papers and notes on methodology

Human plasma lipid exchange protein(s): a method for separation of donor and acceptor lipoproteins by heparin-Sepharose chromatography

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Abstract The transfer or exchange of cholesteryl esters, triglycerides, and phospholipids between plasma very low (VLDL), low (LDL), and high (HDL) density lipoproteins is facilitated by specific lipid transfer proteins. The present report describes a method to separate donor and acceptor lipoprotein pools used in assays for lipid exchange activities. The method is based on the differential binding of lipoproteins to immobilized heparin. At 50 mM NaCl concentration, VLDL and LDL bind to heparin-Sepharose whereas >85% of HDL is unretained; VLDL and LDL are then eluted with 300 mM NaCl, 2% sodium dodecyl sulfate with a recovery ≥85%. The procedure is rapid and quantitative, as judged by a comparison to ultracentrifugation.—**Ellsworth, J. L., L. McVittie, and R. L. Jackson.** Human plasma lipid exchange protein(s): a method for separation of donor and acceptor lipoproteins by heparin-Sepharose chromatography. *J. Lipid Res.* 1982. **23:** 653–659.

Supplementary key words lipid transfer • cholesteryl ester • phospholipid • triglyceride • lipoproteins

The reciprocal transfer of cholesteryl ester and triglyceride between human plasma lipoproteins was first reported by Nichols and Smith (1). Since that initial report, a number of investigators have attempted to isolate and characterize plasma lipid transfer proteins which facilitate the transfer or exchange of cholesteryl esters, triglycerides, and phospholipids between various lipoprotein classes (2–12). The molecular properties of these transfer proteins as well as their role in lipoprotein metabolism have not been established. For example, the reported molecular weight of the cholesteryl ester transfer protein varies from 35,000 (2), to 80,000 (4), to >100,000 (10). One of the problems in defining the properties of these lipid transfer proteins has been the lack of convenient methods for the separation of donor and acceptor lipoprotein pools used in the lipid transfer assays. Most assays have employed very low (VLDL), low (LDL), or high (HDL) density lipoproteins labeled with radioactive lipids. The radiolabeled donor lipoproteins are incubated with unlabeled acceptor lipoproteins in the presence or absence of lipid transfer proteins. After an appropriate time interval, donor and acceptor molecules are separated and radioactivity is determined. Ultracentrifugation (1, 2, 6, 8) and heparin-$MnCl_2$ precipitation of apoB-containing lipoproteins (3–5) have been the most common methods employed for the separation of lipoproteins. However, these methods suffer from several disadvantages. First, ultracentrifugation requires 18–20 hr, precluding it as a routine separatory method for large numbers of assays. Second, heparin-$MnCl_2$ precipitation of previously isolated total apoB-containing lipoproteins is not complete (13).

The purpose of the present study was to develop a rapid and convenient method for the separation of donor and acceptor lipoprotein pools used in lipid transfer protein assays. The method involves the specific ionic strength-dependent binding of lipoproteins to immobilized heparin.

MATERIALS AND METHODS

Materials

[1,2-^{3}H](N)]cholesterol (44 Ci/mol), tri[1-^{14}C]-oleoylglycerol (95 Ci/mol), tri[9,10^{3}H-(N)]-oleoylgly-

cerol (121 Ci/mmol), [choline-methyl-^{14}C]dipalmitoyl phosphatidylcholine (53 Ci/mol), and [^{3}H-G]heparin (100 Ci/mol) were purchased from New England Nuclear. Fatty acid-free bovine serum albumin, fraction V (Sigma), heparin-sodium salt (porcine intestinal mucosa, 169.9 USP K units/mg) and Sepharose CL-4B were obtained from Sigma Chemical Company. Quik-Sep columns were purchased from Isolab Inc.

Preparation of lipoprotein substrates

Plasma was isolated from blood collected in 0.15% (wt/vol) EDTA from normal fasting healthy volunteers. The major lipoprotein classes (VLDL, d < 1.006 g/ml; LDL, d 1.020–1.050 g/ml; HDL, d 1.063–1.210 g/ml) were isolated by ultracentrifugal flotation in KBr at 8°C for 18 hr in a Beckman L8-55 ultracentrifuge using a Beckman 50.2 Ti rotor operated at 48,000 rpm. The HDL density subclass HDL_2 (d 1.063–1.125) was isolated by zonal ultracentrifugation according to Patsch et al. (14) using a Beckman Ti 14 rotor in the Beckman L5-65 ultracentrifuge. After ultracentrifugation, lipoproteins were dialyzed against a standard buffer containing 0.9% NaCl, 10 mM Tris–HCl, 0.001 M EDTA, and 0.02% sodium azide pH 7.4. [^{3}H]Cholesteryl ester-labeled lipoproteins were prepared by the action of endogenous lecithin:cholesterol acyltransferase on radiolabeled cholesterol. In a typical preparation, 500 μCi of [^{3}H]cholesterol was dissolved in 0.25 ml of absolute ethanol and then injected with stirring into 130 ml of freshly isolated plasma; the plasma was incubated at 37°C for 18 hr. The radiolabeled VLDL, LDL, and HDL were isolated between the density limits described above. Radiolabeled HDL was then incubated for 18 hr at 37°C with a 5-fold excess (based on lipoprotein protein) of unlabeled LDL in order to remove radiolabeled free cholesterol; the radiolabeled HDL which was reisolated between d 1.063–1.210 g/ml contained >90% of the radiolabel in cholesteryl ester and 10% in unesterified cholesterol. The specific activity of the [^{3}H]cholesteryl ester-labeled HDL was 611.2 dpm/nmol. LDL was labeled with [^{14}C]dipalmitoyl phosphatidylcholine as described previously (15). VLDL was labeled with tri[^{14}C]oleoylglycerol by the method of Fielding (16). Tri[^{3}H]oleoylglycerol-labeled lipoproteins were prepared by injecting 500 μCi of tri[^{3}H]-oleoylglycerol dissolved in 0.25 ml of ethanol into 60 ml of freshly isolated human plasma with stirring. The plasma was then incubated for 12 hr at 37°C. Radiolabeled lipoproteins were then isolated within the density limits described above. Lipoprotein lipids were extracted by the method of Folch, Lees, and Sloane Stanley (17) and separated by thin-layer chromatography on silica gel 60 HP-TLC plates. Phospholipids were separated in a solvent system of chloroform–methanol–water 75:25:4 (v/v/v) and cholesteryl ester and triglyceride in hexane–ether–acetic acid 86:16:1 (v/v/v). After visualization with iodine, the silica gel was scraped directly into a vial, 10 ml of Budget-Solve was added, and radioactivity was determined in a Beckman LS-8100 scintillation counter equipped with H-number standardization.

Heparin-Sepharose was prepared by a modified procedure of Iverius (18). Sepharose CL-4B (500 ml packed gel) was activated by cyanogen bromide (30 g in 500 ml H_2O); the pH was immediately adjusted to 11.0 with 2 N NaOH. After stirring at room temperature for 12 min, the activated Sepharose was washed on a sintered glass filter with 4 liters of ice-cold 0.1 M $NaHCO_3$. Heparin (5 mg/ml in 500 ml of 0.1 M $NaHCO_3$, pH 8.4, containing 10 μCi [^{3}H]heparin) was then added to 500 ml of activated Sepharose and the gel was stirred overnight at 4°C. To block the unreacted groups on Sepharose, 1 liter of 0.1 M glycine was added and then the gel was stirred for 3 hr at 25°C. The heparin-Sepharose was then sequentially washed with 2 liters of H_2O, 1 liter of 1.0 M NaCl, 2 liters of 0.1 M $NaHCO_3$, and finally 2.0 liters of H_2O. Heparin-Sepharose was stored in 0.9% NaCl, 0.001 M EDTA, 0.01 M Tris-HCl, 0.02% NaN_3, pH 7.4, at 4°C. The heparin-Sepharose contained 0.25 mg heparin/ml packed gel as measured by the incorporation of radioactive heparin.

Heparin-Sepharose chromatography

Columns containing heparin-Sepharose were prepared by adding 1.0–2.0 ml of packed gel to Quik-Sep columns fitted with a sintered glass filter plug. The packed gel size measured 0.8 cm × 2.0 cm with a 5.0 ml above resin volume. The columns were washed and eluted as described in Results. The columns were regenerated by sequential washes with 2.5 ml of elution buffer containing 2% sodium dodecyl sulfate, 0.3 M NaCl, 1 mM EDTA, 10 mM Tris–HCl, pH 7.4, 5.0 ml of H_2O, and, finally, 3.0 ml of equilibration buffer which contained 50 mM NaCl, 1 mM EDTA, 10 mM Tris–HCl, pH 7.4. The columns were stored with their tips in water at 4°C.

Assay of triacylglycerol exchange activity

The protein(s) active in the exchange of lipoprotein, cholesteryl ester, phospholipid, and triacylglycerol, was isolated from human lipoprotein-free plasma by chromatography on phenyl-Sepharose, carboxylmethyl cellulose, and concanavalin-A Sepharose, as previously described (10). The protein-facilitated exchange of triacylglycerol was determined by measuring the transfer of tri[^{3}H]oleoylglycerol from LDL to HDL. Unless stated otherwise, the incubation mixture contained LDL (5 nmol of triacylglycerol, 12220 dpm/nmol), HDL (12 nmol of triacylglycerol), and lipid exchange protein(s)

(10–100 μg of protein) in 0.5 ml of standard buffer. After incubation for 2–3 hr at 37°C, the samples were set on ice and diluted with 0.5 ml of ice cold water. The heparin-Sepharose columns were positioned directly over scintillation vials and 0.5 ml of the diluted assay mixture was applied. When the sample was completely absorbed, the HDL fraction was washed free of the LDL by the addition of 3.5 ml of equilibration buffer; the column eluate containing the HDL was collected directly into the vials. The columns were then placed over another set of scintillation vials, the LDL was removed by elution with 2.5 ml of elution buffer and collected directly into the vials. Ten ml of Budget-solve was added to each vial and radioactivity was determined as described above.

Calculation of rate of exchange of triacylglycerol between LDL and HDL

The rate of exchange of triacylglycerol between LDL and HDL was calculated on the assumption that there was exchange only with no net transfer of mass and that each fraction comprised a single homogeneous pool, all of which was available for exchange. The calculations were based on the formula derived by Barter and Jones (19) as follows:

$$-\ln \frac{S_L(t) - S_{EQ}}{S_L(0) - S_{EQ}} = F\left[\frac{1}{M_H} + \frac{1}{M_L}\right]t$$

where: $S_L(t)$, $S_L(0)$, S_{EQ} represent the specific activity (dpm/nmol) of triacylglycerol in LDL at time t(hr) and at complete equilibration, respectively; M_H and M_L denote the triacylglycerol pool sizes (nmol) in HDL and LDL, respectively, and F denotes the rate of exchange (nmol/hr). If the assumptions are valid, the function describing the plot of $-\ln [S_L(t) - S_{EQ}/S_L(0) - S_{EQ}]$ vs. t should be a single exponential. Once simple exchange of triacylglycerol between LDL and HDL was documented, a simplified formula for the determination of exchange activity was employed, where the exchange rate V (nmol/hr) =

$$\frac{HDL_t^*}{\frac{LDL_0^*}{t}}$$

In this form, HDL_t^* denotes the dpm in HDL at time t and LDL_0^* denotes the specific activity (dpm/nmol) of triacylglycerol in LDL at zero time. Protein-facilitated exchange of triacylglycerol was determined by substracting the exchange activity determined in the absence of exchange protein from that obtained when exchange protein(s) was included in the incubation.

Other methods

Phospholipid phosphorus was determined by the method of Bartlett (20). Protein content was determined by the method of Lowry et al. (21) using bovine serum albumin as standard. Total lipoprotein cholesterol and triglycerides were determined by Autoanalyzer-II (AA-II) techniques (22) at the University of Cincinnati Lipid Research Clinic.

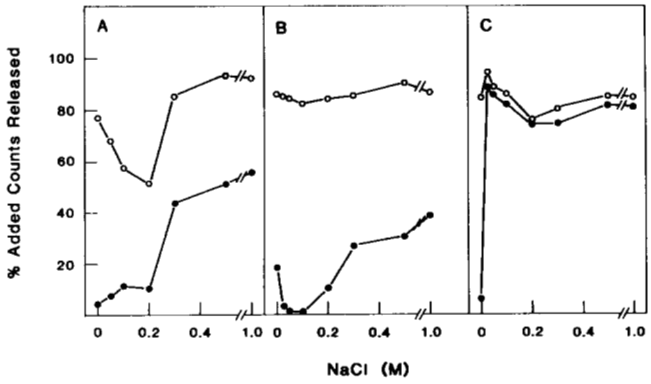

Fig. 1. Effects of NaCl concentration on the binding of lipoproteins to heparin-Sepharose. A, [^{14}C]Dipalmitoyl phosphatidylcholine-labeled LDL (30 μg protein; 1.25×10^5 dpm) in a final volume of 0.5 ml of 10 mM Tris–HCl, 1 mM EDTA, pH 7.4, containing the indicated concentration of NaCl was applied to heparin-Sepharose (2.0 ml packed gel) pre-equilibrated in the Tris buffer containing the same NaCl concentration as the LDL that was applied to the column. After the samples entered the gel, the columns were washed with 2.5 ml of equilibration buffer; lipoprotein was collected and radioactivity was determined (● —— ●). The heparin-Sepharose columns were then eluted with 2.5 ml of elution buffer. The open circles (○ —— ○) correspond to the total radioactivity that eluted from heparin-Sepharose (equilibration wash plus elution wash). B, [^{14}C]Triolein-labeled VLDL (68 μg protein; 5.3×10^4 dpm) was applied to heparin-Sepharose and eluted with conditions identical to those described in A. C, [^{3}H]Cholesteryl ester-labeled HDL (275 μg protein; 1.5×10^4 dpm) was applied to heparin-Sepharose and eluted as described above. The experimental points represent duplicate analyses of three different preparations of each lipoprotein. In all determinations, the duplicates were always within ± 5% of each other.

RESULTS AND DISCUSSION

Chromatography of lipoproteins on heparin-Sepharose

Effects of NaCl concentration. **Fig. 1** shows the effects of NaCl concentration on the chromatography of LDL, VLDL, and HDL on heparin-Sepharose. At low ionic strength (<0.2 M NaCl), 90–95% of the added LDL was retained by heparin-Sepharose. At concentrations >0.2 M NaCl, the amount of LDL bound to heparin-Sepharose decreased; approximately 44% bound at 1.0 M NaCl. Iverius (23) described a critical electrolyte concentration (CEC) as the ionic strength required to elute 50% of the lipoprotein bound to heparin-Sepharose. In the present study, CEC values for three different preparations of LDL were 0.28, 0.23, and 0.25. These values compare favorably with the value of 0.26 reported by Iverius (23). The elution of LDL bound to heparin-Se-

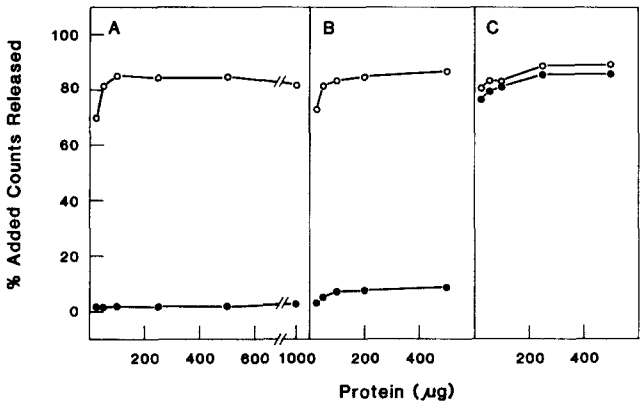

Fig. 2. Effect of lipoprotein-protein concentration on the binding of (A) LDL, (B) VLDL, and (C) HDL to heparin-Sepharose. The heparin-Sepharose columns were equilibrated with 3.0 ml of equilibration buffer. Each lipoprotein fraction, at the indicated protein concentration, was adjusted to 0.5 ml with equilibration buffer; 0.25 ml of each lipoprotein solution was then applied to heparin-Sepharose in duplicate. The columns were washed with 2.5 ml of the equilibration buffer, the lipoprotein fractions were collected directly into scintillation vials, and radioactivity was determined. The columns were then washed with 2.5 ml of elution buffer, the lipoprotein fractions were collected into vials, and radioactivity was determined. The closed circles (● —— ●) represent the % of total lipoprotein-lipid radioactivity removed with equilibration buffer alone; open circles (○ —— ○) represent the total % recovery of radioactivity released with both the equilibration buffer and elution buffer. The experimental points represent duplicate analyses of three different preparations of each lipoprotein.

pharose by sodium dodecyl sulfate was also dependent on ionic strength (Fig. 1A). LDL were most tightly bound between 0.1 and 0.2 M NaCl. However, when LDL were applied to the heparin-Sepharose columns in 25 mM NaCl, >85% of the LDL were eluted with 2% sodium dodecyl sulfate. An ionic strength dependence of the binding of LDL to heparin is consistent with other reports (23–25). Burstein and Scholnick (24) reported that heparin, in combination with metal ions, was more effective in precipitating LDL when the serum had been dialyzed against buffered water. Nakashima et al. (25) also reported that maximal interaction of pyrene-labeled LDL with glycosaminoglycans (GAG) occurred at a salt concentration of 0.14 M NaCl; the interaction was decreased by raising the ionic strength.

Fig. 1B shows the effect of NaCl on the binding of VLDL to heparin-Sepharose. The CEC value of the VLDL-heparin interaction was 0.26 M and is identical to that reported by Iverius (23). The interaction of VLDL and heparin-Sepharose was maximal between 0.025 M and 0.1 M NaCl. In contrast to LDL (Fig. 1A), 2% sodium dodecyl sulfate released approximately 85% of the bound VLDL and was independent of the NaCl concentration. The differences in the ionic strength dependence between VLDL and LDL may be related to their different apoprotein constituents (26, 27).

Fig. 1C shows the NaCl dependence of the binding of HDL to heparin-Sepharose. The chromatographic properties of HDL on heparin-Sepharose were strikingly different from those of LDL or VLDL. When the heparin-Sepharose columns were equilibrated with water, 90% of the HDL was bound. However, as shown in Fig. 1C, a small increase in salt concentration (0 to 25 mM NaCl) had a profound effect on HDL binding. When the columns were equilibrated with 25 mM NaCl, >85% of the HDL was unretained; 2% sodium dodecyl sulfate removed only an additional 4–8%. The tightly bound fraction of HDL may correspond to an apoE subclass (27–29).

Recovery of lipoprotein lipid and protein. **Fig. 2** shows the binding and elution of LDL, VLDL, and HDL from heparin-Sepharose over a range of lipoprotein concentrations. Under the conditions of the chromatography (50 mM NaCl), >90% of LDL and VLDL bound to heparin-Sepharose at all concentrations of lipoprotein tested; 87–92% of the HDL was unretained. The total recovery of lipoprotein-protein and radiolabeled lipids was >80% for all lipoprotein preparations (**Table 1**).

Stability of heparin-Sepharose

The binding characteristics of lipoproteins were compared on freshly prepared heparin-Sepharose and on heparin-Sepharose that was 4 months old and used in transfer assays more than fifty times. The binding and elution of all of the lipoprotein preparations were identical on the new and used heparin-Sepharose columns (**Fig. 3**), indicating that the binding capacity of the resin is unaltered when subjected to reuse.

Comparison of ultracentrifugation and heparin-Sepharose chromatography in the determination of triacylglycerol exchange activity

Table 2 compares heparin-Sepharose chromatography to ultracentrifugation, a standard method used for the separation of lipoproteins. Both separation techniques give essentially identical exchange rates. Recovery of LDL in incubations where the exchange protein(s) was absent was calculated from the LDL fraction collected using each separation technique. For the heparin-Sepharose technique, the dpm recovered in the LDL fraction was 38825 ± 1229 dpm (mean ± S.D., n = 8) which represents 83.03% of the applied radioactivity. For the ultracentrifugation method, the dpm recovered in the LDL fraction was 38496 ± 870 dpm (mean ± S.D., n = 8) which represents 82.40% of the initial LDL radioactivity. These data represent a lower limit for LDL recovery due to the loss of 5% of the initial LDL radioactivity to the HDL fraction during the incubation and is due to non-facilitated exchange of radiolabeled triacylglycerol. Although the two methods for the separation of lipoprotein pools yielded the same activities, ultracen-

TABLE 1. Chromatography of lipoproteins on heparin-Sepharose: recovery of radiolabeled lipid and protein

Lipoprotein	Radiolabeled Lipid[a]	Preparation	Radioactive Lipid[b] Applied	Radioactive Lipid[b] Recovered	Protein[b] Applied	Protein[b] Recovered
			dpm × 10^{-4}		*μg*	
VLDL		I			100.0	101.7 (102)
	[^{3}H]Chol	II	73.02	59.95 (82.1)	100.0	82.6 (82.6)
	[^{14}C]Tg	III	2.78	2.38 (85.6)	46.8	40.0 (85.5)
LDL		I			250.0	259.2 (104)
	[^{3}H]Chol	II	21.10	17.40 (82.5)	100.0	80 (80)
	[^{14}C]PL	III	7.54	6.87 (91.1)	100.0	80 (80)
HDL		I			432.0	379.1 (87.8)
	[^{3}H]Chol	II	2.36	2.05 (86.9)	50.0	45.8 (91.6)

[a] [^{3}H]Chol = [1,2-^{3}H](N)cholesterol; [^{14}C]Tg = tri[1-^{14}C]oleoylglycerol; [^{14}C]PL = [choline-methyl-^{14}C]dipalmitoyl phosphatidylcholine.

[b] The values represent the mean values for each lipoprotein preparation performed in triplicate. All values were within ±5% of each other.

Radiolabeled lipids were incorporated into VLDL, LDL, and HDL as described in Methods. VLDL, LDL, or HDL or the corresponding radiolabeled lipoprotein in a final volume of 0.5 ml of equilibration buffer were applied to heparin-Sepharose equilibrated with the same buffer. The columns were washed and eluted, and the lipoprotein fractions were collected as described in the legend to Fig. 2. The values in parentheses represent the % recovery of protein or radiolabeled lipid for VLDL, LDL, and HDL. The % recovery for VLDL and LDL represents the amount which eluted with elution buffer. The % recovery for HDL is based on the amount which eluted with equilibration buffer.

trifugation suffers from lengthy centrifugation steps and the limited capacity to assay large numbers of samples. In contrast, separation of lipoprotein pools on heparin-Sepharose is achieved within a few minutes, with identical recoveries as compared to ultracentrifugation.

Studies of triacylglycerol exchange between LDL and HDL

The plasma protein-facilitated exchange of triacylglycerol between LDL and HDL using the heparin-Sepharose separation technique is shown in Figs. 4 and 5. **Fig. 4** shows the time course of triacylglycerol exchange between LDL and HDL. The nonfacilitated exchange of triacylglycerol does not change appreciably with time. In contrast, the protein-facilitated triacylglycerol exchange between LDL and HDL increases linearly with time and reaches a value of 7.44 nmol triacylglycerol exchanged/mg of exchange protein at 8 hr. The transfer of radiolabeled triacylglycerol from LDL to HDL represents an exchange process as is shown in Fig. 4 (inset). A semilogarithmic plot of the difference between the specific activity of triacylglycerol in LDL at time t and that at complete equilibration versus time of incubation yields a single exponential suggesting a simple exchange process. The rate of flux of triacylglycerol between LDL and HDL was determined from the slope of the line and calculated to be 0.96 nmol triacylglycerol exchanged/hr or 20.2% of the total LDL triacylglycerol pool. When compared with previous reports (30, 31), this rather fast

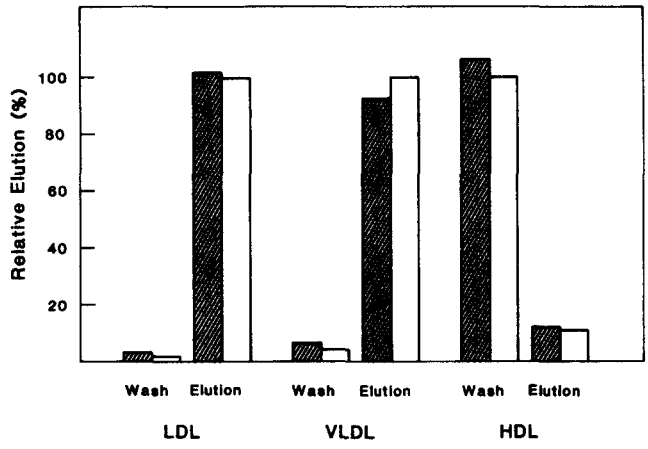

Fig. 3. Effects of reuse on the stability of heparin-Sepharose. [^{14}C]Dipalmitoyl phosphatidylcholine-labeled LDL (30 μg protein; 1.25 × 10^5 dpm), [^{14}C]triolein-labeled VLDL (68 μg protein; 5.3 × 10^4 dpm), or [^{3}H]cholesteryl ester-labeled HDL (275 μg protein; 6.5 × 10^4 dpm) were applied to heparin-Sepharose as described in Fig. 2. The lipoproteins were eluted with 3.0 ml of equilibration buffer (indicated in the figure as wash) and then with 2.5 ml of elution buffer (indicated as elution). The hatched bars (▨) represent freshly prepared heparin-Sepharose. The open bars (□) represent heparin-Sepharose that was 4 months old and had been used >50 times in lipoprotein lipid transfer assays. The values represent the mean of triplicate analyses of single preparations. In all determinations, the triplicates were always within ± 5% of each other. For LDL and VLDL, the relative elution is based on the amount of lipoprotein lipid radioactivity that eluted from the used heparin-Sepharose columns with the elution buffer. This amount of radioactive lipid recovered was set at 100%. For HDL, the relative elution corresponds to the amount of HDL removed from the used heparin-Sepharose columns with the equilibration buffer. This amount of radioactive lipid recovered was set at 100%.

TABLE 2. Comparison of ultracentrifugation and heparin-Sepharose chromatography in the determination of triacylglycerol exchange activity

Method of Separation	Exchange Rate[a] Nonfacilitated (n = 8)	Facilitated (n = 10)
Ultracentrifugation	1.44 ± 0.10	3.63 ± 0.19
Heparin-Sepharose chromatography	1.57 ± 0.12	3.36 ± 0.16

[a] Expressed as nmol lipid exchanged/hr per mg exchange protein ± S.D.

Triacylglycerol exchange activity was determined as described in Methods. For the heparin-Sepharose method, the columns were washed and eluted, and the exchange activity was determined as described above. For the ultracentrifugation method, the incubation mixtures were adjusted to 4.5 ml with standard buffer and the density was adjusted to 1.063 g/ml with solid KBr and overlayed with 1.0 ml of d 1.063 g/ml buffer. The samples were centrifuged at 48000 rpm for 18 hr in a Beckman 50.3 Ti rotor; LDL (top fraction) were removed by aspiration. After removing the clear zone, HDL (bottom fraction) were collected and radioactivity in each fraction was determined.

hourly turnover of lipoprotein triacylglycerol is probably related to the high degree of purity of the exchange protein preparation used in the present studies. To determine if the interaction of LDL with heparin-Sepharose is influenced by the amount of HDL present, the molar ratio of HDL_2/LDL triacylglycerol was increased linearly in the exchange assay up to a 6-fold excess of HDL_2 triacylglycerol. **Fig. 5** shows the dependence of triacylglycerol exchange on the HDL_2/LDL triacylglycerol ratio. The nonfacilitated exchange of triacylglycerol between LDL and HDL did not change appreciably over the entire range of HDL_2/LDL triacylglycerol values. In contrast, the protein-facilitated exchange of triacylglycerol increased to a maximum value of 11.61 nmol/hr per mg exchange protein at an HDL_2/LDL ratio of 1:1 and decreased to a value of 4.91 nmol/hr per mg exchange protein when the HDL_2/LDL triacylglycerol ratio was 6:1. Similar data were reported previously for the transfer of triacylglycerol between rabbit LDL and HDL (19), which were interpreted as a binding of the triacylglycerol exchange protein to HDL but not to LDL, at high HDL concentrations.

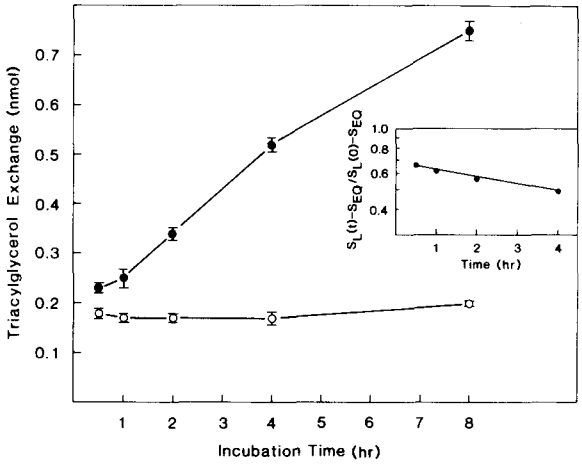

Fig. 4. Time course of triacylglycerol exchange between LDL and HDL. Tri-[9,10-^{3}H(N)]oleoylglycerol-labeled LDL (5 nmol Tg, 12220 dpm/nmol) was incubated with HDL (12 nmol Tg) in the presence or absence of triacylglycerol-exchange protein(s) (67 μg protein) in a final volume of 0.5 ml of standard buffer for the indicated incubation period at 37°C. Protein facilitated (— ● —) and nonfacilitated (— ○ —) transfer of triacylglycerol was determined as described in Materials and Methods. Each point represents the mean ± S.D. for triplicate determinations. Inset, semilogarithmic plot of the difference in the triacylglycerol specific activity in LDL at time t and that at complete equilibration. The triacylglycerol exchange time course data was calculated and plotted as described in Methods.

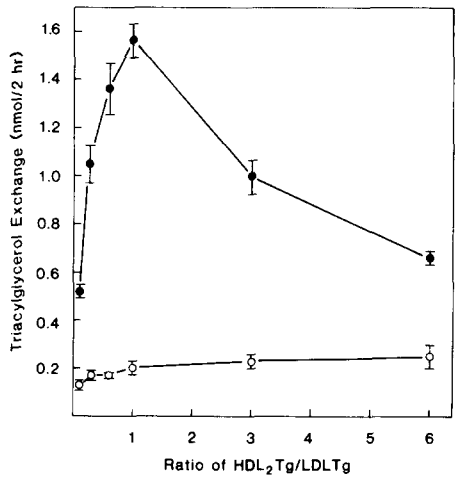

Fig. 5. Effect of the HDL_2-Tg/LDL-Tg ratio on the exchange of triacylglycerol. Tri-[9,10-^{3}H(N)]oleoylglycerol-labeled LDL (5 nmol Tg, 12220 dpm/mol) was incubated with varying amounts of HDL_2 (0.5 → 30 nmol Tg) in the presence and absence of triacylglycerol exchange protein(s) (67 μg protein) in a final volume of 0.5 ml of standard buffer, for 2.5 hr at 37°C. Protein-facilitated (— ● —) and non-facilitated (— ○ —) exchange of triacylglycerol was determined as described in Materials and Methods.

Taken together, the results of the present study demonstrate that the heparin-Sepharose chromatographic procedure is a viable technique for the separation of mixtures of previously isolated lipoproteins. The method is rapid, convenient and quantitative, as judged by a comparison with ultracentrifugation. It is anticipated that this improved method will be of considerable utility in the identification and characterization of the lipid transfer proteins.

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