

Formation of high density lipoproteins containing both apolipoprotein A-I and A-II in the rabbit

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Abstract Human plasma HDLs are classified on the basis of apolipoprotein composition into those that contain apolipoprotein A-I (apoA-I) without apoA-II [(A-I)HDL] and those containing apoA-I and apoA-II [(A-I/A-II)HDL]. ApoA-I enters the plasma as a component of discoidal particles, which are remodeled into spherical (A-I)HDL by LCAT. ApoA-II is secreted into the plasma either in the lipid-free form or as a component of discoidal high density lipoproteins containing apoA-II without apoA-I [(A-II)HDL]. As discoidal (A-II)HDL are poor substrates for LCAT, they are not converted into spherical (A-II)HDL. This study investigates the fate of apoA-II when it enters the plasma. Lipid-free apoA-II and apoA-II-containing discoidal reconstituted HDL [(A-II)rHDL] were injected intravenously into New Zealand White rabbits, a species that is deficient in apoA-II. In both cases, the apoA-II was rapidly and quantitatively incorporated into spherical (A-I)HDL to form spherical (A-I/A-II)HDL. These particles were comparable in size and composition to the (A-I/A-II)HDL in human plasma. Injection of lipid-free apoA-II and discoidal (A-II)rHDL was also accompanied by triglyceride enrichment of the endogenous (A-I)HDL and VLDL as well as the newly formed (A-I/A-II)HDL. **■** We conclude that, irrespective of the form in which apoA-II enters the plasma, it is rapidly incorporated into spherical HDLs that also contain apoA-I to form (A-I/A-II)HDL.—Hime, N. J., K. J. Drew, K. Wee, P. J. Barter, and K. A. Rye. **Formation of high density lipoproteins containing both apolipoprotein A-I and A-II in the rabbit.** *J. Lipid Res.* 2006. 47: 115–122.

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The HDLs in human plasma contain two main apolipoproteins: apolipoprotein A-I (apoA-I) and apoA-II. HDLs are predominantly spherical particles that have been classified on the basis of their apolipoprotein composition into two subpopulations: those that contain apoA-I but not apoA-II [(A-I)HDL] and those containing both apoA-I and

apoA-II [(A-I/A-II)HDL]. Although apoA-I is divided approximately equally between (A-I)HDL and (A-I/A-II)HDL, almost all apoA-II is associated with (A-I/A-II)HDL (1). HDLs that contain apoA-II but not apoA-I [(A-II)HDL] have also been reported in human plasma. However, these particles are present at very low concentrations and constitute only a minor subpopulation of HDLs (2). As apoA-II is known to influence HDL structure and metabolism (3–6), it is important to understand how (A-I)HDL and (A-I/A-II)HDL are formed and how they are related in vivo.

ApoA-I is synthesized in the liver and intestine and secreted into the plasma as a component of discoidal HDLs, which contain phospholipids and unesterified cholesterol (7, 8). Discoidal (A-I)HDL are also assembled in the plasma when phospholipids and unesterified cholesterol from cell membranes, or from triglyceride-rich lipoproteins that are undergoing lipolysis, associate with lipid-free or lipid-poor apoA-I. Irrespective of their origins, discoidal (A-I)HDL are excellent substrates for LCAT (9). When LCAT interacts with discoidal (A-I)HDL, the resulting cholesteryl esters partition into the particle core in a process that converts the discs into spherical (A-I)HDL.

ApoA-II is a hydrophobic apolipoprotein that is synthesized in the liver (10). It is unclear whether apoA-II enters the plasma as a component of discoidal (A-II)HDL or whether it associates with phospholipids and unesterified cholesterol to form discoidal particles after it is secreted (11). Irrespective of the form in which apoA-II enters the plasma, discoidal (A-II)HDL are not substrates for LCAT and are not converted into spherical particles by this enzyme (12, 13). Therefore, it is surprising that most of the apoA-II in human plasma is present as a component

Abbreviations: apoA-I, apolipoprotein A-I; (A-I)HDL, high density lipoproteins containing apolipoprotein A-I without apolipoprotein A-II; (A-I/A-II)HDL, high density lipoproteins containing apolipoprotein A-I and apolipoprotein A-II; (A-II)HDL, high density lipoproteins containing apolipoprotein A-II without apolipoprotein A-I; rHDL, reconstituted high density lipoprotein.

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of spherical (A-I/A-II)HDL. The mechanism by which apoA-II is incorporated into spherical (A-I/A-II)HDL is uncertain. Previous *in vitro* studies from this laboratory have shown that spherical reconstituted high density lipoproteins (rHDLs) that contain apoA-I and apoA-II [(A-I/A-II)rHDL] are formed when LCAT mediates the fusion of discoidal (A-II)rHDL with spherical (A-I)rHDL (14). It is not known whether these events also occur *in vivo*.

The present study investigates the *in vivo* formation of spherical (A-I/A-II)HDL by injecting human apoA-II in either the lipid-free form or as a component of discoidal (A-II)rHDL into rabbits, a species that is naturally deficient in apoA-II and does not have endogenous (A-I/A-II)HDL. Regardless of the form in which it was injected into the animals, the human apoA-II was rapidly and quantitatively incorporated into spherical HDLs that also contained apoA-I. These particles were comparable in size and composition to the spherical (A-I/A-II)HDL that circulate in human plasma. This result is consistent with the LCAT-mediated fusion of discoidal (A-II)rHDL with spherical (A-I)rHDL, which has been shown to generate (A-I/A-II)HDL *in vitro*, also occurring *in vivo*.

MATERIALS AND METHODS

Isolation of human apoA-II

HDLs were isolated by ultracentrifugation from expired, autologously donated samples of human plasma (Gribbles Pathology, Adelaide, Australia) (15). The HDLs were delipidated and subjected to anion-exchange chromatography on a Q-Sepharose Fast Flow column (Amersham Pharmacia Biotech, Uppsala, Sweden) attached to a fast-protein liquid chromatography system (16, 17). The purified apoA-II appeared as a single band after electrophoresis on a homogeneous 20% SDS-polyacrylamide PhastGel (Amersham Pharmacia Biotech) and Coomassie blue staining.

Preparation of discoidal (A-II)rHDL

Discoidal (A-II)rHDL containing 1-palmitoyl-2-oleoyl phosphatidylcholine and human apoA-II were prepared by the cholate dialysis method (18). The (A-II)rHDL were dialyzed against 5×1 liter of endotoxin-free PBS containing 0.008 M Na_2HPO_4 , 0.002 M $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, and 0.15 M NaCl (pH 7.4) before use.

Injection of discoidal (A-II)rHDL and lipid-free apoA-II into rabbits

Male New Zealand White rabbits were maintained on a chow diet with access to food and water *ad libitum*. The left central ear artery of each rabbit was cannulated, and a 5 ml blood sample was collected (0 min). Either lipid-free apoA-II (25 mg) or discoidal (A-II)rHDL (25 mg of apoA-II) was injected into the right marginal ear vein. Given that the average blood volume of a rabbit is 150 ml and the mean endogenous concentration of apoA-I is 0.5 mg/ml, it follows that the apoA-I/apoA-II molar ratio immediately after injection was comparable to that in human plasma (~2:1). Blood samples were drawn from the left central ear artery at 2, 10, 30, 60, and 120 min. Ketamine/xylazine was administered to the rabbits at 180 min. Blood was obtained by cardiac puncture, and plasma was isolated by centrifugation.

Isolation of rabbit HDLs

HDLs were isolated from plasma by ultracentrifugation ($1.07 < d < 1.21$ g/ml) at 100,000 rpm for 16 h in a TLA-100.4 rotor (Beckman Instruments, Fullerton, CA) maintained at 4°C. The HDLs were dialyzed against 3×1 liter of 0.01 M TBS containing 0.15 M NaCl, 0.005% (w/v) EDTA- Na_2 , and 0.006% (w/v) NaN_3 (pH 7.4).

Immunoaffinity chromatography

Ultracentrifugally isolated HDLs (0.4 mg of human apoA-II in animals injected with apoA-II, or 1.0 mg of apoA-I in non-injected animals) were applied to a column containing goat anti-human apoA-II polyclonal antibody (Calbiochem-Novabiochem Corp., San Diego, CA) covalently coupled to CNBr-activated Sepharose 4B (Amersham Pharmacia Biotech). The HDLs that did not bind to the column were eluted with TBS. HDLs that bound to the column were eluted with 0.1 M acetic acid and neutralized immediately with 1 M Tris, pH 7.4 (final concentration, 0.1 M).

Size-exclusion chromatography

Rabbit plasma (0.5 ml) obtained before (0 min) and at 10, 30, 60, 120, and 180 min after injection of either discoidal (A-II)rHDL or lipid-free apoA-II was loaded onto a Superose 6 column (Amersham Pharmacia Biotech). Lipoproteins were eluted with TBS at a flow rate of 0.3 ml/min, and 0.5 ml fractions were collected.

Other techniques

All chemical analyses were carried out on a Hitachi 902 automatic analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Phospholipid, unesterified cholesterol, and triglyceride concentrations were determined enzymatically (19–21). A Roche Diagnostics Kit was used to determine total cholesterol concentrations. Cholesteryl ester concentrations were calculated as the difference between total and unesterified cholesterol concentrations. ApoA-I and apoA-II concentrations were measured immunoturbidometrically (22) using sheep anti-rabbit apoA-I (23) and goat anti-human apoA-II (Calbiochem-Novabiochem Corp.) polyclonal antibodies, respectively. Total protein concentrations were determined using bicinchoninic acid (24).

HDL size was determined by 3–40% nondenaturing polyacrylamide gradient gel electrophoresis and staining with Coomassie blue (25). Agarose gel electrophoresis was carried out at 100 V for 1 h on 0.6% gels. The gels were immunoblotted with rabbit apoA-I and human apoA-II polyclonal antibodies. Two-dimensional gel electrophoresis (agarose gel electrophoresis in the first dimension and nondenaturing gradient gel electrophoresis in the second dimension) was carried out as described (26). The gradient gels were immunoblotted for apoA-I and apoA-II, and the apolipoproteins were detected by enhanced chemiluminescence (Amersham Pharmacia Biotech).

RESULTS

Incorporation of human apoA-II into rabbit HDL after injection of either discoidal (A-II)rHDL or lipid-free apoA-II

Discoidal (A-II)rHDL were injected into each of three rabbits. Before injection, the mean plasma phospholipid, total cholesterol, and triglyceride concentrations were $1,134 \pm 601$, 920 ± 477 , and 592 ± 271 $\mu\text{M/l}$, respectively.

TABLE 1. Effect of injecting discoidal (A-II)rHDL and lipid-free apoA-II on plasma apoA-II and apoA-I concentrations

Apolipoprotein	Time after Discoidal (A-II)rHDL Injection		
	0 min	2 min	180 min
	$\mu\text{g/ml}$		
ApoA-II	0	260 \pm 25	146 \pm 15
ApoA-I	438 \pm 80	495 \pm 103	423 \pm 78
Apolipoprotein	Time after Lipid-Free ApoA-II Injection		
	0 min	2 min	180 min
	$\mu\text{g/ml}$		
ApoA-II	0	246 \pm 13	156 \pm 27
ApoA-I	885 \pm 68	806 \pm 68	775 \pm 67

apoA-II, apolipoprotein A-II; (A-II)rHDL, reconstituted high density lipoprotein containing apolipoprotein A-II without apolipoprotein A-I. Rabbits were injected with either discoidal (A-II)rHDL (25 mg of apoA-II) or lipid-free apoA-II (25 mg). Blood was collected before (0 min) and at 2 and 180 min after the injection as described in Materials and Methods. Values represent means \pm SEM (n = 3).

HDL-cholesterol and non-HDL-cholesterol levels were 591 \pm 13 and 329 \pm 26 $\mu\text{M/l}$, respectively. The mean concentration of apoA-I was 438 \pm 80 $\mu\text{g/ml}$, and there was no detectable apoA-II (Table 1). Injection of discoidal (A-II)rHDL did not affect the concentration of apoA-I. Two minutes after injection, the plasma apoA-II concentration was 260 \pm 25 $\mu\text{g/ml}$. By 180 min, this value had decreased to 146 \pm 15 $\mu\text{g/ml}$. This reduction in the concentration of apoA-II suggests that its turnover in rabbit plasma is rapid.

To ascertain into which lipoprotein fraction the apoA-II had been incorporated, either lipid-free apoA-II (n = 3) or discoidal (A-II)rHDL (n = 3) was injected into rabbits. Blood samples were collected before (0 min) and at 10, 30, 60, and 180 min after injection. Lipoproteins were separated by size-exclusion chromatography. Elution profiles for the 0, 10, and 180 min samples are shown (Fig. 1). The 30 and 60 min profiles (data not shown) were comparable to the 10 min profile. Injection of discoidal (A-II)rHDL did not alter the rabbit apoA-I elution profile (Fig. 1A). When either discoidal (A-II)rHDL (Fig. 1B) or lipid-free apoA-II (Fig. 1C) was injected into the rabbits, all of the apoA-II coeluted with apoA-I. ApoA-II was not detected in either the LDL- or the VLDL-containing fractions.

Characterization of rabbit HDL after injection of either discoidal (A-II)rHDL or lipid-free apoA-II

Spherical (A-I/A-II)HDL migrate to an α position when subjected to agarose gel electrophoresis (14), whereas lipid-free apoA-II (Fig. 2B, track d) and discoidal (A-II)rHDL (Fig. 2B, track e) both have pre β mobility. To determine whether the electrophoretic mobility of lipid-free apoA-II and discoidal (A-II)rHDL changed from pre β to α after injection into rabbits, HDL isolated before and 180 min after injection of apoA-II were subjected to agarose gel electrophoresis. The gels were then immunoblotted for rabbit apoA-I (Fig. 2A) and human apoA-II (Fig. 2B). Before injection of apoA-II, the (A-I)HDL migrated to an α position (Fig. 2A, track a). (A-I)HDL migration was not

affected by injection of either discoidal (A-II)rHDL or lipid-free apoA-II (Fig. 2A, tracks b and c, respectively). The lipid-free apoA-II (Fig. 2B, track d) and the discoidal (A-II)rHDL (Fig. 2B, track e) migrated to a pre β position before injection. After injection, the lipid-free apoA-II and the apoA-II that was injected as a component of discoidal (A-II)rHDL migrated to α positions (Fig. 2B, tracks b and c, respectively). This is consistent with apoA-II being incorporated into spherical, α -migrating HDL irrespective of whether it was introduced into the animals in a lipid-free form or as a component of discoidal (A-II)rHDL.

To determine whether the α -migrating HDLs into which the apoA-II had been incorporated also contained apoA-I, samples of ultracentrifugally isolated HDLs containing 0.4 mg of apoA-II were applied to an anti-apoA-II immunoaffinity chromatography column. As this column binds apoA-II but not apoA-I (3, 14), it follows that any apoA-I that bound to the column must be associated with particles that also contain apoA-II. In the case of HDLs from rabbits infused with discoidal (A-II)rHDL, 1.0 mg of apoA-I bound to the column. When the rabbits were infused with lipid-free apoA-II, 1.2 mg of apoA-I bound to the column (data not shown). This demonstrates that HDLs containing both apoA-I and apoA-II on the same particle are formed when apoA-II is introduced into rabbits either in a lipid-free form or as a constituent of discoidal (A-II)rHDL.

Further evidence that (A-I/A-II)HDL were formed when rabbits were injected with discoidal (A-II)rHDL was obtained by subjecting the samples to agarose gel electrophoresis followed by nondenaturing gradient gel electrophoresis (Fig. 3). Immunoblot analysis of the gradient gels revealed that all of the apoA-II comigrated with apoA-I. This indicated that the apoA-I and apoA-II were associated with HDLs that were comparable in terms of size and charge.

The composition of rabbit HDLs before and after injection of apoA-II is shown in Table 2. Before injection, the percentage mass of HDL phospholipids, cholesteryl esters, unesterified cholesterol, triglycerides, and protein was 24, 15, 1, 10, and 50, respectively. After injection of lipid-free apoA-II and discoidal (A-II)rHDL, the HDLs were depleted of cholesteryl esters and enriched with triglycerides. The cholesteryl ester content of the (A-I)HDL decreased from 15% in the noninjected animals to 7% and 6% in animals injected with discoidal (A-II)rHDL and lipid-free apoA-II, respectively. Before injection, triglycerides accounted for 10% of the total HDL mass. After injection of apoA-II, this value increased to 26–33% in (A-I)HDL and to 14–25% in (A-I/A-II)HDL. The (A-I/A-II)HDLs contained 1.2–1.4 molecules of apoA-II per molecule of apoA-I. As judged by nondenaturing gradient gel electrophoresis, the HDLs were 10.4 nm in diameter both before and after injection of either lipid-free apoA-II or discoidal (A-II)rHDL (data not shown).

Effect of injecting discoidal (A-II)rHDL on plasma lipid profiles

Plasma samples collected from rabbits before (0 min) and at 10, 30, 60, and 180 min after injection of either dis-

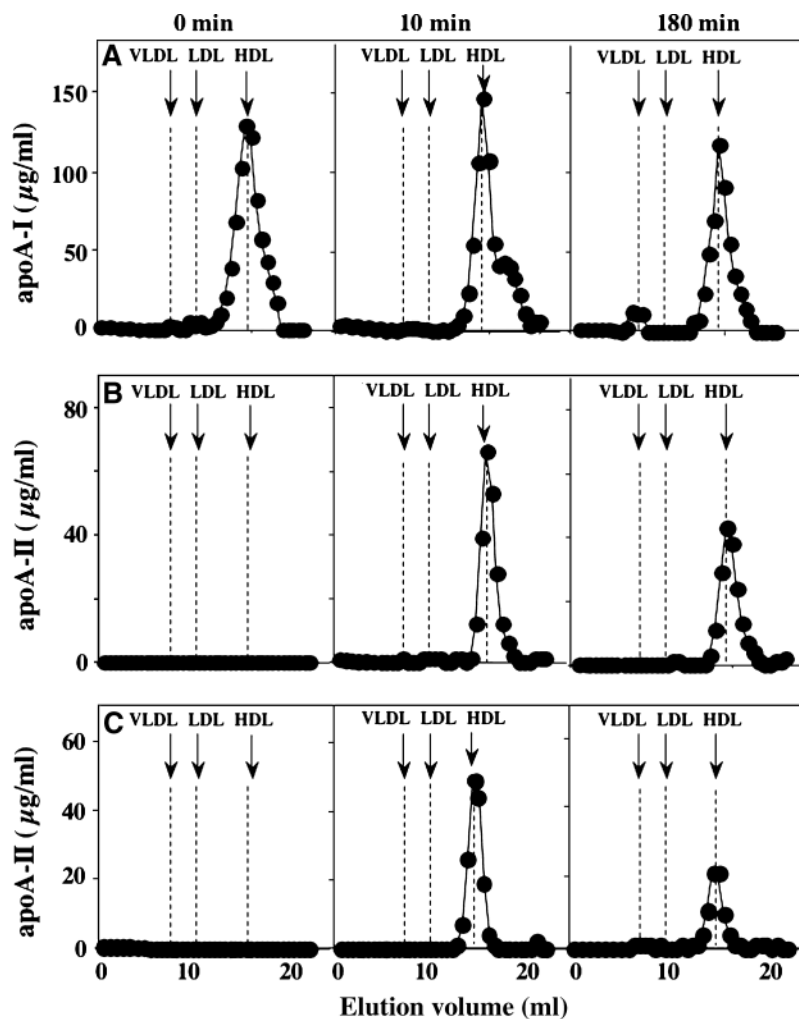


Fig. 1. Size-exclusion chromatography of rabbit plasma after injection of discoidal reconstituted high density lipoproteins containing apolipoprotein A-II without apolipoprotein A-I [(A-II)rHDL] or lipid-free apoA-II. Blood was collected from each of six rabbits (0 min). Either discoidal (A-II)rHDL ($n = 3$) or lipid-free apoA-II ($n = 3$) was injected into the rabbits. Blood was collected at 10, 30, 60, and 180 min after injection, and plasma samples were applied to a Superose 6 column. A: Elution profiles of apoA-I after injection of discoidal (A-II)rHDL. B, C: Elution profiles of apoA-II after injection of discoidal (A-II)rHDL and lipid-free apoA-II, respectively. Each profile is from a single, representative animal. The elution positions of VLDL, LDL, and HDL are indicated.

coidal (A-II)rHDL or lipid-free apoA-II were subjected to size-exclusion chromatography. Phospholipid, cholesteryl ester, and triglyceride elution profiles for a single, representative rabbit at 0 min and 10, 60, and 180 min after injection of discoidal (A-II)rHDL are shown in **Fig. 4**. Cholesteryl ester and triglyceride elution profiles are also shown for a single, representative rabbit that was injected with lipid-free apoA-II. Injection of lipid-free apoA-II had no effect on the distribution of phospholipids (data not shown). In all cases, the 30 min profiles (data not shown) were comparable to those obtained at 60 min.

Injection of discoidal (A-II)rHDL did not affect the LDL phospholipid concentration. VLDL and HDL phospholipid concentrations were increased at 10 min. This increase was sustained for up to 60 min. By 180 min, the VLDL and HDL phospholipid levels were still high but were approaching preinjection levels. Injection of discoidal (A-II)rHDL and lipid-free apoA-II was accompanied by a progressive triglyceride enrichment of the VLDL and HDL and a concomitant reduction in HDL cholesteryl ester levels.

DISCUSSION

The present study was carried out to determine the fate of apoA-II that enters the plasma either in a lipid-free form or as a component of discoidal (A-II)rHDL. This was achieved by injecting either lipid-free apoA-II or discoidal (A-II)rHDL into rabbits, a species that is naturally deficient in apoA-II and therefore does not have endogenous (A-I/A-II)rHDL. The results showed that the lipid-free or lipid-associated apoA-II that was injected into these animals was rapidly and quantitatively incorporated into spherical

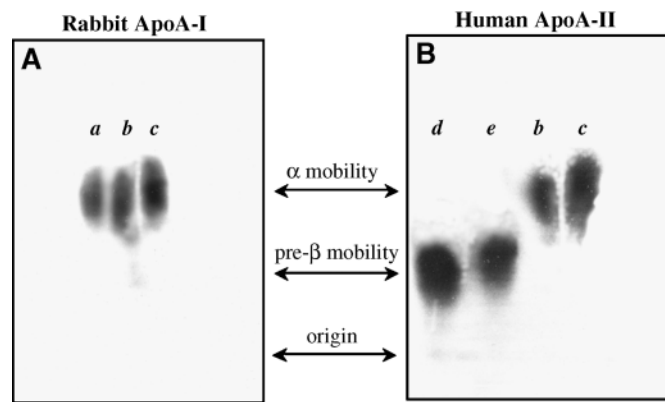


Fig. 2. Agarose gel electrophoresis of rabbit HDLs after injection of discoidal (A-II)rHDL or lipid-free apoA-II. Rabbits were injected with discoidal (A-II)rHDL ($n = 3$) or lipid-free apoA-II ($n = 3$). Blood was collected at 180 min after injection. Blood was also collected from noninjected animals. Ultracentrifugally isolated HDLs were subjected to agarose gel electrophoresis and immunoblotted for either rabbit apoA-I (A) or human apoA-II (B). Results for a single, representative animal are shown. Track a, HDL from a noninjected animal; track b, HDL isolated at 180 min after injection of discoidal (A-II)rHDL; track c, HDL isolated at 180 min after injection of lipid-free apoA-II; track d, lipid-free apoA-II before injection; track e, discoidal (A-II)rHDL before injection.

HDLs that also contained apoA-I. These particles were comparable in size and composition to the spherical (A-I/A-II)HDL in human plasma. Injection of lipid-free and discoidal (A-II)rHDL into rabbits also generated triglyceride-enriched VLDL, (A-I)HDL, and (A-I/A-II)HDL.

The formation of (A-I/A-II)HDL in the present study can be explained in a number of ways. One possibility is that the apoA-II may have bound to preexisting spherical (A-I)HDL without displacing apoA-I. Although lipid-free apoA-II has been shown to bind to discoidal and spherical (A-I)rHDL in vitro (27, 28), this is unlikely to be the case in vivo. As lipid-free apoA-II is extremely hydrophobic, it almost certainly acquires phospholipids and unesterified cholesterol from cell membranes and other lipoproteins as soon as it enters the plasma compartment and is

converted rapidly into discoidal particles. The likelihood of these newly formed discoidal particles binding to preexisting spherical (A-I)HDL is low because the apoA-II no longer has α -helices available for interacting with the (A-I)HDL surface. This is also likely to be the case for the animals that received discoidal (A-II)rHDL.

A more plausible explanation for the present results is that the discoidal (A-II)rHDL were incorporated via particle fusion into preexisting spherical (A-I)HDL to form spherical (A-I/A-II)HDL. Evidence that this may be the case comes from our earlier in vitro studies, in which mixtures of discoidal (A-I)rHDL and discoidal (A-II)rHDL were incubated with LCAT and LDL (14). The results of those experiments established that LCAT converted the discoidal (A-I)rHDL into small, spherical (A-I)rHDL, which subsequently fused with the discoidal (A-II)rHDL to generate large, spherical (A-I/A-II)rHDL. These events were dependent on the discoidal (A-II)rHDL not acting as substrates for LCAT and therefore being available to fuse with the newly formed small, spherical (A-I)rHDL. They are also consistent with numerous reports showing that most of the apoA-II in human plasma is associated with spherical (A-I/A-II)HDL and not discoidal (A-II)HDL. In the case of the animals that were injected with lipid-free apoA-II, the free apolipoprotein most likely acquired phospholipids and unesterified cholesterol from cell membranes and other lipoproteins to form discoidal (A-II)HDL, which were subsequently incorporated into preexisting spherical (A-I)HDL by the mechanism outlined above.

It has also been reported that lipid-free apoA-II can displace all of the apoA-I from spherical (A-I)HDL to generate spherical (A-II)HDL (29, 30). Two lines of evidence indicate that this was not the case in the present study. First, previous work from this laboratory has shown that spherical (A-II)rHDL migrate to a position between that of α -migrating spherical (A-I)rHDL and pre β -migrating, lipid-free apoA-II when they are subjected to agarose gel electrophoresis (30). Particles of this type were not formed in the rabbits that received either lipid-free apoA-II or discoidal (A-II)rHDL (Fig. 2). Second, as discoidal (A-II)rHDL are not substrates for LCAT (13), it is highly

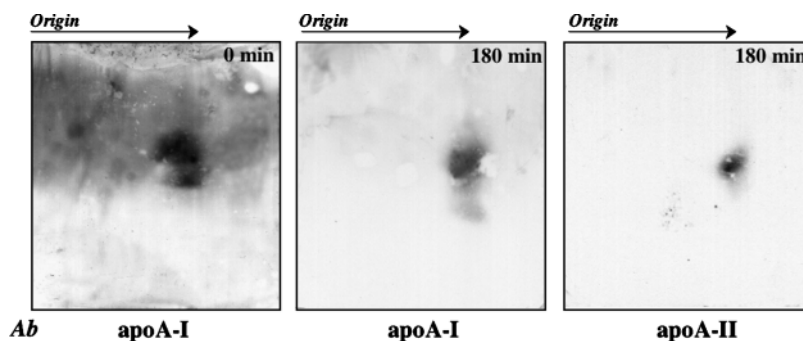


Fig. 3. Two-dimensional gel electrophoresis of rabbit HDLs after injection of discoidal (A-II)rHDL. HDLs were obtained from a rabbit at 180 min after injection with discoidal (A-II)rHDL and subjected to agarose gel electrophoresis followed by nondenaturing gradient gel electrophoresis. The samples were transferred to nitrocellulose membranes and immunoblotted for apoA-I and apoA-II. Migration of the HDLs on the agarose gel is indicated at the top of each panel.

TABLE 2. Composition of (A-I)HDL and (A-I/A-II)HDL after injection of apoA-II into rabbits

Rabbit HDL	Composition						
	Phospholipid	Cholesteryl Ester	Unesterified Cholesterol	Triglyceride	Protein	ApoA-II	ApoA-I
	% mass		% mass			mol/mol	
Preinjected animals							
(A-I)HDL	24	15	1	10	50	0	1
After injection of discoidal (A-II)rHDL							
(A-I)HDL	22	7	1	33	37	0	1
(A-I/A-II)HDL	17	6	0.4	14	62	1.4	1
After injection of lipid-free apoA-II							
(A-I)HDL	19	6	1	26	47	0	1
(A-I/A-II)HDL	19	7	0.6	25	49	1.2	1

(A-I)HDL, high density lipoproteins containing apoA-I without apoA-II. Rabbits were injected with either discoidal (A-II)rHDL (n = 3) or lipid-free apoA-II (n = 3). Blood samples were collected before and 180 min after injection. Ultracentrifugally isolated HDLs were then subjected to anti-apoA-II immunoaffinity chromatography to separate the (A-I)HDL from the (A-I/A-II)HDL. Composition was calculated from means of triplicate determinations that varied by <10%.

unlikely that they would be converted directly into spherical (A-II)rHDL in vivo. This is also consistent with the reportedly low levels of spherical (A-II)HDL in human plasma (2, 31).

One of the most unexpected findings to emerge from this study is that VLDL triglyceride levels increase dramatically when either lipid-free apoA-II or discoidal (A-II)rHDL are injected into rabbits. A similar result has been reported in mice transgenic for human apoA-II (32). In that study, apoA-II had no effect on VLDL production but reduced VLDL catabolism secondary to an inhibition of lipoprotein lipase activity. Although it is tempting to spec-

ulate that the lower VLDL triglyceride hydrolysis in the apoA-II transgenic mice was attributable to displacement of apoC-II, an obligatory cofactor of lipoprotein lipase, by the more hydrophobic apoA-II, this appears not to be the case (32).

The triglyceride enrichment and cholesteryl ester depletion of (A-I)HDL and (A-I/A-II)HDL that occurred when apoA-II was injected into rabbits most likely reflects cholesteryl ester transfer protein-mediated exchanges of core lipids between HDL and the expanded VLDL pool (33, 34). Triglyceride enrichment of HDL has several metabolic consequences. For example, it enhances the

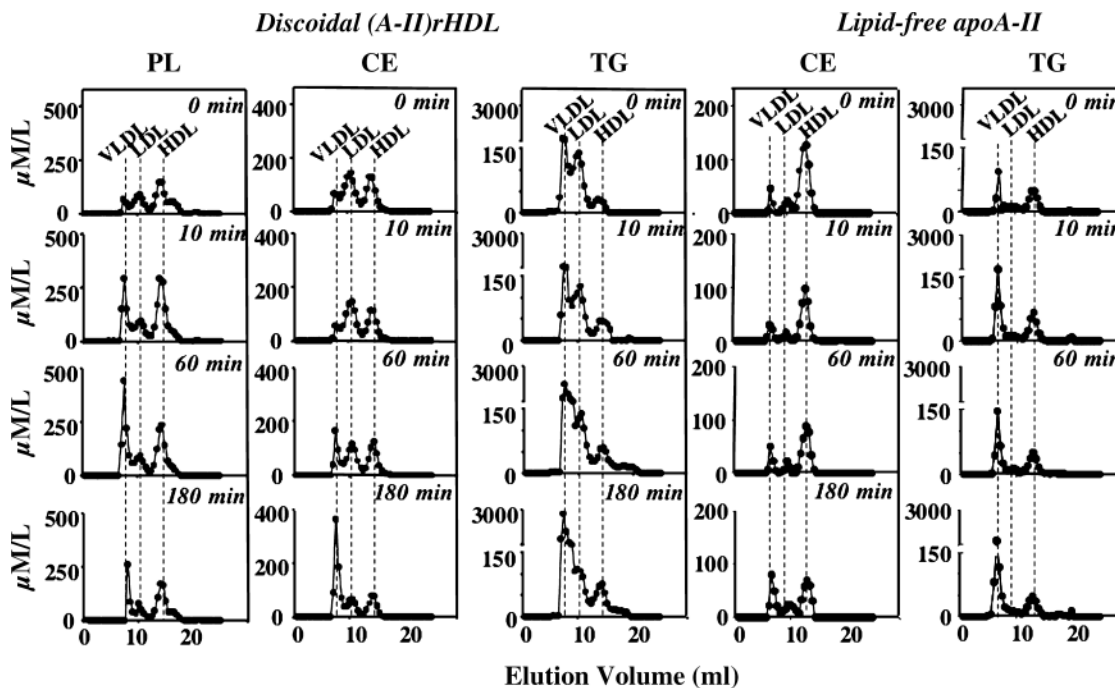


Fig. 4. Effect of discoidal (A-II)rHDL and lipid-free apoA-II on rabbit lipid profiles. Rabbits were injected with an amount of discoidal (A-II)rHDL that contained 25 mg of apoA-II or with 25 mg of lipid-free apoA-II. Blood was collected at 0, 10, 60, and 180 min. Plasma samples were applied to a Superose 6 column. Triglyceride (TG), cholesteryl ester (CE), and phospholipid (PL) elution profiles are shown for a single, representative animal injected with discoidal (A-II)rHDL. Triglyceride and cholesteryl ester elution profiles are also shown for a single, representative animal injected with lipid-free apoA-II. The elution positions of VLDL, LDL, and HDL are shown.

phospholipid transfer protein-mediated remodeling of HDL and generates lipid-free or lipid-poor apoA-I (35, 36). This apoA-I can act as an acceptor of cellular cholesterol in the first step of reverse cholesterol transport. Lipid-free or lipid-poor apoA-I is also generated when triglyceride-rich HDLs are incubated in vitro with hepatic lipase (37). Remodeling of triglyceride-enriched HDLs by the combined activities of cholesteryl ester transfer protein and hepatic lipase has also been reported to enhance the uptake of HDL cholesteryl esters by scavenger receptor class B type I (38) and to increase HDL clearance (39). It should be noted, however, that the latter processes are unlikely to occur in the rabbit, which has very low endogenous levels of hepatic lipase (40). This most likely accounts for the persistent triglyceride enrichment of HDL that was observed in the animals in the current study.

In conclusion, this study shows for the first time that, irrespective of the form in which apoA-II enters the plasma compartment, it is rapidly and quantitatively incorporated into spherical (A-I/A-II)HDL by a mechanism that most likely involves LCAT-mediated particle fusion. The implications of these findings in relation to the cardioprotective properties of HDL remain to be determined. **■**

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