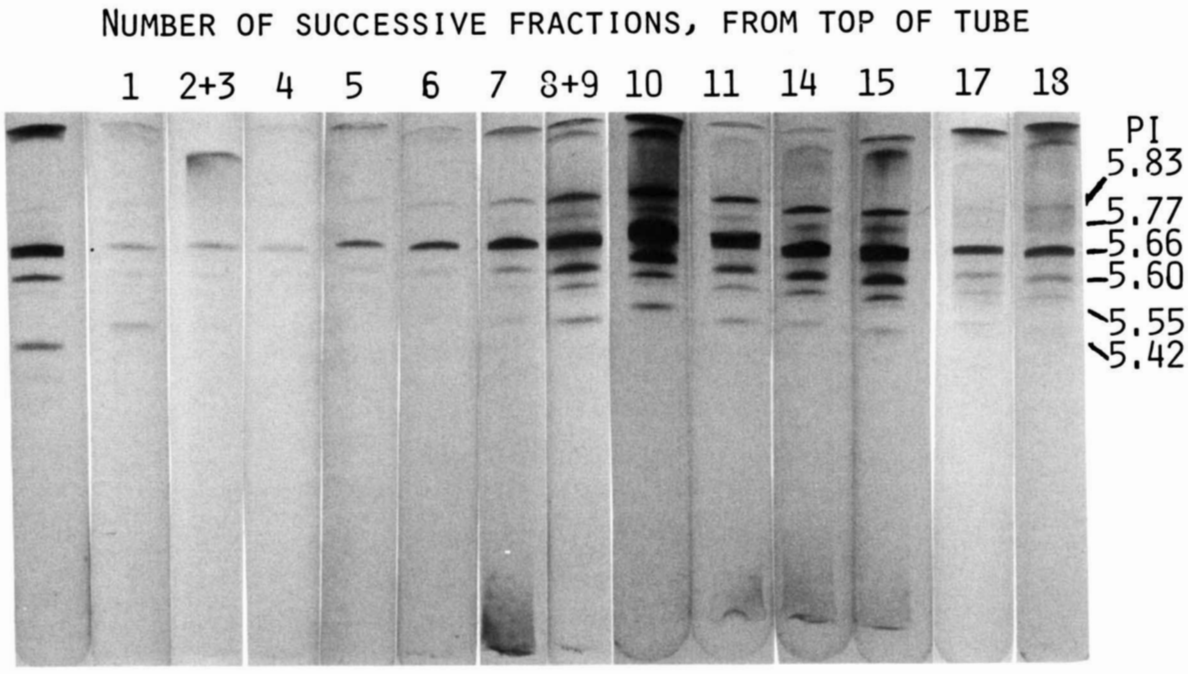

Fig. 5. Analytical isoelectric focussing patterns of the urea-soluble apolipoproteins of goose lipoprotein subfractions. Samples are: gel A, human HDL; gels 1–18, urea-solubilized apolipoproteins of individual gradient subfractions, with the exception of fractions 2 and 3 which, in view of their low protein contents, were pooled; subfractions 12, 13, and 16 were omitted as they were indistinguishable in their focussing patterns from neighboring subfractions, nos. 11, 14, and 15. The density ranges of individual subfractions are provided in Table 2. The pI values were calculated from a calibration curve constructed from a blank gel and are shown at right.

Relative mobility at alkaline pH. The relative mobilities of the urea-soluble, non-apoB apolipoproteins of the major classes of goose lipoproteins were examined in urea-containing gels at alkaline pH, and the electrophoretic patterns so obtained are presented in **Fig. 6.** The VLDL, LDL, and HDL fractions corresponded to colored bands visible on the gradient and of d < 1.013, 1.020–1.040, and 1.055–1.165 g/ml, respectively, while the IDL and F_4 fractions were intermediate in position between VLDL and LDL and between LDL and HDL, respectively, and of d 1.013–1.020 and 1.040–1.055 g/ml.

The electrophoretic pattern for human apoHDL (Fig. 6, gel A) was more complex than that in the goose fractions, displaying a maximum of approximately ten components as compared to approximately seven in the goose. While human apoA-I migrated as an intense band of R_f 0.24, the A-I-like component which predominated in goose HDL migrated with a lower R_f (0.17–0.19) (Fig. 6). The goose A-I-like protein tended to dominate the patterns of the urea-soluble apoproteins of the IDL, LDL, and F_4 fractions as well as that of HDL; it was, however, poorly represented in VLDL. Two more basic apolipoproteins were clearly identifiable as distinct bands above the A-I band, with R_f values of 0.13 and 0.15; the human A-IV protein migrates to a similar position as the latter component. Only the R_f 0.15 band was seen in the goose VLDL and IDL patterns.

Up to four bands, with R_f values in the range of 0.27 and 0.39, migrated in the region of the human C apolipoproteins; of these, only the R_f 0.31 component was detected in all major goose lipoprotein classes and, additionally, in the intermediate fractions IDL and F_4. Thus, the R_f 0.27, 0.31, 0.36, and 0.39 bands were restricted to goose F_4 and HDL; the latter three bands each represented ~ 5% of the total comprising apoHDL on a densitometric basis. Analyses of F_4 and HDL from different animals revealed the variable presence of the R_f 0.39 component. Indeed, approximately one-third of the animals possessed only the R_f 0.31 and 0.36 bands, in which case densitometric scanning showed them to represent ~ 5% and ~ 10% of total apoHDL, respectively. Finally, an additional minor component with R_f 0.44 was occasionally found in goose HDL. Thus, the proportion of the R_f 0.36 band was twofold elevated in the HDL apolipoprotein profiles from animals lacking the R_f 0.39 component.

Gel filtration chromatography. Both nondenaturing gradient gel electrophoresis of native lipoproteins with hydrated densities in the range of 1.020–1.040 g/ml and analysis of their protein moieties suggested the simultaneous occurrence of particles with the physicochemical properties of low density and of high density lipoproteins in this zone. We performed agarose gel filtration chromatography in order to resolve these two particle populations.

The major peak on the OD_{280} elution profile was resolved

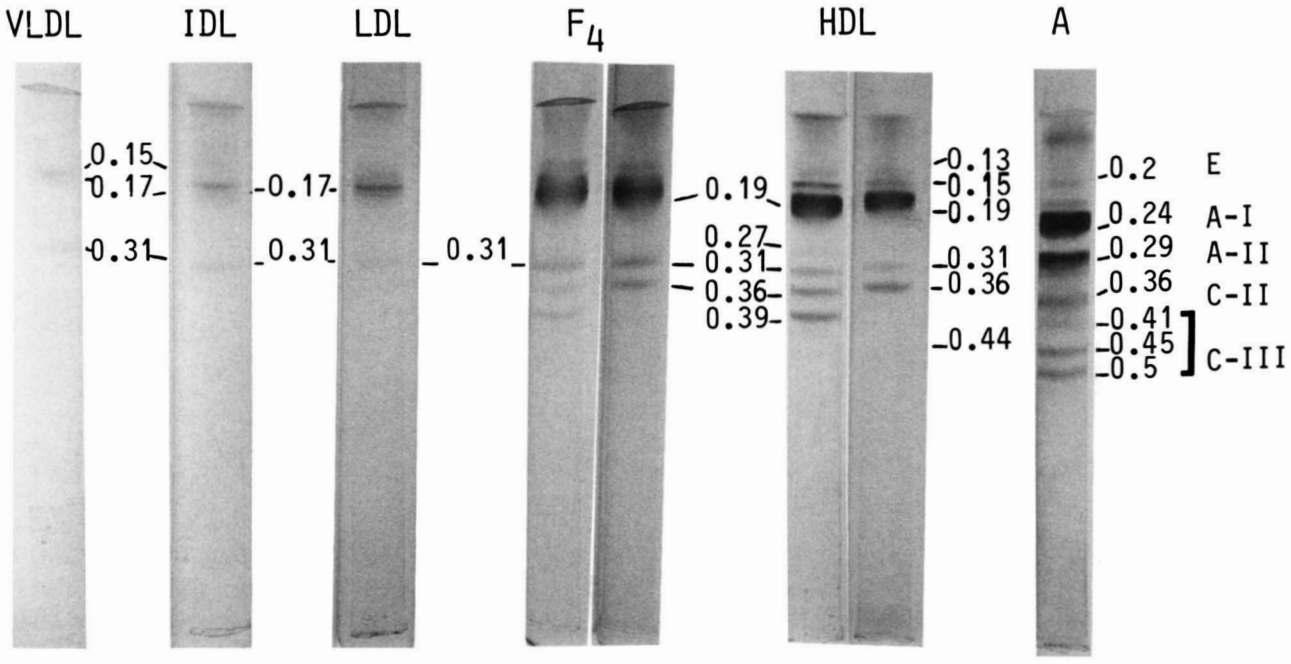

Fig. 6. Electrophoretic patterns in alkaline-urea polyacrylamide gel of urea-soluble apolipoproteins of goose lipoprotein gradient subfractions separated by density gradient ultracentrifugation. Samples (25–100 μg of protein/gel) are: VLDL, d < 1.013 g/ml; IDL, d 1.013–1.020 g/ml; LDL, d 1.020–1.040 g/ml; F_4, d 1.040–1.055 g/ml; HDL, d 1.055–1.165 g/ml and A, human apo-HDL. The R_f values of individual apolipoprotein bands are indicated on each gel and correspond to the ratio between the distance of migration of each band to the migration of the dye front. Gels were stained with Coomassie Brilliant Blue.

into ten fractions, each of which displayed a single size species of diameter 20.5 nm on gradient gel electrophoresis, thereby corresponding to LDL (data not shown). Electrophoresis of the protein moieties of these same fractions in SDS-polyacrylamide gradient gel (3–25% monomer) and of the starting material of d 1.020–1.040 g/ml revealed similar patterns; i.e., the presence of two apoprotein bands, one with M_r 540,000 and the other with M_r 27,000; these apoproteins correspond to the goose apoB-like and apoA-I-like components, respectively.

DISCUSSION

As an animal model in which to study the metabolic perturbations involved in the dietary induction of fatty liver, the goose *(Anser anser)* is perhaps unparalleled. Nonetheless, our knowledge of lipid transport and of lipoprotein homeostatis in this avian species is at most rudimentary, extending to the serum concentrations and chemical compositions of the major lipoprotein classes (VLDL, LDL, and HDL) (12, 37). Furthermore, and as seen earlier in the chicken (14), the hydrodynamic and density criteria originally developed for the analytical ultracentrifugal quantitation and flotational separation of the major lipoprotein classes in man are potentially inadequate in *Anser anser* (16, 18, 37). For these reasons, we undertook to define more accurately the quantitative and qualitative characteristics of the plasma lipoproteins and apolipoproteins in this species. The principal features of the present report concern the resolution of the goose lipoprotein spectrum into subfractions of narrow density range (Table 2) by isopycnic gradient density ultracentrifugation, the marked heterogeneity in particle species and apolipoprotein content across this profile, and the inappropriateness of the 1.063 g/ml density limit as a cutoff point for the separation of low density from high density lipoproteins.

In common with the male pigeon *(Columba sp.)*, male turkey *(Meleagris galapavo galapavo)*, and male chicken *(Gallus domesticus)* (12, 14, 38), the lipoprotein profile of the male goose was dominated by the high density class, which represented ~ 70–80% of the total d < 1.21 g/ml lipoproteins. Indeed, quantitation of goose HDL (as lipoproteins of $F_{1.21}$ 0–9) by analytical ultracentrifugation (Table 1) and by chemical analysis of lipoproteins isolated by gradient centrifugation over the density range 1.055–1.165 g/ml (Table 2) was in good agreement, revealing concentrations in the range of 481–553 mg/dl in the former instance and 447 ± 48 mg/dl in the latter. Quantitation of HDL in goose as the sum of their chemical constituents had previously (37) given a value of 761 mg/dl, a level substantially higher than that seen in our birds.

Very low density lipoproteins were minor components (~ 3–6% of total) in our fasted immature geese, plasma levels ranging from ~ 20 to 50 mg/dl; the values found by Mills and Taylaur (37) were rather higher (total S_f

20–400 lipoproteins, 108 mg/dl). The latter authors did not, however, provide information regarding the number, age, sex, or nutritional status of their bird(s), thus rendering comparison difficult. Moreover, whereas smaller VLDL of S_f 20–100 predominated over those with S_f 100–400 (five-fold less) in the earlier study (37), the prominence of such smaller VLDL was noted in one animal only, while the two VLDL subclasses accounted for equivalent amounts in the other.

Intermediate density lipoproteins (S_f 12–50, and gradient subfractions 2 and 3 of d 1.013–1.020 g/ml) were also poorly represented in our geese (30–36 mg/dl), in contrast to LDL of S_f 0–12 which constituted the major subclass of lipoproteins of S_f 0–400 (~ 54–62%), but tended to vary substantially in absolute level between individuals (~60–137 mg/dl; Table 1). Such variation was confirmed upon chemical analysis of the β-migrating, LDL gradient subfractions (no. 4–7; d 1.020–1.040 g/ml), in which average levels of 92 ± 22 mg/dl were seen in 12 animals. Markedly lower concentrations of S_f 0–12 LDL were, however, detected by Mills and Taylaur (24 mg/dl; ~ 2.5% of total d < 1.21 g/ml lipoproteins), particles of S_f 12–20 predominating (73 mg/dl) (37). Despite such discrepancies however, both the latter authors (37) and ourselves found the peak of the LDL profile to correspond to particles with lower density than those of most mammals (12, 37); thus, in our animals, the peak S_f rate was in the 10.3–10.6 range, and that reported earlier of 12.0 (37).

In their physicochemical properties, goose and chicken VLDL closely resembled each other (12, 14). Goose VLDL, of d < 1.013 g/ml, may contain a small proportion of IDL particles, thereby accounting for the high protein content (14%; Table 2). The average particle diameter of goose VLDL as determined by gradient gel electrophoresis was smaller (22 nm) than that estimated by negative stain electron microscopy (mean 37.9 nm). The origin of this discrepancy may lie in the minimal migration of VLDL into polyacrylamide gradient gels of 4–30%, since application of these two procedures to chicken VLDL (14) revealed a similar discordance in particle size.

Goose IDL (d 1.013–1.020 g/ml) were distinct from VLDL in their elevated cholesterol and diminished triglyceride contents. Indeed, the transition from VLDL to IDL in goose was more abrupt than in chicken, in which it was more gradual (14). Moreover, goose IDL appeared to form a continum with LDL not only in chemical composition, but also in size and apolipoprotein content (see below). Thus, the proportion of cholesteryl ester and phospholipid varied but little (~ 30–33% and ~ 20–21.6%, respectively) along the density interval from 1.016 to 1.040 g/ml, while triglyceride content decreased from ~ 15 to 12% and protein increased from ~ 24 to 27%. It is indeed remarkable that the two particle species which dominated this region of the goose lipoprotein spectrum maintained essentially constant sizes (20.5 nm and 21 nm) in parallel with minor changes in the proportions of both core (neutral lipid) and surface (protein and phospholipid) constituents. An explanation for these findings may lie in the structural organization and degree of hydration of the particles. It is also noteworthy that, in contrast with our present findings, LDL density subfractions in humans exhibit decreasing particle size with increase in density (39).

The protein moieties of goose VLDL, IDL, and LDL (i.e., lipoproteins of d < 1.040 g/ml) were dominated by an apoprotein with physicochemical properties akin to those of human apoB-100 (M_r 549,000; ref. 1); the identity of the goose B-100 protein was confirmed by its immunological cross-reactivity with a monospecific antiserum to chicken apoB-100 (data not shown; chicken apoB-100 antiserum was kindly provided by Dr. D. L. Williams). As in the chicken (12) and pigeon (38), a counterpart to human apoB-48 was undetectable in our fasting geese, suggesting that the liver may not synthesize this protein, in contrast to certain mammalian species such as rat and mouse (40, 41). The low density (d < 1.040 g/ml) lipoprotein subfractions also displayed equivalent proportions of an apoA-I-like protein, exhibiting M_r ~ 26,000, a major isoform with pI 5.66, and a relative mobility of 0.19 in alkaline-urea gels; this apolipoprotein was the major component of goose HDL (see below).

Of the approximately four to seven minor apolipoproteins of low molecular weight detected in goose apoVLDL, apoIDL, and apoLDL, only that of M_r ~ 25,000 was present in amounts comparable to goose apoA-I (Fig. 4). This protein was also found in the same lipoproteins in the chicken (12) and was suggested to represent apoVLDL-II. A counterpart of this component was not detected in the pigeon (38).

The M_r 41,000–45,000 component was distributed asymmetrically across both the low density and high density regions, being detectable in fractions 1 to 5 (d < 1.028 g/ml), 9 to 11 (d 1.048–1.076 g/ml), and 17 to 18 (d 1.046–1.183 g/ml). A protein exhibiting a similar M_r was also detected in both chicken (M_r 45,000) (12) and pigeon (M_r 43,000) (38). In view of its density distribution and size, this protein may represent a counterpart to the mammalian A-IV apolipoprotein (M_r 44,000–46,000) (42). If not, it appears typical of avian species.

The symmetrical distribution profile of low density lipoproteins obtained upon both analytical ultracentrifugation (Fig. 1; as S_f 0–20 lipoproteins) and upon density gradient ultracentrifugation (Fig. 2) gave no indication of their potential heterogeneity. Thus, particles with diameters characteristic of LDL (20.5 and 21 nm) were accompanied in fraction 5 (d 1.024–1.028 g/ml) by a faint, diffusely stained band (Fig. 3) representing HDL-like particles of 17–19 nm. As this latter component became more prominent with increase in density, it increased progressively in size to attain diameters in the range of 10.5–11 nm in fraction 11 (d 1.065–1.076 g/ml). Furthermore, the particle spe-

cies (20.5 and 21 nm) with size typical of LDL were found up to and including fraction 11, in agreement with the detection of trace amounts of apoB-100 in the latter fraction. Such findings, considered together with the presence of significant amounts of both α- and β-migrating lipoproteins in the region of the minima between the LDL and HDL peaks (Fig. 2 and Table 2), were suggestive not only of the occurrence of HDL_1-like particles across the low density range (i.e., d 1.024–1.065 g/ml, fractions 4–10) in goose, but also of the marked overlap of particles with the physicochemical properties of LDL and of HDL over the density interval from 1.028 to 1.076 g/ml. Clearly then, we observed no sharp cutoff between low density, apoB-rich particles and high density, apoA-I-rich substances in the goose. Indeed, the chemical compositions of fractions 8 and 9 (d 1.040–1.055 g/ml) were intermediate between those of LDL and HDL, as were their apolipoprotein contents, which were distinguished by the presence of trace amounts of M_r 100,000, 91,000, 64,000, and 58,000 components; the latter apolipoproteins were otherwise characteristic of goose HDL.

In consequence then of the lack of a clear zone of demarcation between LDL and HDL in the goose, it will be evident that application of a (solvent) density of 1.063 g/ml will not permit net separation of these two lipoprotein classes by flotation procedures. Our present use of an upper limiting density of 1.040 g/ml for the low density interval represents a compromise, since the chemical composition, mobility on agarose gel, and enrichment in apoB-100 demonstrate the low density characteristics of fractions 4–7 (d 1.020–1.040 g/ml), although HDL_1-like particles were detectable over part of this range by gradient gel electrophoresis (Fig. 3).

The question as to the distribution of apoA-I among particles constituting the goose low density class appeared of some relevance. We therefore separated particles with the size of LDL from smaller, HDL-like lipoproteins by agarose gel chromatography. The LDL-sized subfractions presented as a single band on gradient gel electrophoresis, the HDL_1 component having been removed. Nevertheless, both apoA-I and apoB-100 were present as constituents of the protein moieties of the LDL column subfractions, thereby suggesting a role for apoA-I as an integral component of at least some low density, apoB-rich lipoproteins in the goose.

Like LDL, the profile of high density lipoproteins presented a symmetrical distribution, extending over the $F_{1.21}$ range 0–9 and the density range ~ 1.055–1.165 g/ml. These profiles each displayed a single peak, occurring in the analytical ultracentrifugal pattern at $F_{1.21}$ 2.2–2.5 and in the density profile at d ~ 1.100 g/ml. Again, however, the hydrodynamic behavior of these particles concealed the high degree of heterogeneity in their physicochemical properties. Thus HDL subfractions displayed multiple size species. Moreover, a clear transition in HDL particle size from 12–10.5 nm in fraction 10 (d 1.055–1.065 g/ml) to 10.5 nm in fraction 13 (d 1.090–1.0103 g/ml) occurred, accompanied in fractions 10 and 11 by an LDL-like particle species of 20.5 nm diameter containing apoB-100. Two new HDL species (diameters 9.9 and 9.0 nm) appeared in fraction 11 (d 1.065–1.076 g/ml). These latter species were distributed up to a density of 1.205 g/ml and, indeed, were the major HDL species over the density range 1.090–1.205 g/ml. In addition to the HDL subspecies with diameters of 10.5, 9.9, and 9.0 nm in fraction 12 (d 1.076–1.090 g/ml), a fourth species of 8.2 nm appeared in fraction 13 (d 1.090–1.103 g/ml), to be accompanied by a fifth species (diam. 7.5 nm) in fraction 14 (d 1.103–1.120 g/ml). These five HDL species were identified in the density range from 1.103 to 1.183 g/ml and, in fact, maintained constant sizes over this interval (Fig. 3).

The heterogeneity in particle size species was reflected in both the chemical composition and, to a more limited extent, in the apolipoprotein content of HDL subfractions. Thus the chemical composition of successive subfractions showed a progressive decrease in the proportions of each lipid component (with the possible exception of free cholesterol), and a parallel increase in protein content with increase in density (Table 2). Indeed, the compositions of certain of the lighter HDL subfractions tended to resemble that of human HDL_2 (d 1.066–1.100 g/ml) (13) and, in particular, those of fractions 10, 11, and 12 (d 1.055–1.090 g/ml) (Table 2). In this regard, it is pertinent that the hydrated density of human HDL_2 (1.09 g/ml) (33, 34) falls within the density range of these goose subfractions. Furthermore, the chemical compositions of certain of the denser subspecies, and notably subfractions 13, 14, 15, and 16 (d 1.090–1.146 g/ml), resemble that typical of human HDL_3 (d 1.100–1.140 g/ml) (13); again, the hydrated density of the human particles (1.12–1.15 g/ml) (33, 34) falls within the density range of the goose fractions. Finally, the chemical composition of the densest goose HDL subspecies (no. 17 of d 1.146–1.165 g/ml) and that of the dense human HDL subfraction of d 1.140–1.153 g/ml (13) were markedly similar.

It was of interest to calculate the mean weighted sum chemical composition of our series of goose HDL subspecies; such a calculation gave a composition (cholesteryl ester 18.2 ± 1.8%; free cholesterol 3.7 ± 0.3%; triglyceride 6.6 ± 2.2%; phospholipid 25.6 ± 1.6%; and protein 46.3 ± 2.8%) which tended to resemble human HDL_3 (d 1.100–1.140 g/ml) (13) in its cholesteryl ester, free cholesterol, phospholipid, and protein contents more than human HDL_2. Examination of Table 5 in reference 13 clearly illustrates, however, that the composition of human HDL subfractions may vary widely according to the methodology employed for their isolation.

The particle heterogeneity of the goose HDL subfractions was confirmed by electrophoretic analyses of their protein moieties, which revealed both qualitative and quantitative changes along the density interval from 1.055 to

1.165 g/ml. Such alterations primarily involved apolipoproteins of low (M_r < 15,000) and intermediate (M_r ~ 40,000–125,000) size, apolipoprotein A-I predominating in all subspecies. The progressive increase in the proportions of components with M_r ~ 15,000, ~ 18,000, ~ 20,000, 58,000, 64,000, and 91,000 with increase in density is notable; by contrast, the M_r ~ 100,000 polypeptide appeared to be present in rather similar amounts in all eight HDL subfractions, with the exception of fraction 10 in which it was undetectable. Several bands with intermediate molecular weights were also detected in the pigeon, but were more pronounced in LDL than in HDL, suggesting that they could represent degradation products of apoB-100 (38). In the chicken, such components were not detectable by the electrophoretic procedure previously used in our studies (12), but clearly appeared in the same SDS gradient gels as those employed herein for goose apolipoproteins. Thus, four bands were detected with M_r 91,000, 85,000, 71,000 and 69,000, which were more pronounced in HDL subfractions (Hermier, D., P. Forgez, and M.J. Chapman, unpublished data). However, the distribution of these intermediate M_r components differs between the pigeon on the one hand, and both goose and chicken on the other since they predominate in LDL in the former species, and in HDL in the latter.

The component with M_r akin to albumin has been detected in all lipoprotein classes in the goose, pigeon, and chicken (M_r 64,000, 67,000, and 69,000, respectively), but was demonstrated to differ from albumin, at least in the pigeon (38).

As in other avian species including chicken and pigeon, the goose seems to lack a counterpart to mammalian apoE, since no apolipoproteins were detected with M_r 34,000 (42). This raises the question as to whether apoA-I may play a similar role in avian species as apoE in reverse cholesterol transport in mammals, a hypothesis proposed earlier in chicken by Dawson, Schechter, and Williams (43) and Rajavashisth et al. (44). Further studies of the synthesis, structure, and function of apoA-I in goose are clearly needed to shed more light on this question.

The distribution of apoC-like components between the various avian species appears to exhibit considerable variation. Thus, a band with M_r 15,000 has been detected in all HDL subfractions in both goose and chicken (12). By contrast, an M_r 18,000 component was detected throughout the goose lipoprotein profile but was not found in the chicken. Furthermore an M_r 11,000 component was present along the length of the density gradient in the chicken only.

We have presently identified a potential polymorphism of an apolipoprotein migrating in the region of the human C-II and C-III proteins on alkaline-urea polyacrylamide gels. Thus, about one-third of our animals lacked the R_f 0.39 component, in which case the proportion of the R_f 0.36 band was twice that in its presence. Additional investigation will be required to evaluate the potential use of this polymorphism as a marker for susceptibility to induction of fatty liver, especially since the present data on the goose lipoprotein profile reveal no feature indicative of such susceptibility.

To conclude, the density gradient ultracentrifugal approach has provided on overall view of the major qualitative and quantitative characteristics of the lipid transport system in the male goose, *Anser anser*. As such, it constitutes the data base necessary for metabolic studies aimed at understanding the biochemical mechanisms involved in the dietary induction of fatty liver.

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