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HUMAN CHYLOMICRON APOLIPOPROTEIN METABOLISM

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SUMMARY: Chylomicron apolipoprotein metabolism was studied utilizing chylomicrons isolated from the pleural fluid of a patient with a recurrent chylous pleural effusion. Chylomicrons contained apolipoproteins A-I, A-II, B, C-I, C-II, C-III, D, E, and albumin. Following intravenous injection of [^{125}I] chylomicrons, almost all of the A apolipoprotein radioactivity was recovered in high density lipoproteins, while only a small amount of the B apolipoprotein radioactivity was recovered in low density lipoproteins. These observations indicate that intestinal chylomicron A apolipoproteins serve as precursors for plasma high density lipoprotein A apolipoproteins and only a small fraction of chylomicron apolipoprotein B is metabolized to form low density lipoprotein apolipoprotein B.

INTRODUCTION: The human intestine is remarkably efficient in absorbing dietary fat. Medium and short chain fatty acids are absorbed directly into the portal circulation (1). Long chain fatty acids are incorporated into triglyceride, which is associated with cholesterol, phospholipids, and apolipoproteins, is secreted from the intestinal cell as chylomicrons (2-4). Chylomicrons are transported by the lymphatic system, and enter the blood stream via the thoracic duct. Results of animal studies indicate that apolipoporteins synthesized by the intestine may be an important source of apolipoproteins in circulating plasma lipoproteins (5). Data from rat intestinal perfusion studies suggest that apolipoprotein (apo) B, and apoA-I, but not the C apolipoproteins, are synthesized by the intestine (6). ApoA-I appears to be the major apolipoprotein of nascent rat chylomicrons, while the C apolipoproteins appear to be derived from circulating plasma lipoproteins. On entering the plasma, the apolipoprotein composition of rat chylomicrons has been shown to undergo rapid changes, with increases in C and E apolipoproteins, and decreases in apoA-I (7). Chylomicron lipid metabolism occurs rapid in plasma (half life = 4.5 minutes) (8).

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Analysis of nascent human chylomicrons has been limited due to the difficulty in obtaining chylomicrons which have not been significantly altered by interaction with plasma lipoproteins. Chylomicrons isolated from human thoracic duct lymph consisted of 86.4% triglyceride, 8.6% phospholipid, 3.5% cholesterol and 1.5% protein (9). Apolipoproteins in chylomicrons include apoB, 22.5%; apoC-I, 15.2%; apoC-II, 15.1%; apoC-III, 35.6%; apoA-I, 7.4%;and apoA-II, 4.2% (9). The metabolism of human chylomicron apolipoproteins entering the plasma has not been described, and is the subject of this report.

MATERIALS AND METHODS: Chylomicrons were isolated from the pleural fluid of a 27-year-old white female (height 165 cm., weight 52.2 kg) with a recurrent left sided chylous pleural effusion due to lymphangiomyomatosis (10). The patient had plasma cholesterol and triglyceride concentrations of 144 mg % and 136 mg % respectively, a normal lipoprotein pattern on paper electrophoresis, and normal liver, thyroid, and renal function. Informed consent was obtained. Pleural fluid, layered beneath .85% NaCl, .01% EDTA in cellulose nitrate tubes, was centrifuged at 3×10^6 g·min in a Beckman SW 27 swinging bucked rotor (Beckman Instruments, Inc., Palo Alto, CA). Chylomicrons isolated from the supernatant fraction were resuspended in .85% NaCl, .01% EDTA, and recentrifuged twice for 3×10^6 g·min (3).

The isolated chylomicrons were characterized by electron microscopy (11), paper electrophoresis (12), and labeled with [^{125}I] using a modification of the iodine monochloride method as previously reported (13). Assuming a total apolipoprotein molecular weight of 2×10^6 daltons, no more than one atom of iodine was added per mole of protein. Efficiency of labeling was determined by precipitation of the labeled preparation with 10% TCA. Unbound iodine was removed by successive dialysis against 0.85% NaCl, 0.01% EDTA solution. The radiolabeled preparation was tested for pyrogenicity and sterility and analyzed by paper radiochromatography for free iodine and lipoprotein denaturation (13).

The patient was studied while on an isocaloric low fat diet, and supersaturated iodine (1 gram/day) was administered orally before and during the study to prevent [^{125}I] uptake by the thyroid. Chylomicron apolipoprotein kinetics were studied after the intravenous injection of 100 microcuries of [^{125}I] chylomicrons. Blood samples were collected in 0.1% EDTA at 10 minutes, 1, 3, 6, 9, and 12 hours, and then daily for 6 days following injection. Pleural fluid was aspirated on the fifth day of the study. [^{125}I] chylomicrons were incubated (30 min, 37°C) with the patient's plasma (incubation samples) to examine chylomicron apolipoprotein exchange with other plasma lipoproteins. Lipoprotein fractions were isolated from the plasma, pleural fluid, and incubation samples by the following methods: chylomicrons by the method Hazzard et al (14), and very low density lipoproteins (VLDL, d < 1.006 g/ml), intermediate density lipoproteins (IDL, d, 1.006-1.019 g/ml), low density lipoproteins (LDL, d, 1.019-1.063 g/ml), and high density lipoproteins (HDL, d, 1.063-1.21 g/ml) by the method of Havel et al (15) utilizing a Beckman 40.3 rotor (Beckman Instruments, Palo Alto, CA). The composition of lipoprotein fractions was determined. Phospholipids were measured by the method of Chalvardjian et

al (16), cholesterol and triglyceride were quantitated following Folch extraction (17) on the AutoAnalyzer II (18), and protein by the method of Lowry et al (19). Lipid labeling in lipoprotein fractions was measured following Folch extraction (17).

The apolipoprotein content of lipoprotein fractions was assessed by immunodiffusion (20) with antisera specific for apoA-I, apoA-II, apoB, apoC-I, apoC-III, apoD, apoE, and albumin, and by polyacrylamide gel electrophoresis (PAGE) utilizing both the tetramethylurea (TMU) gel system of Kane et al (21) and the SDS PAGE system of Weber et al (22). Lipoprotein apoB content was determined by protein quantitation (19) before and after TMU precipitation (21). TMU PAGE gels were scanned on a Gelman ACD-15 integrating densitometer (Gelman Instruments Co., Ann Arbor, MI). Pleural fluid chylomicron apolipoprotein content was also determined by the gel permeation chromatography on Sephadex G-200 (superfine, 1.2 x 150 cm, Pharmacia Fine Chemicals, Inc., Piscataway, NJ) equilibrated in 0.1 M TRIS HCl, 0.1 M SDS, and 6 M urea, pH 8.2. The effluent was monitored at 280 nm, and analyzed for protein content (11), and apolipoprotein composition by SDS PAGE. Radioactivity of individual apolipoproteins in lipoprotein fractions was determined on gel slices of delipidated apolipoproteins separated by TMU and SDS PAGE. Using both gel systems a clear separation of apoA-I, apoA-II, apoB, apoC-I, apoC-II, and apoC-III was obtained (21, 23).

RESULTS AND DISCUSSION: Pleural chylomicrons had a mean particle size of 1900 Å by electron microscopy, and remained at the origin on paper electrophoresis. The particles were composed (wt %) of 90.4% triglyceride, 5.2% phospholipid, 2.5% cholesterol, and 1.9% protein. Immunodiffusion of delipidated chylomicron revealed apoA-I, apoA-II, apoB, apoC-I, apoC-III, apoD, apoE, and albumin (Figure 1). Antisera for apoC-II was not available for these experiments. The mean weight % of apolipoprotein in pleural fluid chylomicron as determined by column chromatography and PAGE was: apoA-I, 8.9%; apoA-II, 4.4%; apoB, 12.3%; apoC-I, 7.8%; apoC-II, 13.8%; $apoC\text{-}III_1$, 12.2%; $apoC\text{-}III_2$, 16.2%; and albumin, and other proteins, 24.4%. The large quantity of albumin present in the chylomicrons was due, at least in part, to the use of three successive ultracentrifugations instead of the seven employed by Kostner et al (9). The number of ultracentrifugations was kept to a minimum in order to minimize apolipoprotein dissociation during isolation.

Pleural chylomicrons, iodinated with [^{125}I], chromatographed as a single peak on radiochromatography at an apolipoprotein concentration of 1.79 mg/ml, and contained no detectable free iodine. The efficiency of

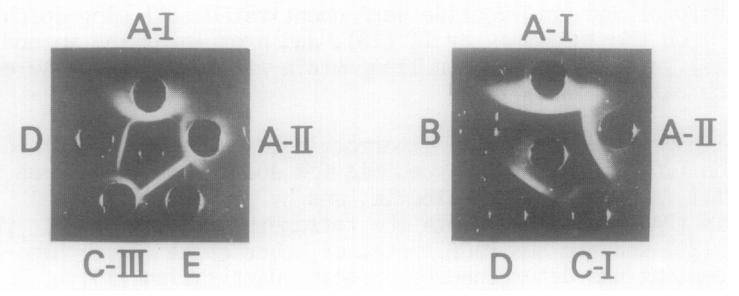

FIGURE 1. Immunodiffusion plates demonstrating the presence of apoA-I, apoA-II, apoB, apoC-I, apoC-III, apoD, and apoE in chylomicron apolipoproteins.

labeling and the lipid labeling were 22.2% and 6.1% respectively. The distribution of radioactivity within labeled chylomicrons is compared with the distribution of radioactivity among lipoprotein fractions (apolipoprotein and lipid components) following in vitro incubation of [^{125}I] chylomicrons with plasma in Table 1. No significant change in apoB distribution occurred with incubation, while there was a change in A apolipoprotein and C apolipoprotein distribution, consistent with the view that in vivo metabolism of chylomicrons is not required for apolipoprotein exchange.

The distribution of labeled apolipoprotein and lipid components in plasma lipoproteins over time following [^{125}I] chylomicron injection is shown in Figure 2. The initial distribution of [^{125}I] chylomicron apolipoprotein and lipid constituents in lipoprotein fractions following intervenous injection is assumed to be that determined following in vitro incubation of [^{125}I] chylomicrons and plasma as shown in table 1. The data shown indicate that changes in labeled apolipoprotein distribution in lipoprotein density fractions over time are not just due to exchange processes, but represent in vivo metabolic changes as well. Almost all A apolipoprotein radioactivity (apoA-I and apoA-II) rapidly disappeared from the chylomicron fraction and was found in HDL (90.2%). Labeled apoA-I and apoA-II were metabolized within HDL at a rate similar to that previously

TABLE 1. DISTRIBUTION OF RADIOACTIVITY OF [^{125}I] CHYLOMICRONS BEFORE AND AFTER IN VITRO INCUBATION IN PLASMA (FRACTION OF TOTAL)

	CHYLOMICRON* SAMPLE	IN VITRO INCUBATION CHYLO.	VLDL	IDL	LDL	HDL	1.21B+
apoA-I	.1230	.0676	.0001	-	-	.0207	-
apoA-II	.0940	.0665	.0004	-	-	.0131	-
apoB	.1080	.1288	.0008	.0001	-	.0001	-
apoC-II	.0430	.0361	.0012	.0001	-	.0380	-
apoC-III	.3290	.1559	.0089	.0006	.0002	.1022	-
albumin and other proteins	.2420	.2069	.0005	-	-	.0008	-
Lipid	.0610	.0487	.0002	-	-	-	-
Total	1.0000	.7109	.0127	.0008	.0002	.1699	.1056

* apoC-I was not labeled since it contains no tyrosine.
+ 1.21B is 1.21 g/ml infranatant, assumed to contain albumin, apoA, and apoC radioactivity.

reported for HDL A apolipoproteins in normal man (23, 24), based on the terminal slope of the decay curve (25). Labeled apoB was isolated in VLDL, IDL, and LDL; however, only 18.2% of the apoB activity initially injected could be isolated in LDL, suggesting that the majority of chylomicron apoB was catabolized prior to reaching LDL.

[^{125}I] ApoC radioactivity (apoC-II and apoC-III) was lost from chylomicrons at a somewhat slower rate than the A apolipoproteins or apoB. Most of the C radioactivity was subsequently isolated in VLDL and HDL. At the 6 hour point, an increase in apoC radioactivity was observed in chylomicrons due to reassociation of apoC proteins with chylomicrons. Chylomicrons and VLDL isolated from the pleural fluid on the fifth day of the study contained significant amounts of labeled C apolipoproteins (79.3% total), and labeled albumin (18.8%), little apoA radioactivity (1.8%) and no apoB activity. These findings are consistent with the view that C apolipoproteins reassociate with newly secreted triglyceride rich particles (26). Labeled chylomicron lipid rapidly disappeared from the chylomicron fraction with only small quantities of labeled lipid being isolated in IDL (1.7%), LDL (4.6%) and HDL (3.2%), suggesting that the majority of lipid is catabolized

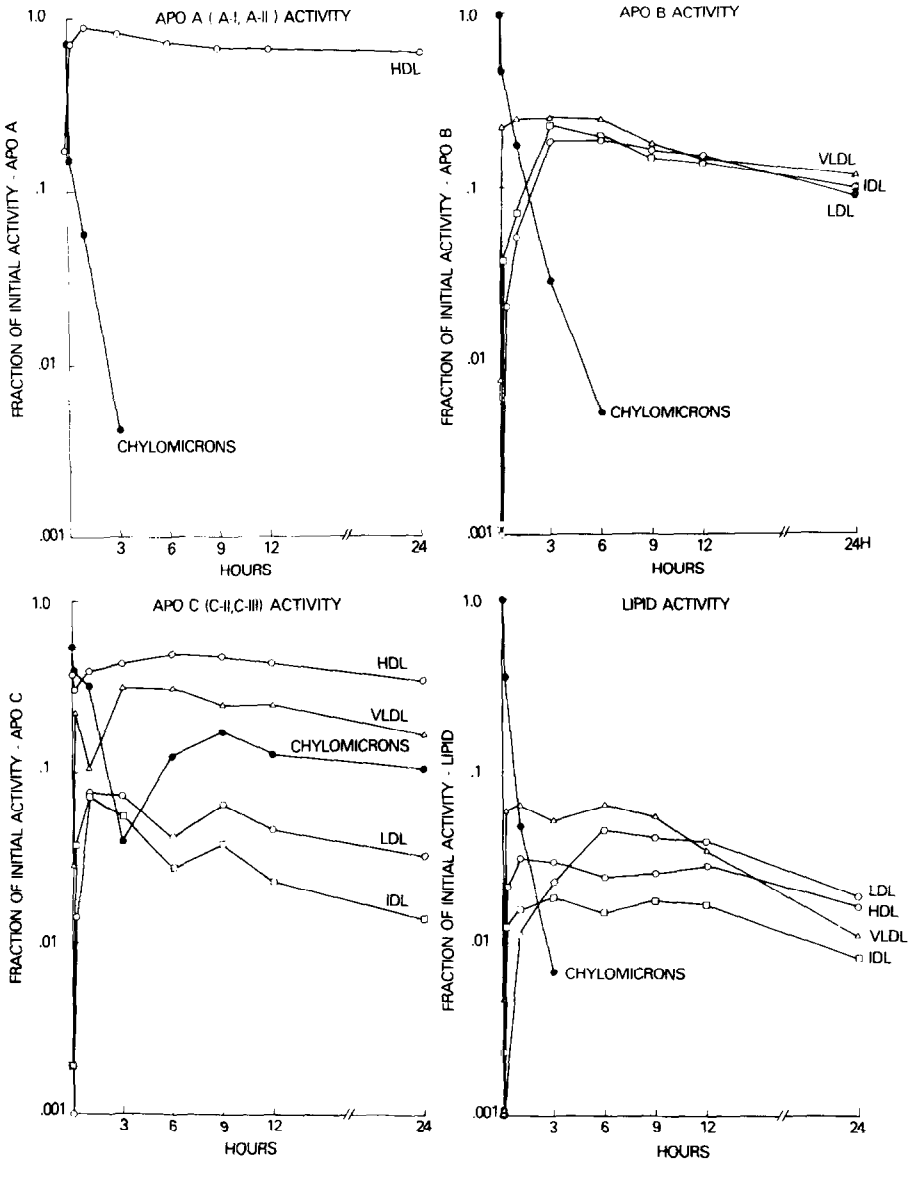

FIGURE 2. Distribution (fraction of initial activity) of A apolipoproteins (apoA-I and apoA-II), B apolipoprotein, C apolipoproteins (apoC-II and apoC-III) and lipid in plasma lipoprotein fractions over time following invenous injection of [^{125}I] chylomicrons.

with chylomicrons and VLDL.

Plasma chylomicrons contain little or no apoA-I and apoA-II in normal man. Chylomicrons isolated from pleural fluid (14) or thoracic duct

lymph (9), however, contain a significant amount of these apolipoproteins. The study of [^{125}I] pleural chylomicrons described in the present report permitted the analysis of the metabolism of chylomicrons which had not previously circulated in plasma. From these results two general conclusions can be drawn:

1) Only a small quantity of radiolabeled apoB on chylomicrons was ultimately isolated in LDL, indicating that only a small fraction of chylomicron apoB appears to be metabolized to LDL apoB. The vast majority of plasma LDL, therefore, appears to be a metabolic product of plasma VLDL secreted by liver as previously reported (13).

2). Chylomicron A apolipoproteins were rapidly transferred to HDL, consistent with the concept that A apolipoproteins in human chylomicron (apoA-I and apoA-II) are precursors for A apolipoproteins in plasma HDL.

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