Characterization of unusual intermediate density lipoproteins

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Abstract We report on the physicochemical properties of unusual lipoproteins isolated from both lymph and blood of ruminating cattle. The densities of most of these particles fall within the range between 1.006 and 1.020 g/ml, although densities of 0.97–0.99 g/ml are calculated from chemical composition, assuming a liquid core. The triglycerides of these particles have a high content of saturated fatty acids. The major apoprotein has a mobility on polyacrylamide-SDS gels consistent with a molecular weight of 40,000. The negatively-stained particles appear flattened and asymmetric in electron micrographs. The particles are very large, with molecular weights in the 20 to 250 million dalton range, and they scatter light strongly. The hydrodynamic frictional ratio is about 1.4, consistent with oblate ellipsoids with axial ratios of about 8 to 1. The flat appearance, asymmetric shape, and anomalous densities of the particles would be explained if these lipoproteins consisted of a core of crystallized triglycerides encapsulated within a phospholipid monolayer. Crystallization of the saturated triglycerides could occur during routine lipoprotein isolation, in which temperatures much lower than the melting points of their core lipids are employed. When protocols are done entirely at 37°C, the unusual structures are not observed in the intermediate density class. Although the saturated fats in these bovine lipoproteins are derived from ruminal fermentation, we feel that any triglyceride-rich lipoprotein highly enriched in saturated fats will behave similarly if isolation temperatures are well below the melting points of the core lipids.—**Puppione, D. L., S. T. Kunitake, R. L. Hamilton, M. L. Phillips, V. N. Schumaker, and L. D. Davis.** Characterization of unusual intermediate density lipoproteins. *J. Lipid Res.* 1982. **23:** 283–290.

Supplementary key words bovine lipoproteins • saturated fats • lymph • inelastic light scattering • lipid crystallization • intermediate density lipoproteins

For many years it has been realized that dietary fats can markedly alter the lipid composition of triglyceride-rich lipoproteins and influence the fluidity of both core and surface components. For example, Zilversmit (1) isolated the triglycerides from cream-fed dogs, and reported, ". . . when sufficient cream chylomicron triglyceride was available to perform a melting point determination, it became evident that this triglyceride did not begin to flow until 41 to 42°C. Apparently the loss from cream of short chain fatty acids, which were absorbed by the portal vein, produces chylomicrons that are semisolid at body temperature."

The triglyceride-rich lipoproteins isolated from the plasma and the lymph of ruminating animals contain unusually high contents of saturated fatty acids in the core lipids (2, 3). This is true even though the dietary plant lipids contain largely linoleic and linolenic acids comprising up to 90% of the total (4). The conversion from unsaturated to saturated fatty acids occurs after the hydrolyzed plant fatty acids undergo an intense biohydrogenation process involving the dense microbial population of the rumen. The predominant product, stearic acid (2), is absorbed by the mucosal cells of the bovine intestine and packaged into triglyceride-rich lipoproteins by processes presumably analogous to those occurring in monogastric animals. Although a small portion of the stearate is desaturated to oleate by the mucosal cells, a high content of saturated fatty acids is found among circulating triglycerides in bovine plasma (2, 3, 5–8).

In this communication we report studies on unusual bovine triglyceride-rich particles isolated from both lymph and serum within the density interval between 1.006 and 1.020 g/ml. We find an anomalous difference between the densities of these particles and their composition, but this difference could be explained if the core contains crystalline triglycerides. Interestingly, these lipoproteins are not present in the IDL class when all procedures are done entirely at 37°C. We believe that exposure either to low temperatures or to a combination of conditions used during routine isolation of these lipoproteins results in the formation of these unusual lipoproteins.

Abbreviations: VLDL, very low density lipoproteins, i.e., lipoproteins with density less than 1.006 g/ml; IDL, intermediate density lipoproteins; apo, apoproteins.

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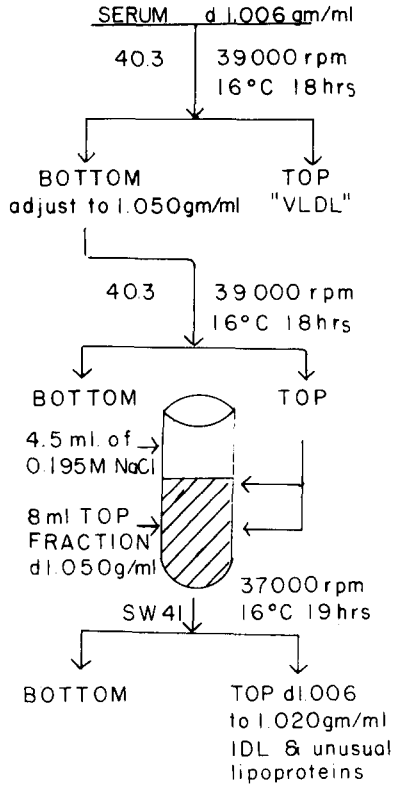

Fig. 1. An outline of the procedure employed for the isolation of the novel particles described in this communication. Lymph samples were spun twice at d 1.006 g/ml.

MATERIALS AND METHODS

Isolation of lipoproteins

The lipoproteins described in this report were isolated separately from the sera obtained from seven lactating, non-pregnant cows (a Jersey, a Guernsey, and five Holstein Friesians). Similar lipoproteins were also isolated from pooled steer serum and from calf lymph plasma samples.

Approximately 300 ml of cow blood were drawn from the jugular vein and kept on ice until the serum was recovered by low speed centrifugation at 4°C. To isolate larger quantities for more detailed studies, 6 liters of pooled blood were obtained from steers at slaughter, and the serum was recovered by centrifugation. Intestinal lymph samples were obtained from two young ruminating steers (a Holstein and a Jersey), surgically prepared as described by Romsos and McGilliard (9). The intestinal lymph duct was cannulated and volumes of 60 to 120 ml were collected into 30-ml plastic conical tubes containing 0.75 ml of an anticoagulant and antibacterial solution of 4% $Na_2 \cdot EDTA$ and 2% NaN_3. Lymph samples were sent refrigerated from Iowa State University in Ames to UCLA.

The cow and pooled steer sera, calf lymph plasma, and all salt solutions used for ultracentrifugation contained $Na_2 \cdot EDTA$ (0.04%), NaN_3 (0.05%), and Gentamycin (0.005%). The densities of the salt solutions employed and of lipoprotein fractions recovered, were measured using a DMA 02D Mettler/Paar Densitometer (Graz, Austria) to an accuracy of ±0.0002 g/ml.

Sequential isolation of bovine lipoprotein is illustrated in **Fig. 1.** The 40.3 rotor was used to isolate the cow serum and calf lymph lipoproteins. Cellulose nitrate tubes were filled with 6 ml of either serum or plasma and centrifuged for 18 hr. After removing the VLDL in the top 2 ml, the subnatant solution was adjusted to a density of 1.050 g/ml. Then, 2-ml fractions of lipoproteins with densities between 1.006 and 1.050 g/ml were isolated under the same ultracentrifugation conditions for the isolation of VLDL, and pooled. Aliquots of 8 ml obtained from this pool were transferred to 1.4 × 8.9 cm cellulose nitrate tubes. These solutions were overlayed with 4.5 ml of 0.195 molal NaCl. Following 18 hr of centrifugation at 37,000 rpm and 16°C in a Beckman SW 41 rotor, the top 3 to 4 ml were removed from each tube. This final top fraction contains lipoproteins ranging in density between 1.006 and 1.020 g/ml. Normally, the lipoproteins in this fraction would be called intermediate density lipoproteins, or IDL. The unusual particles reported in this communication were isolated in this fraction. Complete compositional and physicochemical studies were performed on four preparations of this material.

The 70 Ti rotor was used to isolate lipoproteins from 3 liters of the pooled steer serum. Cellulose nitrate tubes were filled with approximately 38 ml of pooled serum and, after 22–23 hr of centrifugation at 39,000 rpm and 16°C in a Beckman L5-65, the top 18 ml were removed by pipeting. A total volume of 1.2 liters of the pooled bottom fractions from each tube was adjusted to a density of 1.050 g/ml. Cellulose nitrate tubes were filled with this adjusted solution and, after 24 hr of centrifugation, the 1.006–1.050-g/ml fractions of lipoproteins were recovered in the top 6 ml from each tube. A 200-ml pool of these top 6-ml fractions was respun at a density of 1.050 g/ml for 24 hr, and again the top 6-ml fractions were recovered and pooled. Steer lipoproteins isolated from this last spin in the 70 Ti rotor were then separated in an SW 41 rotor under identical conditions as those described above for the cow and calf lipoproteins, yielding a final top fraction ranging in density between 1.006 and 1.020 g/ml and containing a mixture of the large unusual particles and smaller spherical lipoproteins (see Fig. 3).

Gel filtration

For separation of the two types of steer lipoproteins, gel filtration of the pooled top fractions from the swinging

bucket rotor was performed using a 2 × 90 cm Biogel A-15m (Biorad Laboratories, Richmond, CA) column prepared according to Rudel et al. (10). Fifty 4.0-ml fractions were eluted with a 0.1 M NaCl 0.2 M phosphate buffer, pH 7.4, containing 0.01% EDTA and 0.005% NaN_3. The highly light-scattering material, as determined by absorbance at 310 nm, was recovered in the void volume.

Chemical analysis

Total and free cholesterol concentrations were determined enzymatically (11). The enzymatic assay for total cholesterol was compared with a chemical assay and was found to be at least a factor of five times more sensitive. Triglycerides were measured in isopropyl alcohol extracts of these samples using the Technicon AA II (12). Phosphorus analyses were performed as described by Turner and Rouser (13) for phospholipid determinations on lipids that were extracted according to Wulthier (14). Protein concentrations were determined by a modified Lowry procedure employing 1% SDS in a $NaCO_3$ buffer (15). Electrophoresis of the apoproteins in 12% polyacrylamide gels was performed in 0.1% SDS using a modification (16) of the procedure of Laemmli (17). A lipid extract of the pooled column fractions of steer lipiproteins was separated on a 1-g silicic acid column (18). The fatty acid compositions of the cholesteryl esters, triglycerides, and phospholipids were determined after subjecting the isolated lipids to methanolysis by refluxing the lipid with methanol containing 1% sulfuric acid for 7 hr. Fatty methyl esters were separated on a 180 × 0.4 cm Silar 10 C column. The presence of *cis-trans* isomers among the triglyceride fatty acids was determined by using a longer 600 × 0.4 cm Silar 10 C column.

Physicochemical studies

Electron microscopy of cow lipoproteins was performed as described by Hamilton et al. (19), using a Siemans 101 electron microscope and employing phosphotungstate as a negative stain. Calf and steer lipoproteins were photographed in a JEOL 100B electron microscope using uranyl formate as the negative stain. For hydrodynamic studies, lipoproteins isolated from cow plasma and calf lymph lipoproteins, and steer post-Biogel column lipoproteins were dialyzed against a 1.063 g/ml NaBr solution containing $Na_2 \cdot$ EDTA and 0.02% NaN_3. Analytical ultracentrifugation was performed according to the technique of Ma, Schumaker, and Knobler (20) using a Beckman Model E ultracentrifuge equipped with a scanner optical system. Measurements were made at 20,000 rpm and 25°C with the scanner wavelength set at either 265 or 310 nm. Molecular weights, buoyant densities, and frictional coefficients were derived from intensity fluctuation spectroscopy after zone centrifugation, as described by Kunitake et al. (21). Analytical buoyant density gradient experiments were carried out on post-column steer lipoproteins in a 5% Metrizamide (22) solution to obtain estimates of hydrated density. A double sector analytical cell was centrifuged with an appropriate counterbalance in a Beckman An-D rotor at 32,000 rpm for 48 hr at 17°C in a Model E Analytical Ultracentrifuge equipped with both schlieren and scanner optics. Scans were performed at wavelengths of 650 and 350 nm. Metrizamide density gradients were determined from measurements obtained from schlieren patterns photographed at the 'up-to-speed' time and after 48 hr, according to the method of Ifft, Voet, and Vinograd (23).

RESULTS

Compositional analysis

First, the total concentrations of the triglycerides present in the cow and steer sera used as starting materials in our studies were measured and found to range between 10 and 20 mg/dl. Approximately 60% of these triglycerides were found in the VLDL fraction of density less than 1.006 g/ml, and 35% in the IDL range between 1.006 and 1.020 g/ml. Similar distributions of triglycerides were found in studies performed on two separate lymph samples; however, the levels of triglycerides in calf lymph were 10- to 20-fold higher than in the sera of adult animals. Previous bovine studies have demonstrated that the bulk of the circulating triglycerides is transported by lipoproteins with hydrated densities less than 1.019 g/ml (7).

The IDL fractions were separated using the SW 41 rotor, as described in detail in the Materials and Methods section, and their chemical compositions were determined (**Table 1**). These lipoproteins contain relatively high amounts of surface components. Thus, the protein, phospholipid, and cholesterol contents of this material sum to give 30–36% by weight, while the core components, consisting primarily of triglycerides and very little cholesteryl esters, make up the remaining 64–70%. Stead and Welch (8) have reported similar data for the triglyceride-rich lipoproteins in bovine plasma with densities less than 1.019 g/ml.

Electron microscopy

When bovine lipoproteins obtained from the top fractions of the swinging-bucket run were examined by electron microscopy, two populations of particles were seen, as shown in **Fig. 2.** The larger particles were asymmetrical or amorphous in shape and appeared to overlap one another, suggesting a flattened rather than a spherical shape. They ranged in size between 50 and 200 nm with

TABLE 1. Concentrations of the intermediate density lipoprotein fractions following isolation in the swinging bucket rotor

Source	Pro	UC	CE[b]	TG	PL
Holstein cow serum	4.55 ± 0.05[a] (10.9)	3.2 ± 0.0 (7.6)	1.25 ± 0.05 (3.1)	27.8 ± 0.3 (65.9)	5.25 ± 0.43 (12.6)
Guernsey cow serum	4.78 ± 0.22 (15.5)	2.35 ± 0.05 (7.7)	0.65 ± 0.15 (2.3)	19.0 ± 2.0 (61.3)	4.09 ± 0.08 (13.2)
Holstein calf lymph plasma	12.0 ± 1.0 (10.4) n = 3	3.4 ± 0.2 (2.9)	N.D.	81.0 ± 7.0 (70.0)	19.4 ± 0.10 (16.8) n = 4
Jersey calf lymph plasma	13.8 ± 0.7 (8.1) n = 3	15.8 ± 0.2 (9.3) n = 4	2.2 ± 0.2 (1.3) n = 4	111.3 ± 7.3 (65.5) n = 4	26.8 ± 0.5 (15.8) n = 4

[a] Concentrations are given in mg/dl, followed by percent of total mass in parentheses. With the exception of the values for which the number of determinations, n, done on the same sample is given, all other measurements were done in duplicate.

[b] Cholesteryl esters were obtained by subtracting value for unesterified cholesterol from total cholesterol and dividing the difference by 0.6.

Abbreviations used: Pro, protein; UC, unesterified cholesterol; CE, cholesteryl esters; TG, triglycerides; PL, phospholipid; N.D., not detected.

a mean dimension of about 100 nm. A second class of small, spherical particles also was seen which resembled the intermediate density lipoprotein class normally found in human plasma. These particles appeared to be present in much smaller amounts by weight than the large, unusual particles. Two attempts were made to visualize the asymmetric particles by thin-section electron microscopy, but both failed to resolve any structures at all for reasons not entirely clear to us. Perhaps the high content of saturated core lipids (see Table 3) does not alow adequate uptake of osmium tetroxide.

Fig. 2. Electron micrographs of negatively stained bovine lipoproteins. Flattened amorphous particles as well as particles on edge can be seen. Measurements of particle diameter yield a mean of 92.7 nm and a range from 50 to 200 nm. Smaller particles similar in appearance to normal intermediate density lipoproteins are also present. A bar corresponding to 200 nm is in the lower right hand corner.

Gel filtration and detailed chemical analysis

The IDL fraction isolated from pooled steer serum contained both the asymmetric, flattened particles and an abundance of the small spherical particles, as shown in **Fig. 3A.** Compared with the cow IDL, Fig. 2, many more small particles are seen in the steer preparation. This apparent abundance of the small particles in steer sera was due to differences in the isolation procedures. Compositional analysis of the various d 1.006–1.050 g/ml fractions which were placed in the swinging bucket rotor tubes, revealed that the steer fraction had a comparable level of triglycerides, but a 40- to 300-fold higher level of cholesterol than observed in the fractions of the other animals. Thus, the difference in isolation procedures resulted in an abundance of the cholesteryl ester-rich spherical particles in the steer d 1.006–1.050 g/ml fractions. Following swinging bucket isolation, a significant level of these spherical particles was still present.

To reduce the concentrations of the small lipoproteins beyond the point where they would significantly affect the chemical composition determined for the large, light scattering particles, the swinging bucket rotor fraction of steer lipoproteins was further separated on a Biogel A-15m column. Prior to separation, electron microscopy of the pre-column fractions showed both classes of lipoproteins to be present, as shown in Fig. 3A. After passage through the column, electron microscopy of the

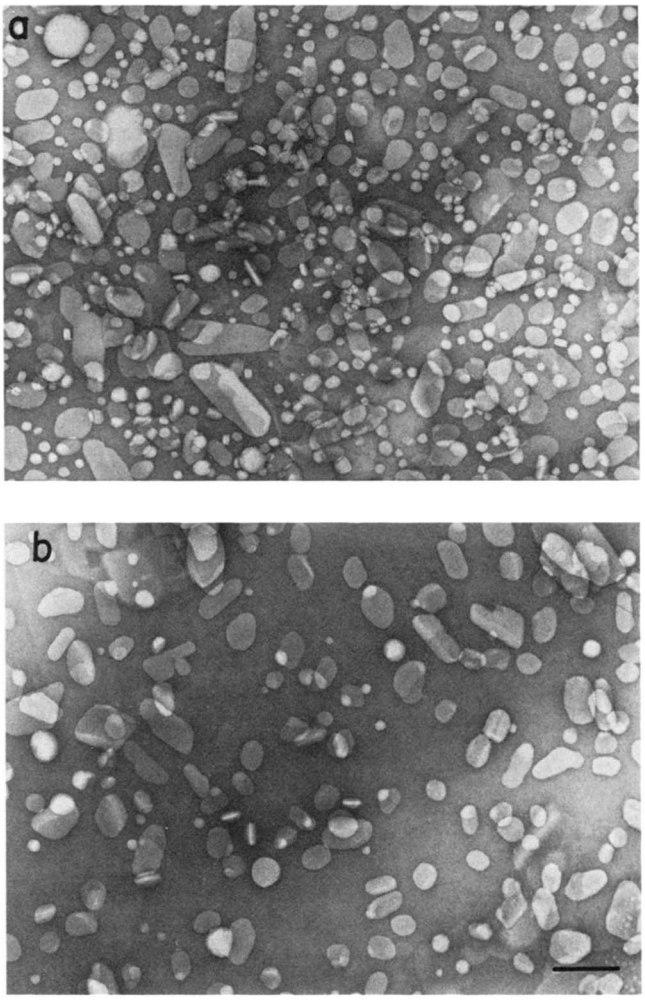

Fig. 3. Negatively stained steer lipoproteins; A., before, and B., after gel filtration to deplete the smaller particles. A bar corresponding to 200 nm is in the lower right hand corner.

post-column, void volume fractions showed fewer of the small, spherical intermediate density particles as shown in Fig. 3B. In **Table 2,** the chemical compositions of the steer lipoproteins are compared before and after gel filtration. A decrease in the content of the cholesteryl esters after passage through the column is consistent with the removal of intermediate density lipoproteins.

In **Table 3** are listed the fatty acid compositions of the lipids extracted from two different preparations of the light-scattering lipoproteins. It is apparent that the degree of saturation is quite high for the triglycerides since the sum of the weights of the 16:0 and 18:0 fatty acids represents over 70% of the fatty acid mass. Separation of the triglyceride methyl esters on the 1.8-meter Silar column revealed a broad oleic acid peak. Separation of the same mixture on a 6-meter column resolved oleic acid and its *trans* isomer in equal amounts.

The post-column compositional data in Table 2 and

TABLE 2. Composition of the light-scattering fraction obtained from pooled steer serum

Fraction	Pre-Column	Post-Column
Protein	6%	4%
Cholesteryl esters	11%	5%
Unesterified cholesterol	13%	12%
Triglycerides	46%	57%
Phospholipids	25%	22%

the fatty acid distribution for triglycerides given in Table 3 are almost identical to the value reported by Stead and Welch (8) for the density less than 1.019 g/ml fraction.

The distributions of the reduced apoproteins obtained from the pre- and post-column fractions are shown in **Fig. 4.** Gels 1 and 2 contain different amounts of the same sample of pre-column apoproteins. Gel 1 emphasizes proteins present in small amounts while gel 2 contained an amount of protein equal in mass to the amount of the post-column fraction applied to gel 3. Small amounts of apoA-I and apoC are seen on gel 2 and less on gel 3. On both gels, a single major apoprotein is seen which migrated in a position corresponding to a protein of molecular weight of 40,000. SDS-polyacrylamide gel analyses were performed on all bovine samples and patterns similar to those shown from the pooled steer serum in Fig. 4, lane 3, were found.

Hydrodynamic studies

Flotation experiments performed in the analytical ultracentrifuge, using the turbidometric technique to follow the migrating boundary (20), revealed that the light-scattering material isolated in the IDL fraction was quite large and highly heterogeneous in size. A spread in flotation rates from S_f 20 to 400 Svedbergs was found, using a solvent density of 1.063 g/ml. Since flotation rate is

TABLE 3. Fatty acid distribution in the three lipid classes of light-scattering IDL

	Steer Post-Column IDL			Holstein Lymph IDL	
	CE	TG	PL	TG	PL
14:0	0.6	1.6	1.2	0.6	1.4
Unknown		1.2	1.3	1.8	13.6
16:0	13.0	25.9	21.5	20.6	17.1
16:1	5.2	2.6			
18:0	16.8	44.7	36.7	52.0	19.1
18:1 *cis*	13.0[a]	10.2	11.9[a]	10.7	22.4[a]
18:1 *trans*		10.2		10.7	
18:2	48.8	3.3	22.8	2.2	13.7
18:3 or 20:1	0.6			1.4	6.4
20:3		0.3	2.2		
20:4	1.2		2.5		3.5

[a] Insufficient material available for analysis on the 8-m column to determine *cis-trans* isomers of 18:1. Abbreviations are the same as in Table 1.

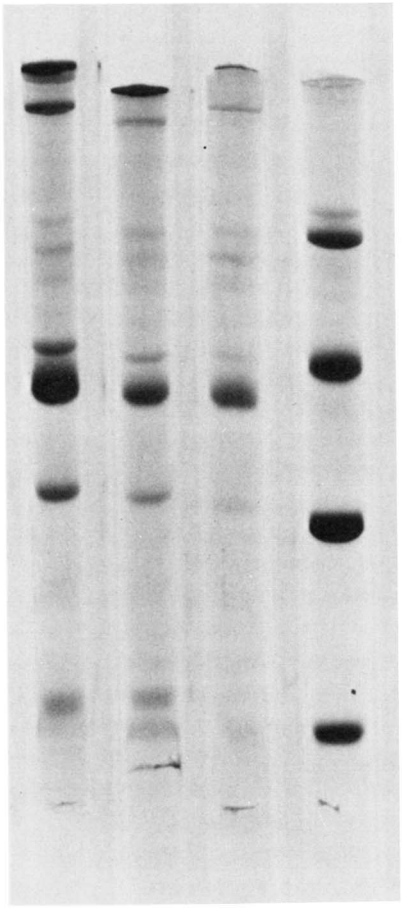

Fig. 4. SDS polyacrylamide gel electrophoretograms of steer light-scattering lipoproteins. From left: gel1, pre-column (36 μg of protein); gel 2, pre-column (9 μg of protein); gel 3, post-column (9 μg of protein); and gel 4, standards (Alb, albumin, Oval ovalbumin, A-I, human A-I apolipoprotein, and Cyt C, cytochrome C). Prior to applications proteins were reduced by incubating with beta mercaptoethanol.

a function of particle density as well as size and shape, we determined the buoyant density by the technique of banding in a density gradient, using the non-ionic, iodinated solute, Metrizamide. The material banded in a sharp peak in the Metrizamide gradient formed after centrifugation for 2 days in the analytical ultracentrifuge at 32,000 rpm. The buoyant density at the center of this sharp peak was calculated to be 1.015 g/ml, a value that falls at the center of the IDL density range. (A small density correction of +0.001 g/ml has already been applied to this value to correct for the 32 atm of pressure at band center.)

A more detailed hydrodynamic analysis can be obtained by the technique of laser scattering after zone centrifugation (21), in which both the flotation coefficient and diffusion coefficient are determined point-by-point along the distribution of macromolecules. By employing two different density gradients in two separate experiments, the distribution of buoyant densities may be obtained as well. Thus, it is possible to combine all of the data to yield the distribution of molecular weights, buoyant densities, and fractional ratios for the lipoprotein sample. The light-scattering material isolated in the IDL fraction has been examined seven times by this technique, and a representative calculation is given in **Table 4.** The smaller spherical lipoproteins, having lower flotation rates, did not float into the gradient and were not analyzed. From Table 4 it may be seen that molecular weights range between 20 and 250 million daltons. Density values, though well within the IDL density range, are a little higher than found in the Metrizamide gradient, averaging about 1.019 ± 0.004 g/ml. Frictional ratios range between 1.3 and 1.4, and indicate that the particles have a substantial amount of asymmetry, consistent with the morphology observed in the electron micrographs. An oblate ellipsoid of axial ratio of 8 to 1 has a frictional ration of 1.37.

Studies on VLDL

In addition to the studies just described on the large light-scattering material isolated in the IDL fraction, we have also looked at the VLDL with densities less than 1.006 g/ml. Electron microscopy, not shown, has revealed the presence of spherical particles as well as some flattened particles similar to those described above. The fatty acid distribution of the VLDL showed a higher content of unsaturated fatty acids than those listed in Table 3 with a 2- to 3-fold increase in the content of both linoleate and oleate and corresponding decrease in the content of stearate.

Effect of temperature on isolation

Preliminary experiments, in which bovine plasma as well as lipoprotein fractions were isolated at 37°C, failed to show the presence of these large light-scattering IDL particles. Yet treatment of the same blood at 16°C as described in Fig. 1 still produced them. Thus, the for-

TABLE 4. Hydrodynamic parameters of bovine lipoproteins measured by fluctuation-intensity spectroscopy

S_f	$D_{20,w}$ (Ficks)	d (g/ml)	Mol wt × 10^{-6}	f/f_0
118	0.33	1.023	260	1.4
114	0.38	1.018	190	1.3
87	0.40	1.024	160	1.4
85	0.44	1.016	120	1.4
47	0.57	1.024	61	1.3
41	0.63	1.015	39	1.4
36	0.71	1.011	28	1.4
26	0.82	1.015	19	1.3

Symbols: S_f, sedimentation coefficient expressed in Svedbergs and corrected to a reference solvent having at 20°C the viscosity and density (d) of NaCl solution (d 1.063 g/ml); $D_{20,w}$, diffusion coefficient expressed in Ficks and corrected to a reference solvent having the viscosity of water at 20°C; Mol wt, molecular weight; f/f_0, frictional ratio.

mation of these lipoproteins appears to be dependent on the choice of temperatures used in their isolation. This experiment at different temperatures has been repeated twice and will be the subject of a forthcoming publication.

DISCUSSION

A large discrepancy exists between the densities of the large, light-scattering particles as determined by hydrodynamic measurements, and the densities which may be calculated from the chemical compositions listed in Table 1. Hydrodynamic measurements of particle densities range between 1.013 and 1.024 g/ml. The densities calculated from compositional data are much lower and give values of 0.972 and 0.989 g/ml if the density of the triglycerides is assumed to have a calculated value of 0.912 g/ml for liquid tristearin at 16°C, using the Handbook (24) value of 0.862 g/ml at 80°C for liquid tristearin and the formula for the thermal expansion of olive oil over the range between 9° and 109°C given in the International Critical Tables. The lipoprotein densities were calculated from the weight percents of protein and the various lipids by assuming additivity of volumes from the expression: lipoprotein density = (% Protein + % CE + % TG + % PL)/(% Protein/1.373 + % CE/0.958 + % C/1.033 + % TG/0.912 + % PL/1.031). The value of 1.373 g/ml for the density of the protein has been calculated from the amino acid composition. Values of densities used by Sata, Havel, and Jones (25) were selected for the other components.

We do not believe the cause of this discrepancy is experimental error. There can hardly be a major error in the hydrodynamic measurements, for the particles were originally isolated by flotation within the density interval between 1.006 and 1.020 g/ml. Moreover, two other hydrodynamic techniques have been used to measure the buoyant densities of these particles, and these methods give similar results. Nor do we believe the error lies in the chemical analyses, which would have to be seriously incorrect to account for the discrepancy.

A possible resolution to this problem was suggested to us by Donald Small.[3] If the saturated triglycerides in the core of these lipoproteins were partially crystalline, then their densities would be much greater. Thus, the density of liquid tristearin would increase to a density of 1.021 g/ml.[3] Similar density changes are found for tripalmitin and trimyristin at their melting points. Therefore, if we recalculate the densities of the lipoproteins using a value of 1.021 g/ml for the density of crystalline triglycerides, we obtain values between 1.064 and 1.032 g/ml. These new values are too high, leading us to conclude that the lipids are only partially crystalline within these large, light-scattering bovine IDL particles.

The hydrodynamic and electron microscopic data clearly indicate that these lipoproteins are non-spherical in shape and polydisperse in size. The molecular architecture and three-dimensional structure still needs to be elucidated; however, if the suggestion of Donald Small is correct, then we would agree with him that "some of the peculiar shapes might be accounted for by the partial crystallization of the glycerides within the partially empty bags".[3]

These intermediate density lipoproteins are not isolated from bovine plasma when all preparative steps are done at 37°C, but they are formed if these same steps are carried out at reduced temperatures. The cores of bovine triglyceride-rich lipoproteins may exist in vivo as super-cooled fluids (26) that crystallize as the temperature is reduced further with a concomitant increase in density. The isolation of these particles by the use of high ultracentrifugal fields might also be a factor in their production. Once crystallized, the solid portions might split away to yield the particles described in this communication. High hydrostatic pressure at the bottom of the centrifuge tube might also be implicated in the formation of these particles.

Although these novel lipoproteins have been isolated from bovine plasma, we feel that rumination is not necessary for their formation. Thus, studies (27) in which sheep were made functionally monogastric through the feeding of encapsulated fat revealed that the distribution of triglyceride-rich lipoproteins between VLDL and IDL would change depending on the saturation of the fed fat. Moreover, IDL, similar in physicochemical properties to what we have described for the bovine, have been isolated from the plasma of a human subject following alimentary absorption of palm oil.[4] We suggest that any circulating triglyceride-rich lipoprotein sufficiently enriched in saturated fats would give rise to intermediate density lipoproteins similar to those we have described if the isolation temperatures are sufficiently low to induce a phase transition. Therefore, it may be important to perform centrifugal isolations of VLDL at or above room temperatures to minimize the possibility of creating the type of abnormal particles described in this communication. Current studies of these conditions are underway.

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[4] Puppione, D. L. Unpublished results.

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