Composition of proteins of mesenteric lymph chylomicrons in the rat and alterations produced upon exposure of chylomicrons to blood serum and serum proteins

Katsumi Imaizumi, Menahem Fainaru, and Richard J. Havel

Cardiovascular Research Institute and Department of Medicine,
University of California, San Francisco, CA 94143

Abstract Protein composition was determined in mesenteric lymph chylomicrons from fat-fed rats. Among the proteins of intermediate molecular weight, apoproteins A-I and the arginine-rich apoprotein accounted for 31% and 4% of the total protein mass, respectively. Apoprotein B and apoprotein A-IV each accounted for about 10% and proteins of low molecular weight (C apoproteins and apoprotein A-II) accounted for most of the remainder. Apoprotein A-I also accounted for more than 30% of the protein mass of mesenteric lymph lipoproteins of density less than 1.006 g/ml ("small chylomicrons") obtained from rats fed glucose. Apoprotein A-I was partially dissociated from chylomicrons during brief ultracentrifugation. Both the arginine-rich apoprotein and the C apoproteins in rat blood serum were transferred to lymph chylomicrons from fat-fed rats during incubation in vitro. Content of arginine-rich apoprotein, determined immunochemically, increased six-fold when chylomicrons were diluted to a final concentration of 500 mg/dl in blood serum. Upon incubation of chylomicrons in equivalent volumes of ultracentrifugal fractions of serum, the increase of the arginine-rich apoprotein was: very low density lipoproteins, 1.5-fold; high density lipoproteins, 1.8-fold; density fraction >1.006 g/ml, 5.0-fold; density fraction >1.21 g/ml, 11-fold. Content of apoprotein A-I, also determined immunochemically, was not altered appreciably by exposure to serum or its ultracentrifugal fractions, whereas content of C apoproteins, estimated from intensity of staining of the low molecular weight protein component in polyacrylamide gel electropherograms, increased in all cases except for the density fraction >1.21 g/ml. The fractional content of apoprotein A-I in the protein of chylomicrons fell after incubation, whereas that of the arginine-rich apoprotein remained constant or rose substantially. The fractional content of apoprotein A-IV in chylomicron-protein tended to follow that of apoprotein A-I, as judged from polyacrylamide gel electropherograms. Transfer of the arginine-rich and C apoproteins to chylomicrons from blood serum was directly related to the volume of serum in which the chylomicrons were diluted and occurred rapidly at room temperature or at 4°C.

Supplementary key words arginine-rich apoprotein · apoA-I · apoA-IV · apoB · VLDL · LDL · HDL · VHDL

Chylomicrons from rat mesenteric lymph contain, in addition to B apoprotein and the C apoproteins of small molecular weight, several proteins of intermediate molecular weight (25,000–60,000) (1–4). Apoprotein A-I (apoA-I) and the arginine-rich apoprotein (ARP) are major and minor components, respectively, of the latter group of proteins in mesenteric lymph chylomicrons from fat-fed rats (5, 6). The protein composition of chylomicrons changes when the chylomicrons are exposed to blood serum or serum lipoproteins (1, 4, 7–9). In particular, C apoproteins are transferred to the chylomicron surface from high density lipoproteins (HDL) (3, 4, 8, 9).

In the present research we have quantified the proteins of rat lymph chylomicrons and have determined the effects of exposing the chylomicrons to blood serum and serum lipoprotein fractions, with particular emphasis upon the proteins of intermediate molecular weight. The results show that these proteins in triglyceride-rich lymph lipoproteins from both fat-fed and carbohydrate-fed rats differ substantially from those of triglyceride-rich very low density lipoproteins (VLDL) of blood serum, and rather resemble those of serum HDL. They also indicate that the com-

Abbreviations: apo, apoprotein; ARP, arginine-rich apoprotein; VLDL, very low density lipoproteins (d < 1.006 g/ml); LDL, low density lipoproteins (1.006 < d < 1.063 g/ml); HDL, high density lipoproteins (1.063 < d < 1.21 g/ml); VHDL, very high density lipoproteins (1.21 < d < 1.25 g/ml); TMU, tetramethylurea; SDS, sodium dodecyl sulfate.

Downloaded from www.jlr.org by guest, on June 19, 2012

position of this group of proteins is substantially altered when chylomicrons are exposed to lipoproteins in blood serum. A preliminary report of some of this work has appeared (10).

METHODS

Preparation of lymph

Male Sprague-Dawley rats, weighing 300–350 g and maintained on standard Purina rat chow (Ralston Purina Co., St. Louis, MO) and tap water, were anesthetized with diethyl ether. A right subcostal incision was made and the main intestinal lymph duct was cannulated with silastic tubing (0.025 ID × 0.047 OD, Dow Corning Corp. Medical Products, Midland, MI) following the method of Bollman, Cain and Grindlay (11). The duodenum was then cannulated with silastic tubing of the same diameter and the rats were placed in restraining cages. A lipid emulsion was infused through the intestinal cannula at a rate of 1.25–3.0 ml/hr from an infusion pump (Harvard Apparatus Co., Inc., Millis, MA). The lipid emulsion contained 0.4% plant lecithin (Practical grade, Eastman Kodak Corp., Rochester, NY), 2.0% triolein (Grade II, Sigma, St. Louis, MO), 0.04% cholesterol (C.P. ash-free, Pfanstiehl Laboratories, Inc., Waukegan, IL), and 2.0% Intralipid triglycerides (Vitrum, Stockholm, Sweden). The mixture without Intralipid was chilled in ice water and sonicated with a probe sonicator three times at 80 W for 3 min (Ultrasonic Inc., Plainview, NY). Lymph was collected overnight in a container placed in crushed ice. The lymph was allowed to clot at room temperature and the fibrin was removed by filtration through glass wool.

Purification of chylomicrons

Chylomicrons were separated by centrifugation of the fresh lymph layered beneath 0.15 M NaCl containing 0.04% disodium EDTA at 12°C in the 40.3 rotor of a Beckman ultracentrifuge (Beckman Instruments Inc., Spinco Div., Palo Alto, CA) for 1.6×10^6 g-av min. The floating chylomicrons were collected, dispersed in saline, and concentrated by centrifugation as before. The partially purified chylomicrons (~1000 mg in 5 ml) were subjected to gel chromatography on a 1.2 × 90 cm column of 2% agarose (Biogel A-50m, 50–100 mesh, Bio-Rad Laboratories, Richmond, CA). The column was equilibrated and eluted at 18–20°C at a rate of 30 ml/hr with 0.2 M NaCl, 0.002 M EDTA (disodium salt), 0.02% sodium azide, pH 7.0. The columns were calibrated with blue dextran 2000 (1% in saline, Vitrum AB, Uppsala, Sweden). Fractions of 1.5 ml were collected, and elution of chylomicrons was monitored by measurement of light scattering (Micronephelometer, Particle Data, Inc., Elmhurst, IL). The elution pattern was essentially the same as that described by Sata et al. (12). The light scattering particles that eluted in the void volume were concentrated by centrifugation as before. Purity of these chylomicron preparations was established by polyacrylamide gel electrophoresis and double immunodiffusion (13) against a monospecific antiserum to rat albumin (6). The chromatographic step was essential to obtain large chylomicrons free of albumin and other serum proteins without extensive centrifugal washing.

To purify triglyceride-rich lipoproteins contained in mesenteric lymph of rats who were not absorbing dietary fat, lymph was collected from rats during intraduodenal infusion of 10% glucose in 0.15 M NaCl at a rate of 2.7 ml/min. The d < 1.006 g/ml lipoproteins (small chylomicrons) were separated at 12°C by centrifugation of the lymph serum at 1×10^8 g-av min and purified by recentrifugation under the same conditions.

Purification of chylomicron proteins

Large chylomicrons were partially delipidated by rapidly dispersing 1 vol of the chylomicron suspension into 20 vol of ice-cold diethyl ether. After 30 min, the mixture was centrifuged at 4°C and the precipitated protein was washed with cold ether. Total delipidation was then accomplished by addition of 20 vol of ethanol–ether 3:1 (v/v) at 4°C. The next day the mixture was centrifuged and the ethanol–ether was decanted. Residual ether was removed under a stream of nitrogen and the precipitated proteins were dissolved in 0.1 M Tris-HCl, pH 8.0, containing 2% sodium decyl sulfate. The solution was applied to a 0.9 × 100 cm column of Sephadex G-200 (Pharmacia AB, Piscataway, NJ) at room temperature and the apoproteins were eluted with 0.015 M Tris-HCl and 6 M urea (freshly deionized), pH 8.2 (6). Fractions of 1.2 ml were collected.

Preparation of serum lipoprotein fractions

Rats selected and maintained as described above were used. Lipoprotein fractions were isolated at 12°C by sequential ultracentrifugation of blood serum at d 1.006, 1.063, 1.21, and 1.25 g/ml in a 40.3 Beckman rotor at 38,000 rpm (14). Centrifugation was for 18 hr at d 1.006 and 1.063, and for 44 hr at d 1.21 and 1.25 g/ml. The lipoprotein fractions were dialyzed exhaustively against 0.15 M NaCl, 0.001 M EDTA, pH 7.0. After dialysis the lipoprotein samples were diluted with 0.15 M NaCl to the initial serum volumes.

Downloaded from www.jlr.org by guest, on June 19, 2012

ASBMB JOURNAL OF LIPID RESEARCH JLR

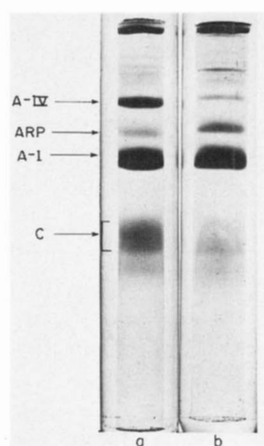

Fig. 1. SDS–polyacrylamide gel electropherograms of apoproteins of large chylomicrons obtained from fat-fed rats (*a*) and small chylomicrons obtained from glucose-fed rats (*b*). About 50 μg of protein was applied to each gel. ApoB is represented by the band close to the top of each gel.

Incubation of chylomicrons with lipoprotein fractions

Freshly purified chylomicrons (0.35–0.45 mg chylomicron protein) were mixed in conical glass tubes with various lipoprotein fractions (2.5–20 ml) in their original serum concentrations or with 0.15 M NaCl. Incubations were usually at 23°C for 60 min with gentle agitation to maintain dispersion of the particles. The incubation was stopped by chilling the tubes in ice water, and the chylomicrons were then separated immediately by centrifugation and column chromatography as described for chylomicron purification. The purity of these chylomicrons was established as before. The composition of chylomicrons after incubation was compared with that obtained after incubation in a comparable volume of 0.15 M NaCl.

Analytical methods

Lipids were extracted in chloroform–methanol 2:1 (v/v) (15), and triglycerides, phospholipids, and cholesterol in the chloroform phase were measured as described previously (16). Protein was determined by the method of Lowry et al. (17); turbidity in the reaction mixture owing to lipid was removed by extraction with diethyl ether or chloroform (16). Content of tetramethylurea (TMU)-soluble and insoluble protein was determined as described by Kane et al. (18). ARP and apoA-I were quantified by radioimmunoassay (5, 6). Chylomicron apoproteins were also estimated from the absorbance at 280 nm of individual apoproteins or groups of apoproteins eluted from columns of Sephadex G-200 as described above.

Sodium dodecyl sulfate (SDS) electrophoresis of apolipoproteins was performed according to Weber and Osborn in 10% polyacrylamide gel (19). The chylomicron samples were partially delipidated as described above and dissolved by heating at 90°C in 0.1 ml of 1% SDS containing 5% β-mercaptoethanol. After electrophoresis the gels were stained with Coomassie brilliant blue in 45% ethyl alcohol and 10% acetic acid, and destained in 10% acetic acid. Isoelectric focusing polyacrylamide gel electrophoresis (pH 3.5–7.0) of apolipoproteins was performed as described by Pagnan et al. (20). Apoprotein bands were identified by comparison with the mobility of purified apoproteins (ARP and apoA-I) (5, 6), by double immunodiffusion (13) against specific antisera (5, 6, 21), and by amino acid analysis (18).

RESULTS

Composition of proteins of mesenteric lymph chylomicrons

The protein mass of the large chylomicrons was less than 1% and their overall composition was similar to that obtained by Mjøs et al. (3) from rats fed corn oil or safflower oil: triglycerides, 92.8 ± 0.7; cholesteryl esters, 0.55 ± 0.23; phospholipids, 5.5 ± 0.49; cholesterol, 0.36 ± 0.10; protein 0.77 ± 0.06 (% wt/wt, mean ± SEM, $n = 3$). In SDS–polyacrylamide gel electropherograms, the pattern of the protein bands resembled that of the apoproteins of HDL in blood plasma (22) except for the presence of a band that barely entered the gel, which presumably represents apoB (**Fig. 1**). ApoB accounted for about 10% of the protein, as estimated from gel chromatography or from protein insoluble in TMU (**Table 1**). The most prominent of the three protein bands with intermediate mobility, representing apoA-I, accounted for

TABLE 1. Protein composition of chylomicrons from mesenteric lymph

Protein	% Total Protein		
	Gel Chromatography[a,b]	TMU Solubility[a]	Radioimmunoassay[c]
ApoB	8.0 ± 1.4	11 ± 0.1	
ApoA-IV	7.6 ± 1.8		
ARP	38.2 ± 3.2 (ARP + ApoA-I)		4.4 ± 0.38
ApoA-I			31.2 ± 2.5
ApoC	37.7 ± 3.0		

[a] Average values from three experiments ± SEM, based upon protein analysis by method of Lowry et al. (17).
[b] Recovery from columns, based upon absorbance at 280 nm, was 91.5 ± 2.4%.
[c] Average values from six experiments ± SEM, based upon absorbance at 280 nm.

Downloaded from www.jlr.org by guest, on June 19, 2012

ASBMB
JOURNAL OF LIPID RESEARCH

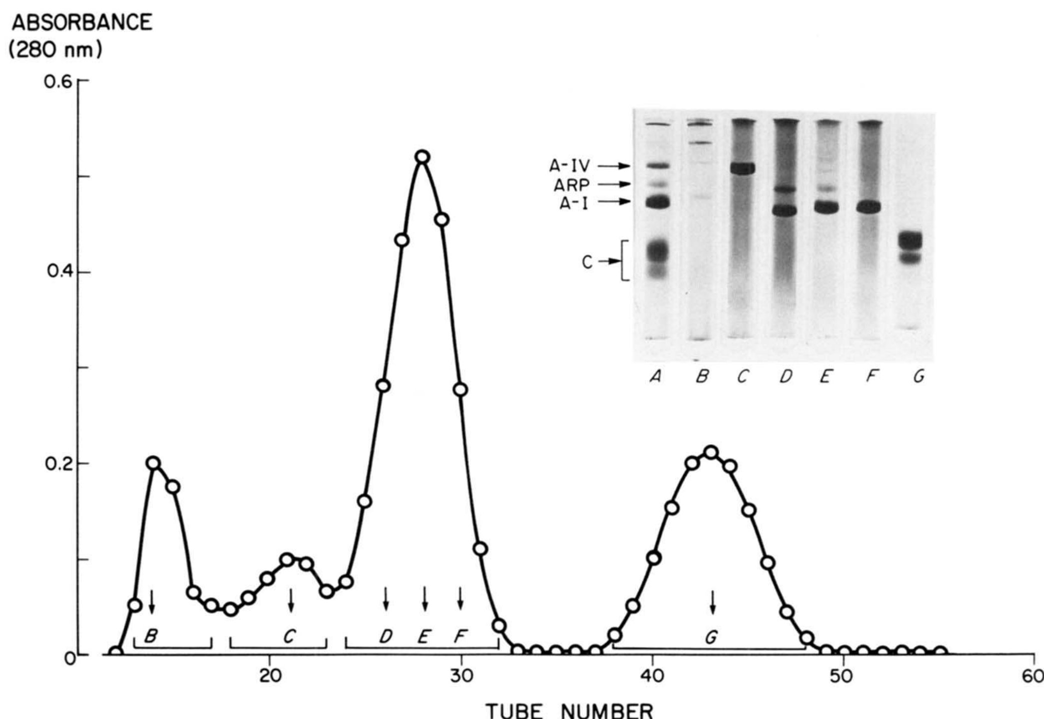

Fig. 2. This diagram illustrates the elution of the total apoprotein component of large lymph chylomicrons on a 0.9 × 100 cm column of Sephadex G-200. A 9.8 mg portion of protein, dissolved in 0.1 M Tris-HCl pH 8.0 containing 2% sodium decyl sulfate was applied and elution was with 0.015 M Tris-HCl pH 8.2 containing 6 M urea. The inset shows SDS–polyacrylamide gel electropherograms of the unfractionated protein (*A*) and the fractions indicated as *B–G*. Fraction *B*, eluted in the void volume, represents mainly apoB. Column volume is at tube 53.

about one-third of the total protein, as determined by radioimmunoassay. The band above it, representing ARP, accounted for only about 4%, also determined by radioimmunoassay. ApoA-I and ARP eluted together from columns of Sephadex G-200. These proteins accounted for 38.2% of the eluted absorbance at 280 nm, in good agreement with the results of immunoassay (Table 1 and **Fig. 2**). The most slowly migrating of the three bands of intermediate mobility in SDS gels comigrated with apoA-IV of plasma HDL. This protein, which accounted for about 8% of the eluted absorbance at 280 nm, was isolated by gel chromatography (Table 1 and Fig. 2). Its identity as apoA-IV was established by immunodiffusion against anti apoA-IV from plasma HDL (**Fig. 3**). This antiserum did not react with apoA-I prepared from plasma HDL or from chylomicrons, nor with ARP prepared from plasma VLDL (**Fig. 4**). The identification of this chylomicron protein as apoA-IV was supported by amino acid analysis (**Table 2**). Because of its low content of tyrosine as compared with that of the other chylomicron apoproteins (21, 23) the value for absorbance at 280 nm underestimates its contribution to the protein mass. Most of the remainder of the chylomicron protein was accounted for by proteins of low molecular weight (apoC and apoA-II (23, 24)) as determined by gel chromatography (Table 1 and Fig. 2). The recovery of applied protein from the gel columns exceeded 90%, excluding the presence of more than minor amounts of other proteins. Small amounts of stainable material with apparent molecular weight exceeding that of apoA-IV were usually visible in SDS gels (Fig. 1).

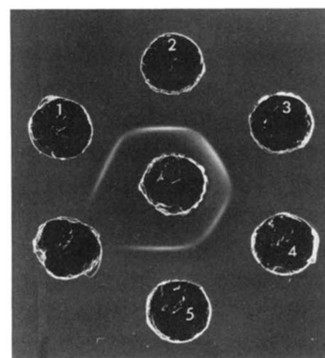

Fig. 3. Double immunodiffusion of apoproteins against anti apoA-IV from rat HDL (center well). Wells 1–5 contained, respectively, apoA-IV from HDL; total apoprotein from large chylomicrons; apo HDL; chylomicron apoA-IV; and apoA-IV from HDL.

Downloaded from www.jlr.org by guest, on June 19, 2012

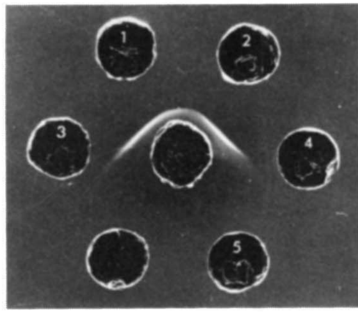

Fig. 4. Double immunodiffusion of apoproteins against anti apoA-IV from rat HDL (center well). Wells 1–5 contained, respectively, total apoprotein from large chylomicrons; apo HDL; apoA-I from HDL; apoA-I from chylomicrons; and ARP from VLDL.

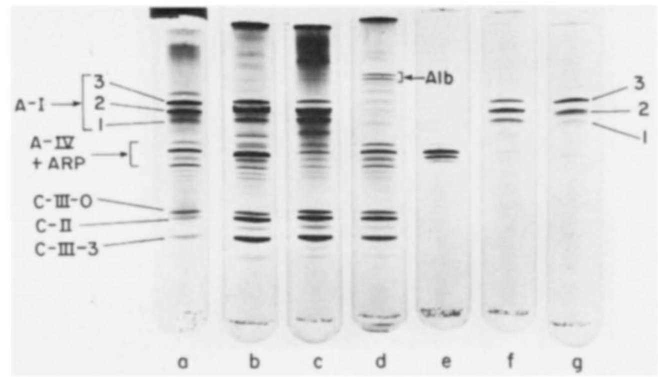

Fig. 5. Isoelectric focusing polyacrylamide gels of apoproteins from lymph and blood serum. *a*, Blood HDL (100 μg); *b*, large lymph chylomicrons (256 μg); *c*, small lymph chylomicrons (240 μg); *d*, blood VLDL (220 μg); *e*, apoA-IV from lymph chylomicrons (15 μg); *f*, apoA-I from lymph chylomicrons (14 μg); *g*, apoA-I from blood HDL (15 μg).

In isoelectric focusing polyacrylamide gel electropherograms, the similarity between the proteins of chylomicrons and HDL was also evident (**Fig. 5**). In this system the polymorphic components of ARP and apoA-IV are not separated (22, 24). However, the polymorphic components of apoA-I are well separated from the other major proteins. The most cationic of the three major bands of apoA-I (band 3) was consistently more prominent in apoA-I of HDL than in chylomicrons; in the latter, band 2 predominated. In chylomicrons and VLDL, apoC-II was generally more prominent than apoC-III-0, whereas the reverse held for HDL (see also ref. 22). A faint band with the mobility of apoA-II (22, 24) was occasionally visible in samples of chylomicron proteins just preceding apoC-III-0 (not visible in Fig. 5).

The composition of the d < 1.006 lipoproteins obtained from lymph of glucose-fed rats was similar to that of the small chylomicrons that were separated from large chylomicrons in lymph of fat-fed rats, as reported by Mjøs et al. (3): triglycerides 73.3 ± 2.6; cholesteryl esters 4.2 ± 1.0; phospholipids 16.4 ± 0.9; cholesterol 1.6 ± 0.2; protein 4.5 ± 0.6 (% wt/wt, mean ± SEM, $n = 3$). The pattern of the soluble proteins in polyacrylamide gels resembled that of large chylomicrons obtained after fat-feeding except that staining of ARP was usually greater and staining of apoA-IV and C apoproteins was usually less in small chylomicrons. By radioimmunoassay, two samples of small chylomicrons contained 40.6 and 37.2% apoA-I and 3.6 and 4.2% ARP (% wt/wt).

TABLE 2. Amino acid composition of apoprotein A-IV

Amino Acid	Chylomicron A-IV[a]	HDL A-IV (Swaney et al. (21))
	mol/10³ mole	
Lysine	73 ± 0.6	67
Histidine	15 ± 0.3	16
Arginine	37 ± 0.7	40
Aspartic Acid	133 ± 3.2	123
Threonine	53 ± 0.9	52
Serine	56 ± 2.1	54
Glutamic Acid	216 ± 7.1	203
Proline	44 ± 1.0	41
Half-Cystine	N.D.	5
Glycine	42 ± 2.2	48
Alanine	63 ± 3.0	65
Valine	60 ± 4.6	64
Methionine	20 ± 4.6	23
Isoleucine	18 ± 0.9	24
Leucine	126 ± 2.3	122
Tyrosine	11 ± 1.8	14
Phenylalanine	34 ± 0.7	33
Tryptophan	N.D.	3

[a] Mean ± SEM, $n = 3$.

Incubation of chylomicrons with serum fractions

Table 3 shows the protein composition of chylomicrons isolated after incubation with serum lipoprotein fractions. Average recovery of triglycerides in chylomicrons after incubation was 72.5 ± 2.3% (mean ± SEM, $n = 23$). Variations in recovery following re-isolation of chylomicrons by repeated centrifugations and gel chromatography are probably the result of small losses at each step. Therefore, content of total protein, A-I and ARP in chylomicrons is expressed as a fraction of the content of triglycerides.

The ratio of protein to triglyceride in chylomicrons incubated with the serum fraction of d > 1.006 g/ml was 2.5 times higher than that of chylomicrons incubated with 0.15 M NaCl. This gain in protein was also evident when chylomicrons were incubated with serum HDL and the serum fraction of d > 1.21 g/ml, but to a lesser extent. Content of ARP, determined by radioimmunoassay, increased substantially when chylomicrons were incubated with serum fractions of d > 1.006 and d > 1.21 g/ml (**Figs. 6,7** and Table 3).

Downloaded from www.jlr.org by guest, on June 19, 2012
ASBMB
JOURNAL OF LIPID RESEARCH

TABLE 3. Composition of chylomicrons after incubation with serum lipoprotein fractions

Incubated Fraction	Total Protein [a] / Triglycerides	Phospholipids [a] / Triglycerides	ARP [a] / Triglycerides	apoA-I [a] / Triglycerides	ARP [a] / Total Protein	apoA-I [a] / Total Protein
No incubation (n = 4)	0.68 ± 0.039	6.32 ± 0.51	0.032 ± 0.005	0.21 ± 0.019	4.57 ± 0.45	29.9 ± 2.72
0.15 M NaCl (n = 5)	0.80 ± 0.036	6.02 ± 0.42	0.032 ± 0.009	0.14 ± 0.021	4.60 ± 0.90	16.4 ± 1.49
P^b	NS	NS	NS	0.06	NS	<0.01
d > 1.006 g/ml (n = 5)	2.07 ± 0.12	4.38 ± 0.29	0.16 ± 0.026	0.12 ± 0.012	7.77 ± 0.83	5.9 ± 0.68
P^c	<0.01	<0.02	<0.05	NS	<0.05	<0.01
LDL (n = 4)	0.85 ± 0.021	5.02 ± 0.18	0.045 ± 0.005	0.11 ± 0.004	5.22 ± 0.47	12.7 ± 0.73
P^c	NS	NS	NS	NS	NS	NS
HDL (n = 5)	1.47 ± 0.11	4.83 ± 0.09	0.057 ± 0.006	0.19 ± 0.046	3.83 ± 0.28	12.3 ± 2.84
P^c	<0.01	<0.05	<0.05	NS	NS	NS
d > 1.21 g/ml (n = 4)	1.17 ± 0.052	4.90 ± 0.07	0.36 ± 0.046	0.13 ± 0.02	30.00 ± 6.10	11.9 ± 2.39
P^c	<0.01	<0.05	<0.01	NS	<0.01	NS

[a] wt/wt × 100; mean ± SEM.
[b] Difference from no incubation by Student's t test (NS, not significant).
[c] Difference from incubation with 0.15 M NaCl.
Lymph chylomicrons (0.35–0.45 mg protein) were incubated with serum lipoprotein fractions from 10 ml of serum (diluted to 10 ml with 0.15 M NaCl), for 1 hr at 23°C. The incubation mixture was centrifuged at 1.6×10^6 g-av min and the chylomicrons were applied to a 1.2 × 90 cm column of 2% agarose gel. The void volume was collected and concentrated by centrifugation as before.

Increments of ARP in chylomicrons incubated with serum protein fractions of d > 1.21 and d > 1.006 g/ml and with HDL were 11-, 5.0-, and 1.8-fold, respectively. The ratio of immunoassayable apoA-I to triglycerides fell in chylomicrons incubated with 0.15 M NaCl. A similar reduction occurred in chylomicrons incubated with the d > 1.006 g/ml and d > 1.21 g/ml fractions, whereas little change occurred upon incubation with HDL. In addition to the increment of ARP in chylomicrons incubated with fractions containing lipoproteins of density <1.21 g/ml, an obvious gain in C apoproteins was evident in SDS–polyacrylamide gels (Figs. 6 and 7).

As a result of these changes, the content of apoA-I as percent of total protein invariably fell in incubated chylomicrons (Table 3). Content of ARP, as percent of total protein, increased significantly in chylomicrons incubated with serum protein fractions of d

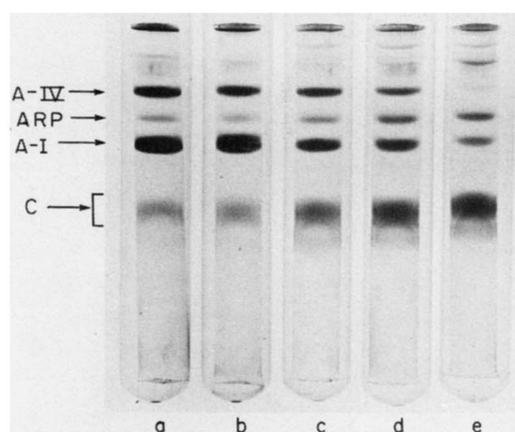

Fig. 6. SDS–polyacrylamide gel electropherograms of apoproteins of large chylomicrons incubated with different amounts of the density fraction >1.006 g/ml from blood serum for 1 hr at 23°C. Chylomicrons containing 450 μg of protein were incubated with: 10 ml of 0.15 M NaCl (*b*); 5, 10, and 20 ml of the density fraction >1.006 g/ml (*c*, *d*, and *e*, respectively), and reisolated by ultracentrifugation and gel chromatography. Gel *a* represents the unincubated control sample. Approximately 50 μg of protein was applied to each gel.

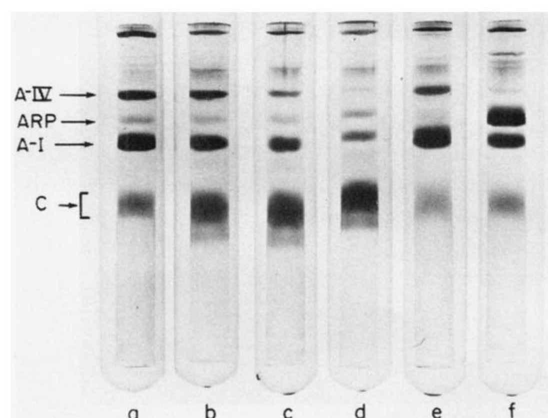

Fig. 7. SDS–polyacrylamide gel electropherograms of apoproteins of large chylomicrons incubated with HDL and the density fraction >1.21 g/ml from blood serum for 1 hr at 23°C. Chylomicrons containing about 400 μg of protein were incubated with HDL from 5, 10, and 20 ml of blood serum (*b*, *c*, and *d*, respectively); 10 ml of 0.15 M NaCl (*e*); 5 ml of the density fraction >1.21 g/ml (*f*) and reisolated by ultracentrifugation and gel chromatography. Gel *a* represents the unincubated control sample. Approximately 50 μg of protein was applied to each gel.

Downloaded from www.jlr.org by guest, on June 19, 2012
ASBMB JOURNAL OF LIPID RESEARCH

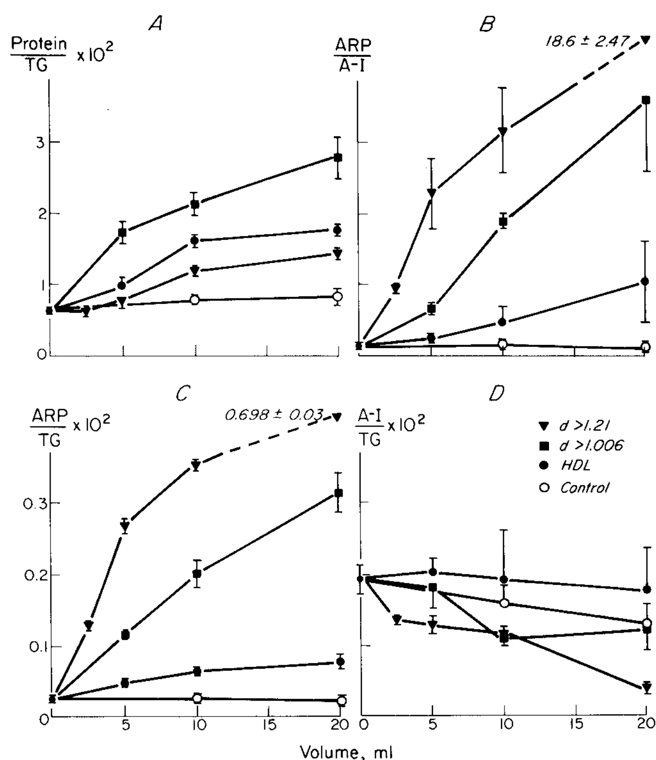

Fig. 8. Apoprotein composition of chylomicrons after incubation with different amounts of serum lipoprotein fractions. Large lymph chylomicrons (450 μg protein) were incubated with 2.5, 5, 10, and 20 ml of serum lipoprotein fractions indicated. Control represents incubation with 0.15 M NaCl. Incubations were as described in Table 3. Each point represents the mean ± SEM for three experiments.

> 1.006 and d > 1.21 g/ml. Apoprotein A-IV, evaluated by densitometric scanning of SDS–polyacrylamide electropherograms, varied directly with apoA-I (Figs. 6 and 7).

Incubation of chylomicrons with LDL (1.006 < d < 1.063 g/ml) (Table 3) or with VHDL (1.21 < d < 1.25 g/ml) (data not shown) did not alter protein composition significantly. The ratio of ARP to triglycerides in chylomicrons incubated with the serum fraction of d > 1.25 g/ml was 11.7 times higher than that of chylomicrons incubated with 0.15 M NaCl ($P < 0.005$).

Incubation of chylomicrons with 0.15 M NaCl altered neither the ratio of phospholipids nor that of protein to triglycerides, but the increased ratio of protein to triglycerides produced by incubation with the d > 1.006 and d > 1.21 g/ml fractions and with HDL was accompanied by a significant reduction in the ratio of phospholipids to triglycerides (Table 3).

Effect of amount of serum lipoprotein fractions on composition of chylomicron proteins

When chylomicrons (0.45 mg protein) were incubated with increasing amounts of serum fractions of d > 1.006 and d > 1.21 g/ml, with HDL, and with 0.15 M NaCl, recovery of triglycerides was unaffected ($P > 0.1$). The increment in total protein and ARP recovered in chylomicrons was a direct function of the amount of serum lipoproteins (expressed as equivalent volume of original serum) in the incubation mixture (Fig. 7 and **Fig. 8**, *A* and *C*). The ratio of apoA-I to triglycerides did not change significantly from that of chylomicrons incubated with 0.15 M NaCl for volumes up to 10 ml of serum equivalent, but the ratio fell when chylomicrons were incubated with 20 ml of the d > 1.21 g/ml fraction (Fig. 8*C*). The ratio of ARP to apoA-I increased substantially with the larger amounts of all serum lipoproteins (Fig. 8*B*). The increment in C apoproteins was also related to volume of serum fractions (Figs. 6 and 7). Again, apoA-IV followed the pattern of apoA-I.

Effect of temperature on the composition of incubated chylomicrons

Lymph chylomicrons were incubated with 10 ml or 20 ml of serum fractions of d > 1.006 g/ml at 4°C and 23°C (**Fig. 9**). The ratio of total protein to triglycerides increased in the incubated chylomicrons with 20 ml (but not with 10 ml) of d > 1.006 g/ml serum fractions at 4°C when compared to that with saline (4- to 4.5-fold). The ratio of ARP to triglycerides in chylomicrons incubated at 4°C for 1 hr increased 5.7- and 6.8-fold (for 10 ml and 20 ml, respectively) when compared to chylomicrons incubated with 0.15 M NaCl, but reached only 0.66 and 0.80, respectively, of the value for chylomicrons incubated at 23°C. Transfer of ARP also occurred when chylomicrons were simply mixed with 10 and 20 ml of ice-cold d > 1.006 g/ml serum fraction and immediately separated by

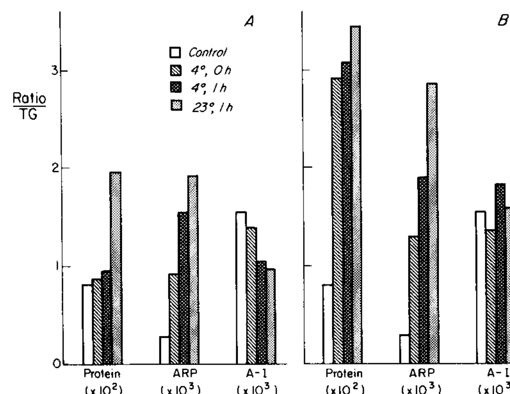

Fig. 9. Effect of temperature and duration of incubation on the protein composition of large lymph chylomicrons exposed to density fraction >1.006 g/ml of blood serum. Lymph chylomicrons containing 450 μg of protein were incubated with 10 ml (*A*) or 20 ml (*B*) of the fraction. Control value is for unincubated chylomicrons.

Downloaded from www.jlr.org by guest, on June 19, 2012

centrifugation at 4°C (3.3- and 4.6-fold increments for 10 ml and 20 ml, respectively). Content of apoA-I changed little at either temperature.

Effect of recombination of the serum fraction of d > 1.21 g/ml with HDL on protein composition of chylomicrons

Because the ratio of ARP to triglycerides was higher in chylomicrons incubated with the d > 1.21 g/ml serum fraction than with the fraction of d > 1.006 g/ml (Table 3, Fig. 6) whereas little change was observed with LDL (1.006 < d < 1.063), we examined the effect of reconstituting a d > 1.063 g/ml fraction by combining HDL with the d > 1.21 g/ml fraction. Recovery of triglycerides in incubated chylomicrons was unaffected ($P > 0.5$). The increment of protein and ARP for chylomicrons incubated with serum fractions of d > 1.006 and d > 1.21 g/ml and with HDL followed the pattern described above (Table 3). The increment of total protein was significantly lower for the reconstituted serum fraction of d > 1.063 g/ml (protein/triglycerides = 0.0081) than for the d > 1.006 g/ml fraction (protein/triglycerides = 0.0141) ($P < 0.005$). By contrast, the increment of ARP was greater for incubation with the reconstituted fraction (ARP/triglycerides = 0.0023) than for the d > 1.006 g/ml fraction (ARP/triglycerides = 0.0012) ($P < 0.005$). The increment of total protein for the reconstituted fraction was similar to that for incubation with HDL, whereas the increment for ARP was virtually identical to that for incubation with the d > 1.21 g/ml serum fraction.

Incubation of chylomicrons with serum or VLDL

Experiments with whole serum or VLDL required that incubated lymph chylomicrons be separated from large triglyceride-rich particles in serum or VLDL. Therefore the serum used in these experiments was centrifuged briefly at 1.6×10^4 g-av min to decrease the number of large particles. VLDL and d > 1.006 g/ml serum fractions were obtained by centrifuging this serum at 1×10^8 g-av min. After the incubation, chylomicrons were separated on a 2.5 × 90 cm column of 2% agarose gel (16). Even with this larger column the elution of chylomicrons slightly overlapped that of VLDL. To avoid contamination by VLDL in the chylomicron fraction, only the first three-quarters of the peak eluting in the void volume was collected. Recovery of triglycerides was therefore lower than in other experiments (52.4 ± 3.0%, mean + SEM, $n = 10$), but it was constant for all incubations. Recovery of total protein again increased in chylomicrons after incubation with serum, VLDL, and the d > 1.006 g/ml fraction (**Table 4**). The increment of protein and ARP in chylomicrons incubated with serum was somewhat higher than that obtained after incubation with the d > 1.006 g/ml fraction. Changes in concentration of A-I were comparable. The apoprotein pattern in SDS–polyacrylamide gel electropherograms was also similar when serum or the d > 1.006 g/ml fraction was used (Fig. 6 and **Fig. 10**). Incubation with VLDL caused a slight increase in content of both total protein and ARP in chylomicrons (Table 4).

Effect of centrifugation on composition of chylomicron apoproteins

It is evident that the concentration of apoA-I was reduced when chylomicrons were incubated with 0.15 M NaCl and re-isolated (Table 3, Fig. 6). To explore this phenomenon, lymph chylomicrons purified by gel chromatography were subjected to sequential centrifugation (1.6×10^6 g-av min) and apoproteins A-I and ARP were quantified by radioimmunoassay in floating chylomicrons and in the infranatant solution. Recovery was 98% for triglycerides and 95% for total protein. The ratio of protein to triglycerides in chylomicrons fell only slightly and that of ARP to triglycerides remained constant, but the ratio of

TABLE 4. Composition of chylomicrons incubated with serum or VLDL

Incubated Fraction	Total Protein / Triglycerides [a]	Phospholipids / Triglycerides [a]	ARP / Triglycerides [a]	apoA-I / Triglycerides [a]	ARP / Total Protein [a]	apoA-I / Total Protein [a]
d > 1.006 g/ml	1.97 ± 0.12	4.07 ± 0.25	0.15 ± 0.013	0.11 ± 0.018	7.41 ± 0.22	5.53 ± 0.78
Whole serum (n = 3)	2.27 ± 0.32	4.32 ± 0.51	0.24 ± 0.039	0.080 ± 0.012	10.52 ± 0.75	3.58 ± 0.23
P [b]	NS	NS	NS	NS	<0.02	NS
VLDL (n = 3)	1.12 ± 0.014	4.72 ± 0.05	0.060 ± 0.002	0.063 ± 0.011	5.43 ± 0.20	5.67 ± 0.87
P [b]	<0.01	NS	<0.01	NS	<0.05	NS

[a] wt/wt × 100; mean ± SEM.
[b] Difference from value for incubation with d > 1.006 g/ml fraction by Student's t test (NS, not significant).
Lymph chylomicrons (0.3 mg protein) were incubated with 10 ml of serum, VLDL, or d > 1.006 g/ml fraction as described in Table 3 and purified on a 2.5 × 90 cm column of 2% agarose gel.

Downloaded from www.jlr.org by guest, on June 19, 2012
ASBMB JOURNAL OF LIPID RESEARCH

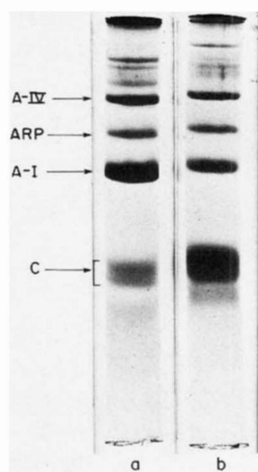

Fig. 10. SDS–polyacrylamide gel electropherograms of chylomicrons incubated with blood serum. Large lymph chylomicrons containing 300 μg of protein were incubated with 5 ml of blood serum for 1 hr at 23°C and reisolated by ultracentrifugation and gel chromatography. *a*, Unincubated chylomicrons; *b*, chylomicrons incubated with blood serum. Approximately 50 μg of protein was applied to each gel.

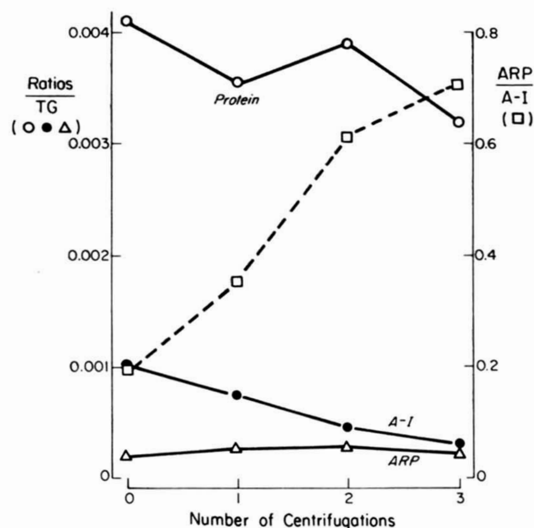

Fig. 11. Effect of centrifugation on chylomicron apoproteins. Large lymph chylomicrons purified by chromatography on 2% agarose gel were subjected to three centrifugations at 1.6×10^6 g-av min. Content of protein, ARP, and A-I in recovered chylomicrons was measured and related to content of triglycerides.

apoA-I to triglycerides decreased progressively (**Fig. 11**). The apoA-I lost from chylomicrons was recovered in the infranatant solution. After three centrifugations, 95 μg of apoA-I had been lost from 1 mg of chylomicron-protein and 91 μg were recovered in the combined infranatant fractions. This loss of apoA-I during centrifugation is further emphasized by the plot of the ratio of ARP to apoA-I with increasing number of centrifugations (Fig. 11).

DISCUSSION

Several reports have indicated the presence of proteins in rat lymph chylomicrons that cross-react with the major immunoreactive protein in blood plasma HDL (2, 25). In HDL, apoA-I is the predominant protein species and, as reported previously (5) and confirmed here, apoA-I is a major protein component of chylomicrons in rat mesenteric lymph. Like VLDL in blood plasma, lymph chylomicrons contain both large molecular weight B apoprotein and small molecular weight C apoproteins, but the components of intermediate molecular weight differ. ARP comprises almost all of the protein of intermediate molecular weight in rat VLDL (5, 6), but only a minor portion of that of lymph chylomicrons.[1] This virtually categorical difference from plasma VLDL holds for both large and small lymph chylomicrons obtained from fat-fed rats (3) and for the total d < 1.006 g/ml lipoproteins from lymph of glucose-fed rats. For this reason, the designation "small chylomicrons" (3) seems more appropriate to us than "lymph VLDL" for the triglyceride-rich particles in lymph that resemble VLDL of blood plasma in size and overall lipid and protein composition.

The same proteins of intermediate molecular weight are present in lymph chylomicrons and plasma HDL, although the latter contain relatively more ARP (6, 21). ApoA-I seems to be actively synthesized by the intestinal mucosa. The major chylomicron protein of intermediate molecular weight has been shown to be rapidly labeled when animals are fed radioactive amino acids together with fat (1, 2), and mucosal scrapings of rat small intestine incorporate radioactive amino acids into apoA-I (27). Recently, specific labeling of apoA-I in mesenteric lymph lipoproteins has been observed in rats given 4-aminopyrazolopyrimidine to prevent hepatic secretion of lipoproteins (28) and we have shown that apoA-I in HDL of blood plasma contributes less than 10% of the apoA-I in mesenteric lymph chylomicrons.[2] The extent to which ARP in lymph chylomicrons is synthesized in the intestine is not clear (1, 2). When mixed with plasma lipoproteins, ARP, like the C apoproteins, is transferred to chylomicrons, so that the small amount found in mesenteric lymph chylomicrons may be derived from lipoproteins that are transported from blood plasma to lymph.

[1] From published data, plasma VLDL of rats contain 30% apoB (26), 19% ARP (6), 0.1% apoA-I (5), and (by difference) 51% C apoproteins (% wt/wt).

[2] Unpublished observations.

Downloaded from www.jlr.org by guest, on June 19, 2012

ASBMB JOURNAL OF LIPID RESEARCH JLR

The results of the present research support the concept that exposure to lipoproteins in blood plasma modifies the surface of triglyceride-rich lipoproteins. As indicated from previous investigations, transfer of C apoproteins contributes to this modification for VLDL (29) as well as chylomicrons (3, 4, 8, 9). We have now shown that ARP is also transferred to chylomicrons. The content of ARP increased six-fold when chylomicrons containing 52 mg of triglycerides were exposed to 10 ml of serum (final concentration of triglycerides ≅500 mg/dl) (Table 4). However, the calculated increase in C apoprotein mass exceeded that of ARP. Of the protein transferred in this experiment, approximately 88% was C apoproteins and this group of proteins comprised about 75% of the total protein of the incubated chylomicrons. Enzymatic activity evidently is not required for these transfers because they occurred with very short incubations at 4°C (Fig. 9). As shown previously (7, 30), the transfer of protein to chylomicrons was accompanied by loss of phospholipids (Table 3), suggesting that the proteins replace phospholipids on the surface of the particle. A similar process of exchange occurs when nascent VLDL obtained from a Golgi apparatus-rich fraction of rat liver are exposed to plasma HDL (29).

Transfer of ARP was greatest when chylomicrons were incubated with the serum fraction of d > 1.21 g/ml. Content of ARP increased more than 10-fold when chylomicrons were incubated with 10 ml of this serum fraction (Table 3) and only about six-fold when incubation was with a comparable amount of serum (Table 4). This significantly greater transfer of ARP is presumably the result of dissociation of ARP from lipoproteins induced by the effect of centrifugation and/or high salt concentration (31). This interpretation is supported by observation that more ARP was transferred from the combined HDL and d > 1.21 g/ml fractions than from the d > 1.006 fraction. Little ARP was transferred from VLDL to chylomicrons; hence, the bulk of the ARP transferred to chylomicrons during incubation with native serum presumably is derived from HDL.

Incubation with serum or lipoprotein fractions reduced the content of apoA-I in chylomicrons, but only to the extent that occurred when incubation was with 0.15 M NaCl. This loss of apoA-I is evidently caused by centrifugation (Fig. 11). The actual content of apoA-I in chylomicrons as they exist in native lymph may therefore be somewhat higher than indicated by the data in Table 1. As in the case of rat HDL (22), the content of apoA-IV in chylomicrons varied with that of apoA-I. Recently Robinson and Quarfordt (32) obtained results that generally confirm ours with respect to reduction of apoA-I and increase of ARP in chylomicrons exposed to serum. However, they did not attribute the absolute loss of apoA-I that they observed to ultracentrifugal dissociation.

The greatest dilution of chylomicrons in serum tested in the present study was to a concentration of about 340 mg triglycerides per dl. Thus our observations may underestimate the extent of the changes that normally occur when chylomicrons enter the bloodstream. As a result of these changes, the protein composition of chylomicrons in blood plasma comes to resemble more closely that of VLDL derived from the liver. However, the original complement of apoA-I evidently remains associated with the chylomicrons. Therefore, if apoA-I transported from the intestine in chylomicrons is transferred to HDL, this transfer must occur in connection with the formation or catabolism of chylomicron remnants.

We thank Agnes Frank, Leila Kotite, Anneliese Merlino, and Ksenia Tomitch for expert technical assistance and John Kane for amino acid analysis of chylomicron apoA-IV. Rat anti apoA-IV, prepared from blood serum HDL as described in reference 21, was the generous gift of John Swaney. Dr. Imaizumi was a Research Fellow of the Bay Area Heart Association. Dr. Fainaru was on leave from the Lipid Research Laboratory, Department of Medicine B, Hadassah Medical School, Jerusalem, Israel and received support from the Hadassah Medical Organization. This work was supported by Arteriosclerosis SCOR Grant HL-14237 from the U.S. Public Health Service.

Manuscript received 16 September 1977; accepted 18 January 1978.

REFERENCES

1. Windmueller, H. G., P. N. Herbert, and R. I. Levy. 1973. Biosynthesis of lymph and plasma lipoprotein apoproteins by isolated perfused rat liver and intestine. *J. Lipid Res.* **14:** 215–223.
2. Glickman, R. M., and K. Kirsch. 1973. Lymph chylomicron formation during the inhibition of protein synthesis. *J. Clin. Invest.* **52:** 2910–2920.
3. Mjøs, O. D., O. Faergeman, R. L. Hamilton, and R. J. Havel. 1975. Characterization of remnants produced during the metabolism of triglyceride-rich lipoproteins of blood plasma and intestinal lymph in the rat. *J. Clin. Invest.* **56:** 603–615.
4. Fielding, C. J., and P. E. Fielding. 1976. Chylomicron protein content and the rate of lipoprotein lipase activity. *J. Lipid Res.* **17:** 419–423.
5. Fainaru, M., R. J. Havel, and T. E. Felker. 1976. Radioimmunoassay of apolipoprotein A-I of rat serum. *Biochim. Biophys. Acta* **446:** 56–68.
6. Fainaru, M., R. J. Havel, and K. Imaizumi. 1977. Radioimmunoassay of arginine-rich apoprotein of rat serum. *Biochim. Biophys. Acta* **490:** 144–155.
7. Lossow, W. J., F. T. Lindgren, and L. C. Jensen. 1967. Net uptake of rat serum protein by S_f > 400 lymph chylomicrons in vitro. *Biochim. Biophys. Acta* **144:** 670–677.

Downloaded from www.jlr.org by guest, on June 19, 2012

8. Havel, R. J., J. P. Kane, and M. L. Kashyap. 1973. Interchange of apolipoproteins between chylomicrons and high density lipoproteins during alimentary lipemia in man. *J. Clin. Invest.* **52:** 32–38.
9. Glickman, R. M., and K. Kirsch. 1974. The apoproteins of various size classes of human chylous fluid lipoproteins. *Biochim. Biophys. Acta* **371:** 255–266.
10. Imaizumi, K., M. Fainaru, and R. J. Havel. 1976. Transfer of apolipoproteins (A-I and ARP) between rat mesenteric lymph chylomicrons and serum lipoproteins. *Circulation* **53–54,** Suppl. II: 134.
11. Bollman, J. L., J. C. Cain, and J. H. Grindlay. 1948. Techniques for the collection of lymph from the liver, small intestine or thoracic duct of the rat. *J. Lab. Clin. Med.* **33:** 1349–1352.
12. Sata, T., D. L. Estrich, P. D. S. Wood, and L. W. Kinsell. 1970. Evaluation of gel chromatography for plasma lipoprotein fractionation. *J. Lipid Res.* **11:** 331–340.
13. Ouchterlony, D. 1968. Handbook of Immunodiffusion and Immunoelectrophoresis. Ann Arbor Science Publishers, Ann Arbor, Mich.
14. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.
15. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226:** 497–509.
16. Sata, T., R. J. Havel, and A. L. Jones. 1972. Characterization of subfractions of triglyceride-rich lipoproteins separated by gel chromatography from blood plasma of normolipemic and hyperlipemic humans. *J. Lipid Res.* **13:** 757–767.
17. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
18. Kane, J. P., T. Sata, R. L. Hamilton, and R. J. Havel. 1975. Apoprotein composition of very low density lipoproteins of human serum. *J. Clin. Invest.* **56:** 1622–1634.
19. Weber, K., and M. Osborn. 1959. The reliability of molecular weight determinations by dodecyl sulfate–polyacrylamide gel electrophoresis. *J. Biol. Chem.* **244:** 4406–4412.
20. Pagnan, A., R. J. Havel, J. P. Kane, and L. Kotite. 1977. Characterization of human very low density lipoproteins containing two electrophoretic populations: double pre-beta lipoproteinemia and primary dysbetalipoproteinemia. *J. Lipid Res.* **18:** 613–622.
21. Swaney, J. B., F. Braithwaite, and H. A. Eder. 1977. Characterization of the apolipoproteins of rat plasma lipoproteins. *Biochemistry* **16:** 271–278.
22. Felker, T. E., M. Fainaru, R. L. Hamilton, and R. J. Havel. 1977. Secretion of the arginine-rich and A-I apolipoproteins by the isolated perfused rat liver. *J. Lipid Res.* **18:** 465–473.
23. Herbert, P. N., H. G. Windmueller, T. P. Bersot, and R. S. Schulman. 1974. Characterization of the rat apolipoproteins. I. The low molecular weight proteins of rat plasma high density lipoproteins. *J. Biol. Chem.* **249:** 5718–5724.
24. Gidez, L. I., J. B. Swaney, and S. Murnane. 1977. Analysis of rat serum apolipoproteins by isoelectric focusing. I. Studies on the middle molecular weight subunits. *J. Lipid Res.* **18:** 59–68.
25. Ockner, R. K., K. J. Block, and K. J. Isselbacher. 1968. Very low density lipoprotein in intestinal lymph: evidence for presence of the A protein. *Science* **162:** 1285–1286.
26. Faergeman, O., T. Sata, J. P. Kane, and R. J. Havel. 1975. Metabolism of apoprotein B of plasma very low density lipoproteins in the rat. *J. Clin. Invest.* **56:** 1396–1403.
27. Rooke, J. A., and R. Skinner. 1976. The biosynthesis of rat serum apolipoproteins by liver and intestinal mucosa. *Biochem. Soc. Trans.* **4:** 1144–1145.
28. Glickman, R. M., and P. H. R. Green. 1977. The intestine as a source of apolipoprotein A-I. *Proc. Natl. Acad. Sci. USA* **74:** 2567–2573.
29. Hamilton, R. L. 1972. Synthesis and secretion of plasma lipoproteins. *In* Adv. Exp. Med. Biol. Vol. 26: Pharmacological Control of Lipid Metabolism. W. L. Holmes, R. Paoletti, and D. Kritchevsky, editors. Plenum Press, New York. 7–24.
30. Minari, O., and D. B. Zilversmit. 1963. Behavior of dog lymph chylomicron lipid constituents during incubation with serum. *J. Lipid Res.* **4:** 424–436.
31. Fainaru, M., R. J. Havel, and K. Imaizumi. 1977. Apoprotein content of plasma lipoproteins of the rat separated by gel chromatography of ultracentrifugation. *Biochem. Med.* **17:** 347–355.
32. Robinson, S. F., and S. H. Quarfordt. 1977. Chylomicron apoprotein alteration after plasma exposure. *Clin. Res.* **25:** 317A.

Downloaded from www.jlr.org by guest, on June 19, 2012