Cholesteryl esters in lymph chylomicrons: contribution from high density lipoprotein transferred from plasma into intestinal lymph

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Abstract Most of the cholesterol in intestinal chyle and chylomicrons is derived from plasma. Our aim was to determine how much plasma low density (LDL) and high density (HDL) lipoproteins contribute to the cholesterol in chyle and chylomicrons, and to examine how plasma cholesterol becomes associated with lymph chylomicrons. Intravenous injection of radioiodinated plasma lipoproteins into two chyluric patients showed that 82% of the HDL plasma pool transferred daily to intestinal chyle, corresponding to 58% of lymph cholesterol; LDL contributed 18% of its plasma pool, corresponding to 18% of lymph cholesterol. When plasma HDL radiolabeled in both the protein and cholesteryl ester moieties was injected, the isotope ratios of plasma HDL and lymph lipoproteins were identical; 85% of the HDL cholesteryl esters transferred to triglyceride-rich lipoproteins, while the apolipoproteins remained largely (70%) in the higher density lipoproteins of the chyle. Incubations of similarly labeled plasma HDL showed preferential transfer of cholesteryl esters to artificial chylomicrons mediated by a factor present in lipoprotein-free plasma. Thus, a sizable portion of plasma HDL enters intestinal lymphatics probably as intact HDL, and then transfers part of their cholesteryl esters to chylomicrons, possibly mediated by transfer proteins. Reverse cholesterol transport may therefore include an extravascular loop via lymph chylomicrons and chylomicron remnants to the liver.—**Oliveira, H. C. F., K. Nilausen, H. Meinertz, and E. C. R. Quintão.** Cholesteryl esters in lymph chylomicrons: contribution from high density lipoprotein transferred from plasma into intestinal lymph. *J. Lipid Res.* 1993. **34:** 1729–1736.

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Lipoproteins in peripheral lymph arise in part by filtration of lipoproteins from plasma (1, 2) and in part from local cellular synthesis (3–6). The lipoproteins of intestinal lymph similarly consist of both plasma-derived lipoproteins and of constituents synthesized locally by the enterocytes (7–15). In addition, they consist of lipids of dietary and endogenous origin absorbed from the intestinal lumen. Studies of intestinal lymph lipoproteins in experimental animals (8, 16, 17) and in human subjects (18, 19) have yielded closely similar results.

Cholesterol transported in the intestinal lymph originates mainly in plasma, as shown both in rats (15, 20) and in chyluric patients (21, 22). In lymph-cannulated rats, the intravenous infusion of radioiodinated plasma HDL showed that all of apoE and part of apoA-I of intestinal lymph lipoproteins were derived from plasma (9, 14, 15). Plasma HDL may therefore be responsible for transfer of cholesterol from the circulation to intestinal lymph.

In human subjects, the distribution of cholesterol among lipoproteins in intestinal chyle is such that by far the greater fraction is associated with the chylomicrons, some is in the very low density lipoproteins (VLDL), and only limited amounts are in the higher density ranges of LDL and HDL (18, 19). As no human studies on the transfer of lipoproteins from the blood to intestinal lymph are available, the first aim of this work was to examine the contribution of the major cholesterol-carrying lipoproteins in plasma to the lipoproteins of mesenteric lymph in chyluric patients. The findings indicated that HDL transferred to the intestinal lymph to a much greater extent than did LDL and thus provided most of the plasma-derived cholesterol in intestinal chyle. This raised the question of how cholesterol was transferred from HDL to chylomicrons, and thus the second aim of this study was to examine whether this came about by a fusion of the two kinds of particles or by a selective transfer of cholesterol from one type of particle to the other.

Abbreviations: LDL, low density lipoproteins; HDL, high density lipoproteins; VLDL, very low density lipoproteins.

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MATERIAL AND METHODS

Material

Triolein, cholesteryl oleate, and cholesterol were purchased from Nu-Chek Prep, Inc. (Elysian, MN) and phosphatidylcholine was from Lipid Products (South Nutfield, Surrey, UK). Reagents for enzymatic determination of total and free cholesterol (Chod Pap) and of triglycerides were obtained from Boehringer (Mannheim, Germany) and Biodiagnostica Ind. (Parana, Brazil), respectively. Phospholipids were measured by the Bartlett procedure (23). The following radioisotopes were obtained from New England Nuclear (Boston, MA): $Na^{125}I$, [1,2-^{3}H(N)]cholesterol, [4-^{14}C]cholesteryl oleate, [1α,2α-^{3}H(N)]cholesteryl oleyl ether; $Na^{131}I$ was from IPEN-São Paulo Atomic Research Institute. Iodo-Gen was from Pierce Chemical Company (Rockford, IL) and Aquasol was from New England Nuclear.

Subjects

Three male patients with chyluria as a sequel of filariasis participated. Patient 1, 37 years old and born in Tanzania, was studied at Righospital, University of Copenhagen. Patient 2, 35 years old and born in Brazil, together with patient 3, 62 years old and born in Japan, were examined at the University of São Paulo Medical School, and their intestinal lymph-renal fistulae were demonstrated by lymphangiography. Patients 1 and 2 received liquid formula diets exclusively; patient 3 ate self-chosen food of unknown quantity and composition during the 4-day study. Patients 1 and 2 had continuous chyluria with a mean daily excretion of urinary fat of 25.2g ± 2.8 (SEM) in case of patient 1, and 5.3g ± 0.8 in case of patient 2. Plasma volume was estimated from height and body weight (24).

Lipoprotein transudation studies

All subjects received an intravenous bolus injection of radiolabeled autologous lipoproteins, which in the case of patient 1 consisted of 37 μCi ^{125}-labeled LDL, for patient 2 was 90 μCi ^{131}I-labeled HDL, and for patient 3 was reconstituted HDL labeled with ^{131}I (93 μCi), [1α,2α-^{3}H(N)]cholesteryl oleyl ether (18 μCi), and [4-^{14}C]cholesteryl oleate (21 μCi). To block the incorporation of radioactive iodine into the thyroid gland, all subjects received potassium iodine, 200 mg per day, orally from 3 days prior to and 4 weeks after the radioactive injection.

Fasting blood samples were drawn daily and mixed with EDTA (1.5 mg/ml); the plasma was dialyzed for 24 h at 4°C against physiological saline EDTA (1 mM) (1 liter × 5). Plasma lipoproteins were fractionated by sequential ultracentrifugation (patient 1) (25) or by discontinuous density gradient ultracentrifugation (patients 2 and 3) (26). For determination of the specific activity of apoLDL (patient 1) and of apoHDL (patient 2), the fractions were respun at their limiting densities.

Urine was collected in EDTA (0.01%) and sodium azide (0.01%); 24-h collections were mixed, the volume measured, and aliquots (100–250 ml) were dialyzed against physiological saline with EDTA (1 mM) and sodium azide (0.01%) (10 l × 4) at 4°C bringing the density to 1.006 g/ml. Urinary lipoproteins were then fractionated by sequential ultracentrifugation. Cholesterol and protein of urinary LDL (patient 1) and HDL (patient 2) were determined. Total fat, cholesterol, and protein were measured by gravimetric (21), gas–liquid chromatographic (27), and colorimetric procedures (28), respectively, in aliquots of total urine and in lymph chylomicrons of the urine.

Transudation of plasma apolipoproteins into enteric lymph was determined for apo-LDL (patient 1) and apo-HDL (patient 2) as the plasma apolipoprotein transported in lymph in mg/day:

$$\frac{\text{24-h urine lipoprotein radioactivity (dpm)}}{\text{sp act of plasma apolipoprotein (dpm/}\mu\text{g)}} \times \frac{\text{fat intake (g/day)}}{\text{24-h urinary fat excretion (g)}}.$$

Total amount of lipoprotein-cholesterol filtered into the lymph was determined as the apolipoprotein transudation multiplied by the cholesterol/apolipoprotein (μg/μg) ratio.

Lipoprotein labeling

Autologous LDL (d 1.019–1.063 g/ml) and HDL (d 1.063–1.21 g/ml) were isolated by sequential ultracentrifugation (26), and the fractions were reisolated at their upper limiting density. The lipoproteins were labeled with $Na^{125}I$ using 1 mCi/mg apolipoprotein by the modified McFarlane method (29) for LDL and with Iodo-Gen for HDL. After iodination, the lipoprotein was dialyzed against physiological saline (EDTA 1 mM), 1 l × 5, for 12 h at 4°C and sterilized by filtration through a 0.22-μm filter (Millipore, Bedford, MA) immediately prior to intravenous infusion. Less than 2% of the radioactivity was lipid-extractable and 96–98% was precipitated by trichloroacetic acid. SDS-polyacrylamide gel electrophoresis of the ^{125}I-labeled HDL showed that 0.3% of the radioactivity was associated with contaminating albumin (30).

Preparation of reconstituted, labeled HDL involved labeling core lipids with [4-^{14}C]cholesteryl oleate and [1α,2α-^{3}H(N)]cholesteryl oleyl ether by a modified procedure of Nestler et al. (31), and of apolipoproteins with ^{131}I. Briefly, the HDL solution (2 ml) was delipidated twice with 40 ml cold ethanol–diethyl ether 3:2 (v/v). After removal of solvent from the lipid phase, a solution of the radioactive steroids was added, and the solvent was removed under N_2 at 4°C. The HDL-apolipoprotein, diluted with phosphate buffer at pH 7.2, was added to the radiolabeled lipids and the mixture was sonicated for 30 min at 70–80 watts, at 37°C, under N_2 (B30 Branson

Sonifier, Branson Sonic Power Co., Danbury, CT). This reconstituted $^{3}H/^{14}C$-HDL (recHDL), and a parallel, unlabeled HDLrec were purified separately by discontinuous density gradient ultracentrifugation (Beckman, SW 41Ti rotor) for 24 h (26). After dialysis, the unlabeled HDLrec was then labeled with $Na^{131}I$ (Iodo-Gen procedure), and both preparations were dialyzed and sterilized by Millipore filtration (0.22 μm). The HDLrec contained 3% cholesterol, 13% cholesteryl ester, 6% triglyceride, 42% phospholipid, and 36% protein by weight. Radioactivity measurement of the iodine label was done directly in the gamma counter, whereas ^{3}H and ^{14}C required prior chloroform–methanol 2:1 (v/v) (plasma) or chloroform (lyophilized urine) extraction. To ensure that the ^{131}I did not interfere with the β-scintillation counting in toluene–phosphor solution (Beckman model LS-6000 TA), the samples were stored for 80 days before measuring ^{3}H and ^{14}C.

Incubation of plasma HDL with chylomicron-like particles

Bulk HDL (d 1.063–1.21 g/ml) prepared from pooled plasma were labeled with $Na^{125}I$ (Iodo-Gen) or with [1,2-^{3}H(N)]cholesterol by the procedure of Gavish, Oschry, and Eisenberg (32). Briefly, [^{3}H]cholesterol (50 μCi in 100 μl ethanol) was added to the HDL solution together with lipoprotein-free plasma, d > 1.21 g/ml (total volume 10 ml), and incubated for 20 h at 37°C. Labeled HDL was then separated from the incubation mixture by ultracentrifugation; analysis of the HDL-lipids by thin-layer chromatography showed that 77–88% of the HDL ^{3}H activity was present as esterified cholesterol.

Chylomicron-like, triglyceride-rich particles were prepared as previously described (33, 34). Briefly, a mixture of cholesterol (2%), cholesteryl oleate (6%), phosphatidylcholine (23%), and triolein (69%) was cosonified in NaCl solution (2.785 M), pH 7.0, d 1.101 g/ml, at 70–80 watts, for 30 min at 37°C under a flow of N_2. The chylomicron-like material was collected at d 1.006 g/ml after discontinuous density gradient ultracentrifugation; it contained 4.5% cholesterol, 6.8% cholesteryl oleate, 9.5% phosphatidylcholine, and 80.3% triolein, by weight.

HDL preparations, labeled with ^{125}I or with [^{3}H]cholesterol, both esterified and free, were incubated together with chylomicron-like particles. Incubation conditions were variable concentrations of HDL together with 500 μg triolein in a total volume of 1.1 ml. The incubation was terminated by the addition of hypertonic potassium bromide solution, d 1.346 g/ml, and then HDL and the chylomicron-like material were immediately separated by discontinuous density gradient ultracentrifugation at d 1.101–1.006 g/ml in an SW 41Ti rotor, at 4°C, for 30 min at 40,000 rpm. The distribution of radioactivity between the top fraction (d < 1.006 g/ml) representing the chylomicron-like material and the infranatant corresponding to the HDL was determined. All assays were done in duplicate or triplicate, together with blank incubations containing no chylomicron-like particles. Less than 1.1% of the total radioactivity in the blank incubations was found in the top fraction (d < 1.006 g/ml). After lipid extraction (chloroform–methanol 2:1) of the two ultracentrifugal fractions, free and esterified cholesterol were separated by thin-layer chromatography on Silica Gel H in hexane–diethyl ether–acetic acid 70:30:1 (v/v/v). The appropriate fractions, identified by iodine vapor, were scraped into scintillation vials containing Aquasol for radioactivity measurement. The findings were expressed either as percent of total radioactivity or as mass that had been transferred to the other type of particle. The transferred mass was calculated as radioactivity (dpm) in the receptor particle divided by the specific activity (dpm/μg) in the donor particle prior to incubation.

RESULTS

Transudation of plasma lipoprotein

After an intravenous injection of ^{125}I-labeled autologous LDL (patient 1) and HDL (patient 2), the entry of apolipoprotein label (^{125}I) into intestinal lymph lipoproteins was measured quantitatively during the subsequent 4–6 days. The amount of plasma apolipoprotein transported daily in the enteric lymph was calculated from the total nondialyzable radioactivity present in the lymph each day divided by the specific activity of plasma LDL and HDL apolipoprotein on the same day. The data showed that 4974 ± 171 mg (mean ± SD) of plasma apoHDL was transported daily in the lymph of patient 2, corresponding to 82% of the plasma pool of HDL, as opposed to 406 ± 44 mg of plasma apoLDL per day, representing only 18% of the plasma pool of patient 1. The amount of apolipoprotein-associated cholesterol was calculated from the cholesterol/apolipoprotein ratio of plasma LDL and HDL, respectively. Thus, assuming that intact lipoprotein particles filtered from plasma to intestinal lymph, 489 ± 55 mg (patient 1) and 892 ± 138 mg (patient 2) of cholesterol in the intestinal lymph originated from plasma LDL and HDL, respectively. These quantities represented 18% and 58%, respectively, of the total cholesterol transport in intestinal lymph so that the combined contribution from the two plasma lipoprotein classes was about 75% of enteric lymph cholesterol. Although these data were obtained in studies of only two individuals, their general validity was supported by our similar findings in previous studies of several chyluric individuals (21).

In plasma, the apolipoprotein label of HDL and LDL remained essentially in the density range into which it was originally introduced for the duration of the study (**Fig. 1**). This was true for patients 1 and 2 who received

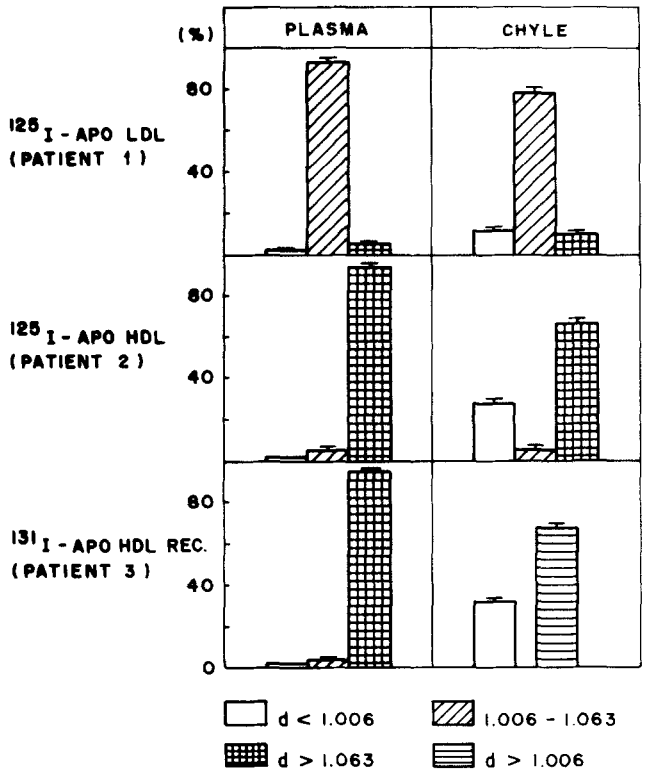

Fig. 1. Percent distribution of radioactivity in plasma and chyle lipoproteins (mean ± SEM) 4–6 days after the intravenous pulse infusion of ^{125}I-labeled apoLDL (patient 1), ^{125}I-labeled apoHDL (patient 2), and reconstituted ^{131}I-labeled apoHDL (patient 3).

lipoproteins radioiodinated in their native state, and also for patient 3 who was injected with a reconstituted HDL preparation. In intestinal lymph, however, more extensive redistribution of the apolipoprotein label took place (Fig. 1). In particular, the HDL apolipoprotein label (^{125}I, patient 2; ^{131}I, patient 3), of which only traces (1–2%) were associated with triglyceride-rich lipoproteins (d < 1.006 g/ml) in plasma, shifted in the intestinal lymph so that about 30% of the radioactivity was in the density range of chylomicrons and VLDL in patients 2 and 3. Compared to HDL, the shift in the intestinal lymph of the LDL apolipoprotein label (^{125}I) was modest in that the fraction associated with triglyceride-rich lipoproteins was 12% (patient 1).

To examine whether the core lipids of plasma HDL transferred in parallel with the apolipoprotein to intestinal chyle and to the individual lymph lipoproteins, autologous reconstituted HDL labeled in the apolipoprotein moiety with ^{131}I and in the core lipids with [^{14}C]cholesteryl oleate and [^{3}H]cholesteryl oleyl ether was injected intravenously into a chyluric subject (patient 3). The ratios of apolipoprotein to core lipid radioactivity of the lymph lipoproteins were virtually identical with those of plasma HDL (**Table 1**). The simplest interpretation of this finding was that the core lipids and the apolipoproteins passed from the circulating blood to the intestinal lymph at identical rates, most likely as constituents of intact HDL particles. Once in the lymph, the HDL core lipids ([^{14}C]cholesteryl ester and [^{3}H]cholesteryl ether) transferred extensively (about 85%) to the triglyceride-rich chylomicrons and VLDL, and only a modest fraction remained in the higher density (d > 1.006 g/ml) lipoproteins (**Fig. 2**). Thus, the shift of cholesteryl esters from HDL to lymph chylomicrons and VLDL was much greater than that of the HDL apolipoprotein (^{131}I), about 85% versus 30% (Figs. 1 and 2), indicating that cholesteryl esters became associated with triglyceride-rich lipoproteins in intestinal lymph mainly by a selective lipid transfer rather than by a simple fusion of HDL particles with chylomicrons.

In plasma, the HDL core lipids ([^{14}C]cholesteryl ester and [^{3}H]cholesteryl ether) likewise transferred extensively to lipoproteins of lower densities, but in plasma the major fraction became associated with LDL rather than with the triglyceride-rich lipoproteins as in the intestinal lymph (Fig. 2). The enrichment of LDL in plasma with labeled cholesteryl ester (^{14}C) and ether (^{3}H) from HDL may explain why the isotope ratio ^{131}I-labeled apoHDL/^{14}C or ^{3}H of total plasma lipoproteins was clearly lower than that of plasma HDL and the lymph lipoproteins (Table 1). Because lipoproteins of lower densities are eliminated faster from circulation than are those of high density, the radioactive cholesteryl esters are preferentially lost from the plasma compartment compared to the HDL-apolipoproteins. The isotope ratio of total lymph lipoproteins, on the contrary, was virtually identical with that of plasma HDL. This suggests that the main source of lymph lipoprotein radioactivity is plasma and that plasma HDL reaches the intestinal lacteals as an intact particle.

Transfer of HDL constituents to artificial chylomicrons

In an attempt to identify factors in intestinal lymph influencing the transfer of HDL constituents to chylomicrons, plasma HDL labeled in the apolipoprotein with ^{125}I and in the lipid moiety with free or esterified [^{3}H]cholesterol was incubated with artificial chylomicrons. Transfer of apolipoprotein label to triglyceride-rich particles was limited to a few percent in an incubation medium of physiological saline, and this process was even inhibited

TABLE 1. Ratios of ^{131}I-labeled HDL to [1α,2α-^{3}H(N)]cholesteryl oleyl ether or [4-^{14}C]cholesteryl oleate in patient 3

Variable	^{131}I/^{3}H	^{131}I/^{14}C
	mean ± SEM	
Plasma HDL	18.5 ± 3.4	14.4 ± 1.5
Total lymph lipoproteins	19.6 ± 4.2	13.7 ± 0.9
Total plasma lipoproteins	12.3 ± 1.5	6.4 ± 0.6

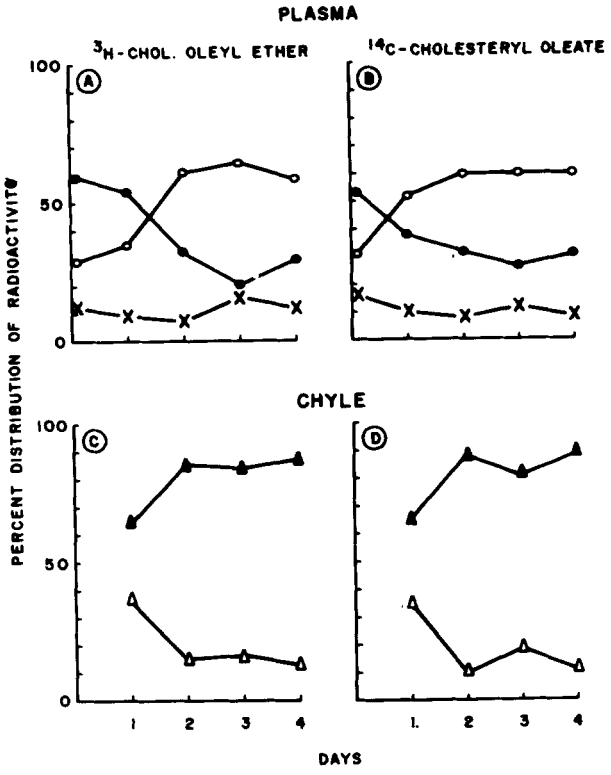

Fig. 2. Percent distribution of radioactivity in lipoprotein fractions in plasma (panels A, B: (○) LDL; (●) HDL; (χ) VLDL) and urine chyle (panels C, D: (▲) d < 1.006 g/ml; (△) d > 1.006 g/ml) of patient 3 1-4 days after the intravenous pulse infusion of autologous bulk HDL simultaneously labeled with [1α,2α-^{3}H(N)]cholesteryl oleyl ether (panels A, C) and [4-^{14}C]cholesteryl oleate (panels B, D).

initially in the presence of albumin or lipoprotein-free plasma (d > 1.21 g/ml) (**Fig. 3, A, B, C**). Free cholesterol transferred rapidly and extensively, unaffected by the composition of the incubation medium (Fig. 3, D, E, F). Transfer of HDL cholesteryl ester was slow initially and increased only gradually with time in saline with or without albumin, but was fast and extensive when the incubation took place in lipoprotein-free plasma, presumably due to the presence of cholesteryl ester transfer proteins; about 80% became associated with chylomicron-like particles after 2 h of incubation (Fig. 3, G, H, I). The marked difference in transfer between HDL apolipoprotein and cholesteryl ester was independent of the concentration of HDL; in saline the fractional transfer of apolipoprotein was less than one-third that of cholesteryl ester (5.5% versus 17.7%), and in lipoprotein-free plasma, apolipoprotein transfer was reduced to one-fifth of that of the saline incubation, whereas cholesteryl ester transfer was increased to about 49% (**Fig. 4**).

DISCUSSION

Cholesterol in intestinal chyle is derived from several sources, namely intestinal absorption, de novo synthesis by the enterocytes, and plasma lipoproteins. Even when intestinal absorption is greatly increased by a heavy dietary load, the contribution from plasma lipoproteins remains the major source of chylous cholesterol (21, 22). In intestinal chyle the triglyceride-rich lipoproteins, especially chylomicrons, carry by far the greater portion of cholesterol, whereas LDL and HDL account for only a minor fraction (21). This study demonstrates that plasma HDL in human subjects is the major source of cholesterol in intestinal lymph, that HDL cholesteryl esters pass from the circulation to the intestinal lymphatics as part of HDL particles, and that most of the cholesteryl esters subsequently are transferred to lymph chylomicrons and VLDL without a parallel transfer of HDL apolipoprotein.

The contribution of plasma lipoproteins to the cholesterol transported in intestinal lymph has, in the present study of human subjects, been evaluated by the intravenous injection of radioactively labeled plasma HDL and LDL in chyluric patients to determine the fate of the labeled lipoprotein constituents in intestinal lymph. Thus, the transfer of cholesterol from plasma to lymph lipoproteins may be viewed as consisting of two discrete steps: the first involves the passage from circulating blood

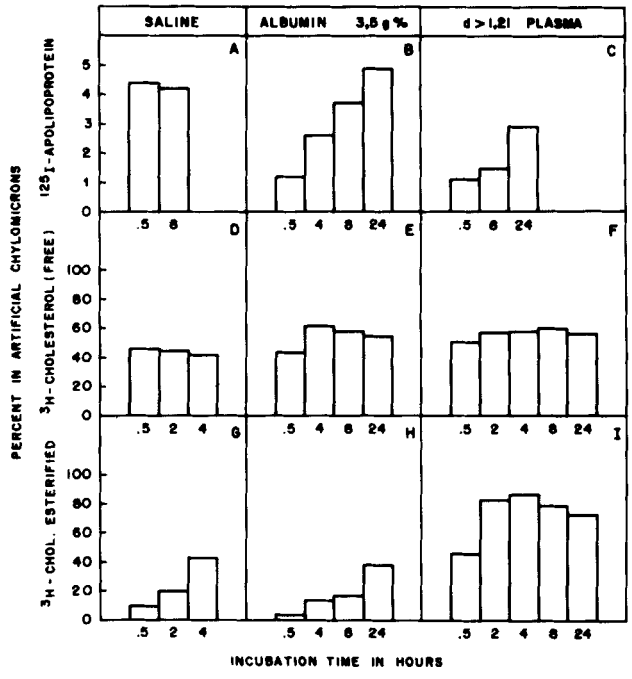

Fig. 3. Transfer with time of ^{125}I-labeled apoHDL (100 μg) or of [1,2-^{3}H(N)]cholesteryl ester and [1,2-^{3}H(N)]cholesterol (unesterified) from HDL to artificial chylomicrons (500 μg TG) in saline (panels A, D, G), albumin solution 3.5 g % (panels B, E, H), or in the plasma d > 1.21 g/ml fractions (panels C, F, I). In vitro ^{125}I and ^{3}H assays were carried out separately. HDL had been labeled with [1,2-^{3}H(N)]cholesterol in the presence of the plasma d > 1.21 g/ml fraction and 77–88% was esterified. In incubated particles, [^{3}H]cholesterol (unesterified) was separated from [^{3}H]cholesteryl ester by thin-layer chromatography. Means of duplicate incubations.

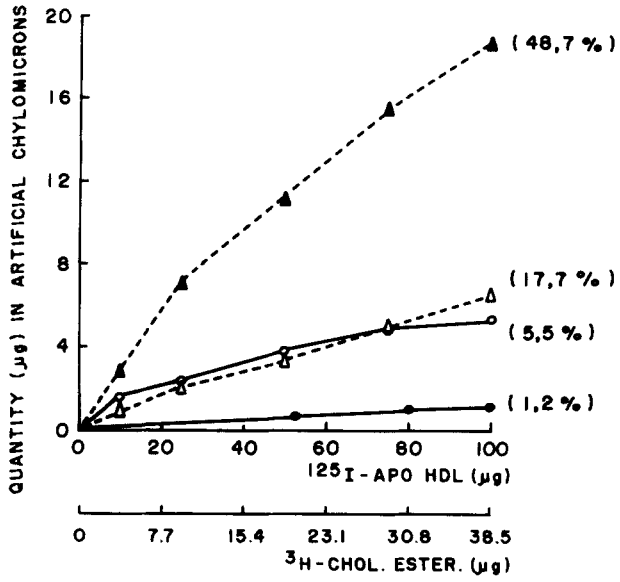

Fig. 4. Transfer to artificial chylomicrons (500 μg TG) of ^{125}I-labeled apoHDL (circles) and of [1,2-^{3}H(N)]cholesteryl ester (triangles) (^{3}H-CE) from HDL either in saline medium (open symbols) or in plasma d > 1.21 g/ml fraction (closed symbols) after incubation periods of 30 min (apoHDL) or 120 min (^{3}H-CE) at 37°C. Incubations were done with increasing quantities of radioactive HDL. Values in parentheses indicate percent of [^{3}H]cholesteryl ester and ^{125}I-labeled apoHDL from the incubation medium that was found in chylomicrons. Results are reported as means of duplicates. Methods are shown in the legend to Fig. 3.

via interstitial tissue to the lymphatics of the intestinal mucosa, and the second concerns the distribution of cholesterol among lipoproteins in the lymph.

Previous studies in the rat have shown that after intravenous injection of radiolabeled plasma HDL, the label not only appears in intestinal lymph (14) but it is recovered in the HDL density range of the lymph lipoproteins (9), as an indication that HDL is transferred as such from the circulating blood to intestinal lymph. Compositional similarities between HDL in plasma and lymph in human subjects (35) and in pigs (8) have also suggested that plasma HDL is an important source of intestinal lymph HDL in these species. In the present study we found that not only radioiodinated HDL but also LDL in plasma passed into intestinal lymph and that the apolipoprotein labels were located predominantly in the HDL and LDL density ranges of lymph lipoproteins, indicating a transfer of plasma HDL and LDL as such to intestinal lymph. In case of plasma HDL this conclusion was further strengthened by our finding that the fractional transfers of core lipid and apolipoprotein labels to the intestinal lymph were virtually identical. In the case of LDL, previous studies have shown close similarity between plasma and lymph lipoproteins in peak flotation rates in human subjects (35) and in composition in the pig (8) in agreement with the conclusion that plasma LDL transfers as such to intestinal lymphatics and is a major source of lymph LDL. The greater fractional transfer of plasma HDL compared to LDL may well be explained by the smaller diameter of the HDL particles.

The amount of cholesterol carried by HDL and LDL from plasma to intestinal lymph was calculated from the amount of transferred apolipoprotein label multiplied by the ratio of cholesterol to apolipoprotein mass in plasma HDL and LDL, assuming that this ratio was valid for the lipoproteins filtered into the lymphatics. To the extent that the lipoproteins passing from plasma to intestinal lymph differ in this ratio from total plasma HDL and LDL, the calculation will be erroneous. However, our finding that transfer of circulating HDL and LDL together accounted for about 75% of total intestinal lymph cholesterol transport agrees very well with our previous studies in chyluric patients, in which the contribution of plasma lipoprotein cholesterol was determined from the transfer of radioactive sterol from plasma to intestinal lymph (21, 22).

Once HDL and LDL from plasma have entered the interstitial tissue and the lymphatics of the intestinal mucosa, they encounter lipoproteins of intestinal origin. Our data show that both apolipoprotein and core lipid labels leave their original density ranges and become associated mainly with the predominant triglyceride-rich lipoproteins of the chyle. In the case of HDL, the fractional transfer of the core lipid labels to triglyceride-rich lipoproteins was nearly complete (85%) and almost three times greater than that of the apolipoprotein label. Thus, most of the cholesteryl ester transferred dissociated from apolipoprotein, suggesting a process mediated by cholesteryl ester transfer protein. Although transfer proteins have not yet been shown to occur in intestinal lymph, they would be expected to pass easily from circulation to intestinal lymphatics whether they exist in plasma unbound to lipoprotein or in association with HDL because their molecular weight is similar to that of plasma albumin (36). If indeed the passage of cholesteryl ester from HDL to chylomicrons is mediated by transfer protein, a reciprocal transfer of triglyceride would take place, and in agreement with this, human HDL from intestinal lymph showed, indeed, a higher content of triglycerides and a lower content of cholesteryl ester compared to circulating HDL in the same patients. An alternative explanation for this compositional difference could, however, be a mass action effect due to the high triglyceride concentration in the chylomicrons (35). The presence of transfer protein in intestinal lymph might also mediate transfer of cholesteryl ester from filtered plasma LDL to triglyceride-rich lymph lipoprotein, analogous to that from HDL, but we have no data to demonstrate such a transfer.

The apolipoprotein labels of plasma HDL and LDL remained in their original density classes while circulating in the blood, but after entry into intestinal lymph some of the labels transferred to lipoproteins of other densities.

About 30% of the HDL apolipoprotein label became associated with the triglyceride-rich lipoproteins, while the corresponding transfer of LDL apolipoprotein was only about 10%. Although we have no data on individual apolipoproteins, a likely explanation for the difference between HDL and LDL in this respect is that apoC, which is a major constituent of HDL apolipoproteins, has been shown to transfer extensively when lymph chylomicrons from the rat were incubated with serum lipoprotein fractions; some apoE transferred as well (16). Similarly, human lymph chylomicrons become enriched in apoC and E when they enter circulating blood (37). How the transfer of about 10% of the LDL apolipoprotein label to triglyceride-rich lymph lipoproteins and another few percent to lymph HDL is to be explained is uncertain in the absence of detailed apolipoprotein analyses. One possibility is that apoC, E, and A were responsible for the transfer of label, as plasma LDL isolated by the presently used procedure generally contains limited amounts of these apolipoproteins (38–40), which are known to transfer readily between lipoprotein classes (37). If, however, the apolipoprotein label represented apoB-100, this would mean that either LDL became trapped by or fused with lipoproteins of lower and higher density classes, or that labeled apoB-100 in some other way became associated with other lymph lipoproteins. Although none of these latter alternatives appear likely, it is noteworthy that lymph chylomicrons from both humans (41) and pigs (8) contain apoB-100, even though the intestinal mucosa may be unable to synthesize this apolipoprotein; but even if the apoB-100 of lymph chylomicrons is of hepatic origin, it is difficult to envision how labeled apoB-100 from plasma LDL could become incorporated into lymph chylomicrons.

The cholesteryl esters of plasma HDL, originally derived from cholesterol of peripheral cells, are to a considerable extent transferred to VLDL and chylomicrons in plasma, mediated by transfer protein, in exchange for triglycerides. They are finally taken up by the liver via endocytosis of LDL and chylomicron remnants, and thus they complete the centripetal transport of cholesterol from peripheral tissues to the liver. As we have presently demonstrated, substantial amounts of plasma HDL enter intestinal lymphatics to interact with large quantities of triglyceride-rich chylomicrons whereby an extensive transfer of HDL cholesteryl esters to chylomicrons occurs. This extravascular interaction between plasma HDL and lymph chylomicrons may be of importance for the efficiency of the reverse cholesterol transport because relatively more cholesteryl ester in that manner ends up in chylomicron remnants, which are removed more efficiently by the liver than LDL.

A number of unresolved questions remain concerning both the transfer of plasma lipoproteins to the intestinal lymphatics and the interactions in intestinal lymph between lipoproteins of intestinal and hepatovascular origin. The heterogeneity of particles in the HDL and LDL density spectra, in terms of size and composition, may well result in the selective passage of some subfractions from plasma to lymph in preference to others. To what extent lipoproteins of density below that of LDL may transfer is uncertain; although VLDL is generally considered too large to pass the endothelial lining of intestinal blood capillaries, the possibility exists that some intermediate density lipoprotein might pass. Extensive transfer or exchange of both lipid and apolipoprotein constituents between plasma and lymph lipoprotein have been observed in previous and in the present studies, but information on how individual apolipoproteins and phospholipids participate is still incomplete. Unresolved also are questions about independent or coupled transfer of different lipoprotein constituents and the role of transfer proteins for these phenomena. Several of these questions may be answered by studies of chyluric patients by the techniques presently used if extended to include analysis of radioactivity and mass of individual apolipoproteins.

In summary, we have provided new evidence that human plasma HDL and LDL are the major sources of cholesterol in intestinal chyle and its major lipoproteins, chylomicrons, and VLDL. The two lipoproteins leave the circulation and enter the intestinal lymph as LDL and HDL, respectively. Cholesteryl esters from HDL, and presumably from LDL, transfer to the triglyceride-rich lymph lipoproteins largely dissociated from apolipoproteins. As cholesteryl esters of plasma HDL are part of the transport system of cholesterol from the periphery to the liver, the present observations demonstrate the existence of an extravascular loop in this pathway which may be of importance for the efficiency of the reverse cholesterol transport.

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REFERENCES

1. Reichl, D., L. A. Simons, N. B. Myant, J. J. Pflug, and G. L. Mills. 1973. The lipids and lipoproteins of human peripheral lymph, with observations on the transport of cholesterol from plasma and tissues into lymph. *Clin. Sci. Mol. Med.* **45:** 313–319.
2. Reichl, D., A. Postiglione, N. B. Myant, J. J. Pflug, and M. Press. 1975. Observations on the passage of apoproteins from plasma lipoproteins into peripheral lymph in two men. *Clin. Sci. Mol. Med.* **49:** 419–426.
3. Dory, L., L. M. Boquet, C. R. Tate, and C. H. Sloop. 1986. Peripheral synthesis and isoform distribution of dog apoprotein E—in vivo approach. *J. Biol. Chem.* **261:** 811–816.

4. Reichl, D., N. B. Myant, D. N. Rudra, and J. J. Pflug. 1980. Evidence for the presence of tissue-free cholesterol in low density and high density lipoproteins of human peripheral lymph. *Atherosclerosis.* **37:** 489–495.
5. Sloop, C. H., L. Dory, R. Hamilton, B. R. Krause, and P. S. Roheim. 1983. Characterization of dog peripheral lymph lipoproteins: the presence of a disc-shaped "nascent" high density lipoprotein. *J. Lipid Res.* **24:** 1429–1440.
6. Sloop, C. H., L. Dory, and P. S. Roheim. 1987. Interstitial fluid lipoproteins. *J. Lipid Res.* **28:** 225–237.
7. Green, P. H. R., R. M. Glickman, C. D. Saudek, C. B. Blum, and A. R. Tall. 1979. Human intestinal lipoproteins—studies in chyluric subjects. *J. Clin. Invest.* **64:** 233–242.
8. Black, D. D., and N. O. Davidson. 1989. Intestinal apolipoprotein synthesis and secretion in the suckling pig. *J. Lipid Res.* **30:** 207–218.
9. Bearnot, H. R., R. M. Glickman, L. Weinberg, P. H. R. Green, and A. R. Tall. 1982. Effect of biliary diversion on rat mesenteric lymph apolipoproteinA-I and high density lipoprotein. *J. Clin. Invest.* **69:** 210–217.
10. Davidson, N. O., and R. M. Glickman. 1985. Apolipoprotein A-I synthesis in rat small intestine: regulation by dietary triglyceride and biliary lipid. *J. Lipid Res.* **26:** 368–379.
11. Fidge, N., and W. Kimpton. 1983. Distribution of C apolipoproteins between the vascular and lymph compartments of the rat. *Biochim. Biophys. Acta.* **752:** 209–213.
12. Green, P. H. R., R. M. Glickman, and J. W. Riley. 1980. Human apolipoprotein A-IV—intestinal origin and distribution in plasma. *J. Clin. Invest.* **65:** 911–919.
13. Hayashi, H., D. F. Nutting, K. Fujimoto, J. A. Cardelli, D. Black, and P. Tso. 1990. Transport of lipid and apolipoproteins A-I and A-IV in intestinal lymph of the rat. *J. Lipid Res.* **31:** 1613–1625.
14. Imaizumi, K., R. J. Havel, M. Fainaru, and J. L. Vigne. 1978. Origin and transport of the A-I and arginine-rich apolipoproteins in mesenteric lymph of rats. *J. Lipid Res.* **19:** 1038–1046.
15. Sipahi, A. M., H. C. F. Oliveira, K. S. Vasconcelos, L. N. Castilho, A. Bettarello, and E. C. R. Quintão. 1989. Contribution of plasma protein and lipoproteins to intestinal lymph: comparison of long-chain with medium-chain triglyceride duodenal infusion. *Lymphology.* **22:** 13–19.
16. Imaizumi, K., M. Fainaru, and R. J. Havel. 1978. Composition of proteins of mesenteric lymph chylomicrons in the rat and alterations produced upon exposure of chylomicrons to blood serum and serum proteins. *J. Lipid Res.* **19:** 712–722.
17. Laplaud, P. M., D. Bauchart, D. Durand, and M. J. Chapman. 1990. Lipoproteins and apolipoproteins in intestinal lymph of the preruminant calf, *Bos* spp., at peak lipid absorption. *J. Lipid Res.* **31:** 1781–1792.
18. Dumont, A. E., and M. H. Witte. 1969. Clinical usefulness of thoracic duct cannulation. *Adv. Int. Med.* **15:** 51–71.
19. Green, P. H. R., and R. M. Glickman. 1981. Intestinal lipoprotein metabolism. *J. Lipid Res.* **22:** 1153–1173.
20. Vasconcelos, K. S., A. M. Sipahi, H. C. F. Oliveira, L. N. Castilho, N. de Luccia, and E. C. R. Quintão. 1989. Origin of intestinal lymph cholesterol in rats: contribution from luminal absorption, mucosal synthesis and filtration from plasma. *Lymphology.* **22:** 4–12.
21. Quintão, E. C. R., A. Drewiacki, K. Stechhahn, E. C. Faria, and A. M. Sipahi. 1979. Origin of cholesterol transported in intestinal lymph: studies in patients with filarial chyluria. *J. Lipid Res.* **20:** 941–951.
22. Doi, S. Q., H. Meinertz, K. Nilausen, E. C. Faria, and E. C. R. Quintão. 1987. Intestinal cholesterol absorption in the chyluria model. *J. Lipid Res.* **28:** 1129–1136.
23. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234:** 466–468.
24. Brown, E., J. Hopper, Jr., L. Hodges, Jr., B. Bradley, R. Wennesland, and H. Yamauchi. 1962. Red cell, plasma and blood volume in healthy women measured by radiochromium cell-labeling and hematocrit. *J. Clin. Invest.* **41:** 2183–2190.
25. 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.
26. 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.
27. Miettinen, T. A., E. H. Ahrens, Jr., and S. M. Grundy. 1965. Quantitative isolation and gas–liquid chromatography analysis of total dietary and fecal neutral steroids. *J. Lipid Res.* **6:** 411–424.
28. 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.
29. Bilheimer, D. W., S. Eisenberg, and R. I. Levy. 1972. The metabolism of very low density lipoprotein I—preliminary "in vitro" and "in vivo" observations. *Biochim. Biophys. Acta.* **260:** 212–217.
30. Connelly, P. W., and A. Kuksis. 1982. SDS-Glycerol polyacrylamide gel electrophoresis of plasma apolipoproteins. *Biochim. Biophys. Acta.* **711:** 245–251.
31. Nestler, J. E., M. Bamberger, G. H. Rothblat, and J. F. Strauss, III. 1985. Metabolism of high density lipoproteins reconstituted with [^{3}H]cholesteryl ester and [^{14}C]cholesterol in the rat, with special reference to the ovary. *Endocrinology.* **117:** 502–510.
32. Gavish, D., Y. Oschry, and S. Eisenberg. 1987. In vivo conversion of human HDL_3 to HDL_2 and apoE-rich HDL_1 in the rat: effects of lipid transfer protein. *J. Lipid Res.* **28:** 257–267.
33. Oliveira, H. C. F., M. H. Hirata, T. G. Redgrave, and R. C. Maranhão. 1988. Competition between chylomicrons and their remnants for plasma removal: a study with artificial emulsion models of chylomicrons. *Biochim. Biophys. Acta.* **958:** 211–217.
34. Zerbinatti, C. V., H. C. F. Oliveira, S. Wechsler, and E. C. R. Quintão. 1991. Independent regulation of chylomicron lipolysis and particle removal rates: effects of insulin and thyroid hormones on the metabolism of artificial chylomicrons. *Metabolism.* **40:** 1122–1127.
35. Andersen, D. W., E. J. Schaefer, T. J. Bronzert, F. T. Lindgren, T. Forte, T. E. Starzl, G. D. Niblack, L. Zech, and H. B. Brewer. 1981. Transport of apolipoproteins A-I and A-II by human thoracic duct lymph. *J. Clin. Invest.* **67:** 857–866.
36. Albers, J. J., J. H. Tollefson, C-H. Chen, and A. Steimetz. 1984. Isolation and characterization of human plasma lipid transfer proteins. *Arteriosclerosis.* **4:** 49–58.
37. Havel, R. J., J. P. Kane, and M. L. Kashyap. 1973. Interchange of apolipoprotein between chylomicrons and high density lipoprotein during alimentary lipemia in man. *J. Clin. Invest.* **52:** 32–38.
38. Lee, D. M., and P. Alaupovic. 1974. Composition and concentration of apolipoproteins in very-low and low-density lipoproteins of normal human plasma. *Atherosclerosis.* **19:** 501–521.
39. Blum, C. B., L. Aron, and R. Sciacca. 1980. Radioimmunoassay studies of human apolipoprotein E. *J. Clin. Invest.* **66:** 1240–1250.
40. Havel, R. J., L. Kotite, J-L. Vigne, P. Tun, N. Phillips, and C. G. Chen. 1980. Radioimmunoassay of human arginine-rich apolipoproteins, apoprotein E. *J. Clin. Invest.* **66:** 1351–1362.
41. Kane, J. P., D. A. Hardman, and H. E. Paulus. 1980. Heterogeneity of apolipoprotein B: isolation of a new species from human chylomicrons. *Proc. Natl. Acad. Sci. USA.* **77:** 2465–2469.