Plasma phospholipid transfer protein enhances transfer and exchange of phospholipids between very low density lipoproteins and high density lipoproteins during lipolysis

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Abstract In order to determine the effects of a plasma phospholipid transfer protein on the transfer of phospholipids from very low density lipoproteins (VLDL) to high density lipoproteins (HDL) during lipolysis, biosynthetically labeled rat ^{32}P-labeled VLDL was incubated with human HDL_3 and bovine milk lipoprotein lipase (LPL) in the presence of the plasma d > 1.21 g/ml fraction or a partially purified human plasma phospholipid transfer protein (PTP). The addition of either the PTP or the d > 1.21 g/ml fraction resulted in a 2- to 3-fold stimulation of the transfer of phospholipid radioactivity from VLDL into HDL during lipolysis. In the absence of LPL, the PTP caused a less marked stimulation of transfer of phospholipid radioactivity. Both the d > 1.21 g/ml fraction and the PTP enhanced the transfer of VLDL phospholipid mass into HDL, but the percentage transfer of phospholipid radioactivity was greater than that of phospholipid mass, suggesting stimulation of both transfer and exchange processes. Stimulation of phospholipid exchange was confirmed in experiments where PTP was found to augment transfer of [^{14}C]phosphatidylcholine radioactivity from HDL to VLDL during lipolysis. In experiments performed with human VLDL and human HDL_3, both the d > 1.21 g/ml fraction and the PTP were found to stimulate phospholipid mass transfer from VLDL into HDL during lipolysis. Analysis of HDL by non-denaturing polyacrylamide gradient gel electrophoresis showed that enhanced lipid transfer was associated with only a slight increase in particle size, suggesting incorporation of lipid by formation of new HDL particles. In conclusion, the plasma d > 1.21 g/ml fraction and a plasma PTP enhance the net transfer of VLDL phospholipids into HDL and also exchange of the phospholipids of VLDL and HDL. Both the transfer and exchange activities of PTP are stimulated by lipolysis. —**Tall, A. R., S. Krumholz, T. Olivecrona, and R. J. Deckelbaum.** Plasma phospholipid transfer protein enhances transfer and exchange of phospholipids between very low density lipoproteins and high density lipoproteins during lipolysis. *J. Lipid Res.* 1985. **26:** 842–851.

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During the lipolysis of triglyceride-rich lipoproteins there is transfer of phospholipids and soluble apoproteins into HDL (1–4). This process may reflect the formation of discs of phospholipid and apoprotein which have the same density as HDL (1), or the addition of phospholipids and apoproteins to pre-existing HDL (2), resulting in their conversion into larger, less dense particles (1, 2). The transfer of phospholipids and apoA-I from triglyceride-rich lipoproteins into HDL during lipolysis has been demonstrated in vitro (1), in the perfused rat heart (2), and also following injection of chylomicrons into rats (3, 4). In humans there is an increase in HDL phospholipids, apoA-I, and apoA-II after a fatty meal, probably reflecting similar transfer of these components from chylomicrons into HDL (5, 6).

The transfer of phospholipids from triglyceride-rich lipoproteins into HDL may be modeled in vitro by incubating phospholipid vesicles with HDL or plasma (7–9). The vesicle phospholipid is transferred into HDL, both by incorporation into pre-existing particles and also by formation of phospholipid/apoprotein discs (7). Recently, we and others (10–12) have observed that the transfer of phospholipids from vesicles into pre-existing HDL may be stimulated by the addition of either the d > 1.21 g/ml fraction or a phospholipid transfer protein (PTP) purified from this fraction. In its purest form, the major component of the PTP had an apparent M_r of 41,000 (11), whereas the cholesteryl ester transfer protein appeared as a major band or a doublet of M_r approximately 63,000 in sodium dodecyl sulfate polyacrylamide gels (11, 13, 14). The present study was designed to test the hypothesis that the PTP would stimulate transfer of phospholipids from

Abbreviations: VLDL, very low density lipoprotein; HDL, high density lipoprotein; LPL, lipoprotein lipase; PTP, phospholipid transfer protein.

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VLDL into HDL during lipolysis. Thus, we examined the effects of adding the plasma d > 1.21 g/ml fraction or the partially purified PTP to a lipolysis system containing VLDL and HDL. The results show that the PTP enhances both transfer and exchange of VLDL phospholipids and that the activity of the PTP is augmented by lipolysis.

MATERIALS AND METHODS

VLDL (d < 1.006 g/ml) and HDL_3 (d 1.125–1.210 g/ml) were prepared from fresh human plasma by preparative ultracentrifugation in a Beckman Ti 50.2 rotor. The d > 1.21 g/ml fraction was prepared by ultracentrifugation of human plasma for 72 hr at density 1.21 g/ml in a Beckman Ti 50.2 rotor. Biosynthetically labeled [^{32}P]-phospholipid-labeled VLDL was prepared by injecting 300-g male Sprague-Dawley rats with $NaH_2{}^{32}PO_4$, as described previously (1). Human HDL_3 containing radiolabeled phospholipid was prepared as follows. Five μCi of 1-palmitoyl, 2-[1,^{14}C]linoleoyl phosphatidylcholine (New England Nuclear, sp act 50 mCi/mmol) was dissolved in 10 μl of ethanol and then slowly injected beneath the surface of a stirred human plasma d > 1.10 g/ml fraction (previously dialyzed against 50 mM Tris-saline, pH 7.4), using a 26-gauge needle. The sample was placed under a stream of nitrogen for 10 min, then incubated for 1 hr at 37°C. Subsequently the HDL fraction (d 1.100–1.210 g/ml) was isolated by preparative ultracentrifugation in a Ti 50.3 rotor; the specific activity of the HDL was 2,000 cpm/μg of phospholipid. Bovine milk lipoprotein lipase (LPL) was prepared by the method of Bengtsson and Olivecrona (15) and stored at −20°C in 10 mM Tris, 1 M NaCl, pH 7.4. The concentration of the LPL was 0.33 mg/ml and its specific activity was 610 units per mg (1 unit = 1 μmol of fatty acid liberated per min). The plasma phospholipid transfer protein was prepared by phenyl-Sepharose and carboxymethylcellulose chromatography of the human plasma d > 1.21 g/ml fraction, as described previously (11). The distribution of phospholipid transfer activity was determined by measuring the ability of column fractions to enhance the transfer of [^{14}C]phosphatidylcholine radioactivity from unilamellar egg phosphatidylcholine vesicles into HDL_3, as described previously (11). The peak of phospholipid transfer activity eluted ahead of the peak of cholesteryl ester transfer activity (11); fractions were pooled so as to minimize contamination of the fractions containing phospholipid transfer activity with cholesteryl ester transfer activity (11). However, the pooled fractions did promote transfer of small amounts radiolabeled cholesteryl esters from HDL to LDL, indicating the presence of the cholesteryl ester transfer protein. Nonetheless, using assays identical to those described previously (11), the ratio of PC radiolabel transfer/CE radiolabel transfer was 5:1 in the pooled fractions containing the PTP and 1:1 under the peak of cholesteryl ester transfer activity. Based on measurements of activity, the phospholipid transfer activity was purified about 500-fold relative to the d > 1.21 g/ml fraction. In previous studies the purest fraction of phospholipid transfer protein contained a protein of M_r 41,000 and was purified about 9,500-fold relative to the d > 1.21 g/ml fraction. The PTP preparation did not contain lecithin:cholesterol acyltransferase, as measured in a standard assay (16).

In order to assess the transfer of VLDL phospholipid mass or radioactivity into HDL, the following components were mixed (in order): VLDL, fatty acid – free albumin (Sigma) (to give an albumin/VLDL triglyceride molar ratio of 0.36–0.48 (17)), heparin (5 Units/100 μg of triglyceride), 200 mM Tris-saline, 0.01% NaN_3, pH 8.5 (buffer A), HDL, and d > 1.21 g/ml fraction or PTP, and/or LPL. In all incubations conducted with the d > 1.21 g/ml fraction, dithionitrobenzoic acid (final concentration 2 mM) was added to buffer A to inhibit lecithin:cholesterol acyltransferase activity. The ratio of PTP to HDL was about 1 mg of PTP/2–7 mg of HDL protein. An amount of LPL was chosen to give 65–80% hydrolysis of VLDL triglycerides, as assessed by disappearance of radiolabeled triglycerides from VLDL lipids (18). Stock solutions of the different components had been dialyzed against or brought up in buffer A. The final incubation volume was 1 ml. The mixtures were incubated in stoppered test tubes for 1–2 hr at 37°C in a metabolic shaker. Lipolysis was stopped by immersing test tubes in ice and by the addition of concentrated NaBr solution to give a final solution density of 1.057 g/ml. In other studies we had verified that ultracentrifugation at d 1.06 g/ml provided an adequate separation of VLDL remnants from HDL (18). Following the incubations HDL was obtained by preparative ultracentrifugation between densities 1.063–1.210 g/ml in a Beckman Ti 50.3 rotor.

RESULTS

Transfer of phospholipid radioactivity from rat ^{32}P-labeled VLDL into other lipoprotein fractions: effects of the d > 1.21 g/ml fraction, PTP, and LPL

Transfer of phospholipid radioactivity from VLDL into HDL was determined in incubations containing LPL and various other components. The effects of addition of human HDL_3, human d > 1.21 g/ml fraction, HDL + d > 1.21 g/ml fraction, PTP, or PTP + HDL to the basic lipolysis system containing VLDL + LPL are shown in **Fig. 1**. Lipolysis of VLDL in the absence of added HDL (Fig. 1, VLDL + LPL) resulted in transfer of 16% of VLDL phospholipid radioactivity into the HDL density range, presumably reflecting formation of new HDL particles as a result of dissociation of phospholipids and soluble

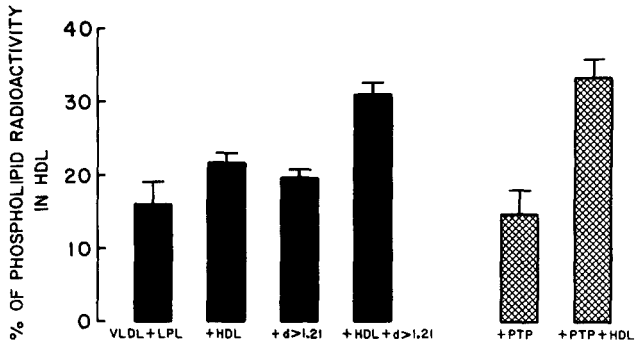

Fig. 1. Percentage of VLDL phospholipid radioactivity transferred into HDL. The transfer into the HDL density range is shown for an incubation containing only VLDL, LPL, and albumin (VLDL + LPL). All the other incubations contained VLDL, LPL, albumin, and additional components, as indicated. The amounts of materials were: biosynthetically labeled rat ^{32}P-labeled VLDL; 100 μg of triglyceride (25,000 cpm); LPL (0.25 μg); human HDL_3 (50 μg of protein); human d > 1.21 g/ml fraction (2 mg); PTP (7.5 μg). The phospholipid/triglyceride weight ratio of VLDL was 0.25 and the phospholipid/protein ratio of HDL_3 was 0.35. The results shown are mean ± SEM (n = 5).

apoproteins from VLDL (1, 17, 19, 20). Addition of either HDL or the d > 1.21 g/ml fraction resulted in slight but statistically insignificant increases in the transfer of VLDL phospholipid radioactivity into the HDL density range (Fig. 1). However, when both HDL and the d > 1.21 g/ml fraction were added, there was about 2-fold greater transfer of phospholipid radioactivity into the HDL density range ($P < 0.02$). When the PTP was added to VLDL + LPL, there was no increase in transfer of phospholipid radioactivity into HDL, but when both PTP and HDL were added there was about 2.5-fold greater transfer of phospholipid radioactivity ($P < 0.01$). Thus, in the presence of HDL, both the d > 1.21 g/ml fraction and the PTP enhance the movement of VLDL phospholipid radioactivity into HDL. In an experiment similar to that shown in Fig. 1, where the samples were incubated at 0°C, there was minimal (<4%) transfer of VLDL phospholipid radioactivity into the HDL density range.

All of the above experiments were conducted in the presence of LPL. To determine whether the enhancement of phospholipid transfer was dependent on lipolysis, similar experiments were performed with or without LPL (**Fig. 2**). In this experiment the addition of LPL resulted in hydrolysis of about 80% of VLDL triglycerides, irrespective of the presence of HDL or PTP. In the absence of added HDL, lipolysis of VLDL resulted in transfer of about 16% of phospholipid radioactivity into the HDL density range. When HDL was added to the lipolysis mixture, a similar amount of phospholipid radioactivity (21%) was transferred into the HDL density range. The addition of HDL + PTP resulted in transfer of 38% of phospholipid radioactivity from VLDL to HDL; this was 17% more than the effect of adding HDL without PTP. When VLDL or VLDL + PTP were incubated in the absence of LPL, there was transfer of less than 3% of phospholipid radioactivity into HDL (not illustrated). In the absence of LPL, the addition of HDL + PTP to VLDL resulted in transfer of only 15% of VLDL phospholipid radioactivity into HDL; this was 7% more than the effect of adding HDL without PTP (Fig. 2). Thus, the PTP stimulated transfer of phospholipid radioactivity from VLDL to HDL, with or without LPL. However, the stimulation of phospholipid transfer by PTP was approximately 2.5-fold greater during lipolysis, indicating an enhancement of the effects of the PTP by lipolysis.

Previous studies have shown that in vitro lipolysis of VLDL results in formation of LDL-like particles, and also transfer of VLDL phospholipids and soluble apoproteins into HDL and into the d > 1.21 g/ml fraction (1, 17, 19, 20). In order to determine the source of the additional phospholipid radioactivity transferred into HDL in the presence of PTP, further experiments were performed to determine the density distribution of phospholipid radioactivity amongst the lipoprotein fractions. The results are shown in **Table 1**. They indicate that the additional phospholipid radioactivity transferred into HDL in the presence of HDL and PTP was derived predominantly from the d 1.006–1.063 g/ml fraction. As noted by others (20), in the presence of LPL a major portion of phospholipid radioactivity was transferred into the d > 1.21 g/ml fraction. However, the phospholipid radioactivity in the d > 1.21 g/ml fraction was not increased as a result of addition of the PTP.

In experiments similar to those shown in Table 1, the lipoprotein fractions were extracted (21) and the distribu-

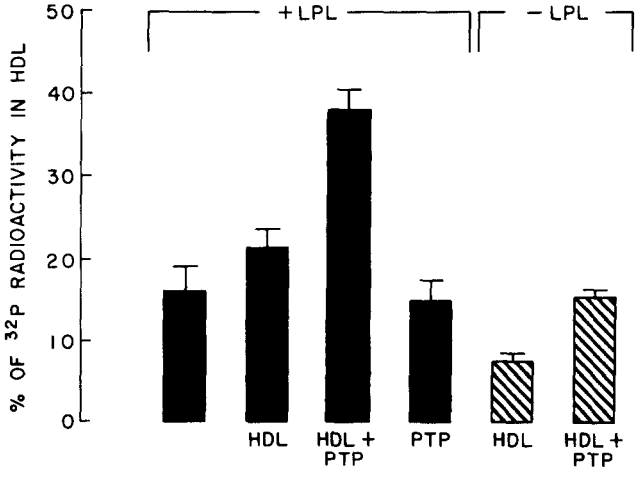

Fig. 2. Percentage of VLDL phospholipid radioactivity transferred into HDL_3 in incubations with (+LPL) or without (−LPL) lipoprotein lipase. All incubations contained VLDL and also the additional components, as indicated. The amounts of materials were: biosynthetically labeled rat ^{32}P-labeled VLDL; 150 μg of triglyceride (30,000 cpm); LPL (0.17 μg); human HDL_3 (50 μg of protein); PTP (15 μg). The incubations were conducted as described in Methods.

TABLE 1. Distribution of phospholipid radioactivity in lipoprotein fractions[a]

	Lipoprotein Fraction			
	d < 1.006	1.006-1.063	1.063-1.210	> 1.210
	%, *mean* ± *SEM*, *n* = 4			
VLDL	85 ± 4	10 ± 2	4 ± 1	0
VLDL + LPL	8 ± 1	44 ± 3	12 ± 2	36 ± 4
VLDL + LPL + HDL	9 ± 1	46 ± 2	15 ± 2	30 ± 2
VLDL + LPL + HDL + PTP	5 ± 1	28 ± 1	36 ± 2	31 ± 2
VLDL + LPL + PTP	4 ± 0	51 ± 4	7 ± 2	37 ± 3
VLDL + HDL	84 ± 2	9 ± 1	8 ± 2	0
VLDL + HDL + PTP	69 ± 4	13 ± 2	19 ± 3	0

[a]As indicated, incubations contained rat ^{32}P-labeled VLDL (150 μg of triglycerides, 30,000 cpm), LPL (0.175 μg), human HDL_3 (50 μg of protein), and PTP (15 μg). Samples were incubated for 2 hr at 37°C, then lipoproteins were separated by preparative ultracentrifugation. Recovery of radioactivity was > 85%.

tion of radioactivity in various phospholipids was determined by thin-layer chromatography. The results for the incubation containing VLDL + LPL + HDL + PTP are shown in **Table 2**. The VLDL added to the incubation contained 83% of its radioactivity in phosphatidylcholine, with smaller amounts in other phospholipids. In the presence of the PTP, the major phospholipid transferred into HDL was phosphatidylcholine. In the d > 1.21 g/ml fraction, most phospholipid radioactivity was in phosphatidylcholine, although the amount in lysophosphatidylcholine was greater than in the other lipoprotein fractions. For the incubation containing VLDL + LPL + HDL (not shown), an almost identical distribution of phospholipid radioactivity was found in HDL (83% of radioactivity in phosphatidylcholine). These results show that the major phospholipid undergoing facilitated transfer in the presence of PTP was phosphatidylcholine.

Facilitated transfer of phospholipid radioactivity is due to both net transfer and exchange of phospholipids

To determine whether the enhanced transfer of phospholipid radioactivity was due to net transfer and/or exchange of phospholipid molecules, the percentage of VLDL phospholipid mass transferred into HDL was compared with that of VLDL phospholipid radioactivity (**Table 3**). Measurements of the mass of phospholipid in HDL showed that the addition of the d > 1.21 g/ml fraction or the PTP to an incubation containing VLDL, HDL, and LPL resulted in a significant increase in phospholipid mass. The presence of the d > 1.21 g/ml fraction resulted in an additional transfer of 7 μg (9%) of VLDL phospholipid mass into HDL, whereas the PTP resulted in an additional 4 μg (5%) mass transfer. However, both the d > 1.21 g/ml fraction and the PTP had more pronounced effects on the transfer of phospholipid radioactivity, resulting in an additional 17% and 11% transfer of VLDL phospholipid radioactivity into HDL, respectively, compared to VLDL + HDL (Table 3, E vs G and I). These results suggest that both net transfer and exchange processes are stimulated by the d > 1.21 g/ml fraction or the PTP.

To examine more directly the possibility that the PTP stimulated exchange of HDL and VLDL phospholipids, rat ^{32}P-labeled VLDL was incubated with human HDL_3 containing [^{14}C]phosphatidylcholine and the distribution of both ^{32}P and ^{14}C radioactivity in the lipoprotein fractions was determined (**Table 4**). The experiments showed that addition of the PTP to VLDL + LPL + HDL resulted in both enhanced transfer of ^{32}P radioactivity from the d < 1.063 g/ml fraction to HDL, and also of ^{14}C radioactivity from HDL to the d < 1.063 g/ml fraction. It was notable that in the absence of LPL, PTP had no effect on the transfer of HDL phospholipid radioactivity into the d < 1.063 g/ml fraction. Similar results were obtained on three separate occasions. These experiments show that, in the presence of LPL, the PTP stimulates exchange of HDL and VLDL phospholipids.

The d > 1.21 g/ml fraction and the PTP stimulate net transfer of phospholipids from human VLDL into HDL

All of the above experiments were performed with biosynthetically labeled rat VLDL. To determine whether

TABLE 2. Percentage distribution of ^{32}P radioactivity amongst lipoprotein phospholipids[a]

	Lipoprotein Fraction			
	VLDL	d < 1.063	1.063-1.210	> 1.210
Phosphatidylethanolamine	3	3	5	0
Phosphatidylcholine	83	72	85	50
Sphingomyelin	9	10	6	15
Lysophosphatidylcholine	5	16	4	35

[a]Incubations were conducted as described in the legend to Fig. 2. The % distribution of ^{32}P amongst the lipoprotein phospholipids is shown for the biosynthetically labeled rat VLDL and also for the various fractions isolated by preparative ultracentrifugation after the incubation. The fractions were extracted (21) and the lipid extract was analyzed by thin-layer chromatography on soft-layer Adsorbosil plates (Alltech) in chloroform-methanol-acetic acid-water 65:25:4:1. Spots were visualized by brief exposure to I_2 vapor, identified by comparison of their migration with that of authentic phospholipid standards, scraped, and subjected to liquid scintillation counting. Recovery of phospholipid radioactivity was 85% during the extraction and >90% through thin-layer chromatography. The results shown are for the incubation containing VLDL, LPL, HDL, and PTP.

TABLE 3. Comparison of the transfer of VLDL phospholipid mass and radioactivity into HDL[a]

	μg Phospholipid in HDL[b]	% Transfer VLDL-PL Mass HDL[c]	% Transfer VLDL [^{32}P]-PL HDL[d]
A. VLDL	2 ± 0	3 ± 0	4 ± 2
B. HDL	28 ± 2		
C. PTP	1 ± 0		
D. d > 1.21 g/ml	1 ± 0		
E. VLDL + HDL[e]	37 ± 2	15 ± 2	22 ± 2
F. VLDL + d > 1.21 g/ml	3 ± 1	2 ± 1	9 ± 1
G. VLDL + HDL + d > 1.21 g/ml	44 ± 3	24 ± 1	39 ± 1
H. VLDL + PTP	3 ± 1	3 ± 1	5 ± 1
I. VLDL + HDL + PTP	41 ± 2	20 ± 1	33 ± 2

[a]As indicated, incubations contained biosynthetically labeled VLDL (60 μg of phospholipid), HDL (35 μg of phospholipid), PTP (20 μg), d > 1.21 g/ml fraction (7.5 mg of protein). All incubations contained LPL (0.17 μg) and were conducted for 2 hr at 37°C. HDL was isolated by preparative ultracentrifugation. The results shown are the mean ± SEM values for six experiments. In each experiment the HDL fraction was analyzed for phospholipid content in triplicate.

[b]Total mass of phospholipid recovered in HDL.

[c]The percentage transfer of VLDL phospholipid mass into HDL due to addition of HDL, the d > 1.21 g/ml fraction, or the PTP was calculated from the increase in mass of HDL phospholipid associated with addition of the various components minus the blank value obtained in the incubation containing VLDL alone (2 μg) (e.g., for VLDL + HDL + d > 1.21 g/ml, the value was (44 − 28 − 2)/60 × 100 = 24%).

[d]Calculated as described in footnote *c*.

[e]Statistical comparisons of HDL phospholipid mass: E versus B, $P < 0.05$; G versus E, $P < 0.05$; I versus E, $P = 0.05$.

stimulated transfer of phospholipid could also be observed using a human triglyceride-rich lipoprotein, human VLDL was incubated with LPL, in the presence of human HDL_3 and/or human d > 1.21 g/ml fraction. All incubations contained 2 mM dithionitrobenzoic acid to inhibit lecithin:cholesterol acyltransferase activity. Following the incubation, the HDL fraction was isolated by preparative ultracentrifugation between d 1.063 to 1.210 g/ml and the mass of its different constituents was determined (**Table 5**). The components that were mixed on the different incubations are shown on the left-hand side of Table 5. The addition of the d > 1.21 g/ml fraction to incubations containing VLDL + HDL + LPL resulted in an increase in HDL phospholipid mass from 97 to 135 μg ($P < 0.05$). Also, the mass of unesterified cholesterol was increased from 18.4 to 22.4 μg, but this was not statistically significant. In the absence of added HDL, the d > 1.21 g/ml fraction did not cause a significant stimulation of transfer of VLDL phospholipid or cholesterol into HDL (Table 5, cf incubations B and D). Thus, in the presence of HDL, the d > 1.21 g/ml fraction stimulated the transfer of phospholipid from VLDL to HDL during lipolysis. When similar experiments were conducted in the absence of LPL (Table 5, F–H), there was no additional transfer of phospholipid or cholesterol into HDL as a result of addition of the d > 1.21 g/ml fraction. This indicates that lipolysis was necessary for the stimulatory effect of the d > 1.21 g/ml fraction to be observed. It was noted that the recovery of HDL lipids was greater for VLDL + HDL, than for VLDL + HDL + LPL. This reflected the transfer of greater amounts of phospholipids, cholesterol, and cholesteryl esters into the d > 1.21 g/ml fraction in the incubation containing LPL (not shown). There was an increase in protein mass in HDL in incubations containing VLDL + LPL, presumably reflecting transfer of apoC peptides from VLDL into HDL (19, 20). The presence of the d > 1.21 g/ml fraction did not result in a significant further increase in the protein mass of HDL. Analysis of HDL proteins by sodium dodecyl sulfate polyacrylamide gel electrophoresis did not show a change in the relative amounts of HDL apoproteins resulting from addition of the d > 1.21 g/ml fraction.

TABLE 4. Effects of PTP on the percentage distribution of VLDL-^{32}P and HDL-^{14}C phospholipid radioactivity[a]

	d < 1.063 g/ml	1.063–1.210 g/ml	d > 1.21 g/ml
		%	
^{32}P-Labeled phospholipid			
VLDL	91	0	9
VLDL + LPL	81	11	9
VLDL + LPL + HDL	67	24	8
VLDL + LPL + HDL + PTP	37	55	8
VLDL + LPL + PTP	88	4	8
VLDL + HDL	90	4	7
VLDL + HDL + PTP	88	5	7
^{14}C-Labeled phospholipid			
VLDL + LPL + HDL	14	82	4
VLDL + LPL + HDL + PTP	26	70	4
VLDL + HDL	9	85	6
VLDL + HDL + PTP	9	84	7

[a]As indicated, incubations contained rat ^{32}P-labeled VLDL (100 μg of triglyceride, 6,000 cpm), LPL (0.08 μg), human ^{14}C-labeled HDL_3 (200 μg of protein, 120,000 cpm), and PTP (30 μg). Samples were incubated for 2 hr at 37°C, then lipoproteins were separated by preparative ultracentrifugation. Recovery of radioactivity was 85–92%. Results are means of triplicate incubations. Individual values varied by less than 10% of the mean values.

TABLE 5. Changes in composition of HDL resulting from incubation with VLDL and/or LPL and/or d > 1.21 g/ml fraction[a]

	Phospholipid	Cholesterol	Cholesteryl Esters	Protein
	mass (μg), n = 4			
A. HDL	89 ± 4	8.6 ± 2.0	79 ± 6	161 ± 9
B. VLDL + LPL	40 ± 4	7.0 ± 2.0	18 ± 4	43 ± 7
C. VLDL + LPL + HDL	97 ± 5	18.4 ± 2.0	78 ± 3	222 ± 12
D. VLDL + LPL + d > 1.21 g/ml	46 ± 8	9.6 ± 0.7	22 ± 6	43 ± 8
E. VLDL + LPL + HDL + d > 1.21 g/ml	135 ± 11	22.4 ± 2.0	74 ± 2	231 ± 12
F. VLDL + HDL	118 ± 5	19.6 ± 2.0	105 ± 9	273 ± 20
G. VLDL + d > 1.21 g/ml	38 ± 11	8.0 ± 1.7	32 ± 12	56 ± 4
H. VLDL + HDL + d > 1.21 g/ml	116 ± 3	15.3 ± 0.6	95 ± 10	222 ± 17

[a]As indicated, incubations contained human VLDL (1.0 mg of triglyceride), HDL_3 (200 μg of protein), d > 1.21 g/ml fraction (25 mg of protein), LPL (1 μg). Samples were incubated for 2 hr at 37°C, in buffer A containing 2 mM DTNB, heparin, and albumin (see Methods). After incubation, HDL was isolated by preparative ultracentrifugation. The results shown are for four different experiments. In each experiment, incubations were performed in triplicate and analyzed in duplicate. Statistical comparisons: Phospholipid: D vs B, NS; C vs A, NS; E vs H, NS; E vs A, $P < 0.03$; E vs C, $P < 0.05$; F vs A, $P < 0.01$; H vs A, $P < 0.05$; H vs F, NS; F vs C, $P < 0.05$. Cholesterol: C, E, F, H vs A, $P < 0.02$; E vs C, NS; E vs F, NS; E vs H, $P < 0.05$. Cholesteryl esters: F vs A, C, E, $P < 0.05$. Protein: C, E, F, H vs A, $P < 0.01$.

Experiments similar to those shown in Table 5 were performed using the partially purified human PTP, instead of the human d > 1.21 g/ml fraction. The mass of lipids in the HDL fraction is shown in **Table 6**. In the presence of LPL and HDL, the PTP resulted in a small but statistically significant increase in the transfer of phospholipid into HDL ($P < 0.05$). In the absence of LPL or HDL, the PTP exerted no significant effect on the mass of HDL phospholipids.

Since the above experiments were performed at high ratios of VLDL/HDL, the effects of variable VLDL/HDL ratio were examined. An increasing mass of HDL was added to an incubation containing a constant amount of VLDL and LPL. The presence of the d > 1.21 g/ml fraction resulted in an approximately constant increase in HDL phospholipid mass at the three VLDL/HDL ratios (**Fig. 3**). Incubations of the d > 1.21 g/ml fraction alone did not result in measurable phospholipid in the HDL density range. Similar results were also obtained for the PTP in experiments performed at two different VLDL/HDL ratios (not shown). Thus, the facilitated phospholipid transfer shown by the d > 1.21 g/ml fraction or the PTP was observed over a range of VLDL/HDL ratios.

To determine whether enhanced lipid transfer was associated with size changes of HDL particles, human HDL was incubated with VLDL, with or without the other components, and then subjected to ultracentrifugation at density 1.21 g/ml. The d < 1.21 g/ml fraction was analyzed by non-denaturing polyacrylamide gradient gel electrophoresis. The scans of the gradient gels from a representative experiment are shown in **Fig. 4**. The scan of the gradient gel of HDL incubated with different components (dashed line) is compared with that of the control HDL preparation (solid line). The addition of VLDL,

TABLE 6. Changes in composition of HDL resulting from incubation with VLDL and/or LPL and/or PTP[a]

	Phospholipid	Cholesterol	Cholesteryl Esters
	mass (μg), n = 4		
A. HDL	89 ± 2	7 ± 1	91 ± 3
B. VLDL + LPL	56 ± 4	11 ± 2	45 ± 2
C. VLDL + LPL + HDL	124 ± 4	21 ± 1	105 ± 2
D. VLDL + LPL + PTP	60 ± 5	13 ± 2	42 ± 2
E. VLDL + LPL + HDL + PTP	146 ± 6	23 ± 1	99 ± 6
F. VLDL + HDL	118 ± 6	23 ± 2	105 ± 8
G. VLDL + PTP	48 ± 3	4 ± 1	30 ± 3
H. VLDL + HDL + PTP	128 ± 3	22 ± 1	115 ± 9

[a]Incubation mixtures were prepared using the components as listed. As indicated, incubations contained VLDL (1.0 mg of triglyceride), HDL_3 (200 μg of protein), PTP (50 μg), LPL (1 μg). Samples were incubated for 2 hr at 37°C. HDL was isolated by preparative ultracentrifugation. The results shown are mean ± SEM values from four different experiments. In each experiment, the incubations were performed in triplicate and analyzed in duplicate. Statistical comparisons: Phospholipids: D vs B, NS; C vs A, $P < 0.02$; E vs A, $P < 0.001$; E vs C, $P < 0.05$; F vs A, $P < 0.01$; H vs A, $P < 0.01$; H vs F, NS; E vs H, $P < 0.05$; F vs C, NS. Cholesterol: B, C, D, E, F, H vs A, $P < 0.01$; E vs C, NS; E vs H, NS.

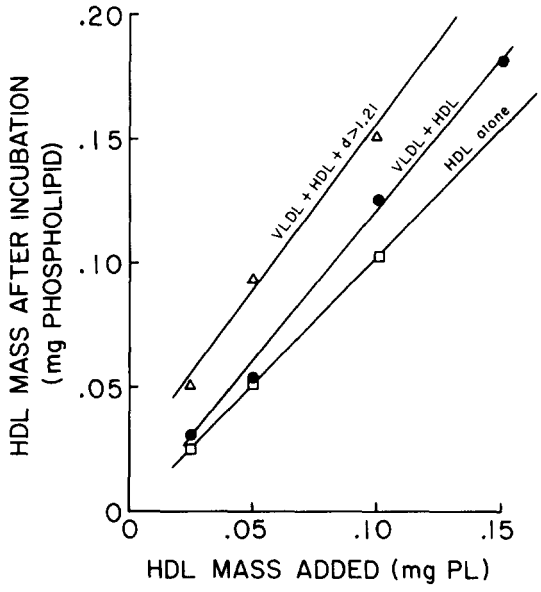

Fig. 3. Transfer of VLDL phospholipids into HDL at different ratios of HDL/VLDL. As indicated, the incubations contained human VLDL (1 mg of triglyceride, 0.25 mg of phospholipid), HDL_3, LPL (1.4 μg), and d > 1.21 g/ml fraction (80 mg of protein). The incubations were conducted as described in Methods.

LPL, or the d > 1.21 g/ml fraction did not result in major changes in the HDL profile. The addition of VLDL + LPL resulted in a small increase in size of the major HDL_3 peak from 9.4 to 9.6 nm. The addition of either the human or the rat d > 1.21 g/ml fraction resulted in a further, more pronounced shift of the HDL towards larger particle size, with peak sizes 9.8 nm (human d > 1.21 g/ml) and 10.1 nm (rat d > 1.21 g/ml). However, in four different experiments, addition of the human d > 1.21 fraction or the PTP resulted in a size increase of the major peak of HDL of only 0 to 0.2 nm. Thus, although the presence of the d > 1.21 g/ml fraction resulted in a pronounced increase in lipid transfer into HDL, there was only a slight change in HDL particle size.

DISCUSSION

In earlier studies we showed that the plasma d > 1.21 g/ml fraction or the PTP were able to enhance the net transfer of phospholipid from unilamellar vesicles of egg phosphatidylcholine into HDL (10, 11). In the present investigation we extent these earlier findings by showing that these plasma fractions also stimulate net transfer of phospholipid from VLDL into HDL during lipolysis. In addition, the d > 1.21 g/ml fraction and the PTP enhanced exchange of VLDL and HDL phospholipids.

Evidence that the PTP enhanced phospholipid exchange between VLDL and HDL was obtained by showing: *1*) the percentage transfer of VLDL phospholipid radioactivity into HDL exceeded that of phospholipid mass and *2*) when [^{32}P]phospholipid-VLDL was incubated with [^{14}C]phospholipid-HDL, the PTP enhanced the transfer of both radiolabels into the other lipoprotein fraction. Thus, although designated as a phospholipid transfer protein, the results suggest that this protein may stimulate both net transfer and exchange of phospholipids. Since the plasma cholesteryl ester transfer protein also enhances exchange of phospholipids between lipoproteins (11, 13, 14), the effects of these two different proteins on phospholipid transfer/exchange may not be distinct. As in the earlier study (11), we found evidence of a peak of phospholipid transfer activity partially separated from that of cholesteryl ester transfer activity during analysis by ion exchange chromatography. Although we were able to completely separate these activities by further purification (11), the instability of the highly purified material made its use impractical in the present study. The separation of two phospholipid exchange activities, one associated with cholesteryl ester exchange activity, the other not, has also been reported by another laboratory; the fraction called "phospholipid transfer protein" in the present studies probably corresponds to "LTP-2" (22). The existence of a separate PTP probably explains why certain species, such as the rat, have abundant phospholipid transfer/exchange activity in their plasma, but very little cholesteryl ester exchange/transfer activity (11, 22). Although the present study has shown that the d > 1.21 g/ml fraction and a 500-fold purified activity obtained from this fraction have similar effects on lipid transfer, it is possible that the results reflect the interactions of several components in these fractions and that the PTP fraction only accounts in part for the lipid transfer properties of the d > 1.21 g/ml fraction.

In the earlier studies we showed that the d > 1.21 g/ml fraction or the PTP enhanced the transfer of phospholipids from vesicles into HDL (10, 11). The activity of these fractions required the addition of HDL to the incubations. Similarly, in the present study the phospholipid transfer activity was only expressed in the presence of added HDL. However, in the earlier study the facilitated transfer of vesicle phospholipid resulted in a pronounced (1.0 nm) increase in HDL particle size, suggesting association of phospholipid molecules with pre-existing HDL particles. Surprisingly, the enhanced transfer of VLDL phospholipids and cholesterol into HDL was not associated with significant increases in particle size. This suggests a more complex mechanism of addition of lipid to the HDL fraction, resulting in formation of new particles similar in size to existing HDL particles; these particles are phospholipid and cholesterol-rich and relatively depleted in cholesteryl esters (Table 5). Nichols et al. (23) have shown that incubation of phospholipid/apoA-I discs with VLDL in the presence of an inhibitor of lecithin:cholesterol acyltransferase results in the formation of spherical particles

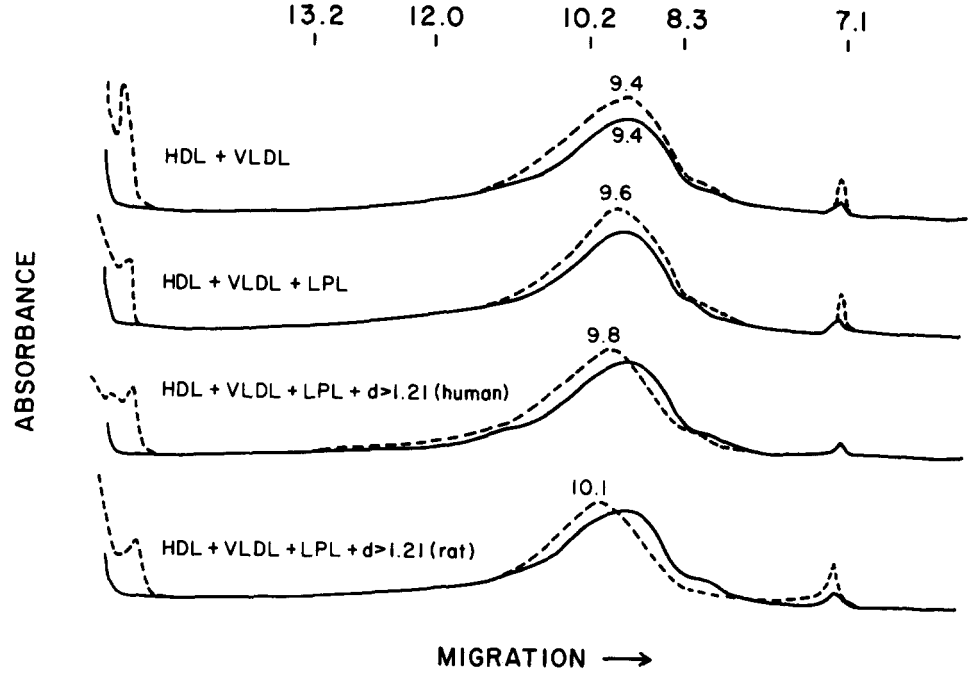

Fig. 4. Gradient gel scans of the d > 1.21 g/ml fraction showing size changes of HDL resulting from lipolysis of VLDL, with or without the d > 1.21 g/ml fraction. The profile of control HDL_3 is shown as a solid line in each section. The dashed lines show the profile of HDL obtained after incubation with the various components, as indicated. The amounts of material were VLDL (1 mg of triglyceride), HDL_3 (400 μg of protein), d > 1.21 g/ml fraction (100 μg of protein), and LPL (1.4 μg). Following incubation the samples were subjected to preparative ultracentrifugation at density 1.21 g/ml for 48 hr at 45,000 rpm in a Ti 50.3 rotor. The top 2 ml was removed, dialyzed, and an aliquot containing 75 μg of protein was analyzed by polyacrylamide gradient gel electrophoresis on 4/30% acrylamide gradient gels. Samples were electrophoresed for 3,000 volt-hours. Gels were stained in Coomassie Blue and scanned at 590 nm. Lipoprotein diameters (nm) are shown above their corresponding peaks. The migration of protein standards of the diameters shown is indicated at the top of the figure; from left to right the standards were thyroglobulin, apoferritin, catalase, LDH, and albumin.

similar in size to HDL_{3b} (about 8 nm). These particles consist of phospholipid, cholesterol, and protein. It is conceivable that a similar transformation of HDL is associated with the facilitated transfer of VLDL lipids into HDL.

The additional phospholipid transferred into HDL in the presence of the d > 1.21 g/ml fraction or the PTP was derived predominantly from the d 1.006–1.063 g/ml fraction (Table 1). This fraction contains VLDL remnants as well as excess surface components of VLDL in the form of sac-like liposomes (17). We speculate that the source of the additional phospholipid transferred into HDL may have been the excess surface material present in this fraction. The PTP-mediated stimulation of both net transfer and exchange of phospholipid was found to be dependent in large part on the presence of lipoprotein lipase. The dependence on lipolysis suggests that the surface phospholipids of VLDL are more available for PTP-mediated transfer or exchange, once the VLDL has undergone lipolysis. This could reflect altered physical properties of the phospholipids, or a variety of compositional changes such as the presence of small amounts of lysophospholipids or fatty acids formed during lipolysis.

We have recently found that lipolysis enhances cholesteryl ester transfer protein-mediated transfer of cholesteryl esters from HDL to VLDL (18). This phenomenon probably accounts for the decrease in HDL cholesteryl esters in the presence of LPL and the d > 1.21 g/ml fraction noted in the present study (Table 5). The stimulation of cholesteryl ester transfer protein-mediated transfer and exchange by lipolysis is probably due to increased binding of the cholesteryl ester transfer protein to VLDL remnants and HDL as a result of accumulation of products of lipolysis in these lipoproteins (24). Based on studies of the kinetics of cholesteryl ester exchange between LDL and HDL, Ihm et al. (25) proposed that the cholesteryl ester transfer protein mediates the formation of a collision complex between HDL and acceptor lipoproteins. They proposed that the free energy of formation of the collision complex would be decreased if cholesteryl ester transfer protein entered into the complex already bound to lipoprotein, since it would convert the reaction from third order to second order (25). It is conceivable that similar considerations may govern the PTP-mediated transfer or exchange of phospholipids, i.e., that lipolysis enhances the binding of PTP to VLDL and/or HDL, with resultant

more efficient phospholipid transfer or exchange.

It is likely that the phospholipid transfer activity shown by the d > 1.21 g/ml fraction and the PTP plays a role in the physiological transfer of phospholipids from chylomicrons or VLDL into HDL during lipolysis. When phospholipid vesicles are incubated with isolated HDL, the $t_{1/2}$ of phospholipid transfer is several hours; under comparable conditions, when vesicles are incubated in plasma, the $t_{1/2}$ of transfer into HDL is about 30 min (7, 10). Following injection of a bolus of ^{32}P-labeled chylomicrons into a rat, there is rapid (within 10 min) transfer of phospholipid radioactivity into HDL (3). These considerations indicate that the kinetics of physiological phospholipid transfer are similar to those obtained in vitro in the presence of phospholipid transfer protein.

The phospholipid transfer process may be regarded as a method of disposing of the excess surface materials resulting from lipolysis of triglyceride-rich lipoproteins. The transfer of phospholipids and cholesterol into HDL may provide substrate molecules for the lecithin:cholesterol acyltransferase reaction. The phospholipid and cholesterol-rich HDL particles formed as a result of enhanced phospholipid transfer are probably good substrates for lecithin:cholesterol acyltransferase (23). Phospholipid exchange between VLDL and HDL may also result in net transfer of certain molecular species of phospholipid into HDL, provided VLDL and HDL contain phospholipids of different fatty acid composition. Since lecithin:cholesterol acyltransferase uses polyunsatured fatty acids derived from the C-2 position of phosphatidylcholine, phospholipid exchange between HDL and triglyceride-rich lipoproteins could result in replenishment of HDL phospholipids containing polyunsaturated fatty acids. Thus, the enhancement of both the net transfer and exchange of VLDL phospholipids, by PTP during lipolysis, may play a role in providing substrate for lecithin:cholesterol acyltransferase. The overall effects of lipolysis and lipid transfer activities are *1*) to enhance the transfer of surface lipids of triglyceride-rich particles into HDL, and *2*) to promote the movement of HDL cholesteryl esters into remnants of triglyceride-rich lipoproteins (18).

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