Lipoprotein metabolism in the suckling rat: characterization of plasma and lymphatic lipoproteins

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Abstract Suckling rat plasma contains (in mg/dl): chylomicrons (85 ± 12); VLDL (50 ± 6); LDL (200 ± 23); HDL_1 (125 ± 20); and HDL_2 (220 ± 10), while lymph contains (in mg/dl): chylomicrons (9650 ± 850) and VLDL (4570 ± 435) and smaller amounts of LDL and HDL. The lipid composition of plasma and lymph lipoproteins are similar to those reported for adults, except that LDL and HDL_1 have a somewhat higher lipid content. The apoprotein compositions of plasma lipoproteins are similar to those of adult lipoproteins except for the LDL fraction, which contains appreciable quantities of apoproteins other than apoB. Although the LDL fraction was homogeneous by analytical ultracentrifugation and electrophoresis, the apoprotein composition suggests the presence of another class of lipoproteins, perhaps a lipid-rich HDL_1. The lipoproteins of lymph showed low levels of apoproteins E and C. The triacylglycerols in chylomicrons and VLDL of both lymph and plasma are rich in medium-chain-length fatty acids, whereas those in LDL and HDL have little or none. Phospholipids in all lipoproteins lack medium-chain-length fatty acids. The cholesteryl esters of the high density lipoproteins are enriched in arachidonic acid, whereas those in chylomicrons, VLDL, and LDL are enriched in linoleic acid, suggesting little or no exchange of cholesteryl esters between these classes of lipoproteins. The fatty acid composition of phosphatidylcholine, sphingomyelin, and lysophosphatidylcholine were relatively constant in all lipoprotein fractions, suggesting ready exchange of these phospholipids. However, the fatty acid composition of phosphatidylethanolamine in plasma chylomicrons and VLDL differed from that in plasma LDL, HDL_1, and HDL_2. LDL, HDL_1, and HDL_2 were characterized by analytical ultracentrifugation and shown to have properties similar to that reported for adult lipoproteins. The much higher concentration of triacylglycerol-rich lipoproteins in lymph, compared to plasma, suggests rapid clearance of these lipoproteins from the circulation.—**Fernando-Warnakulasuriya, G. J. P., M. L. Eckerson, W. A. Clark, and M. A. Wells.** Lipoprotein metabolism in the suckling rat: characterization of plasma and lymphatic lipoproteins. *J. Lipid Res.* 1983. **24:** 1626–1638.

Supplementary key words apoproteins • lipid composition • fatty acid composition • analytical ultracentrifugation

Milk lipids, predominately triacylglycerols, provide about 70% of the energy intake in the suckling rat (1). In spite of the importance of lipid digestion, absorption, and transport during the neonatal period, relatively few studies have been directed towards characterization of these processes. Recent studies on fat digestion and absorption have provided data which show that the processes operative during the suckling period are different in several details from the well-studied processes in adults (2, 3). Lipoprotein metabolism has been extensively studied in man and the adult rat, but only one study on the suckling rat has appeared, in which lipoprotein composition was examined solely by gel electrophoresis (4). As a first step in investigating lipoprotein metabolism in suckling rats, we have isolated and characterized the lipoproteins present in lymph and plasma.

MATERIALS AND METHODS

Sprague-Dawley rats, 14–15 days old, from a colony maintained by the Division of Animal Resources, were used in all experiments (2). Animals were used within 30 min of removal from the mother to maintain the nearly continuous fed-state characteristic of suckling rats (1). Blood was collected under diethyl ether anesthesia via aortic puncture using EDTA as the anticoagulant, and stored at 4°C until all samples were collected. Plasma was separated by centrifuging the blood in a clinical centrifuge for 20–30 min at 4°C. Mesenteric lymph was collected into chilled tubes which contained 0.1 ml of a solution containing 0.4% sodium azide, 1 mM diisopropylfluorophosphate, and 0.1 M EDTA as described (5). Generally lymph was collected for 2–3 hr.

Lipoprotein isolation

Solutions of different densities were prepared according to Redgrave, Roberts, and West (6). Chylomicrons

Abbreviations: LCAT, lecithin:cholesterol acyltransferase; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.

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were separated from plasma or lymph by centrifugation using a Beckman SW-60 (Beckman Instruments, Inc., Palo Alto, CA) rotor for 3×10^6 *g*-min at 5°C, and the centrifuge was stopped without braking. The tube was punctured at the bottom and chylomicron-free plasma or lymph was removed. The chylomicrons remaining in the tubes were transferred into vials using a solution of d 1.006 g/ml. The chylomicron suspension was diluted to 10 ml with the same salt solution, adjusted to a d 1.30 g/ml with KBr, and placed in a 39-ml seal-top tube for the VTi 50 vertical rotor (Beckman). This solution was overlaid with a d 1.006 g/ml NaCl solution and centrifuged in an L8-70 centrifuge (Beckman) at 4°C and 50,000 rpm for 4.5 hr. The rotor was accelerated using the slow accelerate mode and the brake was turned off when the rotor had slowed to 2000 rpm. The chylomicrons, free of plasma contaminants, were collected from the meniscus of the tube.

The lipoproteins in the chylomicron-free plasma were isolated in two ways. For total analysis of the lipoproteins, they were separated by density gradient centrifugation using a modification of the method of Redgrave et al. (6). Three ml of chylomicron-free plasma (or lymph) was placed in the bottom of a centrifuge tube (SW-41) and mixed with 0.975 g of KBr. This solution was successively overlaid with 3.2 ml of d 1.063 g/ml, 3.2 ml of d 1.019 g/ml, and 2.6 ml of d 1.006 g/ml solutions. The sample was centrifuged at 34,000 rpm for 48 hr at 5°C (we obtained better separation of lipoproteins if the centrifugation was carried out at 5°) and stopped without braking. One-ml fractions were collected starting from the top of the tube. This was the only procedure used to isolate lymph lipoproteins.

For preparative isolation of plasma lipoproteins, separations were carried out in a vertical rotor using a modification of the procedure of Chung et al. (7). Ten ml of chylomicron-free plasma was adjusted to a density of 1.30 g/ml with KBr and placed in a 39-ml seal-top centrifuge tube for the VTi 50 rotor (Beckman). This solution was overlaid with a d 1.21 g/ml KBr–NaCl solution and centrifuged as described above for chylomicron purification. As in the case of the density gradient separation, we achieved better separation if the centrifugation was carried out at a low temperature. Total plasma lipoproteins were recovered from the top 7–10 ml of the gradient. The solution was adjusted to a density of 1.30 g/ml with KBr (in some cases 60 μl of a 1 mg/ml solution of Sudan Black in ethylene glycol was added to aid in visualization of the lipoprotein bands), made to a volume of 11.5 ml, overlaid with a d 1.006 g/ml NaCl solution, and centrifuged in the vertical rotor as described above. The seal at the top of the tube was removed, lipoprotein bands were located using indirect light (or via Sudan Black staining), and the bands were removed sequentially starting from the top. The tube was punctured with an 18-gauge needle just below the band at the meniscus and the material was removed from the tube with a syringe. The fluid in the tube between the bottom of the band and the top of the next band was then removed. This process was repeated until all bands were recovered. Adult (300-g animals) lipoproteins were isolated in a similar manner. In order to increase the content of adult plasma chylomicrons, the animals were given 3 ml of corn oil by stomach tube 2 hr before bleeding.

The density of each fraction from the SW-41 density gradient was measured in a 1-ml pycnometer at 5°C using samples from gradients run without lipoproteins.

Analyses

For compositional analyses, the lipoprotein fractions were dialyzed against 0.15 M NaCl, 1 mM EDTA (pH 7.0) at 4°C overnight using a BRL microdializer (Bethesda Research Laboratories, Inc., Gaithersburg, MD). In all cases the data presented represent the results of analyses carried out on three to five separate lipoprotein preparations. Lipoproteins were characterized by polyacrylamide gel electrophoresis according to the method of Naito et al. (8). The refractive index of each lipoprotein fraction was measured relative to the dialysate, using a Brice-Phoenix Differential Refractometer (VirTis, Gardiner, NY), in order to determine the concentration of lipoproteins (9). Protein was assayed according to Kashyap, Hynd, and Robinson (10) using bovine serum albumin as a standard, except that samples containing chylomicrons were extracted with peroxide-free diethyl ether after color development. Phosphorus content was measured by the Bartlett method (11). Total cholesterol was measured using gas–liquid chromatography by the following method. The sample was dried in a screw-capped tube, 50–100 μl of stigmasterol (a gift from Dr. Henry Kircher) (1 mg/ml) in ethanol was added as an internal standard followed by 500 μl of 2 M sodium methoxide solution in methanol. The mixture was allowed to react at room temperature for 90 min (these conditions gave complete hydrolysis of cholesteryl esters). After adding 1 ml of water and 2 ml of isooctane, the isooctane layer was analyzed using a Hewlett-Packard 403 gas chromatograph equipped with a 3380A intergrator. The columns (glass, 20 in, i.d. ⅛ in) were filled with 3% JXR 100/120 Gas-Chrom Q (Applied Science Labs, State College, PA) and separations were carried out at 230°C using helium gas as the carrier at a flow rate of 50 ml/min. Lecithin:cholesterol acyltransferase (LCAT) activity was assayed as described by Pinon, Bridoux, and Laudat (12).

All the lipoprotein fractions, except chylomicrons, were extracted for lipid analyses by the method of Bligh and Dyer (13). The chylomicron fractions were extracted by the method of Folch, Lees, and Sloan Stanley (14). The

lipids of each fraction were separated into cholesteryl ester, triacylglycerol, cholesterol, and phospholipid as described (2). The phospholipid fraction was separated into phosphatidylethanolamine, phosphatidylcholine, sphingomyelin, and lysophosphatidylcholine by thin-layer chromatography using silica gel G plates and chloroform–methanol–acetic acid–water 50:30:8:3 (15). The lipids were analyzed for fatty acid composition and quantitated by the addition of internal standards as described (2). Sphingomyelins were transmethylated in 7% H_2SO_4 in anhydrous methanol using 10–200 nmol of sphingomyelin, a reaction volume of 0.5 ml, and heating at 60–63°C for 12–16 hr (16).

For apoprotein analysis, the lipoproteins were dialyzed against 10 mM ammonium carbonate, 1 mM EDTA, lyophilized, and extracted twice with chloroform–methanol 3:1 (v/v) (17). The apoproteins were suspended in buffer (0.125 M Tris, pH 6.8, 4% SDS, 20% glycerol, and 10% 2-mercaptoethanol) and heated in a boiling water bath for 15 min. The apoproteins were separated in a gradient slab gel system using the method of Laemmli (18). The gels (140 × 160 × 1.5 mm, Bio-Rad Laboratories, Richmond, CA) contained 30 ml of a linear gradient of 4–15% polyacrylamide, and 10 ml of a 4.0% stacking gel. The gels were run at 50 mA for 3 hr at 4°C and stained for 60 min at 40°C in 0.125% Coomassie Blue R-250 in 50% methanol and 10% acetic acid. They were destained in 50% methanol and 10% acetic acid for 18 hr, and in 5% methanol and 7% acetic acid until clear. Molecular weight standards were obtained from Bio-Rad, Pharmacia Fine Chemicals (Piscataway, NJ), and BRL. Apoproteins were identified by molecular weight and correspondence to apoproteins from adult lipoproteins.

Analytical ultracentrifugation

Total plasma lipoproteins (minus chylomicrons) were isolated using the vertical rotor system described above. Total plasma lipoproteins or lipoprotein fractions isolated by the vertical rotor method were dialyzed against either a d 1.21 g/ml NaBr or d 1.063 g/ml NaCl solution. Analytical ultracentrifugation was carried out in a Beckman Model E equipped with electronic speed control at 25°C and 52,000 rpm. Schlieren photographs were taken and data were analyzed as described by Ewing, Freeman, and Lindgren (19) and Lindgren (9).

RESULTS

Since plasma lipoproteins from suckling rats had not been extensively investigated, it was felt that the use of sequential centrifugations at fixed densities would not necessarily yield pure lipoprotein fractions. Therefore, we used the SW-41 density gradient centrifugation to obtain an initial characterization, and later developed the vertical rotor separation for preparative isolation of lipoproteins. The fractions obtained from the SW-41 gradient were essentially free of plasma contaminants as judged by the low level of albumin and other plasma proteins after electrophoresis of the apoproteins. However, in the case of the vertical rotor, a preliminary isolation of total plasma lipoproteins was necessary before the gradient separation in order to obtain fractions with low levels of albumin. Even if the same gradient system used in the SW-41 rotor was used in the vertical rotor, we found all lipoprotein fractions heavily contaminated with albumin. In the case of plasma chylomicrons, a second centrifugation in the vertical rotor was necessary to obtain albumin-free material.

The separation of lipoproteins from chylomicron-free plasma achieved by these methods is shown in **Fig. 1** and **Fig. 2.** Starting from the top of the tube there are four bands identified as VLDL, LDL, HDL_1, and HDL_2 (20, 21). The densities (g/ml) of the fractions collected from the SW-41 gradient were as follows: 1 = 1.015, 2 = 1.018, 3 = 1.022, 4 = 1.028, 5 = 1.037, 6 = 1.050, 7 = 1.066, 8 = 1.087, 9 = 1.113, 10 = 1.141, 11 = 1.173, and 12 = 1.202. Polyacrylamide gel electrophoresis of the fractions obtained from the SW-41 gradient separation

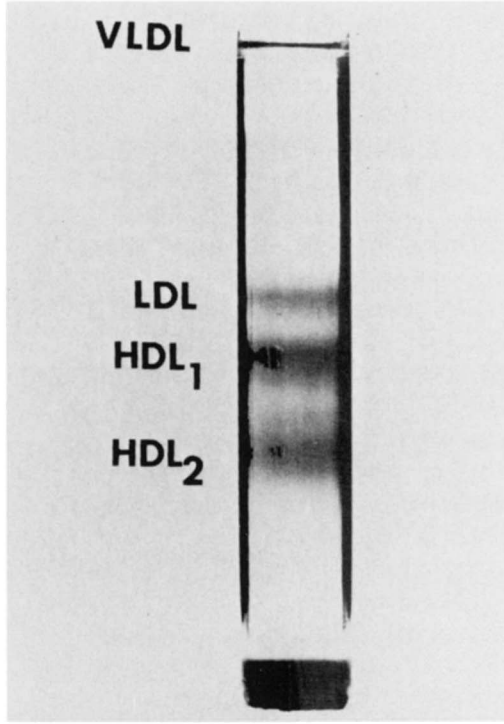

Fig. 1. Distribution of suckling rat lipoproteins from chylomicron-free plasma after centrifugation in the SW-41 rotor. Three ml of plasma, containing Sudan Black, was centrifuged as described in the text. The various lipoprotein are labeled. The material at the bottom of the tube is lipoprotein-free plasma.

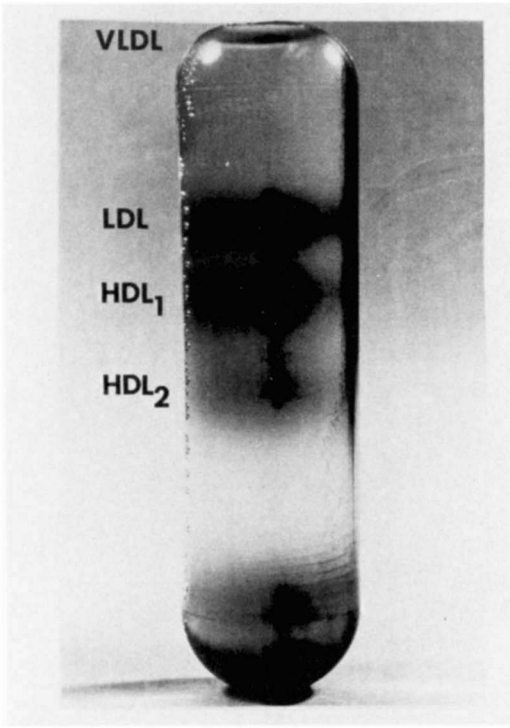

Fig. 2. Distribution of suckling rat lipoproteins from chylomicron-free plasma after centrifugation in the VTi-50 vertical rotor. Ten ml of plasma, containing Sudan Black, was centrifuged as described in the text. The various lipoproteins are labeled. The material at the bottom of the tube is lipoprotein-free plasma.

using plasma are shown in **Fig. 3,** patterns of the lipoproteins isolated using the vertical rotor are in **Fig. 4.** Although it appears that good separation of the four lipoprotein classes is achieved using the SW-41 rotor (Fig. 1), it is apparent from Fig. 3 that pure fractions were not obtained in all cases when the gradient was removed from the tube. On the other hand, the fractions obtained from the vertical rotor separation appear pure by electrophoresis (Fig. 4), although it may be noted that the HDL_2, and perhaps the HDL_1, fractions appear to contain two components.

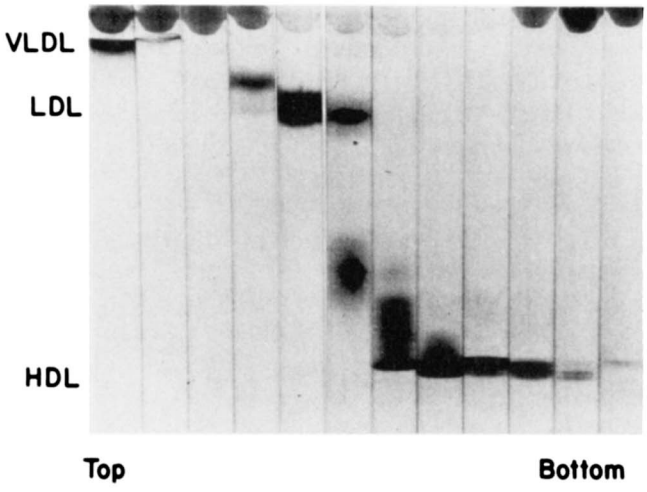

Fig. 3. Polyacrylamide gel electrophoresis of suckling rat plasma lipoproteins separated in the SW-41 rotor. The lipoproteins in each fraction were analyzed by polyacrylamide gel electrophoresis using Sudan Black to stain the lipoproteins. Electrophoresis was from top to bottom and the top of the gradient is to the left of the figure. The various lipoproteins are identified.

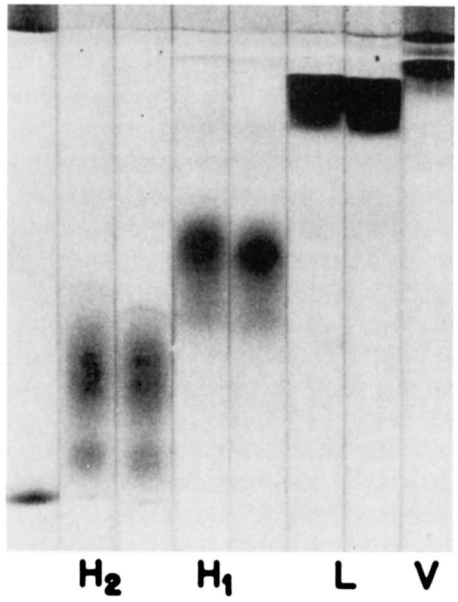

Fig. 4. Polyacrylamide gel electrophoresis of suckling rat plasma lipoproteins isolated using the VTi-50 vertical rotor. Electrophoresis was carried out as in Fig. 3. Lane 1, serum albumin; lanes 2 and 3, HDL_2 fraction; lanes 4 and 5, HDL_1 fraction; lanes 6 and 7, LDL fraction; lane 8, VLDL fraction.

The lipid and protein content of plasma chylomicrons and the plasma lipoproteins from the SW-41 density gradient separation are given in **Table 1** (fraction 12 from the gradient contains only traces of lipid and is not included in this or other tables). In the case of the density gradient fractions, we measured the density and lipoprotein content. As judged by electrophoresis and composition, the density gradient gives reasonably clean separation of VLDL (fractions 1–3) and LDL (fractions 4–6). HDL_1 and HDL_2 were not cleanly separated by this procedure (fractions 7–11). Based on these data the concentration of lipoproteins in plasma are as follows: VLDL ($d < 1.015$ g/ml), 52 ± 7 mg/dl; LDL (d 1.028–1.066 g/ml), 220 ± 29 mg/dl; $HDL_1 + HDL_2$ (d 1.066–1.173 g/ml), 310 ± 32 mg/dl.

In the case of the vertical rotor separation of plasma lipoproteins, the concentrations of the lipoproteins were as follows: VLDL 48 ± 10 mg/dl; LDL 175 ± 20 mg/dl; HDL_1 144 ± 15 mg/dl; and HDL_2 218 ± 20 mg/dl. In the case of plasma chylomicrons, the turbidity of the solutions was too great to allow measurement by refractometry. Their approximate concentration can be in-

TABLE 1. Concentration of components in suckling rat plasma lipoproteins isolated by density gradient centrifugation[a]

Sample	TG	CE	CH	PC	PE	SP	LPC	PR
CHY	750 (108)	28 (12)	30 (6)	52 (1.2)	7.6 (5.2)	3.7 (1.5)	6.1 (3.0)	73 (6.5)
Frn #1	328 (71)	29.2 (6.6)	100 (25)	124 (11.8)	13.1 (9.2)	16.6 (10.8)	4.6 (1.5)	24 (2)
#2	74 (14)	12.9 (0.7)	12.2 (6.7)	41.6 (14.5)	2.0 (0.9)	3.7 (1.6)	1.7 (1.2)	4.3 (1.0)
#3	12.0 (6.3)	16.6 (3.8)	11.6 (3.1)	28.5 (1.6)	1.5 (1.4)	3.8 (3.3)	2.6 (0.8)	11.2 (4.0)
#4	16.3 (4.8)	86.7 (30.7)	37.0 (2.8)	41.0 (9.0)	4.0 (1.9)	11.0 (7.7)	3.2 (1.1)	14.9 (6.3)
#5	34.9 (10.8)	371 (151)	180 (17)	247 (120)	5.9 (0.1)	36.0 (22.8)	10.4 (2.7)	164 (3.0)
#6	18.7 (6.8)	265 (108)	181 (27)	297 (169)	17.1 (11.9)	38.0 (24.3)	10.1 (3.0)	132 (2.8)
#7	4.4 (2.1)	220 (69)	159 (26)	315 (178)	18.0 (10.3)	40.0 (22.2)	10.7 (3.1)	184 (21)
#8	2.7 (0.7)	250 (35)	171 (8)	478 (208)	24.7 (14.4)	37.0 (16.8)	22.4 (12.9)	398 (10)
#9	1.9 (1.6)	153 (35)	85 (15)	372 (116)	10.4 (3.3)	30.0 (3.4)	11.7 (2.8)	302 (18)
#10	0.7 (0.6)	44.8 (10.0)	14.5 (3.9)	99 (60)	2.5 (1.3)	5.6 (2.7)	8.8 (8.3)	84.8 (2.2)
#11	0.6 (0.4)	13.2 (2.2)	3.4 (1.3)	31.0 (22.0)	TR	3.7 (1.9)	2.1 (1.9)	40.4 (9.8)

[a] Concentration for lipid components is μmol/l of plasma, and protein content is in mg/l of plasma. Values are means with standard deviation in parentheses. TR present at <0.1 μmol/l. CHY, chylomicrons; Frn #'s refer to density gradient fractions. TG, triacylglycerol; CE, cholesteryl ester; CH, free cholesterol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; SP, sphingomyelin; LPC, lysophosphatidylcholine; PR, protein.

ferred by comparing the triacylglycerol content to that of VLDL, which gives a value of 85 ± 12 mg/dl.

For comparison to previously reported compositional studies on adult rat plasma lipoproteins, we have determined the composition of suckling rat plasma chylomicrons and lipoproteins purified in the vertical rotor. These data are presented in **Table 2.** All plasma lipoproteins have compositions rather similar to that of adult lipoproteins (20, 21), except for a somewhat higher lipid content.

Further characterization of suckling rat plasma lipoproteins was carried out using analytical ultracentrifugation. By use of this technique we were unable to detect chylomicrons or VLDL even when the rotor speed was 20,000 rpm and/or the density of the solution adjusted to 1.006 g/ml. Whether this results from aggregation of the triacylglycerol-rich lipoproteins during isolation or is an inherent property of these lipoproteins is unknown. When plasma lipoproteins were chromatographed on Bio-Gel A 50-m (exclusion limit 50×10^6), all the triacylglycerol-rich lipoproteins eluted in the void volume. This result would also suggest a very large size, but does not eliminate the possibility of aggregation during isolation.

We were able to characterize plasma LDL, HDL_1, and HDL_2 by analytical ultracentrifugation. **Fig. 5** presents the results of ultracentrifugation of total plasma lipoproteins carried out at d 1.21 g/ml. These data show three lipoproteins with flotation rates at d 1.21 g/ml ($F_{1.21}$) between 0 and 40. Based on the flotation properties of the isolated lipoproteins shown in **Fig. 6,** these can be identified as LDL ($F_{1.21}$ = 31.2, range 22.5–40); HDL_1 ($F_{1.21}$ = 10.5, range 3.5–17.5); and HDL_2 ($F_{1.21}$ = 4.4, range 0.5–8.5). Judging by the patterns in Fig. 6, each fraction is pure, although the HDL_1 fraction shows some heterogenity. When ultracentrifugation was carried out with total plasma lipoproteins or purified lipoproteins at d 1.063 g/ml, only LDL and HDL_1 floated (data not shown). Because only HDL_1 was detected in this experiment, it was possible to determine its concentration in plasma and therefore to determine the concentration of

TABLE 2. Suckling rat lipoprotein composition (% by weight)[a]

Lipoprotein	TG	CH	CE	PL	PR
A. Plasma					
Chylomicrons	79.4 (2.4)	1.2 (0.1)	1.8 (0.2)	10.8 (0.7)	6.8 (0.2)
VLDL	73.3 (1.9)	5.3 (0.2)	3.7 (0.1)	11.4 (0.5)	6.3 (0.4)
LDL	21.2 (0.5)	13.2 (0.4)	28.8 (0.8)	20.4 (0.7)	16.4 (0.7)
HDL_1	12.1 (0.4)	16.7 (0.3)	19.6 (0.3)	28.1 (1.2)	23.5 (0.9)
HDL_2	0.1 (0.1)	8.2 (0.4)	23.8 (0.5)	31.0 (0.9)	36.9 1.2)
B. Lymph					
Chylomicrons	95.0 (1.4)	1.1 (0.2)	0.1 (0.01)	2.2 (0.5)	1.6 (0.2)
VLDL	92.7 (1.8)	2.9 (0.3)	1.2 (0.1)	1.5 (0.2)	1.7 (0.1)

[a] Lymphatic lipoproteins were isolated by SW-41 density gradient centrifugation and plasma lipoproteins by vertical rotor centrifugation. Values are means with standard deviation in parentheses. TG, triacylglycerol; CH, unesterified cholesterol; CE, cholesteryl ester; PL, phospholipid; PR, protein.

HDL_2 by subtracting the value for HDL_1 from the total for HDL_1 and HDL_2 obtained from centrifugation at d 1.21 g/ml (Fig. 5). These results are presented in **Table 3** along with the properties of the isolated lipoproteins determined from ultracentrifugal measurements. For LDL the values for the molecular weight (2.3×10^6), and hydrated density (1.028 g/ml) compare favorably to values reported for adult LDL; 2.36×10^6 (20); and

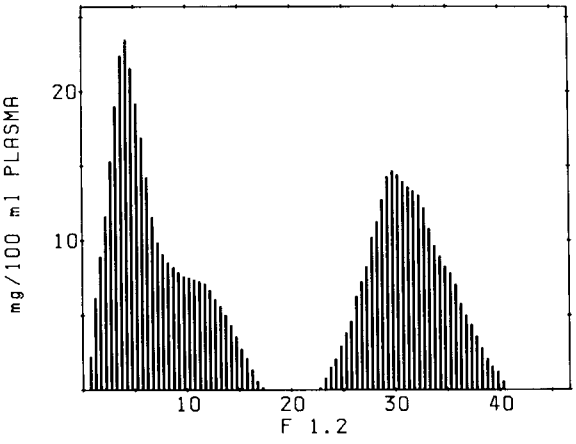

Fig. 5. Analytical ultracentrifugation of suckling rat plasma lipoproteins. Total plasma lipoproteins were centrifuged in a double sector cell at 52,000 rpm and 25°C at a density of 1.2 g/ml (NaBr). A schlieren photograph was taken at 20 min. The difference between the sample pattern and the baseline was measured every 0.5 F (flotation rate at density = 1.2 g/ml). Concentration was calculated and corrected for radial dilution and concentration dependence (9, 19).

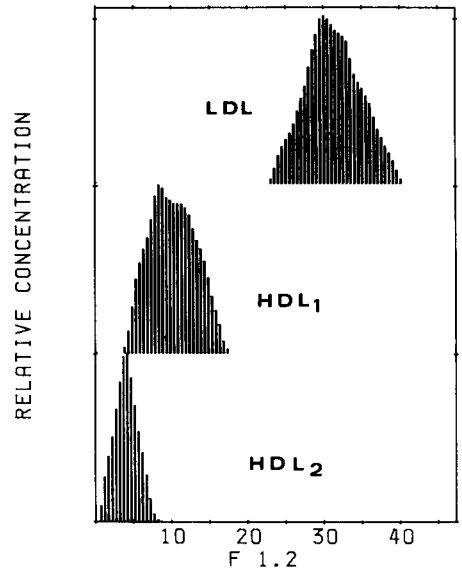

Fig. 6. Analytical ultracentrifugation of isolated suckling rat lipoproteins. The LDL, HDL_1, and HDL_2 fractions isolated using the VTi-50 vertical rotor were analyzed as described in Fig. 5.

1.031 (20), respectively. The diameter (19.2 nm) is somewhat smaller than reported for adult LDL; 28.0 nm (20) and 21.9 nm (21). For HDL_1 the values for the molecular weight (1.28×10^6) and hydrated density (1.034) are also comparable to adult values; 1.30×10^6 (20) and 1.054 (20), respectively. The lower value for the hydrated density of suckling rat HDL_1 reflects the higher lipid content. As is the case for LDL, the diameter (15.7 nm) is somewhat different from values reported for the adult; 20.0 nm (20) and 12.7 nm (21). There was good agreement between the lipoprotein concentration in suckling rat plasma determined by the various methods, and the average values are presented in **Table 4.**

The lipid and protein content of lymph chylomicrons and lymphatic lipoproteins from the SW-41 density gradient fractions are presented in **Table 5.** All lipoprotein fractions from the gradient separation of lymph contained high levels of triacylglycerol, probably due to contamination by the very high levels of VLDL. For this reason we do not have much confidence in the lipid composition data for the LDL and HDL fractions; however, we feel that the data for the chylomicrons and VLDL (fractions 1–3) are correct. These data are presented in Tables 2 and 4. The concentration of VLDL (Table 4) was determined by refractometry, and that of chylomicrons was inferred from the triacylglycerol content as for plasma chylomicrons. The lipid compositions of lymph chylomicrons and VLDL (Table 2) are similar to values reported for adults (22), but have a higher triacylglycerol and a lower phospholipid and protein content. The distribution of lipid and protein across the gradient (Table

TABLE 3. Properties of plasma lipoproteins from suckling rat plasma as determined by analytical ultracentrifugation[a]

Lipoprotein	mg/dl Plasma	S_f^{0b}	D (Å)[c]	Mol. Wt.[d]	σ[e]
LDL	202 ± 30[f]	7.0 ± 0.1	192 ± 8	23 ± 2	1.028 ± 0.003
HDL_1	108 ± 5[f]	2.0 ± 0.05	157 ± 10	12.8 ± 1.6	1.034 ± 0.005
HDL_2	224 ± 10[g]	[h]	[h]	[h]	[h]

[a] The concentration of the lipoproteins was determined from ultracentrifugation of total plasma lipoproteins isolated from chylomicron-free plasma. The properties of the lipoproteins were determined by ultracentrifugation of lipoproteins isolated from the vertical rotor separation of plasma lipoproteins.
[b] Calculated from second moment of schlieren pattern and corrected to ρ = 1.063.
[c] Diameter (D) calculated from $S_f^0 = D^2 (1.063\text{-}\sigma)/184.7$ (9).
[d] Molecular weight $\times 10^{-5} = 0.3153D^3\ \sigma$ (9).
[e] Hydrated density (σ) determined from a plot of $\eta_t F^0$ vs. ρ (9).
[f] Average of runs at ρ = 1.21 and 1.063.
[g] Run at ρ = 1.21 only.
[h] Cannot be determined since this species did not float at ρ = 1.063 g/ml.

5), suggests that LDL and HDL are also present in lymph. It is noteworthy that lymph HDL (fractions 7–11) has a high content of esterified cholesterol relative to free cholesterol, suggesting that they are not nascent lipoprotein particles (23).

The distribution of apoproteins across the SW-41 density gradient separation of plasma lipoproteins is shown in **Fig. 7.** The VLDL fractions (fractions 1–3) contains $apoB_{335}$, $apoB_{240}$, and apoE as the major apoproteins. LDL (fractions 4 and 5) contains predominately $apoB_{335}$. Although the high density lipoproteins are not cleanly separated by this system, it can be seen that the apoE-rich HDL_1 is found in fractions 6 and 7, while HDL_2 is at the bottom of the gradient (fractions 8–11). The HDL_2 fractions do not have a homogeneous apoprotein composition, with the denser fractions being enriched in apoA-IV and the lighter fractions in apoA-I. This difference in apoprotein composition may be related to the two bands observed on electrophoresis of the intact lipoprotein (Fig. 4). The apoprotein composition of fractions isolated by the vertical rotor method are shown in **Fig. 8** along with

TABLE 4. Concentration of lipoproteins in suckling rat plasma and lymph[a]

Lipoprotein	Concentration
	mg/dl
Plasma	
Chylomicrons	85 ± 12
VLDL	50 ± 6
LDL	200 ± 23
HDL_1	125 ± 20
HDL_2	220 ± 10
Lymph	
Chylomicrons	9650 ± 750
VLDL	4570 ± 60

[a] The data for plasma lipoproteins represent the average for samples isolated by the vertical rotor and from analytical ultracentrifuge studies. The values for lymph represent values from SW-41 preparations. The values for chylomicrons are based on triacylglycerol content, the others are from refractometric measurements.

TABLE 5. Concentration of components in suckling rat lymph lipoproteins isolated by density gradient centrifugation[a]

Sample	TG	CE	CH	PL	PR
CHY	93450 (1350)	15 (2)	461 (99)	1930 (470)	1740 (130)
Frn #1	15100 (2400)	67 (40)	144 (34)	350 (120)	640 (80)
#2	17300 (1800)	320 (180)	44 (30)	90 (40)	150 (15)
#3	5500 (3100)	480 (30)	21 (9)	120 (20)	129 (3)
#4	2000 (500)	140 (10)	130 (10)	100 (20)	123 (50)
#5	1700 (700)	430 (120)	22 (6)	360 (80)	155 (11)
#6	4000 (250)	350 (40)	20 (7)	990 (420)	130 (13)
#7	1000 (500)	360 (40)	12 (5)	810 (80)	138 (6)
#8	2600 (1000)	870 (80)	6 (2)	1650 (120)	145 (2)
#9	2300 (1200)	360 (120)	6 (2)	3710 (440)	153 (45)
#10	500 (200)	720 (140)	8 (3)	1950 (750)	120 (12)
#11	400 (200)	770 (90)	3 (1)	750 (200)	370 (190)

[a] Concentration for lipid components is μmol/l of lymph, and protein content is in mg/l of lymph. Values are means with standard deviation in parentheses. CHY, chylomicrons; Frn #'s refer to density gradient fractions. TG, triacylglycerol; CE, cholesteryl ester; CH, free cholesterol; PL, total phospholipid; PR, protein.

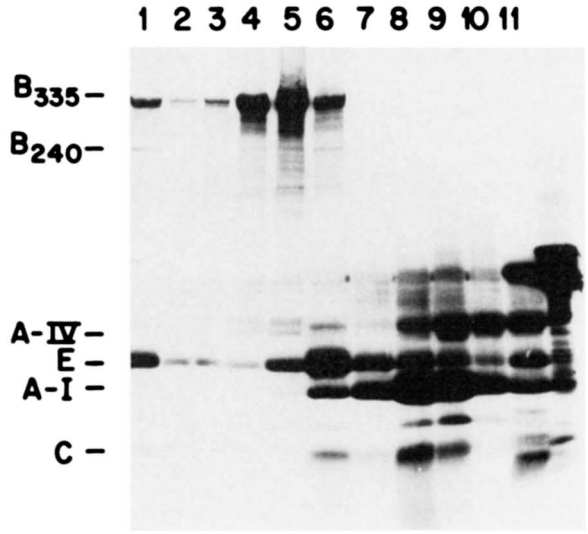

Fig. 7. Apoprotein composition of fractions from the SW-41 rotor separation of suckling rat plasma lipoproteins. Slab gel electrophoresis was carried out with a linear gradient of 4–15% polyacrylamide. The numbers at the top of the figure refer to gradient fractions. The position of the various apoproteins is shown to the left.

plasma chylomicrons. The apoproteins of adult lipoproteins isolated by the same method are also shown in this figure (we were not able to isolate sufficient LDL from adult plasma for analysis). The apoprotein composition for chylomicrons, VLDL, HDL_1, and HDL_2 are similar to those shown for the adult and reported in the literature (20, 21). The LDL fraction, although apparently pure by electrophoresis and analytical ultracentrifugation, is not pure as judged by apoprotein composition, since it contains significant quantities of apoproteins other than $apoB_{335}$. It is unknown whether these additional apoproteins are contributed by an HDL_1 particle with an abnormally high lipid content.

The distribution of apoproteins across the SW-41 density gradient separation of lymph lipoproteins is shown, along with lymph chylomicrons, in **Fig. 9.** It can be seen that both lymph chylomicrons and VLDL contain only $apoB_{240}$. It should be noted that lymph LDL (fraction 6) contains predominately $apoB_{335}$, and that none of the lymphatic lipoproteins contain appreciably amounts of apoC.

The fatty acid composition of triacylglycerols, cholesteryl esters, phosphatidylcholines, phosphatidylethanolamines, sphingomyelins, and lysophosphatidylcholines of the plasma and lymph lipoproteins are presented in **Tables 6 to 11.** Consistent with earlier reports on the fatty acid composition of milk triacylglycerols (2), the triacylglycerol-rich lipoproteins of lymph and plasma are enriched in medium-chain-length fatty acids (8:0 + 10:0 + 12:0), whereas LDL and HDL do not contain appreciable amounts of these fatty acids. Although the triacylglycerols from milk and triacylglycerol-rich lipoproteins contain relatively small amounts of polyunsaturated fatty acids (20:4 + 22:5 + 22:6), it is interesting to note that plasma LDL triacylglycerols contain high

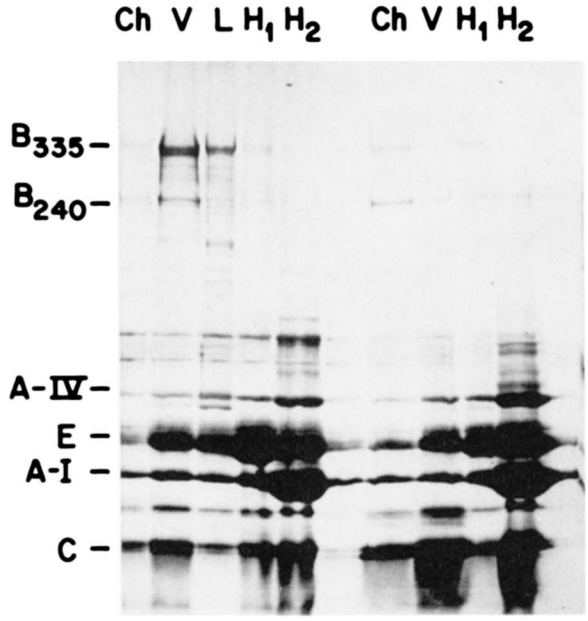

Fig. 8. Apoprotein composition of lipoproteins isolated using the VTi-50 vertical rotor. Slab gel electrophoresis was carried out with a linear gradient of 4–15% polyacrylamide. CH, chylomicrons; V, VLDL; L, LDL; H_1, HDL_1; H_2, HDL_2. The samples on the left are from suckling rat plasma and those on the right from adult rat plasma.

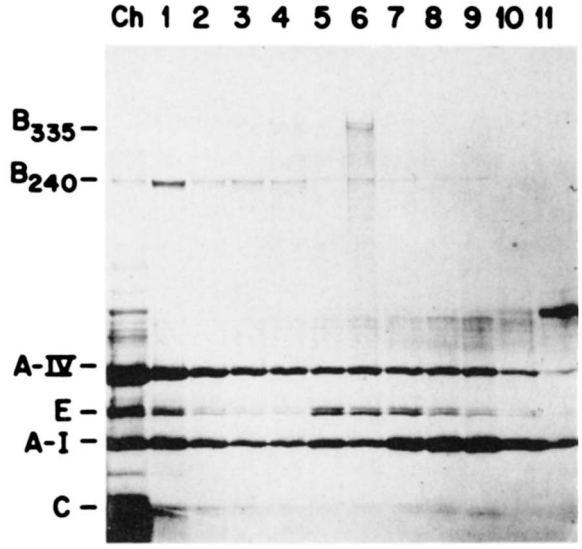

Fig. 9. Apoprotein composition of fractions from the SW-41 rotor separation of suckling rat lymph lipoproteins. Slab gel electrophoresis was carried out with a linear gradient of 4–15% polyacrylamide. Ch, chylomicrons; the numbers at the top of the figure refer to gradient fractions. The position of the various apoproteins is shown to the left.

TABLE 6. Fatty acid composition of triacylglycerols from suckling rat lipoproteins[a]

Sample	8:0	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4	22:5	22:6
L CHY	1.3	8.4	15.6	13.8	22.0	1.9	2.2	14.5	16.5	1.7	1.0	0.3	0.8
	(0.1	0.9	1.2	0.7	0.9	0.2	0.1	1.1	1.4	0.1	0.1	0.1	0.2)
L VLDL	0.8	5.5	11.4	11.6	25.3	1.7	3.1	14.8	17.8	1.8	2.9	0.9	2.3
	(0.0	0.4	1.2	1.2	0.3	0.2	0.3	1.8	1.2	0.2	1.4	0.8	1.6)
P CHY	1.6	9.6	15.8	13.2	21.0	1.7	2.3	14.3	17.0	1.7	0.9	0.4	0.6
	(0.2	1.5	2.0	1.0	1.2	0.2	0.1	1.9	1.2	0.1	0.2	0.1	0.1)
P VLDL	0.8	5.6	10.3	10.4	24.5	1.5	2.4	15.9	20.4	1.6	2.2	1.3	3.3
	(0.4	1.5	2.0	1.0	1.9	0.1	0.1	1.5	2.6	0.1	0.3	0.3	1.0)
P LDL	ND	TR	0.8	2.8	19.8	0.8	2.7	16.8	25.8	0.9	11.8	3.9	13.7
	(		0.6	0.7	2.2	0.1	0.2	3.3	2.2	0.2	2.0	0.5	1.7)
P HDL_1	ND	ND	3.1	6.7	21.5	1.2	3.5	15.5	20.8	1.1	11.1	4.7	9.7
	(		2.0	2.2	2.1	0.1	0.4	2.1	2.5	0.4	3.6	1.3	1.5)
P HDL_2	ND	ND	3.8	7.7	24.4	2.4	4.4	14.6	19.1	1.2	5.8	6.7	7.9
	(		2.9	1.5	2.3	0.8	1.1	2.9	0.9	0.3	0.3	1.1	3.2)

[a] Mean values of mol % with standard deviation in parentheses. TR, fatty acid present at <0.1 mol %; ND, not detected. L, lymph; CHY, chylomicron; P, plasma.

proportions of these fatty acids. Thus, LDL, which contains only 8.2% of plasma triacylglycerol, carries 46% of all plasma triacylglycerol polyunsaturated fatty acids.

In lipoprotein cholesteryl esters, the fatty acid composition is relatively constant in all classes except for *1*) a notable alteration in the ratio of 18:1/20:4 as the density of the lipoproteins increase, and *2*) the fact that plasma chylomicrons have a higher content of 20:4 than lymph chylomicrons or VLDL. In lower density lipoproteins, 18:1 predominates, whereas in high density lipoproteins 20:4 predominates. A similar observation has been made for adult lipoproteins (21). In preliminary experiments, we have found more than 95% of the LCAT activity recovered from the SW-41 density gradient is found in fractions 9–11. Although the assays have not been optimized for the different cholesterol concentration in each fraction, these data would suggest that the origin of 20:4 in cholesteryl esters of HDL is through the action of LCAT. The lack of cholesteryl ester exchange protein in rat plasma (24) would account for these 20:4 enriched cholesteryl esters remaining in the HDL. We have no explanation for the differences in fatty acid composition between lymph chylomicrons and plasma chylomicrons.

Neither phosphatidylcholines, lysophosphatidylcholines, nor sphingomyelins show any significant differences in fatty acid composition between the various lymph and plasma lipoproteins. On the other hand, there are some differences between the fatty acid composition of lipo-

TABLE 7. Fatty acid composition of cholesteryl esters from suckling rat lipoproteins[a]

Sample	8:0	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4	22:5	22:6
L CHY	ND	ND	0.8	2.8	21.2	3.1	4.5	32.3	24.6	2.4	6.2	0.9	1.1
	(		0.2	0.8	0.9	0.2	0.0	0.8	2.9	0.4	3.6	0.1	0.2)
L VLDL	ND	ND	0.9	2.3	27.8	3.8	5.9	26.1	21.5	2.9	7.0	0.5	1.3
	(		0.3	0.4	0.6	0.4	1.0	0.6	1.4	1.0	3.7	0.4	0.4)
P CHY	ND	ND	0.8	4.4	16.8	2.3	3.5	17.0	20.8	1.6	23.6	TR	0.1
	(		0.4	2.0	3.0	1.2	0.5	1.4	3.4	0.6	4.1		0.1)
P VLDL	ND	ND	0.3	3.6	21.1	2.4	4.0	30.5	23.4	1.9	12.1	0.4	1.3
	(		0.4	1.2	0.5	0.2	0.2	2.4	3.2	0.2	1.8	0.3	1.1)
P LDL	ND	ND	0.1	2.4	21.4	2.1	2.0	15.4	28.1	0.8	25.7	0.2	1.9
	(		0.0	0.3	1.3	0.2	0.0	1.7	1.4	0.2	0.5	0.1	0.3)
P HDL_1	ND	ND	0.2	3.1	15.5	2.2	0.5	4.9	28.1	0.2	43.4	0.1	1.9
	(		0.0	0.4	0.7	0.3	0.1	1.0	1.1	0.1	1.3	0.0	0.2)
P HDL_2	ND	ND	0.1	2.8	14.3	2.2	0.4	3.9	28.1	0.2	45.7	0.1	1.7
	(		0.1	0.1	0.3	0.5	0.0	0.9	1.3	0.1	1.0	0.0	0.7)

[a] Mean values of mol % with standard deviation in parentheses. TR, fatty acid present at <0.1 mol %; ND, not detected. L, lymph; CHY, chylomicron; P, plasma.

TABLE 8. Fatty acid composition of phosphatidylcholines from suckling rat lipoproteins[a]

Sample	8:0	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4	22:5	22:6
L CHY	ND	ND	0.1	1.3	25.4	0.8	17.8	5.2	34.3	0.5	12.5	0.2	2.0
	(		0.0	0.2	0.4	0.1	0.3	0.4	0.5	0.0	0.7	0.0	0.3)
L VLDL	ND	ND	0.1	1.1	25.9	0.8	17.5	4.9	31.6	0.5	15.4	0.2	2.1
	(		0.0	0.1	0.8	0.1	0.4	0.7	1.5	0.1	2.1	0.0	0.0)
P CHY	ND	ND	0.1	1.3	27.3	0.5	18.9	4.1	28.1	0.2	13.3	0.7	5.4
	(		0.0	0.1	0.9	0.0	0.3	0.1	0.8	0.0	0.9	0.1	0.4)
P VLDL	ND	ND	0.1	1.3	28.3	0.5	18.3	4.1	27.0	0.3	14.3	0.5	5.4
	(		0.0	0.3	0.3	0.0	0.6	0.2	0.4	0.0	0.2	0.1	0.1)
P LDL	ND	ND	TR	1.5	28.8	0.5	18.1	4.2	27.9	0.2	12.8	0.7	5.3
	(			0.2	0.8	0.0	0.4	0.1	0.5	0.0	1.0	0.1	0.1)
P HDL_1	ND	ND	0.1	1.4	28.3	0.5	18.0	4.3	27.4	0.3	13.9	0.5	5.5
	(		0.0	0.1	0.8	0.0	0.5	0.1	0.6	0.1	0.9	0.0	0.1)
P HDL_2	ND	ND	0.1	1.2	27.8	0.5	17.8	4.4	27.0	0.3	14.4	0.7	5.8
	(		0.0	0.1	0.5	0.0	0.3	0.1	0.5	0.1	0.6	0.1	0.1)

[a] Mean values of mol % with standard deviation in parentheses. TR, fatty acid present at <0.1 mol %; ND, not detected. L, lymph; CHY, chylomicron; P, plasma.

protein phosphatidylethanolamines. Thus, lymph chylomicron and VLDL and plasma chylomicron phosphatidylethanolamines have higher contents of 18:0 and 18:2 and lower 22:6 when compared to plasma VLDL, LDL, HDL_1, and HDL_2. These differences may reflect the different origins (intestinal vs. liver) for these two groups of lipoproteins. None of the phospholipids contain significant quantities of medium-chain-length fatty acids.

DISCUSSION

The data presented in this paper represent the first comprehensive investigation of the lipoproteins of suckling rat lymph and plasma. The procedures adopted for lipoprotein isolation represent the best compromise between obtaining the maximal amount of information, pure fractions, and the small amount of material available. It is now well established that the use of sequential centrifugation for the isolation of plasma lipoproteins from the rat does not work well (20, 21), however, the vertical rotor method described here appears to give reasonably pure fractions.

Striking features of these data are the very high concentration of triacylglycerol-rich lipoproteins in lymph compared to plasma (about 100-fold higher), and the high concentration of all lipoproteins in plasma, especially an LDL-like material. The high concentration of tri-

TABLE 9. Fatty acid composition of phosphatidylethanolamines from suckling rat lipoproteins[a]

Sample	8:0	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4	22:5	22:6
L CHY	ND	ND	0.1	0.3	11.4	0.4	30.5	4.6	16.9	0.4	24.4	1.3	9.6
	(		0.1	0.1	0.2	0.0	0.3	0.2	0.3	0.0	0.7	0.3	0.6)
L VLDL	ND	ND	0.2	0.4	11.6	0.5	28.7	4.2	13.4	0.4	26.4	1.2	13.1
	(		0.2	0.0	1.1	0.0	0.9	0.6	1.5	0.1	0.8	0.2	0.3)
P CHY	ND	ND	0.3	0.8	8.9	0.5	26.8	3.1	13.6	0.3	21.5	3.5	18.8
	(		0.1	0.5	0.9	0.3	2.5	0.4	2.1	0.0	1.7	1.1	2.1)
P VLDL	ND	ND	TR	3.9	12.3	0.7	13.3	4.3	12.1	0.6	16.0	6.5	24.3
	(			3.9	6.5	0.5	4.1	4.7	5.6	0.7	6.2	5.4	14.1)
P LDL	ND	ND	0.1	0.5	8.9	0.4	12.8	2.8	8.2	0.2	20.3	8.3	37.5
	(		0.1	0.2	2.2	0.2	3.4	0.7	1.6	0.1	2.4	1.0	5.4)
P HDL_1	ND	ND	0.2	0.6	7.5	0.2	13.3	2.2	7.8	0.2	21.5	8.0	38.5
	(		0.1	0.7	1.3	0.0	2.7	0.4	1.4	0.0	1.8	0.7	4.9)
P HDL_2	ND	ND	TR	0.5	8.0	0.3	15.4	2.6	7.7	0.1	22.6	7.6	35.2
	(			0.4	1.0	0.2	1.9	0.3	1.4	0.1	4.2	1.3	1.1)

[a] Mean values of mol % with standard deviation in parentheses. TR, fatty acid present at <0.1 mol %; ND, not detected. L, lymph; CHY, chylomicron; P, plasma.

TABLE 10. Fatty acid composition of sphingomyelins from suckling rat lipoproteins[a]

Sample	12:0	14:0	16:0	17:0	18:0	18:1	18:2	20:0	22:0	23:0	24:0	24:1	24:2
P CHY	0.6	1.5	29.5	0.9	12.7	2.0	1.4	1.3	3.6	8.4	10.6	17.6	9.3
	(0.2	0.1	1.6	0.2	1.3	0.1	0.2	0.3	0.3	2.3	1.1	1.5	1.5)
P VLDL	0.4	2.1	26.3	0.6	12.9	5.0	2.3	0.8	3.4	10.4	9.9	14.8	10.7
	(0.5	0.8	6.7	0.0	5.1	4.2	1.2	0.1	0.2	3.2	0.5	0.7	6.5)
P LDL	0.3	1.9	28.4	0.7	13.4	1.2	1.3	1.0	4.2	8.9	13.6	18.9	6.2
	(0.2	1.2	0.9	0.0	3.5	0.3	0.6	0.2	0.5	2.4	2.7	3.6	1.2)
P HDL_1	0.5	2.2	27.7	0.7	12.1	1.3	1.6	1.0	4.7	9.2	13.4	19.2	6.4
	(0.3	1.2	3.8	0.1	4.9	0.5	1.9	0.3	1.3	3.6	3.9	5.0	1.8)
P HDL_2	0.5	2.0	29.7	0.9	11.4	1.9	1.0	1.1	4.2	6.9	13.4	20.1	7.0
	(0.2	1.0	2.7	0.1	1.5	0.3	0.0	0.0	0.2	1.9	0.5	0.8	0.8)

[a] Mean values of mol % with standard deviation in parentheses. TR, fatty acid present at <0.1 mol %; ND, not detected. CHY, chylomicron; P, plasma. There was not sufficient sphingomyelin in the lymph lipoproteins for analysis.

acylglycerol-rich lipoproteins in lymph is consistent with earlier analyses on lymph (2). Several studies have shown that the adult rat has an efficient system for removal of chylomicrons and their remnants from plasma (25–28). It is apparent from our studies that the suckling rat also has a very efficient system. Thus, we have shown that triacylglycerol-rich lipoproteins are secreted from the suckling intestine (14-day-old rats) at a rate of about 0.55 mg/min (5), which, in a steady state situation, would mean that lymphatic triacylglycerol-rich lipoproteins are cleared from the plasma with a half-life of about 1.3 min. A further indication of the rapid removal of lymphatic triacylglycerol-rich lipoproteins is that the total plasma pool (based on a total plasma volume of 2 ml in a 14-day-old rat (29)) is 2.7 mg, which is only 5-fold higher than the per-minute entry rate.

Lymph also contains HDL and an LDL-like lipoprotein. Whether both HDL_1 and HDL_2 are present cannot be determined from the available data. The apoprotein and lipid composition of lymph LDL are consistent with a plasma origin. Small amounts of LDL have been isolated from adult mesenteric lymph (30). The fact that the lymph HDL fraction has low levels of apoE may suggest that the HDL are of intestinal origin, since, in adults, it has been shown that the intestine makes only very small amounts of apoE (31). On the other hand, the high content of cholesteryl esters in lymph HDL is not consistent with intestinal origin, since Green, Tall, and Glickman (23) have shown in the adult rat that lymph HDL is secreted as nascent particles lacking a cholesteryl ester core. The action of LCAT on the phosphatidylcholine and cholesterol of the nascent HDL creates the cholesteryl ester core. It is possible that the HDL of lymph is derived from plasma and loses the apoE by exchange to lymph chylomicrons, which are being produced at a rapid rate.

Suckling rat plasma contains a high level of an LDL-

TABLE 11. Fatty acid composition of lysophosphatidylcholines from suckling rat lipoproteins[a]

Sample	8:0	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4	22:5	22:6
L CHY	ND	ND	0.3	0.6	30.6	0.5	28.3	6.1	16.4	0.4	12.6	0.7	3.4
	(		0.1	0.3	4.6	0.5	1.8	0.3	0.3	0.0	3.4	0.0	0.4)
P CHY	ND	ND	0.3	0.6	32.6	1.0	36.6	5.5	12.2	0.3	6.1	TR	2.6
	(		0.1	0.3	3.0	0.1	1.4	0.4	0.8	0.1	1.6		1.3)
P VLDL	ND	ND	0.3	0.5	33.1	1.3	43.2	5.3	8.0	TR	5.3	1.6	1.4
	(		0.1	0.1	1.5	0.7	1.7	2.5	1.4		4.4	0.1	1.2)
P LDL	ND	ND	TR	0.5	29.9	0.5	41.7	6.9	10.2	0.6	7.1	1.0	2.1
	(			0.1	6.4	0.4	2.8	3.0	3.3	0.2	3.1	1.1	1.4)
P HDL_1	ND	ND	TR	0.8	31.5	0.9	40.8	5.1	9.7	0.3	8.1	0.8	2.1
	(			0.1	2.0	0.7	1.6	0.9	1.4	0.1	2.9	0.4	0.7)
P HDL_2	ND	ND	TR	0.6	31.6	0.5	32.7	6.0	11.8	0.5	8.9	1.0	2.8
	(			0.3	5.5	0.1	3.1	0.9	1.1	0.1	3.2	0.7	1.9)

[a] Mean values of mol % with standard deviation in parentheses. TR, fatty acid present at <0.1 mol %; ND, not detected. L, lymph; CHY, chylomicron; P, plasma.

like lipoprotein (about 10-fold higher than adult plasma). An earlier report, using electrophoretic analysis, also showed high levels of LDL during the fetal and suckling period in the rat (4). Assuming that LDL should contain predominately apoB_{335} (21), it is clear that the LDL fraction is not pure, in spite of the fact that its physical properties are consistent with those reported for LDL. The apoprotein composition across the SW-41 gradient does show an apoB_{335}-rich fraction (fraction 4, Fig. 7), which suggests that LDL in the suckling rat is similar to adult LDL. The nature of the "contaminant" in the LDL fraction is unknown at present. It is clearly not HDL_1, since we have isolated a lipoprotein with the correct physical and compositional properties for this lipoprotein. Since the origin of HDL_1 is unknown at present, it is possible that the lipoprotein present in the LDL fraction represents a precursor of HDL_1, which is only detected in suckling rat plasma due to the rapid rate of lipoprotein metabolism. It does not seem likely that the material in the LDL fraction arises directly from the triacylglycerol-rich lipoprotein of lymph since the fraction contains apoB_{335}, but not apoB_{240}. Furthermore, there is a significant difference in the fatty acid composition of LDL triacylglycerol, compared to VLDL triacylglycerol (in particular the presence of polyunsaturated fatty acids and the absence of medium-chain-length fatty acids). Attempts to subfractionate the LDL fraction are underway. In general terms, the composition of suckling rat plasma lipoproteins is similar to that of adult plasma lipoproteins (20, 21). In particular, the lack of cholesteryl ester exchange protein found in the adult (24) can also be inferred to be the case in the suckling, since the cholesteryl esters of VLDL and LDL are significantly different than HDL_{1+2}, as is found in the adult (21). In the suckling rat, plasma and lymphatic chylomicrons and VLDL seem to be very large, and represent an extreme extension of recent observations in the adult (21). An HDL_3 fraction has not been observed in adult rats (21); however, there is some evidence for a heavy HDL fraction in suckling rat plasma, with an electrophoretic mobility and apoprotein composition different than HDL_2. This material is present in small amounts and was not further characterized.

Considering that per 100 g of body weight, the suckling rat consumes 10–20 times as much fat as the adult (5), it is remarkable that there are such minor differences between the composition of lipoproteins from the suckling and adult. Furthermore, considering the difference in fat intake, it is also remarkable that the concentrations of lipoproteins in the suckling are not higher than observed. These considerations, and those described above, suggest that lipoprotein metabolism in the suckling rat must be occurring at a rapid rate, and that the suckling rat will be a valuable model in which to study lipoprotein metabolism under conditions of high fat intake.

This research was supported by National Institutes of Health Grants HD 10954 and AM 28337.

Manuscript received 11 July 1983.

REFERENCES

1. Henning, S. J. 1981. Postnatal development of feeding: coordination of feeding, digestion, and metabolism. *Am. J. Physiol.* **241:** G199–G214.
2. Fernando-Warnakulasuriya, G. J. P., J. E. Staggers, S. C. Frost, and M. A. Wells. 1981. Studies on fat digestion, absorption, and transport in the suckling rat. I. Fatty acid composition and concentrations of major lipid components. *J. Lipid Res.* **22:** 668–674.
3. Staggers, J. E., G. J. P. Fernando-Warnakulasuriya, and M. A. Wells. 1981. Studies on fat digestion, absorption, and transport in the suckling rat. II. Triacylglycerols: molecular species, stereospecific analysis, and specificity of hydrolysis by lingual lipase. *J. Lipid Res.* **22:** 675–679.
4. Schlag, B., and L. Winkler. 1978. Untersuchungen zur Entwicklung der Serumlipoproteinverhältnisse vom Fetalstadium bis zum Erwachsenenalter der Ratte. *Acta Biol. Med. Ger.* **37:** 239–244.
5. Frost, S. C., W. A. Clark, and M. A. Wells. 1983. Studies on fat digestion, absorption, and transport in the suckling rat. IV. In vivo rates of triacylglycerol secretion by intestine and liver. *J. Lipid Res.* **24:** 899–903.
6. Redgrave, T. G., D. C. K. Roberts, and C. E. West. 1975. Separation of plasma lipoproteins by density-gradient ultracentrifugation. *Anal. Biochem.* **65:** 42–49.
7. Chung, B. H., T. Wilkinson, J. C. Geer, and J. Segrest. 1980. Preparative and quantitative isolation of plasma lipoproteins: rapid, single discontinuous density gradient ultracentrifugation in a vertical rotor. *J. Lipid Res.* **21:** 284–291.
8. Naito, H. K., M. Wada, L. A. Ehrhart, and L. A. Lewis. 1973. Polyacrylamide-gel electrophoresis as a screening procedure for serum lipoprotein abnormalities. *Clin. Chem.* **19:** 228–234.
9. Lindgren, F. T. 1974. Serum lipoproteins: isolation and analysis with the preparative and analytical ultracentrifuge. *In* Fundamentals of Lipid Chemistry. R. M. Burton, and F. C. Guerra, editors. BI-Science Publications Division, Webster Groves, MO. 475–510.
10. Kashyap, M. L., B. A. Hynd, and K. Robinson. 1980. A rapid and simple method for measurement of total protein in very low density lipoproteins by the Lowry assay. *J. Lipid Res.* **21:** 491–495.
11. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234:** 466–468.
12. Pinon, J-C., A. M. Bridoux, and M-H. Laudat. 1980. Initial rate of cholesterol esterification associated with high density lipoproteins in human plasma. *J. Lipid Res.* **21:** 406–414.
13. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37:** 911–917.
14. 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.
15. Skipski, V. P., R. F. Peterson, and M. Barclay. 1964. Quantitative analysis of phospholipids by thin-layer chromatography. *Biochem. J.* **90:** 374–377.

16. Nelson, G. J. 1962. Studies on human serum lipoprotein phospholipids and phospholipid fatty acid composition by silicic acid chromatography. *J. Lipid Res.* **3:** 71–79.
17. Ghiselli, G., H. B. Brewer, and H. G. Windmueller. 1982. Apolipoprotein A-I isoprotein synthesis by the perfused rat liver. *Biochem. Biophys. Res. Commun.* **107:** 144–149.
18. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature.* **227:** 680–685.
19. Ewing, A. M., N. K. Freeman, and F. T. Lindgren. 1965. The analysis of human serum lipoprotein distributions. *Adv. Lipid Res.* **3:** 25–61.
20. Lusk, L. T., L. F. Walker, L. H. DuBien, and G. S. Getz. 1979. Isolation and partial characterization of high-density lipoprotein HDL_1 from rat plasma by gradient centrifugation. *Biochem. J.* **183:** 83–90.
21. Oschry, Y., and S. Eisenberg. 1982. Rat plasma lipoproteins: re-evaluation of a lipoprotein system in an animal devoid of cholesteryl ester transfer activity. *J. Lipid Res.* **23:** 1099–1106.
22. Chapman, M. J. 1980. Animal lipoproteins: chemistry, structure, and comparative aspects. *J. Lipid Res.* **21:** 789–853.
23. Green, P. H. R., A. R. Tall, and R. M. Glickman. 1978. Rat intestine secretes discoid high density lipoprotein. *J. Clin. Invest.* **61:** 528–534.
24. Barter, P. J., and J. I. Lally. 1978. The activity of an esterified cholesterol transferring factor in human and rat serum. *Biochim. Biophys. Acta.* **531:** 233–236.
25. Redgrave, T. G. 1970. Formation of cholesteryl ester-rich particulate lipid during metabolism of chylomicrons. *J. Clin. Invest.* **49:** 465–471.
26. Eisenberg, S., and D. Rachmilewitz. 1973. Metabolism of rat plasma very low density lipoprotein. I. Fate in circulation of the whole lipoprotein. *Biochim. Biophys. Acta.* **326:** 378–390.
27. Eisenberg, S., and D. Rachmilewitz. 1973. Metabolism of rat plasma very low density lipoprotein. II. Fate in circulation of apoprotein subunits. *Biochim. Biophys. Acta.* **326:** 391–405.
28. 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.
29. Travnickova, E., and J. Heller. 1963. Plasma and blood volumes of infant rats during the first postnatal month. *Physiol. Bohemoslov.* **12:** 541–547.
30. Riley, J. W., R. M. Glickman, P. H. R. Green, and A. R. Tall. 1980. The effect of chronic cholesterol feeding on intestinal lipoproteins in the rat. *J. Lipid Res.* **21:** 942–952.
31. Wu, A-L., and H. G. Windmueller. 1979. Relative contributions by liver and intestine to individual plasma apolipoproteins in the rat. *J. Biol. Chem.* **254:** 7316–7322.