ApoA-IV metabolism in the rat: role of lipoprotein lipase and apolipoprotein transfer

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Abstract Factors influencing the association of apoA-IV with high density lipoproteins (HDL) were investigated by employing a crossed immunoelectrophoresis assay to estimate the distribution of rat plasma apoA-IV between the lipoprotein-free and HDL fractions. Incubation of rat plasma at 37°C resulted in the complete transfer of lipoprotein-free apoA-IV to HDL within 45 min. When plasma obtained from fat-fed rats was incubated at 37°C in the presence of postheparin plasma as a source of lipolytic activity, there was a complete transfer of HDL apoA-IV to the lipoprotein-free fraction within 30 min. With extended incubation (120 min), lipoprotein-free apoA-IV began to transfer back to HDL. Similar patterns of apoA-IV redistribution were seen when plasma from fat-fed rats was incubated with postheparin heart perfusate or was perfused through a beating heart. Incubations conducted with plasma obtained from fasted rats showed similar but markedly attenuated apoA-IV responses. Similar observations were found in vivo following intravenous heparin administration. To determine whether the transfer of apolipoproteins from triglyceride-rich lipoproteins to HDL was partially responsible for the lipolysis-induced redistribution of apoA-IV, purified apoA-I, apoE, and C apolipoproteins were added to plasma from fasted rats. When added to plasma, all of the apolipoproteins tested displaced apoA-IV from HDL in a dose-dependent manner. Conversely, apolipoproteins were removed from HDL by adding Intralipid to plasma from fasted rats. With increasing concentrations of Intralipid, there was a progressive loss of HDL apoC-III and a progressive increase in HDL apoA-IV. Intravenous injection of a bolus of Intralipid to fasted rats resulted in a transient decrease of HDL apoC-III and concomitant increase in HDL apoA-IV. From these studies, we conclude that the binding of apoA-IV to HDL is favored under conditions that result in a relative deficit of HDL surface components, such as following cholesterol esterification by LCAT or transfer of apolipoproteins to nascent triglyceride-rich lipoproteins.—**Lefevre, M., M-Y. Chuang, and P. S. Roheim.** ApoA-IV metabolism in the rat: role of lipoprotein lipase and apolipoprotein transfer. *J. Lipid Res.* 1986. **27:** 1163–1173.

Supplementary key words crossed immunoelectrophoresis • free apolipoprotein • HDL • Intralipid • lecithin:cholesterol acyltransferase (LCAT) • lipolysis • surface remnants • triglyceride-rich lipoproteins

ApoA-IV was first described by Swaney et al. (1, 2) as a component of rat plasma HDL. It has since been described in the plasma of dogs (3) and humans (4). Unlike the majority of HDL apolipoproteins, a significant portion of apoA-IV is found unassociated with plasma lipoproteins. In humans, a majority of plasma apoA-IV (more than 80%) is found in the lipoprotein-free fraction (4–6), while in rat plasma, apoA-IV is approximately equally distributed between HDL and the lipoprotein-free fraction (7). The precise distribution of apoA-IV between the lipoprotein and lipoprotein-free fractions has been difficult to assess due to the ease with which apoA-IV is dissociated from lipoproteins during ultracentrifugation (4, 5).

In a previous study (8), we showed that the distribution of rat apoA-IV between the HDL and lipoprotein-free fraction could be influenced by the action of lecithin:cholesterol acyltransferase (LCAT). By employing agarose gel filtration chromatography, it was shown that incubation of rat plasma at 37°C for 4 hr led to the quantitative transfer of apoA-IV from a peak eluting after albumin (lipoprotein-free fraction) to a peak eluting in the size range of rat HDL. The inhibition of LCAT by 5′5-dithiobis (2-nitrobenzoic acid) (DTNB) or 56°C heat-inactivation of rat plasma resulted in the redistribution of apoA-IV from the HDL to the lipoprotein-free fraction within 4 hr. Direct evidence for LCAT involvement was demonstrated when the addition of a semipurified preparation of LCAT to heat-inactivated plasma resulted in the transfer of apoA-IV from the lipoprotein-free fraction to HDL.

The lipid and apolipoprotein environments on the lipoprotein surface most likely determine the degree to which apoA-IV associates with lipoprotein. The LCAT reaction results in the net loss of two HDL surface components (free cholesterol and phosphatidylcholine) and the formation of a new core component (cholesteryl ester). Therefore, it was hypothesized (8) that the binding of apoA-IV to HDL was favored under conditions where there was a relative deficit of HDL surface components.

Abbreviations: HDL, high density lipoproteins; apo, apolipoproteins; LCAT, lecithin:cholesterol acyltransferase; DTNB, 5′5-dithiobis(2-nitrobenzoic acid); LPL, lipoprotein lipase; TRL, triglyceride-rich lipoproteins; XIE, crossed immunoelectrophoresis.

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In this study, we test this hypothesis directly by examining the distribution of rat plasma apoA-IV following manipulation of HDL composition in vitro and in vivo. We demonstrated that *1*) only a small degree of plasma cholesterol esterification is necessary for lipoprotein-free apoA-IV to completely associate with HDL; *2*) lipolysis of triglyceride-rich lipoproteins (TRL) by lipoprotein lipase (LPL) causes apoA-IV to dissociate from HDL; *3*) the dissociation of apoA-IV from HDL following lipolysis is due in part to the transfer of apolipoproteins to HDL; and *4*) transfer of apolipoproteins from HDL to model TRL particles (Intralipid) causes lipoprotein-free apoA-IV to associate with HDL.

MATERIALS AND METHODS

Plasma collection

Male Sprague-Dawley rats (275–300 g) were maintained on standard rat chow. Eighteen hours prior to the initiation of the experiments, food was removed from the cages. On the day of the experiment, animals were gavaged with 3 ml of a 1:1:1 mixture of olive oil–heavy cream–milk (fat-fed) or saline (fasted). Animals were left in cages with free access to drinking water. Three hours after gavage, rats were lightly anesthetized with ether and blood was collected from the abdominal aorta into syringes containing EDTA (final concentration, 1 mg/ml). The blood was kept on ice, and the plasma was obtained by centrifugation at 1200 *g* at 4°C. Care was taken at all times to keep the blood or plasma below 4°C to prevent any LCAT-dependent redistribution of apoA-IV prior to the beginning of the incubations.

In vitro experiments

LCAT studies. Pooled plasma samples were divided in half. To one sample, DTNB was added to a final concentration of 1.5 mM to inhibit LCAT. Each pool was then further subdivided into five equal aliquots corresponding to different incubation times. Each aliquot was kept on ice until incubated at 37°C for times ranging between 5 and 120 min. Initiations of the incubations were staggered so that all samples could be analyzed simultaneously at the end of the experiment. One sample (0 min) from each group was kept on ice throughout the course of the experiment. The distribution of plasma apoA-IV did not change when kept at 0°C for as long as 4 hr.

Lipase studies. Experiments similar in design to the LCAT studies were conducted with lypolytic activity obtained either from postheparin plasma or postheparin heart perfusate. Postheparin plasma was obtained 10 min after injection of 100 U/kg heparin (Upjohn, Kalamazoo, MI) into the tail vein of fasted rats and had a specific activity of 58.6 U/ml (66% NaCl-sensitive lipase). Postheparin heart perfusate was obtained following a single pass of 3 ml of oxygenated Kreb's Ringer bicarbonate buffer saline (pH 7.40) containing 7 U/ml of heparin through a beating rat heart. Postheparin rat heart perfusate had a specific activity of 24.5 U/ml (96% NaCl-sensitive lipase). Collections of postheparin plasma or postheparin heart perfusate were pooled, aliquoted, and stored at −70°C.

Pooled plasma samples from fasted and fat-fed rats were aliquoted and incubated as described for the LCAT studies. Prior to initiation of the incubations, 0.2 volume of either postheparin plasma or postheparin heart perfusate was added to one set of tubes, while the other set received an equal amount of phosphate-buffered saline.

Heart perfusion. Heart perfusions were carried out essentially as described by Pedersen and Schotz (9). The perfusate consisted of oxygenated (95% O_2, 5% CO_2) serum obtained from fat-fed rats. The heart rates were greater than 150 beats/min during the 30-min perfusion period. Controls consisted of serum perfused simultaneously through a parallel apparatus without the heart in place.

In vitro addition of apolipoproteins and Intralipid. Purified rat apolipoproteins (apoA-I, apoE, or C apolipoproteins) or Intralipid were prepared in phosphate-buffered saline and added to fasted rat plasma. An equal amount of phosphate-buffered saline alone was added to control incubations. Plasma was kept at 4°C and was analyzed immediately (less than 5 min) after the additions.

In vivo experiments

Heparin administration. To assess the effect of lypolytic activity on apoA-IV distribution in vivo, fasted and fat-fed rats were injected with 100 U/kg heparin to enhance plasma lipolytic activity. Following injection, serial blood samples (200–300 μl) were obtained from the tail vein for times ranging between 5 and 120 min.

Intralipid administration. Two hundred microliters of 10% Intralipid (Cutter Labs, Berkeley, CA) was administered intravenously through tail veins of fasted, unanesthetized rats; controls received saline. Serial blood samples were obtained from the tail vein 2 to 60 min after injection.

Analytical methods

ApoA-IV crossed immunoelectrophoresis (XIE). To assess the distribution of apoA-IV between the HDL and lipoprotein-free fractions, we utilized the observation made by Beisiegel and Utermann (4) that human lipoprotein-free apoA-IV migrates with α_2 mobility in agarose electrophoresis. Rat plasma (15 μl) was electrophoresed in 0.5% agarose essentially as described by Noble (10). Following electrophoresis, the agarose strip was embedded into a

precast agarose plate (1.2% agarose and 2% dextran, mol wt 81,600) containing apoA-IV antiserum. The agarose plate was electrophoresed at 2 V/cm for 18 hr, perpendicular to the initial direction of electrophoresis.

Crossed immunoelectrophoresis of rat plasma demonstrated at least two distinct populations of apoA-IV particles with a single line of identity (**Fig. 1**). The identity of these particles was confirmed by performing an additional crossed electrophoresis in which the second dimension consisted of a Pharmacia PAA 4/30 gradient gel to separate the lipoproteins by size (11). The proteins were electrophoretically transferred from the gels to nitrocellulose (12) and the apoA-IV particles were immunolocalized with ^{125}I-labeled IgG prepared from apoA-IV antiserum. With this procedure, we were able to demonstrate that the peak with the slowest electrophoretic mobility corresponded to a particle migrating below albumin (lipoprotein-free apoA-IV) while the faster migrating peak corresponded primarily to HDL-associated apoA-IV.

The areas under each of the curves were digitized with the aid of a HiPlot digitizing pad (Houston Instruments) interfaced to an Apple IIe computer. Both the total area under the curves and the percentage of apoA-IV appearing in the lipoprotein region were determined. During the course of the incubations, the total area under the curves did not change significantly.

Apolipoprotein preparation and antisera preparation. Rat apolipoproteins were isolated from d < 1.21 g/ml lipoproteins by a combination of gel filtration chromatography and heparin-affinity chromatography (13).

Antisera to rat apolipoproteins A-I, A-IV, C-III, and E were prepared in goats as previously described (14). Their monospecificities were established by protein blot analysis (15) using both rat plasma and d < 1.21 g/ml lipoproteins.

Other analytical methods. Apolipoprotein concentrations were determined by electroimmunoassay (16), as modified by Dory and Roheim (17). Cholesterol and triglycerides were assayed enzymatically (18, 19). Lipase activity was determined by the method of Nilsson-Ehle and Schotz (20). (One unit of lipase activity was defined as the release of 1 mmol of free fatty acid per hr at 37°C.) Lipoprotein lipase and hepatic lipase activities were determined by their sensitivity to 1 M NaCl (21).

Data presentation. All experiments were repeated a minimum of three times. Data presented in the figures are taken from typical experiments.

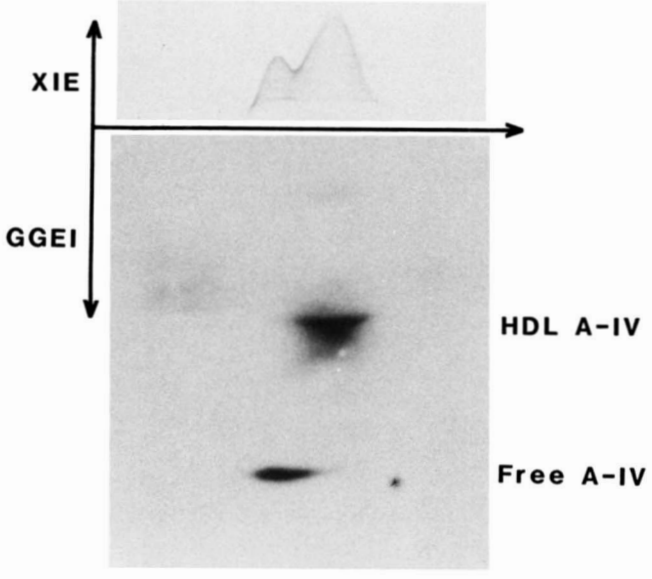


Fig. 1. Identification of plasma apoA-IV particles separated by crossed immunoelectrophoresis (4). Plasma lipoproteins were separated by conventional agarose electrophoresis as indicated by the horizontal arrow. One agarose strip was embedded into an agarose plate containing anti-rat apoA-IV antiserum and electrophoresed in a direction perpendicular to the first electrophoresis. A second, identical strip was placed on top of a Pharmacia PAA 4/30 gradient gel (GGE) and electrophoresed, blotted, and immunolocalized as described in the Methods section to determine the size of the apoA-IV particles. Crossed immunoelectrophoresis demonstrates the existence of two apoA-IV particles. The slower migrating peak (left peak) corresponds to apoA-IV particles with diameters smaller than albumin. The faster migrating peak (right peak) was comprised of particles having sizes similar to that of rat HDL.

RESULTS

We have previously shown that incubation of rat plasma at 37°C results in an LCAT-dependent transfer of apoA-IV from the lipoprotein-free fraction to HDL (8). We further examined the time course of this phenomenon with crossed immunoelectrophoresis. Incubation of rat plasma (from fasted and fat-fed animals) at 37°C resulted in a progressive decrease in the area associated with the slower-migrating apoA-IV peak (lipoprotein-free apoA-IV) and an increase in the area associated with the faster-migrating peak (HDL apoA-IV) (**Fig. 2**). By 60 min, no lipoprotein-free apoA-IV could be detected. There was no apparent difference in the rates of the LCAT-dependent redistribution of apoA-IV between the fasted and fat-fed groups.

Data from six separate experiments were averaged and are presented graphically in **Fig. 3.** In the absence of DTNB, there was a quantitative transfer of lipoprotein-free apoA-IV to HDL, which was essentially complete by 45 min. In contrast, when DTNB was present to inhibit LCAT, apoA-IV was transferred in the opposite direction, i.e., from HDL to the lipoprotein-free fraction. This reaction occurred much more slowly than the LCAT-dependent reaction, requiring almost 4 hr to go to completion.

Since a decrease of HDL surface components presumably led to a redistribution of apoA-IV from the lipopro-

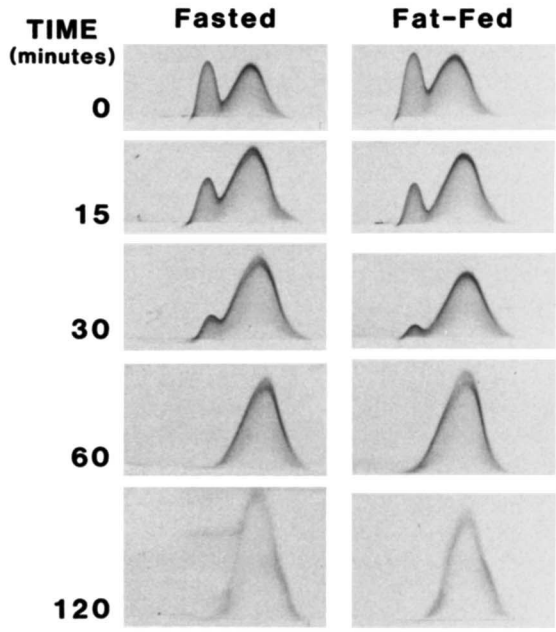


Fig. 2. Redistribution of apoA-IV during in vitro incubation at 37°C as determined by crossed immunoelectrophoresis. Origins of agarose electrophoresis are to the left. The faster migrating peaks are HDL apoA-IV, and the slower migrating peaks are lipoprotein-free apoA-IV.

tein-free fraction to HDL, we postulated that surface components transferred to HDL might result in dissociation of apoA-IV from HDL. To test this hypothesis, we made use of the excess surface components generated through hydrolysis of triglyceride-rich lipoproteins by adding postheparin plasma (as a source of lipolytic activity) to plasma obtained from fasted and fat-fed rats.

When postheparin plasma was incubated with plasma obtained from fat-fed rats, HDL apoA-IV was almost completely transferred to the lipoprotein-free fraction by 30 min (**Fig. 4a and b**). However, at 120 min, apoA-IV began to transfer back to HDL. Incubations with plasma from fasted rats showed a small initial transfer of HDL apoA-IV to the lipoprotein-free fraction at 10 min, followed by a transfer back to HDL by 30 min. After 120 min of incubation, all of the apoA-IV in the plasma of fasted rats was in HDL. When incubations with postheparin plasma were conducted in the presence of DTNB to inhibit LCAT, the transfer of lipoprotein-free apoA-IV to HDL seen at the later time points was inhibited (data not shown).

ApoA-IV distribution during in vivo lipolysis was studied following intravenous injection of heparin. Intravenous heparin injection produced a rapid decline in plasma triglycerides and was accompanied by the transfer of apoA-IV from HDL to the lipoprotein-free fraction (**Fig. 5**). The transfer of apoA-IV was far more pronounced in fat-fed rats than in fasted rats, similar to the observation made in the in vitro experiments. A transfer of apoA-IV from the lipoprotein-free fraction back to HDL occurred for both fasted and fat-fed rats and returned to the approximate preinjected distribution at 45 min when plasma TG concentrations were still depressed.

Postheparin plasma contains both lipoprotein lipase (LPL) and hepatic lipase activities. To test whether LPL alone was sufficient to cause the redistribution of apoA-IV, we added postheparin heart perfusate, which contains only LPL activity, to plasma from fasted or fat-fed rats (**Fig. 6**). Without the addition of postheparin heart perfusate, lipoprotein-free apoA-IV was completely transferred to the HDL in both fasted and fat-fed groups after 45 min of incubation. When postheparin heart perfusate was included in the incubation, the changes in apoA-IV distribution were similar to those seen with postheparin plasma. In plasma obtained from fat-fed rats, apoA-IV completely redistributed from HDL to the lipoprotein-free fraction, while in plasma obtained from fasted rats, apoA-IV showed a small initial shift to the lipoprotein-free fraction followed by a shift back to the HDL.

Circulating lipoproteins are exposed in vivo to lipoprotein lipase action as they come in contact with membrane-bound lipoprotein lipase present on the surface of the endothelial cell. Therefore, we perfused serum obtained from fat-fed rats through an isolated heart preparation to determine whether membrane-bound LPL behaved similarly to isolated LPL in influencing apoA-IV redistribution. **Fig. 7** shows that during the 30 min of

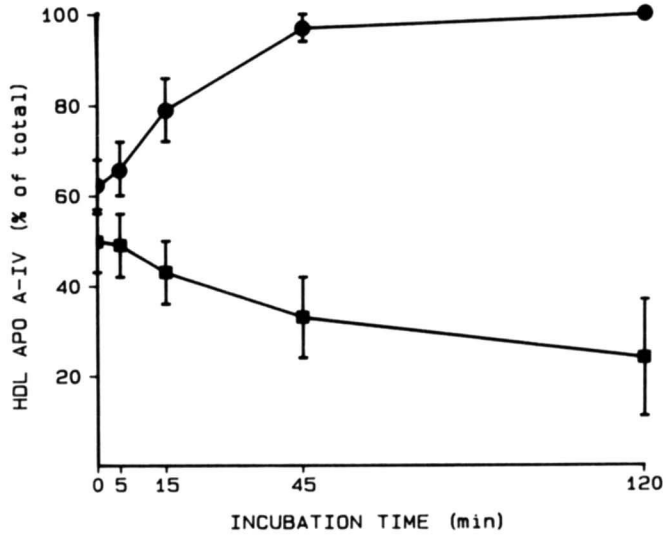
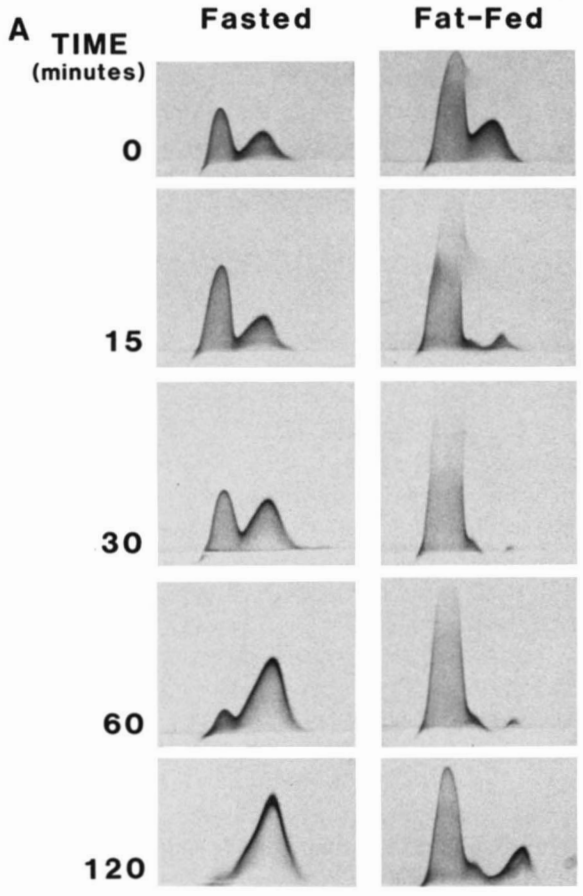


Fig. 3. Change in plasma apoA-IV distribution during in vitro incubation in the presence (■) or absence (●) of 1.5 mM DTNB. Plasma apoA-IV distribution was determined by crossed immunoelectrophoresis. The areas under each of the curves were determined, and the percent of total plasma apoA-IV associated with HDL was calculated. The total area under the curves was not significantly influenced by the incubation. Data are mean ± SD of six rats.



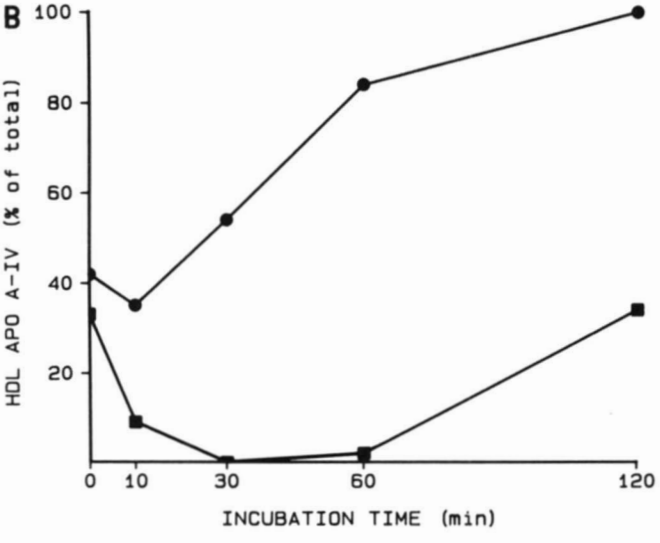


Fig. 4. A. Effect of adding postheparin plasma to plasma from fasted and fat-fed rats on the distribution of apoA-IV. Postheparin plasma (0.2 volumes) was added to induce lipolysis of triglyceride-rich lipoproteins. Origins of agarose electrophoresis are to the left. The faster migrating peaks are HDL apoA-IV, and the slower migrating peaks are lipoprotein-free apoA-IV. B. Graphic presentation of data presented in panel A. Data are presented as a percent of total plasma apoA-IV associated with HDL; (●) fasted; (■) fat-fed.

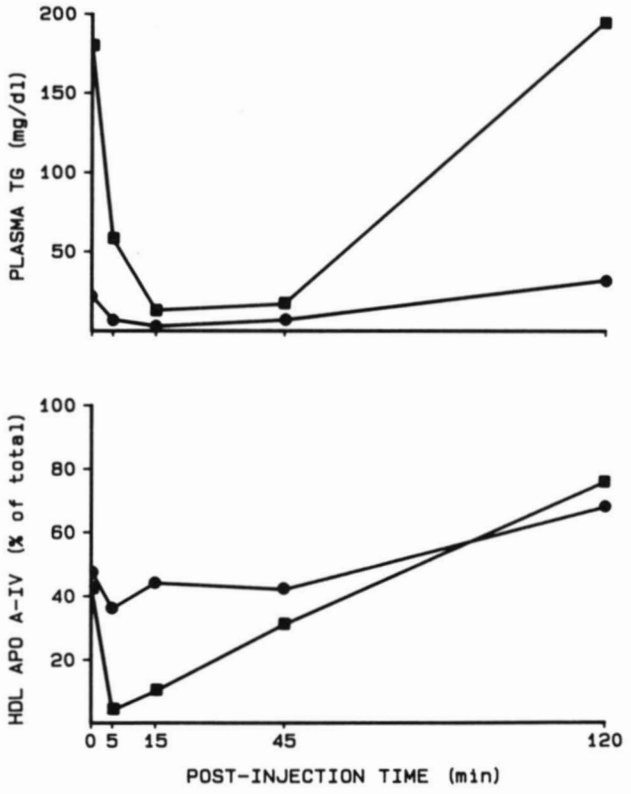


Fig. 5. In vivo heparin administration (100 U/kg body weight) to fasted (●) and fat-fed (■) rats. Upper panel, triglyceride concentrations; lower panel, percent of total plasma apoA-IV associated with HDL.

heart perfusion, there was a continuous, gradual transfer of apoA-IV from HDL to the lipoprotein-free fraction. In contrast, the control perfusion without the heart resulted in the redistribution of apoA-IV in the opposite direction, reflecting the LCAT-dependent redistribution.

We wanted to determine whether the transfer of apoA-IV from HDL to the lipoprotein-free fraction was due to the transfer of apolipoproteins from triglyceride-rich lipoproteins to HDL during lipolysis. In initial studies, we examined the changes in apoA-IV distribution after the addition of purified apoA-I, apoE, or C apolipoproteins to rat plasma. Determinations of apoA-IV distribution were performed immediately after the addition of individual apolipoproteins to plasma kept at 4°C. Analysis of the incubated plasma by nondenaturing gradient gel electrophoresis followed by electrophoretic transfer and immunolocalization indicated that the added apolipoproteins were incorporated into pre-existing lipoproteins with no new lipoprotein particle formation (data not shown). ApoA-IV was displaced from HDL in a dose-dependent manner by all apolipoproteins tested (**Fig. 8**). While the addition of apoA-I and the C apolipoproteins displaced HDL apoA-IV in a linear manner, the same was not true for apoE. The displacement of HDL apoA-IV at the lowest amount of apoE was proportionately less than that ob-

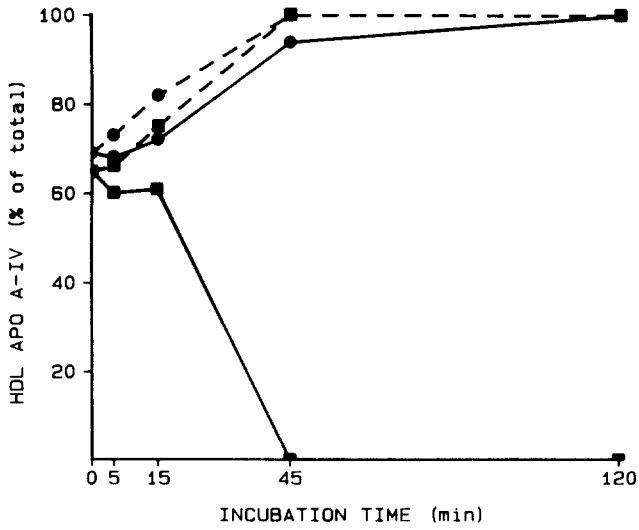


Fig. 6. Effect of lipoprotein lipase on plasma apoA-IV distribution. Plasma from fasted (●) or fat-fed (■) rats was incubated at 37°C with (——) or without (– – –) 0.2 volume of postheparin heart perfusate as a source of lipoprotein lipase activity. Data are presented as a percent of total plasma apoA-IV associated with HDL.

served at the higher concentration of apoE, where HDL apoA-IV was displaced in a linear manner. In comparisons of mass, C apolipoproteins were more effective in displacing HDL apoA-IV than either apoA-I or apoE.

Since the addition of apolipoproteins to HDL displaced HDL-associated apoA-IV, then removal of apolipoproteins from HDL may favor the reassociation of lipoprotein-free apoA-IV with HDL. We tested this possibility by adding Intralipid to plasma from fasted rats. Partial transfer of HDL apolipoproteins to Intralipid particles was demonstrated by the reduction in HDL apoC-III as determined by crossed immunoelectrophoresis (**Fig. 9**). We found that by achieving a final concentration of 0.5% Intralipid in the plasma, we had decreased HDL apoC-III by 44%. This was associated with the complete transfer of lipoprotein-free apoA-IV to HDL.

Finally, to confirm in vitro experiments as well as to simulate the entrance of triglyceride-rich lipoproteins into the circulation, we injected Intralipid intravenously into fasted rats. We observed no change in apoA-IV or apoC-III distributions in saline-injected controls (**Fig. 10**). In contrast, a rapid decrease in HDL apoC-III and a reciprocal increase in apoA-IV in HDL were observed in experimental groups. At 60 min, we observed that apoC-III was transferred back to HDL, and a gradual decrease in HDL apoA-IV was evident.

DISCUSSION

The distribution of apoA-IV between HDL and the lipoprotein-free fraction can be influenced by many factors. Factors such as nutritional status (22) (fasted vs. fed), diet (7), and age (23) have all been shown to influence apoA-IV distribution. Furthermore, sample handling can also influence plasma apoA-IV distribution. When samples are allowed to stand at room temperature for 15 to 30 min (e.g., for serum collection), apoA-IV redistributes quickly during this time, resulting in an increase of HDL apoA-IV. Therefore, in these studies, steps were taken to collect plasma samples on ice and to use plasma as soon as possible.

Methods used to determine apoA-IV distribution can also result in a redistribution of apoA-IV. It has been observed that ultracentrifugation causes apoA-IV to be removed from lipoproteins and results in overestimation of lipoprotein-free apoA-IV (4, 5). Molecular sieve column chromatography provides a better estimation of apoA-IV distribution. However, with column chromatography, the number of samples that can be processed at one time is limited, making kinetic studies of apoA-IV redistribution very difficult.

Crossed immunoelectrophoresis (XIE) was found to be a fast and convenient method for studying apoA-IV redistribution. XIE does not require large sample sizes, thus allowing frequent sampling of the same animal. Furthermore, we could routinely process 20 samples in a 24-hr period. Therefore, it can be used to study apoA-IV redistribution over time in a single animal under a variety of physiological conditions.

During in vitro incubation of rat plasma, apoA-IV is transferred from the lipoprotein-free fraction to HDL as a consequence of the LCAT reaction (8). This process occurs rapidly and results in complete association of apoA-IV with HDL after approximately 45–60 min of incu-

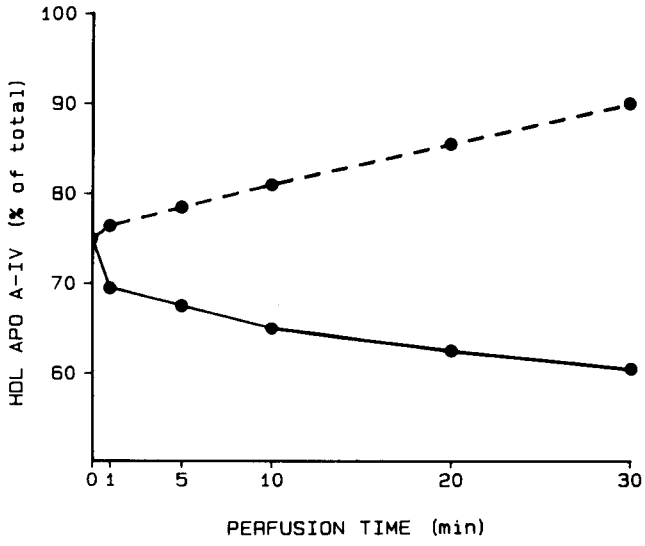


Fig. 7. Rat apoA-IV redistribution in plasma perfusate in the presence (——) or absence (– – –) of a rat heart. Heart perfusions were carried out at 37°C with oxygenated fat-fed rat serum for 30 min. Data are presented as percent of total plasma apoA-IV associated with HDL.

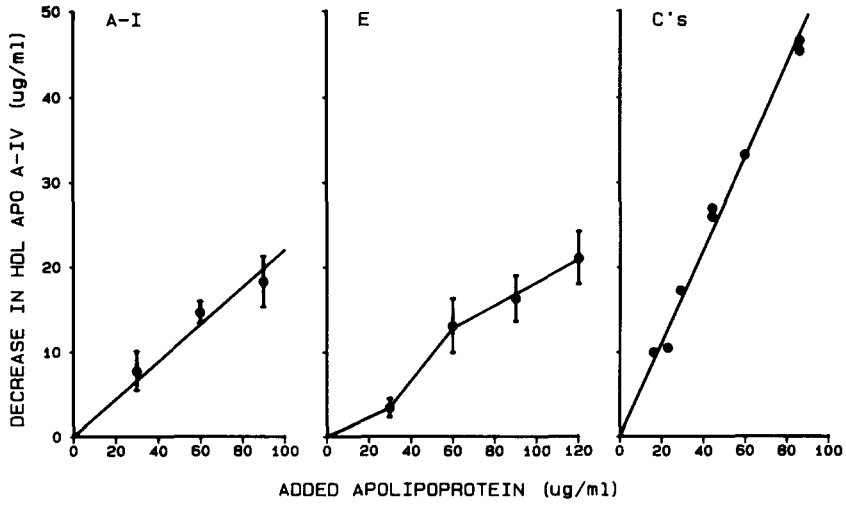


Fig. 8. Displacement of HDL apoA-IV by apoA-I, apoE, and C apolipoproteins. Isolated apolipoproteins were added to plasma from fasted rats and the distribution of apoA-IV was immediately determined. The displacement of apoA-IV from HDL was calculated by multiplying the plasma concentration of apoA-IV by the percent of total apoA-IV associated with HDL, followed by subtracting the value obtained in the presence of added apolipoproteins from the value obtained in control plasma.

bation. During this time, approximately 30 nm/ml of free cholesterol (approximately 25% of the free cholesterol associated with apoA-I HDL) is esterified (unpublished observation). Preliminary calculations based on the model provided by Shen, Scanu, and Kezdy (24) suggest that this degree of cholesterol esterification, when uniformly distributed among all HDL particles, would not increase the available HDL surface area sufficiently to fully accommodate an apolipoprotein the size of apoA-IV. This apparent discrepancy can be explained by *1*) the association of apoA-IV with a subpopulation of HDL that has undergone a greater degree of cholesterol esterification than the bulk of HDL; or *2*) the transfer of HDL apolipoproteins to other density classes during cholesterol esterification, thus providing the additional available surface area required for apoA-IV binding. Investigations into these two possibilities are currently in progress.

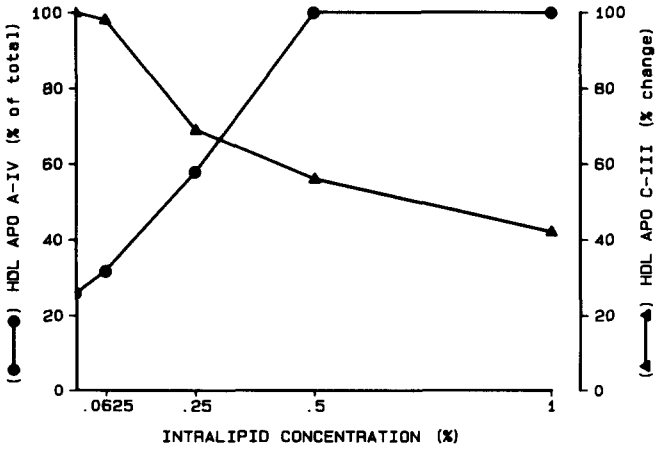


Fig. 9. Reciprocal changes in HDL apoA-IV and apoC-III following in vitro addition of Intralipid to plasma. Varying amounts of Intralipid were added to fasted plasma to give the final Intralipid concentration indicated in the figure. Immediately following the addition of the Intralipid, the distribution of both apoA-IV and apoC-III was determined by crossed immunoelectrophoresis. Data for apoA-IV are presented as percent of total plasma apoA-IV associated with HDL. Data for apoC-III are presented as percent of starting (no Intralipid) HDL apoC-III concentration.

LCAT is continuously active in the circulation and thus favors apoA-IV association with HDL. The fact that we observed large percentages of apoA-IV in the lipoprotein-free fraction in fresh plasma samples suggested that other mechanisms must compensate for the LCAT-dependent apoA-IV redistribution. DeLamatre et al. (8) postulated that since the LCAT reaction reduces surface components and increases core components in its substrate lipoproteins, the surface-to-core ratio may influence the affinity of apoA-IV for lipoproteins. In the case of the LCAT reaction, a reduction of the surface-to-core ratio presumably increases the affinity of apoA-IV with the HDL. In the present study, we also looked at the converse conditions, i.e., do reactions involved in increasing surface-to-core ratios of lipoproteins cause apoA-IV to dissociate from HDL? If so, these reactions may be the possible compensating mechanisms that maintain apoA-IV distribution in vivo. Since lipolysis of triglyceride-rich lipoproteins results in the transfer of surface components from triglyceride-rich lipoproteins to HDL (25–27), we investigated the effect of this reaction on apoA-IV distribution.

In initial studies, postheparin plasma was used as a source of lipolytic activity. Incubation of postheparin plasma with rat plasma showed a clear effect of lipolytic

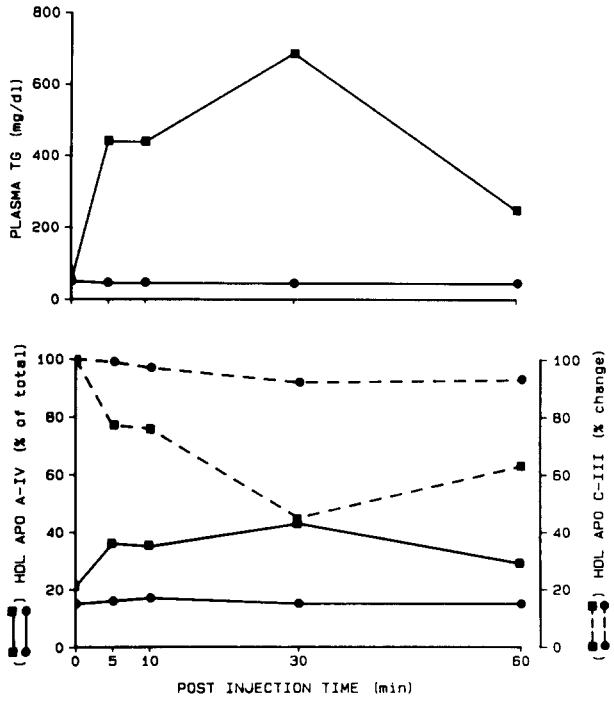


Fig. 10. Effect of Intralipid administration on the distribution of apoA-IV and apoC-III in vivo. Two hundred μl of 10% Intralipid (■) or saline (●) were injected into the tail veins of fasted rats. At the times indicated, small aliquots of blood were obtained from the tail veins and the distribution of apoA-IV (——) and apoC-III (– – –) was determined. Data for apoA-IV are presented as percent of total apoA-IV associated with HDL; data for apoC-III are presented as percent of starting (0 time) HDL apoC-III concentration.

activity on apoA-IV distribution. With plasma obtained from fat-fed animals, HDL apoA-IV was transferred quickly to the lipoprotein-free fraction and remained in this fraction for about 60 min. At later time points, apoA-IV in the lipoprotein-free fraction began to transfer back to HDL. A similar, but much less pronounced, redistribution of apoA-IV occurred when postheparin plasma was incubated with the plasma of fasted rats.

In vitro observations were confirmed in vivo using intravenous heparin injections. Plasma triglyceride levels of fat-fed rats indicated that large amounts of triglyceride-rich lipoproteins were present. Heparin injection decreased plasma triglyceride-rich lipoproteins rapidly and resulted in a simultaneous transfer of HDL apoA-IV to the lipoprotein-free fraction. As with the in vitro studies, the effects of heparin injection in fasted animals were much less pronounced.

Postheparin plasma contains at least two triglyceride hydrolases, lipoprotein lipase and hepatic lipase. Studies with postheparin heart perfusate demonstrated that the presence of lipoprotein lipase was sufficient to bring about a redistribution of apoA-IV. Preliminary studies with postheparin liver perfusate as a source of hepatic lipase were uninterpretable, since hepatic lipase appeared to be inhibited in vitro by lipoproteins (21, 28) and/or apolipoproteins (28, 29) in the plasma.

Since in vivo lipoprotein lipase is membrane-associated at the luminal side of capillary endothelium (30), we wanted to determine whether membrane-bound lipoprotein lipase also had the same influence as released lipoprotein lipase. Rat heart perfusions with oxygenated rat serum indicated that membrane-bound lipoprotein lipase had an influence similar to that of isolated lipoprotein lipase. Thus, with these in vitro and in vivo experiments, we have demonstrated that lipolysis of TRL produces changes in the distribution of apoA-IV between the HDL and lipoprotein-free forms, and that lipoprotein lipase participates in the maintenance of lipoprotein-free apoA-IV in the plasma.

In the above experiments, studies done with plasma from fasted rats showed less extensive transfer of HDL apoA-IV to the lipoprotein-free fraction than those done with plasma from fat-fed rats. This was not due to differences in the rate of the competing LCAT-dependent transfer of lipoprotein-free apoA-IV to HDL, since studies of this reaction with plasma from fasted and fat-fed rats showed no differences in the rate of transfer. This suggests that the redistribution of apoA-IV seen in the presence of LPL was dependent upon the presence of substrate for LPL.

The rate of dissociation of apoA-IV from HDL as a result of TRL lipolysis was much greater than that observed during simple LCAT inhibition (15–30 min vs 4 hr to complete the transfer of all HDL apoA-IV to the lipoprotein-free fraction). Thus, inhibition of LCAT by the products of lipolysis (31) could not account for the accelerated transfer of HDL apoA-IV to the lipoprotein-free fraction. Therefore, it seems reasonable to speculate that surface remnants transferred to HDL as a result of lipolysis promoted the dissociation of apoA-IV from HDL.

During lipolysis of TRL, both lipids and apolipoproteins are transferred from TRL to HDL (9–11). Weinberg and Spector (32) have shown that apoA-IV bound to triglyceride-rich particles (Intralipid) could be displaced by C apolipoproteins. More recently, Rifici, Eder, and Swaney (13) demonstrated that apoA-IV associated with dimyristolphosphatidylcholine complexes could be displaced by apoA-I. We, therefore, wanted to determine whether the transfer of apolipoproteins alone from triglyceride-rich lipoproteins to HDL could displace HDL apoA-IV under physiological conditions.

When apoA-I, apoE, or apoCs were added to rat plasma, the distribution of apoA-IV shifted toward the lipoprotein-free form. The displacement of apoA-IV by either apoA-I or apoCs was a linear function of the mass of the added apolipoproteins. This suggests that these apolipoproteins either bind exclusively to apoA-IV-containing HDL or bind to HDL with or without apoA-IV

with similar affinities. The displacement of apoA-IV from HDL by apoE, however, was not a simple linear function of mass. At low concentrations of added apoE, proportionately less apoA-IV was displaced than at the higher concentrations. This could be explained by the preferential binding of apoE to lipoproteins not containing apoA-IV.

In a final series of experiments, we wanted to determine whether the removal of apolipoproteins from HDL might promote apoA-IV binding to HDL. Previous studies by Robinson and Quarfordt (33) have shown that incubation of unfractionated Intralipid with rat plasma results in the transfer of HDL-associated apolipoproteins to the Intralipid particles. In our studies, we used changes in HDL apoC-III as our marker for HDL apolipoprotein transfer to Intralipid particles. When increasing amounts of Intralipid were incubated with fasted rat plasma, increasing amounts of HDL apoC-III were transferred to the Intralipid particles (**Fig. 9**). This was associated with the redistribution of apoA-IV from the lipoprotein-free fraction to HDL. In general, in vivo injection of Intralipid confirmed the observations made in vitro. When the plasma triglycerides were at their highest point, apoC-III in HDL was at its lowest level, and the distribution of apoA-IV favored the HDL fraction. At later time points, lipolysis became more pronounced, reflected by the declining plasma triglyceride levels, apoC-III gradually returned to HDL, and apoA-IV redistributed toward the lipoprotein-free fraction.

It should be pointed out that in vitro incubation of human HDL_2 with Intralipid particles of $S_f > 400$ also results in the transfer of free cholesterol from HDL_2 to the Intralipid particles and the reciprocal transfer of phospholipid from Intralipid particles to HDL_2 (32, 34). If in our system, using unfractionated Intralipid and rat plasma, the decrease in surface components resulting from the loss of free cholesterol is not compensated for by the increase in phospholipids, then an alternative mechanism, independent of HDL apolipoprotein loss (i.e., loss of free cholesterol), may account for the transfer of lipoprotein-free apoA-IV to HDL during incubation with Intralipid.

The relationship between lipolysis, apolipoprotein transfer, and apoA-IV redistribution is presented in schematic form in **Fig. 11.** Circulating HDL can be made deficient in surface components by two independent mechanisms. One mechanism, involving the loss of free cholesterol and phospholipids and the expansion of the HDL core, is catalyzed by LCAT. A second mechanism involves the transfer of surface components, including apolipoproteins, to nascent, triglyceride-rich lipoproteins. The modified HDL (mHDL), with deficits in surface components, readily associates with lipoprotein-free apoA-IV, forming an apoA-IV-containing HDL. Changes in HDL physiochemical properties following reacquisition of surface components decreases the affinity of HDL for apoA-IV, resulting in the transfer of apoA-IV to the lipoprotein-free fraction. This can be brought about by the transfer of surface components (lipids and apolipoproteins) to HDL following lipolysis of triglyceride-rich lipoproteins by LPL. Other enzymatic reactions that modify HDL lipid and apolipoprotein composition, such as phospholipid and cholesteryl ester transfer, are also likely to have an effect on apoA-IV distribution.

Many physiological roles for apoA-IV have been postulated. ApoA-IV has been shown to activate LCAT (35). Plasma apoA-IV increases during fat absorption (36) and after feeding (7), suggestive of a role in triglyceride transport. Furthermore, a role of apoA-IV in reverse choles-

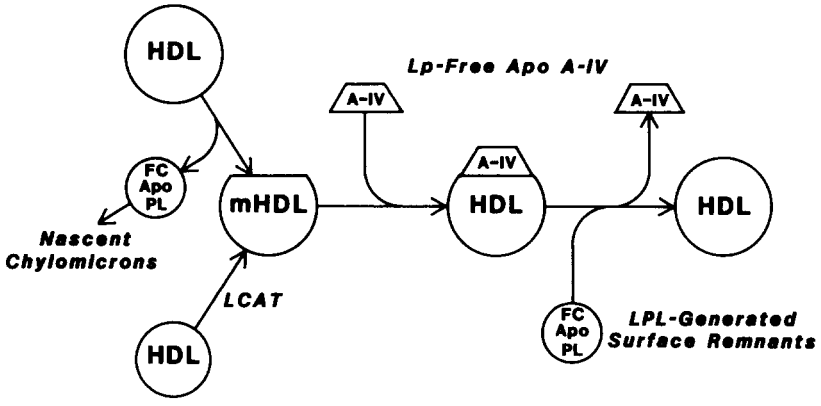


Fig. 11. Simplified scheme of apoA-IV redistribution and metabolism. Surface-deficient modified HDL (mHDL) can be produced either by the action of LCAT or the transfer of surface components to nascent chylomicrons. The modified HDL has an increased affinity for lipoprotein-free apoA-IV, resulting in the formation of an apoA-IV-containing HDL. ApoA-IV dissociates from HDL following the reacquisition of surface remnants generated by the action of LPL on triglyceride-rich lipoproteins. Abbreviations: FC, free cholesterol; HDL, high density lipoproteins; mHDL, modified HDL; LCAT, lecithin:cholesterol acyltransferase; LPL, lipoprotein lipase; PL, phospholipids; TRL, triglyceride-rich lipoproteins.

terol transport has been postulated (8, 37). Plasma lipoprotein-free apoA-IV filtered into the extravascular space appears to participate in the peripheral assembly of free cholesterol-enriched discoidal particles found in interstitial fluid (38, 39).

Based on our observations on plasma apoA-IV redistribution, an additional role may be postulated. Lipoprotein-free apoA-IV may represent an important physiological reservoir of apolipoproteins that can reversibly bind to HDL rendered deficient in surface components, either as the result of LCAT modification or the transfer of apolipoproteins to TRL during triglyceride transport. Loss of apolipoproteins from the surface of HDL has been shown to lead to fusion of HDL particles (40, 41), and HDL fusion and rearrangement has been suggested to account for the formation of discrete products following in vitro incubation of LCAT with small spherical HDL obtained from LCAT-deficient patients (42). Recently, apoA-I has been shown to prevent the fusion of phospholipid microemulsion with LDL (43). Given the structural similarity between apoA-IV and apoA-I (44), apoA-IV may similarly serve to prevent fusion of surface-deficient HDL with other lipoproteins.

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