Distribution of apolipoprotein A-IV between the lipoprotein and the lipoprotein-free fractions of rat plasma: possible role of lecithin:cholesterol acyltransferase

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Abstract Plasma samples were incubated under various conditions to study the effect of in vitro incubation on apolipoprotein A-IV distribution between the lipoprotein and lipoprotein-free fractions. When plasma was fractionated immediately after bleeding, apolipoprotein A-IV was present in equal concentrations in the lipoprotein and lipoprotein-free fractions. After a 4-hr, 37°C incubation, >90% of total plasma apolipoprotein A-IV was present in the lipoprotein fraction and the percentage of plasma cholesterol present as cholesteryl ester increased from 58% to 74%. When plasma was incubated for 4 hr at 37°C in the presence of 1.5 mM 5,5-dithiobis(2-nitrobenzoic acid) (DTNB), >90% of total plasma apoA-IV was present in the lipoprotein-free fraction, whereas plasma cholesteryl ester concentration did not change. Incubating heat-inactivated plasma for 4 hr also resulted in the redistribution of apolipoprotein A-IV from the lipoprotein fraction to the lipoprotein-free fraction, concurrent with no change in cholesterol esterification. When heat-inactivated plasma was incubated in the presence of a purified lecithin:cholesterol acyltransferase preparation, cholesterol esterification was restored and apolipoprotein A-IV was redistributed from the lipoprotein-free fraction to the lipoprotein fraction in such a manner that >90% was present in the lipoprotein fraction. No changes in apolipoprotein A-I and apolipoprotein E distributions were found under any of the above conditions. Thus, the in vitro plasma incubations show that apolipoprotein A-IV can move bidirectionally between lipoprotein and lipoprotein-free fractions; the direction of this movement depends on the condition of the incubation. These data also suggest that the LCAT reaction can influence apolipoprotein A-IV distribution in the plasma.—**DeLamatre, J. G., C. A. Hoffmeier, A. G. Lacko, and P. S. Roheim.** Distribution of apolipoprotein A-IV between the lipoprotein and the lipoprotein-free fractions of rat plasma: possible role of lecithin:cholesterol acyltransferase. *J. Lipid Res.* 1983. **24:** 1578–1585.

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Apolipoprotein (apo) A-IV is found primarily in high density lipoproteins (HDL) and the d > 1.21 g/ml fraction in rat plasma (1–6). In the rat, apoA-IV is synthesized by both the intestine and liver (7). Turnover studies in the rat suggest that apoA-IV, which enters the plasma as a chylomicron component, transfers to the lipoprotein-free fraction (d > 1.21 g/ml) and then moves to HDL (4). Previous studies from this laboratory have demonstrated a reciprocal relationship between apoA-IV concentration in HDL vs. the lipoprotein-free fraction; when rats are fed high-cholesterol, high-fat diets, the apoA-IV concentration in HDL decreases at the same time that apoA-IV concentration in the lipoprotein-free fraction increases (6). We postulated that apoA-IV moves from the lipoprotein-free fraction to HDL in association with the esterification of free cholesterol due to the action of lecithin:cholesterol acyltransferase (LCAT). The present study provides direct evidence that the LCAT reaction can influence the distribution of apoA-IV, and shows that apoA-IV can transfer from the lipoprotein-free fraction to the lipoprotein fraction or vice versa in vitro.

MATERIALS AND METHODS

Plasma collection

Male Sprague-Dawley rats, 275–300 g, were maintained on laboratory rat chow. On the day of the experiments, two to four rats were bled in the morning from the abdominal aorta while under light ether anes-

Abbreviations: HDL, high density lipoproteins (d 1.063–1.21 g/ml); LCAT, lecithin:cholesterol acyltransferase (EC 2.3.1.43); DTNB, 5,5-dithiobis (2-nitrobenzoic acid); LDL, low density lipoproteins (d 1.03–1.063 g/ml); SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; apo, apolipoprotein; EDTA, ethylenediaminetetraacetic acid.

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thesia. Syringes contained 300 mM ethylenediaminetetraacetic acid (EDTA) in an amount that would result in a final concentration of 3 mM EDTA. Blood was placed on ice until plasma was separated by centrifugation at 1500 rpm for 20 min and immediately pooled. The pooled plasma was divided into two samples, one of which served as the control for the other sample. In this manner, each experimental run consisted of a paired experimental and control group.

Lipoprotein isolation

Column chromatography. Immediately after the plasma was separated, or after incubation, the control and experimental samples were fractionated by agarose gel column chromatography using two calibrated column sets that were run simultaneously and had similar elution patterns. A 0.9 × 100-cm column of 10% agarose (Bio-Gel 0.5 m, 200–400 mesh, Bio-Rad Laboratories, Richmond, CA) was used with a buffer of 0.15 M NaCl, 0.1% EDTA, and 0.1% sodium azide, pH 7.4. The column was operated at 4°C with a flow rate of 4.5 ml/hr. Fractions of 25 drops/tube were collected. These columns separated the plasma into three general areas: *a*) the void volume that contained lipoproteins having a size approximately that of low density lipoprotein (LDL) and larger; *b*) "HDL" size lipoproteins; and *c*) lipoprotein-free fraction. In each column run, ^{125}I-labeled LDL and ^{125}I-labeled albumin were used as internal standards to mark the void volume and lipoprotein-free fractions, respectively, as previously described (5, 6). The distribution of apoA-I was such that it was between the two markers; therefore, the "HDL" region was designated as beginning one fraction after the LDL peak and ending two fractions before the albumin peak. This consistently corresponded with the apoA-I distribution which was determined in each experiment. In the data shown in Table 1 and subsequent tables, region A consisted of the fractions that contained the void volume; regions B and C were "HDL" fractions with pool B being the first half of those fractions (large HDL) and pool C being the second half (small HDL); and region D consisted of the lipoprotein-free or non-lipoprotein fractions. In this manuscript, the lipoprotein-free fractions refer to those fractions that eluted in the same region as albumin and were distinctly separated from fractions containing lipoproteins.

Ultracentrifugal separation of lipoproteins. In some experiments, lipoproteins were further isolated from agarose column fractions (regions B and C) by ultracentrifugation. Samples were adjusted by the addition of KBr to 1.21 g/ml (8); after that, at least 2 ml of KBr solution of d 1.21 g/ml was layered over the samples which were then ultracentrifuged for 48 hr in a Beckman L5-50 preparative ultracentrifuge at 40,000 rpm using an SW-41 rotor.

In vitro incubations

The following in vitro experiments were done using paired plasma samples: *a*) 3–5 ml of plasma samples were incubated for 4 hr at 37°C in either the presence or absence of 1.5 mM 5,5-dithiobis(2-nitrobenzoic acid) (DTNB), an agent that is known to inhibit LCAT activity at this concentration (9). After the incubations, DTNB was added to the samples that did not contain DTNB during the incubation, and samples were immediately subjected to column chromatography. *b*) Plasma samples were either applied to the columns immediately after separation or after incubation for 4 hr at 37°C in the presence of 1.5 mM DTNB. Just before column fractionation, DTNB was also added to samples not incubated. *c*) Whole blood was collected and plasma was separated either immediately after bleeding or after incubation for 4 hr at 37°C and applied to agarose columns as described above. *d*) Pooled plasma was heat-inactivated by incubation at 56°C for 30 min (9–11); this was either not incubated or incubated for 4 hrs at 37°C and immediately applied to agarose columns. *e*) Heat-inactivated plasma samples were incubated for 4 hr at 37°C in the presence or absence of a purified LCAT preparation and immediately applied to agarose columns. The human LCAT preparation used in this study gave a single band on SDS-PAGE and contained no detectable apolipoproteins; however, the presence of minor contaminants could not be ruled out.

Analytical methods

Apolipoprotein concentrations were determined by the electroimmunoassay technique of Laurell (12), as modified by Dory and Roheim (13). Antisera used in this study were prepared as previously described (13). Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was done using a system described by Laemmli (14) in a 3–20% polyacrylamide slab gel gradient. Cholesterol concentrations of plasma and plasma fractions were determined by a gas–liquid chromatography method previously described by Davis, Showalter, and Kern (15). Cholesteryl ester concentrations were calculated by subtracting free cholesterol from total cholesterol and expressed as a percentage of total cholesterol. For statistical analysis, the paired sample *t*-test was used (16).

RESULTS

Effect of in vitro incubation on apolipoprotein A-IV distribution between lipoproteins and lipoprotein-free fractions

The percentage of total plasma cholesterol present as cholesteryl ester was determined at several time points

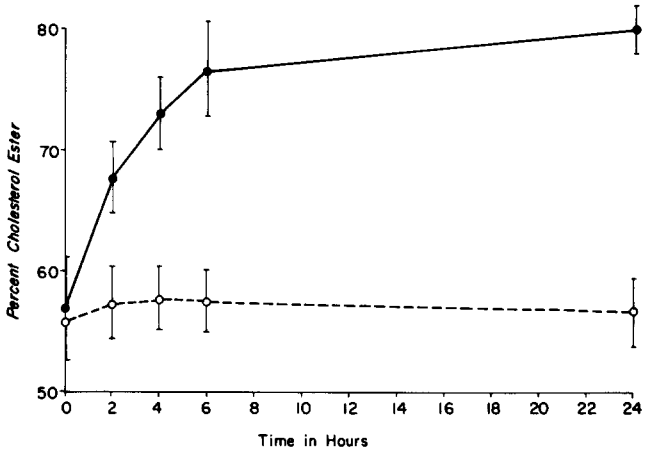

Fig. 1. Cholesterol esterification in rat plasma during incubation with and without DTNB. Plasma was incubated at 37°C in the presence (○ – – – ○) or absence (● – – – ●) of 1.5 mM DTNB. Samples were taken at several time points during a 24-hr incubation and immediately extracted in chloroform–methanol 2:1. The percentage of total plasma cholesterol present as esterified cholesterol was determined. Each value represents the mean ± SEM of four separate experiments.

in a 24-hr incubation. The data in **Fig. 1** show that just before the beginning of the incubation (0 hr), 58% of total plasma cholesterol was present as cholesteryl ester. In samples incubated without DTNB, this amount increased to 76% at 6 hr and was not significantly different at 24 hr. If 1.5 mM DTNB was included in the incubation mixture, the percentage of total plasma cholesterol present as cholesteryl ester did not change throughout the 24-hr incubation. Fig. 1 shows that at 4 hr the percentage of total plasma cholesterol present as cholesteryl ester averaged 73% in samples incubated without DTNB and 58% in samples incubated with 1.5 mM DTNB. Based on these data we chose 4 hr as our time of incubation in subsequent studies.

Experiments were done to determine how incubation of plasma with or without DTNB influences apoA-IV distribution. The data presented in **Table 1** and **Fig. 2** show that when plasma was incubated in the presence of 1.5 mM DTNB, practically all of the apoA-IV (>90%) was present in the lipoprotein-free fractions. When plasma was incubated without DTNB, however, practically all (>90%) of the apoA-IV was present in the lipoprotein fraction. More than 50% of the lipoprotein ApoA-IV was present in region B, the fraction which contains large HDL. No significant changes in the distribution of apoE or apoA-I were observed. Lipoproteins observed by agarose gel column chromatography were further purified by ultracentrifugation. When SDS-PAGE was run on these samples, the difference in their apoA-IV content was similar to the difference observed by immunochemical determinations (data not shown); the lipoproteins derived from plasma incubated without DTNB contained considerably more apoA-IV than the samples from plasma incubated with DTNB.

TABLE 1. Effect of a 4-hr, 37°C incubation of plasma in the presence and absence of DTNB on the distribution of apoA-IV, apoE, apoA-I, cholesterol, and cholesteryl ester

		Region			
		A VLDL and LDL	B Large HDL	C Small HDL	D Lipoprotein-Free
		% of total plasma[a]			
ApoA-IV	+DTNB	0	1 ± 1	8 ± 3	91 ± 4
	−DTNB	19 ± 8[b]	52 ± 7[b]	19 ± 2[b]	10 ± 7[b]
ApoE	+DTNB	32 ± 7	50 ± 5	18 ± 2	1 ± 1
	−DTNB	27 ± 6	59 ± 3	14 ± 3	0
ApoA-I	+DTNB	1 ± 0.4	55 ± 0.5	43 ± 2	1 ± 0.3
	−DTNB	3 ± 0.5	60 ± 3	37 ± 4	0
Total cholesterol	+DTNB	35 ± 3	38 ± 1	25 ± 2	1 ± 0.5
	−DTNB	27 ± 2[b]	50 ± 2[b]	22 ± 1[b]	1 ± 0.2
Percent cholesteryl ester	+DTNB	38 ± 1	70 ± 2	75 ± 4	0
	−DTNB	56 ± 7[b]	85 ± 1[b]	84 ± 1[b]	0
Total cholesterol		53 ± 2 mg/dl			
Percent cholesteryl ester					
+DTNB		58 ± 4			
−DTNB		74 ± 4[b]			

[a] Values shown for the pooled fractions express the parameter as a percentage of the total plasma concentration except for the percent cholesteryl ester values, which represent the percentage of that region's total cholesterol that is esterified. Values represent the mean ± SEM for three experiments.

[b] Significantly different from paired observations ($P < 0.05$).

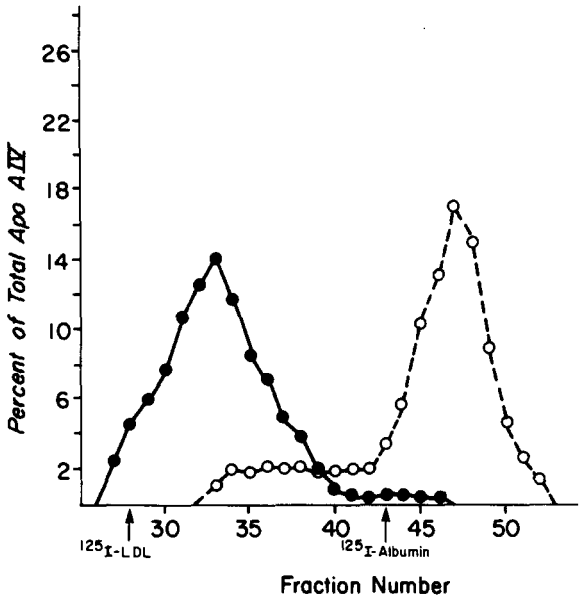

Fig. 2. ApoA-IV distribution in plasma incubated with or without DTNB. Paired plasma samples were incubated for 4 hr at 37°C either in the presence (O – – – O) or absence (● – – – ●) of 1.5 mM DTNB. Samples were then simultaneously fractionated by column chromatography using 10% agarose gel. Standards of ^{125}I-labeled LDL and ^{125}I-labeled albumin were included with each run to mark the void volume and lipoprotein-free fraction, respectively. Arrows indicate where the peak fraction for each standard was found. ApoA-IV concentrations were then determined by electroimmunoassay. The data shown depict two representative paired samples.

The distribution of total cholesterol as well as the percentage of cholesterol present as cholesteryl ester differed among the plasma fractions depending on whether the plasma was incubated with or without DTNB (Table 1). When plasma was incubated without DTNB, the total cholesterol decreased in regions A and C whereas the percentage of cholesterol present as cholesteryl ester increased, reflecting a decrease of free cholesterol in these fractions. In region B, however, total cholesterol as well as percentage of cholesterol present as cholesteryl ester increased. In addition to the largest increase in esterified cholesterol occurring in large HDL (region B), apoA-IV concentrations were highest in this region.

To better clarify this phenomenon (change in apoA-IV distribution), we determined the apolipoprotein distribution in plasma fractionated immediately before the incubation (0 hr) and compared the result with the apolipoprotein distribution of plasma fractionated after a 4-hr incubation in the presence of DTNB. The data in **Fig. 3** and **Table 2** show that immediately before the incubation, apoA-IV was distributed in roughly equal concentrations in the lipoprotein (HDL) and lipoprotein-free fractions. This distribution most closely approximates the distribution of apoA-IV normally present in vivo in circulating plasma. In contrast, when plasma was fractionated after it had been allowed to incubate for 4 hr in the presence of DTNB, 87% of the apoA-IV was found in the lipoprotein-free fractions. As in previous experiments, no significant changes in apoE or apoA-I distribution were observed.

Whole blood was incubated to more closely approximate in vivo conditions. Blood was separated and fractionated either immediately (O hr) or after a 4-hr 37°C incubation. **Fig. 4** shows the effect of incubating whole blood on the distribution of apoA-IV. This experiment provided information as to whether the presence of available free cholesterol and phospholipids in the red cells would affect apoA-IV distribution. We observed that most of the apoA-IV was present in the lipoprotein fraction after a 4-hr incubation compared with data from 0 hr, where the apoA-IV was about equally distributed between the lipoprotein and lipoprotein-free fractions. These data are practically identical to data obtained by incubation of whole plasma alone.

Effect of heat-inactivation and LCAT activity on apolipoprotein A-IV distribution

These observations suggest that the LCAT reaction may influence the apoA-IV distribution between the lipoprotein and lipoprotein-free fractions. Because DTNB

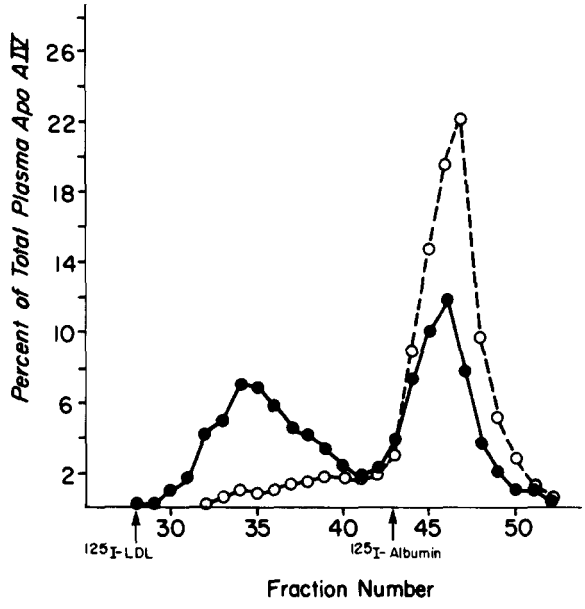

Fig. 3. ApoA-IV distribution in vivo and the effect of incubating plasma in the presence of DTNB. Paired plasma samples were fractionated by column chromatography (using 10% agarose gel) either immediately before an incubation (● – – – ●) or after a 4-hr, 37°C incubation in the presence of 1.5 mM DTNB (O – – – O). Standards of ^{125}I-labeled LDL and ^{125}I-labeled albumin were included with each run to mark the void volume and lipoprotein-free fractions, respectively. Arrows indicate where the peak fraction for each standard was found. ApoA-IV concentrations were then determined by electroimmunoassay. The data shown depict two representative paired samples.

TABLE 2. Effect of a 4-hr, 37°C incubation of plasma in the presence of DTNB on the distribution of apoA-IV, apoE, and apoA-I[a]

		Region			
		A VLDL and LDL	B Large HDL	C Small HDL	D Lipoprotein-Free
ApoA-IV	0 hr	0.3 ± 0.1	20 ± 3	25 ± 0.3	55 ± 3
	4 hr	0	4 ± 3[c]	9 ± 3[c]	87 ± 5[c]
ApoE	0 hr	18 ± 3	55 ± 2	25 ± 4	2 ± 2
	4 hr	17 ± 3	55 ± 5	27 ± 4	0.1 ± 0.1
ApoA-I	0 hr	0	23 ± 5	71 ± 4	5 ± 4
	4 hr	0.1 ± 0.1	29 ± 6	70 ± 6	0.6 ± 0.3

[a] DTNB was added to plasma and plasma was separated either immediately (0 hr) or after a 4-hr, 37°C incubation (4 hr).
[b] Values shown for the pooled fractions express the parameter as a percentage of the total plasma concentration. The values represent the mean ± SEM for three experiments.
[c] Significantly different from paired observations ($P < 0.05$).

could possibly have an effect on plasma lipoproteins other than the inhibition of LCAT activity, experiments were carried out in which LCAT activity was inhibited by a means other than DTNB. Incubation of plasma at 56°C for 30 min (heat inactivation) is known to eliminate LCAT activity (9–11); therefore, plasma was heat-inactivated and fractionated both immediately (0 hr) and after a 4-hr incubation. The results were practically identical (data not shown) to the results obtained when the same experimental protocol was used for DTNB-treated samples (Fig. 3, Table 2). Thus, the net movement of apoA-IV from the lipoprotein to lipoprotein-free fractions was demonstrated in both DTNB-treated samples and heat-inactivated samples. As with DTNB-treated samples, no changes in apoE or apoA-I distribution occurred.

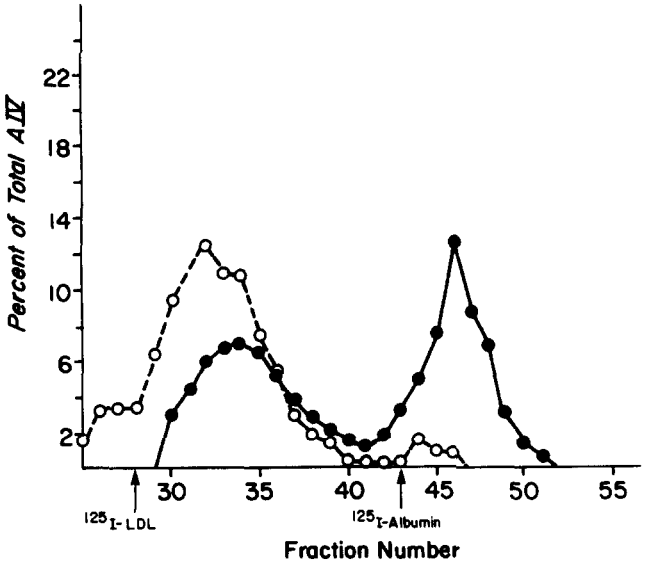

Fig. 4. ApoA-IV distribution after incubation of whole blood. Paired plasma samples were fractionated by column chromatography (using 10% agarose gel) either immediately after bleeding (0 hr) (● – – – ●) or after a 4-hr 37°C incubation (○ – – – ○). Standards of ^{125}I-labeled LDL and ^{125}I-labeled albumin were included with each run to mark the void volume and lipoprotein-free fractions, respectively. Arrows indicate where the peak fraction for each standard was found. ApoA-IV concentrations were then determined by electroimmunoassay. The data shown depict two representative paired samples.

The role of the LCAT reaction was assessed by adding a purified LCAT preparation to heat-inactivated plasma and determining the distribution of apoA-IV. The data in **Fig. 5** and **Table 3** show that when the heat-inactivated plasma alone was incubated, only 15% of apoA-IV was in the lipoprotein fractions. When purified LCAT was added to the heat-inactivated plasma, however, more than 90% of apoA-IV was found in the lipoprotein form. As in all other experiments reported here, no changes in apoE and apoA-I distributions were observed. The increase in the percentage of plasma cholesterol present as cholesteryl ester (57% to 67%) that resulted from the LCAT addition was of a similar magnitude to the difference seen between plasma incubated with (58%) vs. without (74%) DTNB (Fig. 1). Thus, the data provide direct evidence that LCAT activity can influence the net movement of apoA-IV from the lipoprotein-free fraction to the lipoprotein fraction.

DISCUSSION

The presence of apolipoproteins in the lipoprotein-free fraction of plasma and other biological fluids has long been suggested (2, 17, 18). Subsequent experiments showed that in the human (19, 20), rat (5, 6), and dog,[2]

[2] Roheim, P. S., et al. Unpublished observation.

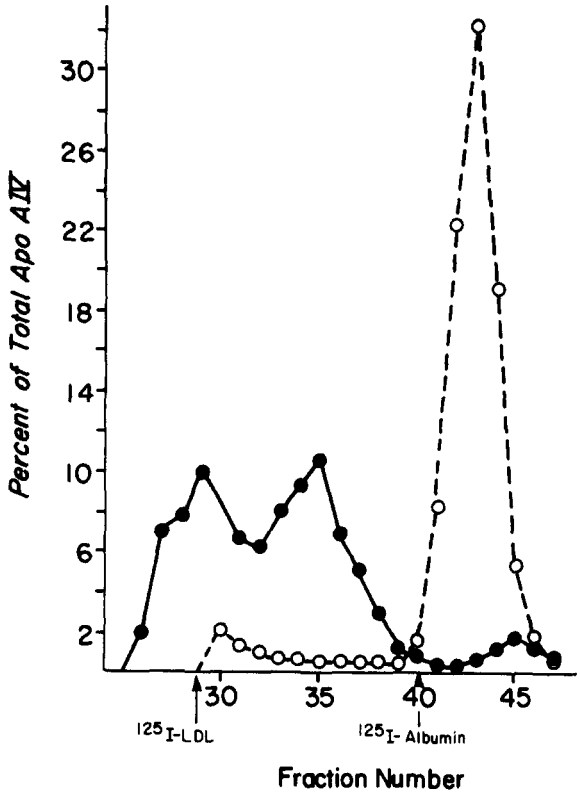

Fig. 5. ApoA-IV distribution in heat-inactivated plasma incubated either in the presence or absence of a purified LCAT preparation. Plasma was heat-inactivated by incubation at 56°C for 30 min and samples were incubated for 4 hr at 37°C either in the presence (● - - - ●) or absence (○ - - - ○) of a purified LCAT preparation. Samples were then simultaneously fractionated by column chromatography using 10% agarose gel. Standard ^{125}I-labeled LDL and ^{125}I-labeled albumin were included with each run to mark the void volume and lipoprotein-free fractions, respectively. Arrows indicate where the peak fraction for each standard was found. ApoA-IV concentrations were then determined by electroimmunoassay. The data shown depict two representative paired samples.

apoA-IV is unequivocally present in the lipoprotein-free fraction; however, the amount of apoA-IV present in the lipoprotein fraction of these animals is variable depending on the species as well as the diet (6) and age (5). These observations prompted us to study whether the distribution of apolipoproteins (between the lipoprotein- and lipoprotein-free fraction) can be influenced in vitro.

To eliminate ultracentrifugal artifacts, we used 10% agarose column chromatography for the separation of the lipoprotein fractions from lipoprotein-free fractions (5, 6, 21). Apolipoprotein concentrations were assessed by both immunochemistry and SDS-PAGE. The in vitro experiments were designed so that all observations were paired with their respective controls. In that way, the results of two different experimental conditions on a plasma sample could be directly compared. In this study, plasma was used instead of serum to avoid the introduction of factors that may be released during the clotting process. However, the same experiments were also done using serum (data not shown), and the results were essentially the same as those obtained with plasma.

These experiments suggest that a bidirectional reaction that influences the apoA-IV distribution takes place in plasma. One direction is inhibited by DTNB as well as by heat inactivation and results in the net movement of apoA-IV from the lipoprotein-free fraction to the lipoprotein fraction; that direction appears to be related to LCAT-activity. The other direction is not inhibited by DTNB or heat inactivation and results in the net movement of apoA-IV from the lipoprotein fraction to the lipoprotein-free fraction. Experiments in which the plasma was fractionated immediately (Figs. 3 and 4, Table 2) indicate that in the circulating rat plasma, apoA-IV is equally distributed between the lipoprotein and lipopro-

TABLE 3. Effect of a 4-hr, 37°C incubation of heat-inactivated plasma to which a purified LCAT preparation was added on the distribution of apoA-IV, apoE, and apoA-I[a]

		Region			
		A VLDL and LDL	B Large HDL	C Small HDL	D Lipoprotein-Free
		% of total plasma[b]			
ApoA-IV	−LCAT	0	10 ± 8	5 ± 3	85 ± 11
	+LCAT	24 ± 5[c]	45 ± 4[c]	24 ± 4[c]	8 ± 1[c]
ApoE	−LCAT	24 ± 7	70 ± 8	6 ± 2	0
	+LCAT	28 ± 5	64 ± 5	8 ± 1	0
ApoA-I	−LCAT	2 ± 1	37 ± 8	61 ± 9	0.2 ± 0.2
	+LCAT	1 ± 1	46 ± 2	53 ± 2	0.2 ± 0.2

[a] Plasma was heat-inactivated by incubation at 56°C for 30 min. A purified human LCAT preparation was then added to half the pool and dialysis buffer to the other half. The purity of the LCAT preparation was tested by SDS-PAGE and it showed only one major band.

[b] Values shown for the pooled fractions express the parameter as a percentage of the total plasma concentration. The values represent the mean ± SEM for three experiments.

[c] Significantly different from paired observations ($P < 0.05$).

tein-free fractions. Thus, in vivo, the reactions are balanced so that the movement of apoA-IV onto the lipoproteins is about equal to the movement of apoA-IV from the lipoproteins to the lipoprotein-free fraction. The observation that apoA-IV moved from the lipoprotein-free fraction to the lipoprotein fraction when LCAT was added to a heat-inactivated sample directly demonstrated that LCAT can influence the apoA-IV distribution between the lipoprotein and lipoprotein-free fraction. It should be noted that "purified" human LCAT preparation was used to demonstrate the relationship of apoA-IV movement to LCAT activity; however, the presence and possible role of minor contaminants cannot be ruled out.

In vitro incubation at 37°C also influences the distribution of apoA-IV within lipoprotein fractions. At 0 hr, i.e., in vivo, practically all the lipoprotein A-IV was present in the HDL fraction (Table 2); but after incubation without DTNB, a substantial amount was found on the larger particles (Table 1, region A). A similar increase of apoA-IV was observed on the larger particles when heat-inactivated plasma was incubated in the presence of purified LCAT (Table 3). Thus, it appears that movement of apoA-IV from the lipoprotein-free fraction to the lipoprotein fraction is not limited to HDL only. The same incubations did not influence the distribution of apoA-I and apoE; by agarose chromatography, >98% of these apoproteins were present in the lipoprotein form under all conditions studied. These data suggest that this phenomenon is specific for apoA-IV and, most likely, does not apply to apoA-I and apoE.

The basic substrates for the LCAT reaction, free cholesterol and lecithin, are known to reside on the lipoprotein surface (22). LCAT cleaves the two-position fatty acid chain of lecithin and transfers it to free cholesterol, thereby forming the two products, lysolecithin and cholesteryl ester (11). Lysolecithin does not remain with HDL, but binds to albumin (23) whereas cholesteryl ester moves into the lipoprotein core. Thus, the LCAT reaction results in the net loss of two lipoprotein surface materials, free cholesterol and lecithin, creating a need for surface material. Adding to this need for surface material is the fact that, as the lipoprotein core expands, the surface, in turn, expands to reflect the increase in core volume. We speculate that apoA-IV moves (with or without phospholipid) to the lipoprotein fraction to fulfill this need for surface material that is created as a result of the LCAT reaction. Therefore, we postulate that the presence of apoA-IV in the lipoprotein or lipoprotein-free fractions is likely to be influenced by the physical-chemical state of the lipoprotein particle.

At the present time, we can only speculate as to the mechanism for the movement of apoA-IV from the lipoprotein fraction to the lipoprotein-free fraction. It is possible that, in vivo, when the transfer of apoA-IV to the lipoprotein fraction occurs, apoA-IV may transfer phospholipid to the lipoprotein surface. The phospholipid that is transferred could be derived from other lipoproteins or cell surfaces. If by the transfer of this surface material the stability of the lipoprotein particle is reestablished, then a physical-chemical state may be created whereby the binding of apoA-IV to the lipoproteins is not favored and apoA-IV moves to the lipoprotein-free fraction. Alternately, the stability of the lipoprotein particle may be reestablished after phospholipid is transferred to the lipoprotein as a result of phospholipid transfer proteins present in the $d > 1.21$ g/ml fraction of plasma (lipoprotein-free fraction) (24). Another possibility is that the transfer of surface material to HDL in the form of phospholipid-rich surface remnants derived from the metabolism of triglyceride-rich lipoproteins (25, 26) may act to alter the HDL particle.

Whatever the exact mechanism, we postulate that apoA-IV transfers from the lipoprotein fraction to the lipoprotein-free fraction after the physical-chemical state that previously favored the binding of apoA-IV to the lipoproteins is altered, and a new physical-chemical state that does not favor the binding of apoA-IV to the lipoproteins is established. Thus, the distribution of apoA-IV between the lipoprotein fraction and the lipoprotein-free fraction may be the result of an equilibrium between those fractions that is determined by the physical-chemical state of the lipoproteins. We speculate that the physical-chemical state of the lipoproteins that determines apoA-IV binding may be greatly influenced by the phospholipid content of these lipoproteins. The question is whether this phenomenon is related to any of the known physiological reactions. This phenomenon is possibly related, in addition to LCAT activity, to other known physiological mechanisms such as phospholipid exchange (27, 28) or transfer (24, 28, 29).

The possible physiological role of this phenomenon is suggested from studies showing that, in old rats, the apoA-IV concentration in the lipoprotein-free fraction is much greater than in young adult animals (5). This observation is consistent with the suggestion that LCAT activity is decreased during aging (30, 31). Moreover, after feeding rats cholesterol, the apoA-IV concentration in the lipoprotein-free fraction is increased (6), which is consistent with the observation that LCAT activity is decreased in the cholesterol-fed rat plasma (32). These data suggest that, in vivo, cholesterol esterification or the LCAT reaction could play a role in influencing the balance between the amount of apoA-IV in lipoprotein or lipoprotein-free fractions.

In conclusion, these experiments show that in vivo in the normal control rat, about the same amount of apoA-IV is present in the lipoprotein fraction as in the lipoprotein-free fraction. The data also show that apoA-IV can transfer from the lipoprotein-free fraction to the lipoprotein fraction or vice versa in vitro, depending on

the incubation conditions. It is postulated that the relative concentration of apoA-IV in these fractions may be determined by the ability of apoA-IV to bind to the lipoproteins and that this ability is a function of the physical-chemical state of the lipoproteins. the lipoproteins.

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