Effect of temperature and plasma on the exchange of apolipoproteins and phospholipids between rat plasma very low and high density lipoproteins

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Abstract The effect of temperature and plasma on the exchange of apoprotein C and phospholipids between VLDL and HDL was studied using ^{125}I-labeled and ^{32}P-labeled VLDL. Temperature affected the exchange of apoproteins and phospholipids similarly, and both were enhanced by increasing the temperature of incubation to 20°C and higher. The exchange of apoC was almost complete within 5 minutes of incubation and was not influenced by the addition of plasma to the incubation mixture. The exchange of phospholipids occurred much more slowly and was enhanced 5- to 10-fold in the presence of plasma. These results indicate that the exchange of both phospholipids and apoC is dependent on the temperature of the incubation, but that they exchange independently, at least in part. It is suggested that the exchange of apoC occurs through dissociation of the apoprotein from the lipoprotein surface to the water phase, whereas the exchange of phospholipids is mediated mainly by a carrier molecule present in plasma and absent in lipoproteins.

Supplementary key words ^{125}I-labeled very low density lipoprotein · ^{125}I-labeled apolipoprotein C · [^{32}P]phosphatidylcholine · plasma-induced phospholipid exchange

The lipid-binding properties of apolipoproteins have been studied extensively during the last 5 years (1–10). In these studies, a remarkable and specific interaction between apoproteins and phospholipids has been established and shown to result in the formation of stable complexes. Yet, it is not known to what extent apoproteins and lipids are associated in intact lipoproteins.

Apoproteins (apoC in particular) and phospholipids are in dynamic equilibrium among lipoproteins through two different pathways: *a*) exchange among lipoproteins (11–14) and *b*) removal of apoC from either chylomicrons or very low density lipoproteins (VLDL) to high density lipoproteins (HDL) during degradation of the triglyceride-rich lipoproteins (15–18). The pathway of the exchange reaction was used here to study possible associations of apoproteins and phospholipids in intact lipoproteins. To this end, the effect of temperature on the exchange of ^{125}I-labeled apoproteins between VLDL and HDL was investigated and compared to the exchange of ^{32}P-labeled phospholipids.

MATERIALS AND METHODS

Preparation of lipoproteins and labeling procedures

VLDL and HDL were isolated from rat serum containing 1 mg/ml ethylenediamino tetraacetate (EDTA) at $d < 1.006$ g/ml and $d < 1.085$–1.21 g/ml respectively, as described previously (19, 20). The two lipoproteins were washed twice in NaCl solution of $d = 1.006$ g/ml (VLDL) and KBr solution of $d = 1.21$ g/ml (HDL), and the KBr was removed by exhaustive dialysis against 4-l changes of 0.9% NaCl, 0.01% EDTA solution over a 24-hr period. VLDL and HDL were shown to be free of contamination with other lipoproteins and plasma proteins by three different procedures: immunodiffusion techniques using antisera specific to rat plasma VLDL, HDL, and serum proteins; lipoprotein electrophoresis; and polyacrylamide gel electrophoresis of apoproteins (19–21). Lipoprotein isolation was carried out at 4°C. Very low density lipoprotein was iodinated by slight modifications (22) of MacFarlane's iodine monochloride procedure (23). Labeled lipids were extracted in chloroform–methanol 2:1 (v/v) and assessed for radioactivity content. 41 ± 1.7% of the radioactivity (11 iodinations) was associated with lipids and about 60% with apoproteins. Very low density lipoprotein labeled with ^{32}P was prepared biosynthetically. Male rats (150–250 g body weight) were injected intravenously with 2 mCi of $H_3{}^{32}PO_4$ and plasma was obtained from the abdominal aorta 16–18 hr after the injection.

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

VLDL was isolated at a plasma density of 1.006 g/ml by a single spin in the Beckman L3-50 ultracentrifuge using the 50Ti rotor and 50,000 rpm for 18 hr.

Delipidation, polyacrylamide gel electrophoresis, and gel filtration

Delipidation, with ethanol–diethyl ether and diethyl ether was carried out at 4°C on freeze-dried lipoprotein samples (20). Apoproteins were solubilized in 0.2 M Tris buffer, pH 8.2, containing 0.1 M sodium decyl sulfate. Recovery of protein and radioactivity ranged between 89 and 103%. Apoproteins were separated by a discontinuous polyacrylamide gel electrophoresis, using 10% acrylamide and a buffer system containing 6 M urea, following standard procedures (24). The gels were stained in 0.05% Coomassie blue (25). The pattern of apoproteins of VLDL and HDL was indistinguishable from that described previously (20). To determine the distribution of radioactivity among apoproteins, the gels were sliced by hand, and the slices were assayed for radioactivity (16, 20, 22). Apoproteins were also separated by gel filtration on Sephadex G-150 (particle size 40–120μm) using a 0.2 M Tris buffer, pH 8.2, containing 6 M urea and 0.002 M sodium decyl sulfate (16, 20). The column, 0.9 × 60 cm, was loaded with 1–2 mg of protein and was run at a rate of about 4 ml/hr. With these columns, two protein peaks were observed (see Fig. 4). Peak I contained apoB and arginine-rich protein (VLDL), or apoA-I (HDL). Peak II contained the C proteins; it also contained small quantities of apoA-II (in HDL) estimated by polyacrylamide gel electrophoresis to contribute about 5–10% of total peak II protein. Samples from individual tubes containing peak II proteins were taken for polyacrylamide gel electrophoresis. The gels were stained with Coomassie blue, inspected visually for protein bands, sliced, and the gel zones were assayed for radioactivity. Tubes containing only apoC were combined, dialyzed against 0.005 M Tris, 0.002 M sodium decyl sulfate buffer, pH 8.2, and assayed for protein and radioactivity content. ^{125}I-Labeled apoC specific activity was calculated from these values.

Analytical procedures

Protein was determined by the method of Lowry et al. (26) and phospholipid–phosphorus by the method of Bartlett (27). Phospholipids were extracted in chloroform–methanol 2:1 (v/v) according to the procedure of Folch, Lees, and Sloane Stanley (28), and were separated on silica gel G or HR silica gel plates using thin-layer chromatography and a solvent system containing chloroform–methanol–glacial acetic acid–water 100:50:18:8 (v/v/v/v). ^{32}P was determined in a Packard Auto-Gamma scintillation spectrometer, Model 544. ^{125}I was determined in a Packard Auto-Gamma scintillation spectrometer, Model 578, and the resulting cpm were corrected for absorption of radioactivity by concentrated salt and high density organic solutions (29).

^{125}I (as Na^{125}I, carrier-free) for protein iodination was purchased from the Radiochemical Centre, Amersham, England, and ^{32}P-orthophosphate, carrier-free, from the Israel Atomic Energy Commission, Beer-Sheba, Israel.

Experimental procedures

Incubations were carried out in 6.5-ml cellulose nitrate ultracentrifuge tubes at a final volume of 5 ml in a thermostated bath with constant monitoring of temperature. At the end of the incubation, the tubes were transferred on to crushed ice, and centrifugation was carried out at 4°C. The tubes were not allowed to warm up at any stage, either before or after centrifugation. To study the effect of temperature on the exchange of apoproteins and phospholipids between VLDL and HDL, the lipoproteins (0.5 mg of VLDL protein and 5 mg of HDL protein, or as indicated in the text) were pipetted to tubes containing 0.9% NaCl. When plasma was used, VLDL was added to freshly prepared rat plasma at a final volume of 5 ml.

RESULTS

Exchange of apoproteins

Recentrifugation of ^{125}I-labeled VLDL incubated in 0.9% NaCl resulted in separation of 96.2% of the radioactivity with VLDL ($d < 1.019$ g/ml) at either 0°C or 37°C, and only 3.8% with the fraction of $d > 1.019$ g/ml. An additional 6.8% of the VLDL radioactivity was separated with HDL when ^{125}I-labeled VLDL (0.5 mg of protein) was suspended for 60 min in ice-cold (0–2°C) 0.9% NaCl solution containing HDL (5 mg of protein) and centrifuged at 4°C (**Fig. 1**). Almost identical values were obtained at shorter (5–30 min) incubation periods. Incubations of ^{125}I-labeled VLDL with HDL at higher temperatures (10–37°C) resulted in a progressive transfer of radioactivity from VLDL to HDL (Fig. 1). The transfer of radioactivity from VLDL to HDL was more rapid during the first 5 min of the incubation than during the subsequent 25 min Fig. 1). A maximum transfer of about 25% of the VLDL radioactivity to HDL was achieved at 30 and 37°C following 30 min of incubation, and it did not change further when the incubation was carried out for an additional 30 min (see **Table 1**).

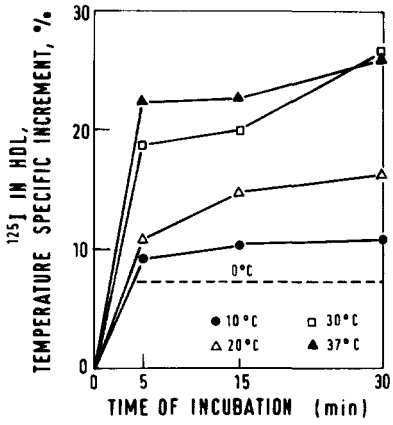

Fig. 1. ^{125}I-Labeled VLDL (0.5 mg of protein) was incubated with unlabeled HDL (5 mg of protein) for different time intervals (5–30 min) and at different temperatures (0–37°C) in 6.5-ml cellulose nitrate ultracentrifuge tubes. At the end of the incubation, the tubes were transferred onto crushed ice. VLDL and HDL were separated by a single spin at d < 1.019 g/ml as described in Methods, and assayed for radioactivity content. In the absence of HDL, only 3.8% of the radioactivity was separated as d > 1.019 g/ml and this value was subtracted from each experimental value. The horizontal line shows the percent of radioactivity separated with HDL when the samples were kept in crushed ice for 60 min and then subjected to ultracentrifugation for 24 hr (see symbols in the figure). Results are means of three experiments.

Radioiodinated VLDL contained about 40% of the radioactivity attached to lipids and 60% to proteins. The extent of apoprotein- and lipid-bound radioactivity in VLDL and HDL was determined in all samples, and is shown in Table 1 for the different temperatures (incubation time 30 min) and at different time intervals (at 37°C). As seen in the table, temperature or time affected the extent of transfer of ^{125}I-labeled apoproteins from VLDL to HDL, but not of lipids. Consequent to the uneven transfer of labeled apoproteins and lipids from VLDL to HDL, the ratio of labeled apoproteins to labeled lipids in HDL was much higher than that in VLDL. Temperature-specific increments of ^{125}I-labeled apoproteins and ^{125}I-labeled lipids in HDL were calculated on the basis of the data shown in the table and represent the percentage of labeled apoproteins and labeled lipids transferred to HDL. The ^{125}I-labeled apoprotein increments increase from 15.3% to 0°C to 47.5–46.4% at 30–37°C; increments for ^{125}I-labeled lipid were relatively low and did not change much with the change of temperature.

Similar results were obtained when ^{125}I-labeled VLDL was incubated with 5 ml of plasma (**Table 2**). At 0°C about 80% of the radioactivity was isolated with VLDL (at density less than 1.019 g/ml), 8.5–9.2% with the fraction of density 1.04–1.21 (predominantly HDL), and 9.4–11.0% with the ultracentrifuge fraction of density greater than 1.21 g/ml.More than one-half of the radioactivity in this latter fraction was dialyzable and not precipitable with 10% trichloroacetic acid; it represented, in part, small amounts of free

TABLE 1. Effects of temperature and time on the distribution of ^{125}I-labeled apoproteins and ^{125}I-labeled lipids between VLDL and HDL

	^{125}I-Labeled Apoproteins			^{125}I-Labeled Lipids[a]		
	VLDL	HDL	Increment[b]	VLDL	HDL	Increment[b]
			% total radioactivity			
Experiment 1						
	57.2 ± 0.6[c]			39.0 ± 0.5[c]		
0°C	46.0 ± 0.6	8.3 ± 0.8	15.3	43.4 ± 1.4	2.3 ± 0.2	5.0
10°C	44.2 ± 0.5	12.7 ± 0.6	22.3	39.1 ± 2.8	4.3 ± 0.9	9.9
20°C	36.5 ± 0.7	22.3 ± 1.1	37.9	38.9 ± 2.4	2.3 ± 0.2	5.6
30°C	31.5 ± 0.9	28.5 ± 1.6	47.5	37.2 ± 1.7	2.8 ± 0.7	7.0
37°C	31.8 ± 0.9	27.5 ± 1.4	46.4	38.3 ± 1.9	2.4 ± 0.1	5.9
Experiment 2						
0 min	47.4 ± 0.2	9.0 ± 0.4	16.0	41.7 ± 2.1	1.8 ± 0.1	4.1
5 min	34.7 ± 1.1	24.2 ± 2.5	41.1	39.3 ± 0.4	2.0 ± 0.1	4.8
15 min	31.7 ± 0.7	24.2 ± 1.3	43.3	41.8 ± 0.5	2.3 ± 0.1	5.2
30 min	34.7 ± 1.8	27.4 ± 3.6	44.1	35.6 ± 1.0	2.3 ± 0.1	6.1
60 min	33.3 ± 1.1	27.4 ± 3.3	45.1	36.3 ± 2.3	3.0 ± 0.2	7.6

[a] Chloroform–methanol extractable radioactivity.

[b] The increment value represents the percent of ^{125}I-labeled apoproteins or ^{125}I-labeled lipids transferred from VLDL to HDL. It is calculated from the distribution of radioactivity between VLDL and HDL, as presented in their appropriate columns of the table.

[c] Samples incubated with 0.9% NaCl at either 0°C or 37°C for 30 min.

Experiment 1. ^{125}I-labeled VLDL (0.5 mg of protein) was incubated with HDL (5 mg of protein) at different temperatures for 30 min. Final volume was 5 ml. After the incubation, samples were transferred to ice-cold water, and lipoproteins were separated by ultracentrifugation at 4°C as described in Methods. VLDL and HDL were separated at d 1.019 g/ml as described in Methods, dialyzed against 0.9% NaCl solution containing 0.01% EDTA, and assayed for radioactivity content. Labeled lipids were extracted with chloroform–methanol 2:1 (v/v). Results are mean ± SD of six experiments.

Experiment 2. Incubations were carried out at 37°C, following the procedures described above, experiment 1. Results are mean ± SD of three experiments.

TABLE 2. Effect of temperature on the distribution of radioactivity among lipoproteins after incubation of ^{125}I-labeled VLDL with plasma

Temperature (°C)	Distribution of ^{125}I Among Ultracentrifugally Separated Density (g/ml) Fraction (%)					
	^{125}I-Labeled Lipoproteins				^{125}I-Labeled Lipids	
	d < 1.019	d 1.019–1.040	d 1.040–1.21	d > 1.21	d < 1.019	d 1.04–1.21
0	79.6 ± 4.0	0.2 ± 0.1	9.2 ± 2.2	11.0 ± 1.9	41.7 ± 2.7	6.8 ± 1.0
10	81.9 ± 0.4	0.2 ± 0.1	8.5 ± 0.6	9.4 ± 0.1	36.0 ± 3.9	7.7 ± 1.9
20	72.1 ± 0.7	0.3 ± 0.1	17.6 ± 0.4	10.0 ± 0.8	44.0 ± 2.1	8.8 ± 1.1
30	62.0 ± 0.4	0.3 ± 0.1	28.0 ± 0.5	9.7 ± 0.1	53.7 ± 3.7	4.2 ± 0.8

Mean ± SD of four experiments.
^{125}I-Labeled VLDL (0.5 mg of protein) was incubated with 5 ml of rat plasma at the indicated temperatures for 30 min. Lipoproteins were separated by ultracentrifugation as described in Methods. Labeled lipids were extracted with chloroform–methanol 2:1 (v/v).

iodide. Minimal amounts of radioactivity were associated with the fraction of 1.019–1.04 g/ml (predominantly LDL). No change occurred in the distribution of radioactivity among the lipoprotein fractions on increasing the temperature to 10°C; however, at higher temperatures (20°C and 30°C) up to 28% of the radioactivity introduced with VLDL was isolated with HDL.

The nature of the apoproteins transferred from VLDL to HDL at the 30-min time interval was first determined by polyacrylamide gel electrophoresis. ApoC was the predominant apoprotein group transferred from VLDL to HDL at temperatures of 20°C or higher, and accounted for about 70% of the HDL radioactivity (**Fig. 2**). At 37°C an almost maximal transfer of labeled apoC from VLDL to HDL had been achieved already at the 5-min time interval (**Fig. 3**). At lower temperatures, however, about one-half of the radioactivity present in HDL was associated with several apoprotein groups isolated at the upper part of the polyacrylamide gels (fraction VS-2, Fig. 2).

To determine the mode of transfer of apoC between lipoproteins, net transfer or exchange phenomenon, apoprotein groups were separated by gel filtration on Sephadex G-150 (**Fig. 4**). ApoC, separated with the second peak, was isolated and assayed for protein and radioactivity content (sp act curve, **Fig. 5**). ApoC specific activity did not change much between 0°C and 10°C, but started to decline in VLDL and to increase in HDL when the incubation was carried

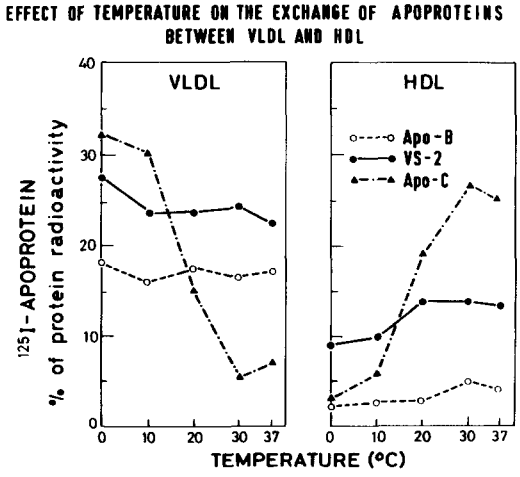

Fig. 2. Labeled apoproteins in VLDL and HDL after incubation of ^{125}I-labeled VLDL (0.5 mg of protein) with unlabeled HDL (5 mg of protein) at different temperatures. Incubations were carried out for 30 min, and lipoproteins were isolated in the ultracentrifuge. Lipoproteins were delipidated, solubilized in 0.2 M Tris, 0.1 M sodium decyl sulfate pH 8.2 buffer, and separated by polyacrylamide gel electrophoresis. Apoproteins B, VS-2 (predominantly the arginine-rich protein) and C were sliced off the gel and assayed for radioactivity. The amount of radioactivity associated with the various apoproteins is expressed as percent of total protein-bound radioactivity.

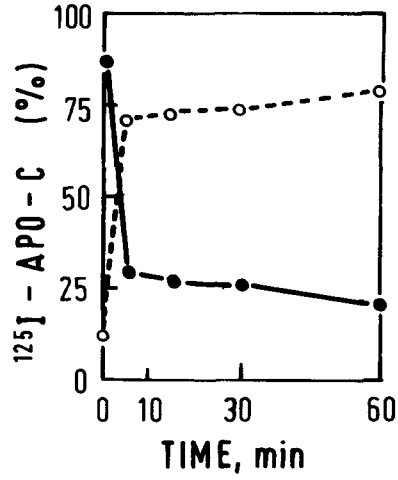

Fig. 3. Percent distribution of ^{125}I-labeled apoC between VLDL and HDL after incubation of ^{125}I-labeled VLDL (0.5 mg of protein) with unlabeled HDL (5 mg of protein) for different time intervals. Incubations were carried out at 37°C, and were terminated by transfer of the incubation mixture onto crushed ice. VLDL and HDL were separated at 4°C in the ultracentrifuge and apoproteins were separated after delipidation, solubilization in 0.2 M Tris, 0.1 M sodium decyl sulfate, pH 8.2, buffer and polyacrylamide gel electrophoresis. The protein bands corresponding to apoC were sliced off the gels and assayed for radioactivity content. ApoC radioactivity in both VLDL and HDL was taken as 100% of ^{125}I-labeled apoC, and was used as the basis for calculating the percent distribution of ^{125}I-labeled apoC between VLDL and HDL.

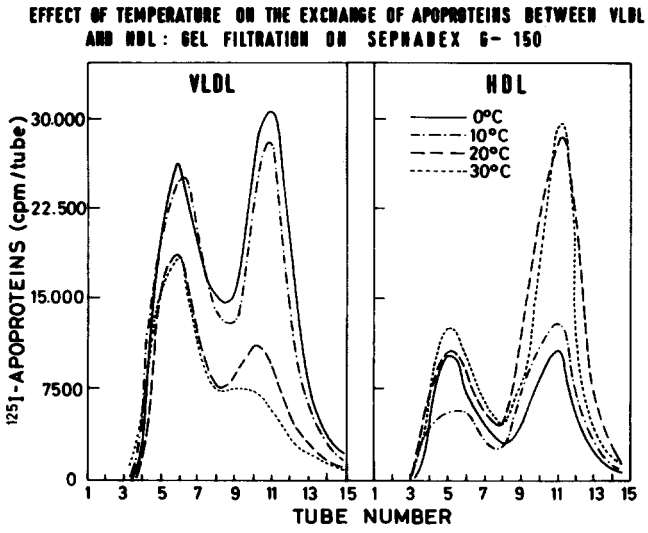

Fig. 4. Gel filtration pattern of labeled apoproteins in VLDL and HDL after a 30-min incubation of ^{125}I-labeled VLDL (0.5 mg of protein) with unlabeled HDL (5 mg of protein) at different temperatures. Solubilized apoproteins (1–2 mg) were applied to a Sephadex G-150 0.9 × 60 cm column, and apoproteins were eluted with 0.2 M Tris, 6 M urea, 0.002 M sodium decyl sulfate buffer, pH 8.2. The eluate was collected in 2-ml fractions, and radioactivity was determined in individual tubes. The protein content of the fractions was monitored by absorbency at 280 nm and was similar to that published previously (19–21). The first peak represented apoB and the arginine-rich protein (VLDL) or apoA-I (HDL). The second peak contained apoC.

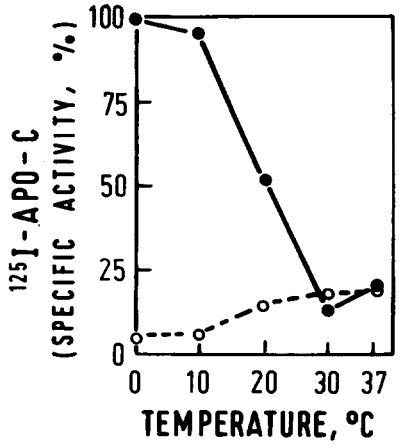

Fig. 5. Specific activity of ^{125}I-labeled apoC in VLDL and HDL after a 30-min incubation of ^{125}I-VLDL (0.5 mg of protein) with unlabeled HDL (5 mg of protein). The specific activity values were calculated on the basis of radioactivity and protein content of apoC, isolated by gel filtration on Sephadex G-150 as shown in Fig. 4. The specific activity of ^{125}I-labeled-apoC in VLDL incubated with HDL at 0°C is expressed as 100%.

out at 20°C (Fig. 5). Yet, apoC specific activity in VLDL was still 2- to 3-fold higher than in HDL. The specific activity of apoC in VLDL approached that of HDL only at 30°C, and no further change was observed upon increasing the temperature of incubation to 37°C.

Using the apoC specific activity data and the amount of apoC radioactivity in each lipoprotein as shown in Fig. 2, the ratio of apoC mass in VLDL to HDL could be calculated. At the five different temperatures studied (1, 10, 20, 30, and 37°C), this ratio remained constant and was 0.27, 0.25, 0.22, 0.25, and 0.26, respectively, thus demonstrating a pure exchange reaction with no net transfer of apoC between VLDL and HDL.

Exchange of phospholipids

Labeled phosphatidylcholine constituted 85–90% of total ^{32}P-labeled phospholipids of the biosynthetically labeled VLDL. Initially, phospholipid exchange between VLDL and HDL was determined using conditions similar to those described for apoproteins. To this end, ^{32}P-labeled VLDL was incubated with isolated unlabeled HDL in 0.9% NaCl solution, at a protein ratio of 1:10 (phospholipid ratio of 1:4) and an incubation period of 30 min. Minimal exchange of ^{32}P-labeled phospholipids between VLDL to HDL was observed (**Table 3**). Only a small increase of phospholipid exchange occurred when the ratio of phospholipid–phosphorus between VLDL and HDL was changed to 1:10 and the incubation was carried out for 120 min (Table 3).

In an attempt to increase the extent of exchange of phospholipids between lipoproteins, the experiment was repeated using ^{32}P-labeled VLDL and 5 ml of rat plasma (**Table 4**). Exchange of small amounts of [^{32}P]phosphatidylcholine between VLDL and the fraction of density 1.04–1.21 g/ml (predominantly HDL) was observed already at 0°C and 10°C; it increased markedly at temperatures of 20°c and 30°C,

TABLE 3. Phospholipid exchange between isolated VLDL and HDL

Temperature (°C)	Distribution of ^{32}P between VLDL and HDL (%)			
	30 min[a]		120 min[b]	
	VLDL	HDL	VLDL	HDL
	97.7 ± 0.5[c]	2.3 ± 0.5[c]		
0	95.1 ± 0.4	4.9 ± 0.4	94.2 ± 0.4	5.8 ± 0.4
10	95.3 ± 0.1	4.7 ± 0.1	92.8 ± 1.3	7.2 ± 1.3
20	94.5 ± 0.7	5.5 ± 0.7	92.4 ± 0.7	7.6 ± 0.7
30	93.0 ± 0.2	7.0 ± 0.2	87.6 ± 0.4	12.4 ± 0.4
37	91.4 ± 0.1	8.6 ± 0.1	87.5 ± 0.7	12.5 ± 0.7

[a] Phospholipid–phosphorus ratio (VLDL:HDL) of 1:4.
[b] Phospholipid–phosphorus ratio (VLDL:HDL) of 1:10.
[c] Samples incubated with 0.9% NaCl, at 37°C.
Mean ± SE of three experiments. VLDL labeled biosynthetically with ^{32}P-labeled phospholipids was incubated with HDL at different temperatures for either 30 min or 120 min and at a final volume of 5 ml. VLDL and HDL were separated in the ultracentrifuge at d 1.019 g/ml. Lipids were extracted with chloroform–methanol 2:1 (v/v) and assayed for radioactivity content.

TABLE 4. Effect of temperature and time on the distribution of ^{32}P-labeled phospholipids between VLDL and HDL after incubation of ^{32}P-labeled VLDL with plasma

	^{32}P-Phospholipids % of Radioactivity		[^{32}P]Phosphatidylcholine Sp act (cpm/μg P)	
	d < 1.019[a]	d 1.04–1.21[b]	d < 1.019[a]	d 1.04–1.21[b]
Experiment 1				
0°C	93.2 ± 0.6	6.8 ± 0.5	71.7 ± 5.2	3.3 ± 0.5
10°C	86.9 ± 2.8	13.1 ± 0.5	63.0 ± 2.3	5.9 ± 0.5
20°C	66.7 ± 1.2	33.3 ± 4.0	48.9 ± 2.5	14.9 ± 1.6
30°C	47.1 ± 1.1	52.9 ± 2.7	32.0 ± 0.5	22.8 ± 0.8
37°C	44.5 ± 2.8	55.4 ± 0.5	30.8 ± 0.8	24.0 ± 2.2
Experiment 2				
0 min	92.4 ± 3.1	7.6 ± 1.3	85.9 ± 3.2	1.8 ± 0.3
5 min	86.0 ± 3.8	14.0 ± 2.6	65.6 ± 0.7	5.0 ± 1.6
15 min	71.3 ± 4.5	28.7 ± 5.5	56.4 ± 0.4	9.5 ± 2.2
30 min	57.2 ± 3.5	42.8 ± 4.2	43.4 ± 0.6	15.0 ± 3.1
60 min	39.2 ± 1.9	60.8 ± 2.5	29.9 ± 4.6	27.2 ± 4.8

[a] Predominantly VLDL.
[b] Predominantly HDL.

Experiment 1. VLDL labeled biosynthetically with ^{32}P-labeled phospholipids (0.5 mg of protein) was incubated with 5 ml of rat plasma at the indicated temperatures for 30 min. Phospholipids:phosphorus ratio in rat plasma VLDL and HDL was approximately 1:2 (19). VLDL and HDL were isolated at d < 1.019 g/ml and d 1.04–1.21 g/ml by ultracentrifugation as described in Methods. ^{32}P-Labeled phospholipids were extracted with chloroform–methanol 2:1 (v/v) and separated into phospholipid classes by thin-layer chromatography. Specific activity of [^{32}P]phosphatidylcholine was determined after determination of phosphorus and radioactivity content after extraction from the silica gel. Results are mean ± SD of six experiments.

Experiment 2. Incubations were carried out at 37°C, following the procedures described above, experiment 1. Results are mean ± SD of three experiments.

but did not change further at 37°C. The exchange of [^{32}P]phosphatidylcholine in VLDL and HDL was thus affected by temperature similarly to that of apoproteins. Calculation of the phospholipid mass ratio between VLDL and HDL, based on the radioactivity and specific activity data, demonstrated again a constant ratio of 0.63, 0.62, 0.61, 0.63, and 0.63 at 0, 10, 20, 30,and 37°C, respectively.

The time course of the exchange of phospholipids was determined at 37°C (Table 4). A complete exchange (equal specific activity of [^{32}P]phosphatidylcholines in VLDL and HDL) was achieved only after a 60-min incubation period.

DISCUSSION

In the present investigation we have studied the effect of temperature on the exchangeability of apolipoproteins (apoC in particular) and phospholipids between rat plasma VLDL and HDL. The ratio of VLDL to HDL used was similar to that of rat plasma of the Hebrew University strain of 10 and 100 mg% protein, respectively (19, 30). When complete exchange was observed (30°C and 37°C, vide infra), the distribution of ^{125}I-labeled apoproteins between the lipoproteins (Fig. 3) paralleled the ratio of the mass of apoC in VLDL and HDL of about 1:3 (0.3 mg for VLDL and 1.0 mg for HDL).[1] These results are similar to previously published observations (14) demonstrating parallelism between the distribution of radioiodinated apoC and the mass of apoC in VLDL and HDL when using several different ratios of the two lipoproteins.

Variable amounts of ^{125}I radioactivity (predominately in apoproteins) were found at d > 1.019 g/ml under all incubation conditions. These amounts were very small (3.8%) when ^{125}I-labeled VLDL was suspended in 0.9% NaCl. They increased slightly (to 10.6%, increment of 6.8%) upon the addition of HDL and when the incubation mixture was kept at 0°C for 60 min and centrifuged at 4°C for an additional 24 hr. However, even brief exposure of the incubation mixture to higher temperatures, especially 30°C and 37°C, resulted in a pronounced change in the distribution of radioactivity between VLDL and HDL. Thus, at 30°C and 37°C, more than 45% of the protein-bound radioactivity was isolated with HDL, as compared to only 15–16% at 0°C. Since the concentration of lipoproteins and the experimental conditions were identical, this marked increased of the transfer of ^{125}I-labeled apoproteins to HDL must reflect the effects of temperature and temperature alone.

The major labeled apoprotein transferred from VLDL to HDL was apoC. The specific activity determinations indicated transfer by an exchange reaction, i.e., decreased specific activity in VLDL and increased specific activity in HDL. The present study represents the first report where apoC specific activities were determined in VLDL and HDL during the course of an exchange reaction. It was therefore of interest to determine whether all of the apoC molecules (labeled and unlabeled) in the lipoproteins were present in one exchangeable pool. We found that at the points of complete exchange (30°C and 37°C, 30-min incubation) the specific activity of apoC in VLDL and in HDL was identical. It is thus concluded that iodinated and uniodinated apoC in VLDL represent one miscible pool, and that all of the apoC molecules are easily exchangeable, as is the biosynthetically labeled phosphatidylcholine (17).

[1] Calculated as 60% of VLDL apoproteins (16) and 20% of HDL apoproteins (31, 32).

Temperature has recently been shown to have a profound effect on phospholipid binding by apoproteins. While studying the binding of apoC-III to dimyristoyl lecithin, Pownall et al. (33) have found that specific binding was taking place predominately at temperatures above the transition phase of the phospholipid. Similar conclusions were reported by Trauble, Middelhoff, and Brown (34) using dimyristoyl-, dipalmitoyl-, and distearoyl-lecithins. In the present study, it was shown that the exchange of both apoC and phosphatidylcholine between VLDL and HDL is also profoundly affected by the temperature of incubation. The mechanism by which temperature influences the exchange reaction of either apoC or phosphatidylcholine between lipoproteins has not been elucidated here. One possible explanation is that the physical state(s) of the phospholipids and/or apoproteins in the lipoproteins might be altered by temperature. The altered physical state(s) of the lipoprotein lipids and apoproteins may then affect their exchangeability between the lipoproteins. In this context it is interesting to note that Jonas and Jung (35) have recently reported a profound effect of temperature on the fluidity of bovine serum HDL. Thus, at 0, 25, and 40°C the microviscosity (a reciprocal of fluidity) of the HDL was 19.0, 6.1, and 2.6 poise, respectively. Similar temperature-induced decrements of the microviscosity of rat plasma VLDL were recently found by us.[2] Whether the fluidity of the lipoproteins, however, affects the exchange of apoproteins and phospholipids among lipoproteins is unknown.

Although the exchange of apoC and phosphatidylcholine was affected similarly by temperature, it differed fundamentally in two respects. ApoC exchange occurred within a few minutes of incubation at 37°C and proceeded at similar rates when the labeled VLDL was incubated with either HDL or plasma. The exchange of phosphatidylcholine was considerably slower and almost did not proceed at all in the absence of plasma. Plasma evidently must contain a carrier factor that enhances the exchange of phospholipids between VLDL and HDL. Since this factor is not separated with the ultracentrifugally purified lipoproteins, it is probably not an apolipoprotein. Analogous conclusions were reported previously by Illingworth and Portman (13) who showed a marked effect of a pH 5.1 supernatant fraction prepared from liver cells on the exchange of biosynthetically labeled phospholipids but not of apoproteins between total low density lipoproteins (VLDL + LDL) and HDL. Both studies therefore indicated that the exchange of apoC between VLDL and HDL occurs independently of that of the bulk of the lipoprotein phospholipid. They further indicate that phospholipid exchange occurs through the presence of a specific cofactor (molecule?) present in liver cell cytosol and plasma, but not in lipoproteins. This cofactor may functionally be similar to the phospholipid carrier protein described originally in the hepatocytes (12, 36, 37). With regard to apoC, no definite mechanism for exchange has as yet been described. It is interesting, however, to note that a poorly lipidated apoC form of $d > 1.21$ g/ml is a predominant product of lipoprotein lipase-induced lipolysis of rat plasma VLDL when plasma or HDL is not present in the incubation system (38).

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[2] Barenholz, Y., A. Gafni, and S. Eisenberg. Unpublished observations.

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