Acute Effects of Intravenous Infusion of ApoA1/Phosphatidylcholine Discs on Plasma Lipoproteins in Humans

M.N. Nanjee, J.E. Doran, P.G. Lerch, N.E. Miller

Abstract—To investigate the metabolism of nascent HDLs, apoA1/phosphatidylcholine (apoA1/PC) discs were infused IV over 4 hours into 7 healthy men. Plasma total apoA1 and phospholipid (PL) concentrations increased during the infusions. The rise in plasma apoA1 was greatest in small preβ-migrating particles not present in the infusate. Total HDL unesterified cholesterol (UC) also increased simultaneously. After stopping the infusion, the concentrations of apoA1, PL, HDL UC, and small preβ HDLs decreased, whereas those of HDL cholesteryl ester (CE) and large α-migrating apoA1 containing HDLs increased. ApoB-containing lipoproteins became enriched in CEs. Addition of apoA1/PC discs to whole blood at 37°C in vitro also generated small preβ HDLs, but did not augment the transfer of UC from erythrocytes to plasma. We conclude that the disc infusions increased the intravascular production of small preβ HDLs in vivo, and that this was associated with an increase in the efflux and esterification of UC derived from fixed tissues. The extent to which the increase in tissue cholesterol efflux was dependent on that in preβ HDL production could not be determined. Infusion of discs also reduced the plasma apoB and apoA2 concentrations, and increased plasma triglycerides and apoC3. Thus, nascent HDL secretion may have a significant impact on preβ HDL production, reverse cholesterol transport and lipoprotein metabolism in humans. (Arterioscler Thromb Vasc Biol. 1999;19:979-989.)

Key Words: apolipoprotein A1 ■ cholesterol ■ HDLs ■ lecithin ■ phosphatidylcholine

Plasma HDL concentration is a risk factor for atherosclerosis. The HDLs are a family of particles of differing size and composition.1 In most HDLs the principal protein is apoA1, and the major phospholipid (PL) is phosphatidylcholine (PC). Most HDLs are spheroidal particles, in which the apoA1, PLs and unesterified cholesterol (UC) enclose a core composed mostly of cholesteryl esters (CEs). On agarose gel electrophoresis these particles have α-mobility. A few HDLs contain no core lipids, and have preβ electrophoretic mobility.2 Three size subclasses of preβ HDLs have been described: preβ1, which are the smallest and contain 1 molecule of apoA1; preβ2, which are PL-rich discs containing 3 apoA1 molecules; and preβ3, which contain lecithin:cholesterol acyltransferase (LCAT) and cholesteryl ester transfer protein (CETP) in addition to apoA-1.2,3 Unlike some α-HDLs, preβ HDLs do not contain apoA2.3

Lipoproteins containing apoA1 play a central role in cholesterol transport from tissues (reverse cholesterol transport, RCT).3 Native plasma HDLs and reconstituted apoA1/PL discs promote efflux of UC from cultured cells.4,5 When fibroblasts containing radiolabeled cholesterol are exposed to plasma, most of the radioactivity released initially enters the preβ1 HDLs.2 Cell-derived UC then appears sequentially in the preβ2 and preβ3 HDLs, wherein it is esterified by LCAT. The resultant CEs are transferred by CETP to α-HDLs. Some are then transferred to triglyceride-rich lipoproteins (TGRLs) in exchange for triglycerides (TGs).1,3 Preβ1 HDL concentration appears to be rate-limiting for UC efflux from cultured fibroblasts.6 Some apoE- and apoA4-containing HDLs also remove UC from cultured cells.7,8

The origin of the preβ HDLs is unclear. Similar particles are formed when lipid-free apoA1 recruits PLs and UC from cultured cells.9 In vitro lipid-free apoA1 and/or small preβ1-like particles are released by the actions of CETP and hepatic lipase (HL) on α-HDLs.10 and by fusion of α-HDLs with each other or with discoidal lipoproteins.11 In addition to the preβ2/3 HDLs, several other discoidal lipoproteins have been described: apoE/PL particles secreted by macrophages12; the surface remnants of lipolyzed TGRLs13; and nascent HDLs, secreted by liver and small intestine, composed of PL, UC, and pro-apoA1.14

Experiments in vitro have indicated that after entering plasma nascent HDLs probably acquire UC and associate with LCAT, leading to their conversion into α-HDLs with
was essentially no free PC or free apoA1. The larger discs contained 3 or 4, and the smaller discs 2 or 3, apoA1 molecules. They were efficient cofactors for LCAT in vitro, and promoted cholesterol efflux from cultured cells.

**Subjects and Clinical Procedures**

Seven healthy males were studied (Table). Subjects were excluded if they had allergies, alcoholism or hemolactic, hepatic, renal, cardiovascular, endocrine or inflammatory disease; if they had taken any medication within 2 weeks; if they were HIV positive; or if they had received blood products during the past year. The study was approved by the local Ethics Committee. All subjects gave informed consent.

Clinical procedures were carried out in a metabolic ward (Clin-Pharma Research AG, Birsfelden, Switzerland), to which the subjects were admitted after an overnight fast (12 to 14 hours). The apoA1/PC discs were infused into a forearm vein. Each bottle was dissolved (5 minutes, room temperature) with 40 mL sterile water (final protein concentration, 20 mg/mL). The appropriate amount was mixed with sterile 0.15 mmol/L NaCl to provide the required dose in 300 mL. Three subjects were given 25 mg/kg, and four were given 40 mg/kg. Each infusion lasted 4 hours: 40 mL/h for the first 0.5 hours, and then 80 mL/h for 3.5 hours. Fat-free meals were provided 4.5 and 10.5 hours after the start of the infusion. Blood for measurements of lipoproteins and cholesteryl esters was collected into plain glass tubes, serum for routine clinical chemistry and hematology was collected from the contralateral arm into plain glass tubes, and deproteinized plasma was collected 0.5 hours, 1 hour, 2 hours, 4 hours, 6 hours, 8 hours, and after 24 hours. After the 24-hour blood draw, blood was collected in ethylenediaminetetraacetic acid (EDTA) tubes, centrifuged, and the plasma was frozen. Serum and plasma samples were stored at -70°C until the end of the study. All samples were analyzed in random order.

Clinical Details of Study Subjects

<table>
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<tr>
<th>No</th>
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<th>Weight (kg)</th>
<th>Triglycerides (mmol/L)</th>
<th>Cholesterol (mmol/L)</th>
<th>HDL Chol (mmol/L)</th>
<th>ApoA1 (mg/dL)</th>
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<td>0.84</td>
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<td>0.78</td>
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**Mean** 24.4 71.4 1.06 4.57 0.98 123

**SD** 3.1 4.0 0.37 0.48 0.36 14

All subjects were apparently healthy males. Subjects 1 to 3 were given the low-dose infusion of apoA1/PC discs (25 mg apoA1/kg body weight over four hours) and subjects 4 to 7 were given the high dose (40 mg/kg).

**Methods**

**Apolipoprotein A1/Phosphatidylcholine Discs**

The apoA1/PC discs were prepared from human apoA1 and soybean PC by cholate dialysis in ZLB Central Laboratory. Parenteral grade PC containing 0.2% α-tocopherol as antioxidant (Phospholipon 90) was obtained from Rhône-Poulenc Rorer, Nattermann Phospholipid. Sucrose (final concentration, 10% wt/vol) was added to stabilize the discs, and the protein concentration was adjusted to 2% (wt/vol). After filtration (0.22 μm) the particles were dispersed in glass bottles (1 g apoA1), lyophilized, sealed under vacuum, and stored at 4°C. Their properties have been described in detail. The discs contained no pyrogens. They were prepared in the absence of oxygen, and no evidence of phospholipid peroxidation was detected. They were stable for at least 2 years at 4°C or 30°C. When redissolved in water (2% wt/vol with respect to protein), they were stable for at least 60 days at 4°C, and had a pH of 7.5, a sodium concentration of 17 mmol/L, a cholate concentration of 13 mmol/L, and an osmolality of 450 mosmol/kg. On electron microscopy there were two main populations, 12.6 ± 2.8 and 17.7 ± 4.2 nm (mean ± SD) in diameter, each 4.8 ± 0.3 nm thick (Figure 1). The elution profiles of apoA1 and PC by high performance–size exclusion chromatography (HP-SEC) appear in Figure 1. The larger particles contained most of the apoA1 (~77%), and had a lower mean apoA1/PC molar ratio (~1:100 versus 1:200). There were 2 main populations, 12.6 ± 2.8 and 17.7 ± 4.2 nm (mean ± SD) in diameter, each 4.8 ± 0.3 nm thick (Figure 1). The elution profiles of apoA1 and PC by high performance–size exclusion chromatography (HP-SEC) appear in Figure 1. The larger particles contained most of the apoA1 (~77%), and had a lower mean apoA1/PC molar ratio (~1:100 versus 1:200). There was essentially no free PC or free apoA1. The larger discs contained 3 or 4, and the smaller discs 2 or 3, apoA1 molecules. They were efficient cofactors for LCAT in vitro, and promoted cholesterol efflux from cultured cells.

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Laboratory Procedures

Lipids and Apolipoproteins

All assays were performed in duplicate. Plasma total cholesterol, UC, TG, and choline-containing PLs were quantified using commercial enzymes (Sigma) and Trinder-type reagents (Research Organics) in a microtiter plate spectrophotometer20, CE was calculated by difference. The TG measurements were not corrected for endogenous free glycerol. Precinorm L® (Boehringer-Mannheim GmbH) was used as calibrator. HDL lipids were measured after precipitation of apoB-containing lipoproteins with polyethylene glycol (PEG) 6000 (8% wt/vol, final concentration),21 ApoA1, apoA2, apoB, and apoC3 were quantified by rocket immunoelectrophoresis in the presence of PEG (3% wt/vol) and Tween 20 (0.2% vol/vol).22 All apoAssays were standardized using dilutions of Precinorm L.

Crossed Immunoelectrophoresis

Nonsieving charge-based electrophoresis in the first dimension was performed through a 1% (wt/vol) low electroendosmosis agarose slab gel (SeaKem LE, FMC Bioproducts) at 30 V/cm for 2 hours at 4°C, using 63 mmol/L Tris, 27 mmol/L Tricine, 1 mmol/L calcium lactate, 3 mmol/L sodium azide (pH 8.6) as the electrophoresate, in a Bio-Phoresis flat-bed chamber (Bio-Rad Labs Ltd). The second dimension was through the same gel matrix impregnated with 0.5% (vol/vol) goat polyclonal anti-human apoA1 serum (INCSort Corp, Stillwater, Minn.), 0.2% (vol/vol) Tween 20 and 3% (wt/vol) PEG 6000, at 15 V/cm for 16 hours at 4°C using the same buffer. After removal of reactants by soaking in 150 mmol/L NaCl, antigen-antibody complexes were visualized with 0.5% (wt/vol) Coomassie Blue R250 in ethanol:acetic acid:water (9:2:9 vol/vol).21

Size Exclusion Chromatography

To separate VLDLs, LDLs, and HDLs, plasma (100 μL) was passed through a Superose 6 column (10 mm×300 mm) (HR 10/30, Pharmacia) in series. Collection of fractions (200 μL) was monitored by in-line absorbance at 280 nm and by rocket immunoelectrophoresis for apoA1 and apoB. Recoveries were >90%. Fractions 4 to 7 contained apoA1 and apoB. Fraction 5 (1.020 g/mL) was further cut into 10 fractions (fractions 41 to 55). With use of the same eluant as in the Superose 6 method (flow rate, 0.75 mL/min), 50-μL aliquots of plasma were chromatographed through Superdex 200 and Superdex 75 gel permeation columns (HR 10/30, Pharmacia) in series. Collection of fractions (200 μL) and immunossay of apoA1 were as described above. The different subclasses isolated by this method have been fully characterized (E.A. Brinton and M.N. Nanjee, manuscript in preparation). [The smallest particles (fractions 41 to 55) all have preβ electrophoretic mobility, normally account on average for 9% of plasma apoA1, have a molecular weight range of 40 to 60 kDa, contain no apoA2, and contain no detectable CE, UC, or TG. Their concentration was increased in hypertriglyceridemic, LCAT-deficient, and postheparin plasma. When plasma was incubated at 37°C, this subclass first disappeared (by 1 to 4 hours) and then reappeared in excess during long-term incubation. These particles contain no detectable PL by our assay, indicating that there are fewer than 5 molecules of PL per particle. Thus, some of them may be lipid-free apoA1 dimers. The major population of particles (fractions 20 to 40) has a molecular weight range from 60 to 500 kDa, has α-electrophoretic mobility, contains apoA2 in addition to apoA1, is rich in CEs, and also contains UC, TG, and PL. The very largest particles (fractions 10 to 15) have preβ mobility, a molecular weight of >500 kDa, and are rich in PL. Some of these data have been presented.22]

Other Methods

Antibodies to lipid-free human apoA1 and apoA1/PC discs in serum were sought by immunoblotting, passive hemagglutination, and an ELISA, as previously described.22 Cholate was quantified using a commercial enzymatic colorimetric kit (Merck).

Statistical Analyses

Changes relative to Time 0 were examined by repeated-measures 2-way ANOVA and Fisher’s protected least significant difference test; P<0.05 was considered statistically significant.

Results

No evidence of an acute-phase response or other clinical effects was observed: the subjects experienced no symptoms, developed no rashes, and showed no significant changes in pulse rate, blood pressure, or temperature. Nor were any significant changes observed in routine clinical chemistry or hematology. No glycosuria, proteinuria, or hemoglobinuria occurred. No viral antibodies, HBsAg positivity, or antibodies to lipid-free apoA1 or apoA1/PC discs developed.

Plasma Total ApoA1, Phospholipids, and Cholate

During the infusions plasma total apoA1 concentration increased linearly by 22.9±2.7% and 43.3±8.3% (mean±SEM) at the low and high doses, respectively (Figure 2). Thereafter, plasma apoA1 decayed in an apparently bi-exponential manner, although there were insufficient data for mathematical modeling. Plasma total PL increased during the low and high doses by 74.4±4.2% and 129.6±3.5%, respectively, and then decreased at a rate which always exceeded that of apoA1 (Figure 2). The infused cholate was cleared rapidly (Figure 2).

Lipoprotein Lipids

Except where otherwise stated, in this and subsequent sections, the low-dose infusions had effects that were qualitatively similar to, but smaller than, those produced by the high dose. Changes in total cholesterol, UC, CE, TG, and PL in whole plasma, HDLs and non-HDL lipoproteins are shown in Figure 3. Almost all the increment in plasma total PL was in the HDLs. This was accompanied by a rise in HDL UC. After the infusion, HDL UC decreased in association with a rise in HDL CE. There was little effect on the non-HDL UC or non-HDL CE concentrations. During the 4 hours after the infusion, plasma TG increased on average by 46%, owing to a rise in non-HDL TG.

ApoA2, -B, and -C3

The low-dose infusions had no significant effects on plasma total apoA2 or apoB. However, the high-dose transiently decreased both apoA2 and apoB (Figure 4). The reduction of
apoB concentration was accompanied by a substantial increase in the non-HDL CE/apoB ratio (Figure 5). All infusions increased plasma total apoC3 (Figure 4).

Size Exclusion Chromatography
The distributions of apoA1, apoB, apoC3, UC, CE, and PL in size subclasses of lipoproteins were studied. After separation through Superdex 200 and 75, a major increase in the concentration of the smallest apoA1-containing particles was observed throughout each infusion (Figure 6). Thereafter, their concentration declined, although it was still above baseline at 24 hours. The major population of apoA1-containing particles increased in size and concentration.

Results obtained with Superose 6 appear in Figure 7. In the HDL subclasses the increments in UC and CE were initially greater in the region where the larger particles eluted. UC and CE in the region of large LDLs also increased, whereas those in the small LDL region decreased. The apoB profiles showed that apoB-containing particles of all sizes were reduced in number during the first 6 hours. The increase in plasma total apoC3 was confined to the HDL size range.

Crossed Immunoelectrophoresis
A striking increase in preβ-migrating apoA1 was observed during each infusion (Figure 8); thereafter, preβ apoA1 declined. The concentration of apoA1 in α-migrating HDLs was also increased, but to a proportionately lesser degree.

Experiments in Vitro
Several experiments were carried out in vitro to clarify the mechanisms of the changes observed in vivo. The extent to which the increase in HDL cholesterol in vivo might have reflected uptake of UC from erythrocytes and/or non-HDL lipoproteins was examined by incubating whole EDTA-blood at 37°C with or without the addition of apoA1/PC discs at Time 0. In the absence of discs the major changes during incubation were increases in the plasma total cholesterol and total CE concentrations, reflecting increases in both HDL CE and non-HDL CE (Figure 9). Addition of discs at Time 0 had no effect on the rise in plasma total cholesterol or total CE during subsequent incubation (except perhaps beyond 10 hours of incubation). This was documented at disc apoA1 concentrations of up to 50 mg/dL, about the average increment in plasma apoA1 achieved in vivo with the high-dose infusions. However, the distribution of CE between HDLs and non-HDLs was altered, such that the HDL CE/non-HDL CE ratio was higher in the presence of discs. These findings, obtained using PEG 6000 to separate lipoproteins, were confirmed in 1 experiment in which Superose 6 was used instead (data not shown).

In other experiments the effects of the discs on incubation-induced changes in size subclasses of apoA1-containing HDLs were studied, using HP-SEC through Superdex 200 and 75. In the absence of discs incubation of whole EDTA-blood at 37°C was associated first with a decrease in the concentration of the smallest particles, which had disappeared by 2 hours, followed by their reappearance and increase in concentration between 4 and 24 hours (Figure 10). In contrast, when discs were added at Time 0, the small apoA1-containing particles increased during the first 4 hours, followed by a progressive decline. Similar results were obtained when EDTA-plasma was incubated with or without discs (data not shown). In the
same experiments \( \text{pre}\beta \)- and \( \alpha \)-migrating apoA1s were measured by crossed immunoelectrophoresis. The results for \( \text{pre}\beta \) apoA1 paralleled those seen in the smallest apoA1 particles by HP-SEC (Figure 11).

In similar incubations of blood or plasma apoC3 was assayed in Superose 6 size subclasses. Addition of discs induced a transfer of apoC3 from non-HDLs to HDLs. This was evident within 15 minutes, and increased during further incubation up to 60 minutes (data not shown).

**Discussion**

The apoA1/PC discs used in this study were similar in size and composition to some intestinal and hepatogenous nascent HDLs.\(^{14,15,24,25}\) We have shown that they are good substrates for LCAT in vitro\(^{17}\) and that they promote cholesterol efflux from cultured cells.\(^{19}\) The increments in plasma total apoA1 during the infusions were compatible with the known distribution volume of endogenous HDL apoA1 in humans.\(^{26-30}\) Although there were insufficient time points for kinetic analysis, the rate of decline of total apoA1 after infusion was compatible with the known fractional catabolic rate (FCR) of endogenous HDL apoA1.\(^{26-30}\) Malmendier et al\(^{31}\) also found that the kinetics of radioiodinated apoA1 in apoA1/PC discs were essentially identical the kinetics of apoA1 in endogenous HDLs in humans. No evidence of an allergic, immunologic, or acute-phase response was seen, and no changes in routine clinical chemistry or hematology occurred. Therefore, it is reasonable to assume that the discs behaved in vivo like endogenous nascent HDLs.

Although the discs contained some cholate, this was rapidly cleared from plasma. The presence of some cholate has probably been a feature of most similar proteoliposomes used for in vitro and tissue culture work.\(^{5,31,40-42,50}\) We undertook no studies using an aqueous cholate infusion as a control. Because the cholate in the apoA1/PC disc preparations is bound to the particles, not in aqueous solution, the effects of such cholate infusions, if any, would not have been relevant.

**Figure 3.** Changes in the concentrations of total cholesterol, UC, CE, TG, and PL in whole plasma, HDLs and non-HDL lipoproteins in the 4 subjects given the high-dose infusion. Results (means±SEM) are the differences between the concentration at each time point and the baseline concentration; *\( P < 0.05 \). HDLs and non-HDL lipoproteins were separated by PEG. Baseline concentrations (mean±SEM, mmol/L): Whole plasma: total cholesterol, 4.3±0.25; UC, 1.2±0.07; CE, 3.1±0.20; TG, 1.1±0.15; PL, 2.5±0.04. HDL: total cholesterol, 0.75±0.10; UC, 0.09±0.02; CE, 0.66±0.08; TG, 0.03±0.01; PL, 0.98±0.05. Non-HDL lipoproteins: total cholesterol, 3.6±0.27; UC, 1.2±0.07; CE, 2.4±0.21; TG, 1.07±0.15; PL, 1.5±0.09.
The more rapid decline in plasma PL than in plasma apoA1 concentration after infusion presumably reflected the transfer of PLs to tissues and/or the actions of LCAT, HL, and other phospholipases. The increase in HDL UC during the infusions, the reciprocal changes in HDL UC and HDL CE after infusion, and the rise in non-HDL CE/apoB ratio were all consistent with increased uptake and esterification of UC by HDLs, followed by transfer of CEs to apoB-containing lipoproteins by CETP. This result contrasts with the failure of infused lipid-free apoA1 to increase HDL UC or HDL CE in humans.\textsuperscript{22} Theoretically, the UC entering the HDLs during the disc infusions could have come from several sources: other lipoproteins, erythrocytes, and/or fixed tissues. Other lipoproteins are unlikely to have been a major source, as non-HDL UC decreased only slightly (Figure 3), and such a mechanism could not explain the rise in plasma total cholesterol concentration that occurred.

To clarify the origin of the new UC entering the HDLs in vivo several experiments were carried out in vitro. In the absence of discs, incubation of whole EDTA-blood at 37°C increased the concentrations of HDL CE and non-HDL CE, without any major decrease in UC. This phenomenon is well recognized and is attributable mostly to the transfer of UC from erythrocytes to HDLs, driven by the LCAT reaction.\textsuperscript{32} Although addition of discs to blood at Time 0 augmented the subsequent incubation-induced increase in HDL CE, it tended also to reduce that in non-HDL CE, and had no significant effect on the rise in plasma total cholesterol or total CE concentration. This presumably reflected a decrease in the rate of transfer of newly formed CEs from HDLs to other lipoproteins, with no change in the efflux of UC from erythrocytes. Bruce et al\textsuperscript{33} have shown that the binding affinity of CETP for HDLs is influenced by their size, shape, and lipid composition. Thus, the rise in HDL cholesterol observed in vivo must have reflected an increase in the efflux of UC from fixed tissues, with little or no contribution from erythrocytes. It is not possible from these results to determine which fixed tissues were the sources of the new cholesterol that appeared in the plasma HDLs.

The disc infusions also had significant effects on apoB-containing lipoproteins. The rise in plasma TG concentration was similar in magnitude to, but about 4 hours later than, that seen with lipid-free apoA1 infusion, when it was attributed to inhibition of lipolysis of TGRLs.\textsuperscript{22} The decrease in plasma total apoB that occurred in the present study, but not after lipid-free apoA1,\textsuperscript{22} involved all classes of apoB-containing particles. This might have been secondary to an increase in
the activity of LDL receptors consequent on reduction of intracellular UC, and/or to an increase in the binding of VLDL remnants to hepatic receptors secondary to the transfer of apoC3 to HDLs which the discs induced. The rise in the plasma concentration of apoC3 (catabolized mostly as a component of TGRLs and their remnants) may also have been a consequence of its transfer to HDLs. The mechanism of the decline in plasma apoA2 concentration is not apparent. It is unlikely to have resulted from release of apoA2 from endogenous HDLs, because apoA2 is more resistant to displacement from HDLs than is apoA1.

In all subjects infusion of apoA1/PC discs initially raised the plasma concentration of preβ-migrating apoA1. After the infusions, preβ apoA1 declined, accompanied by an increase in α-migrating apoA1. Crossed immunoelectropherograms of the discs before infusion showed a major peak whose mobility was slightly faster than that of normal plasma preβ apoA1, and a minor peak with mobility between those of preβ and α-apoA1 in plasma (Figure 1). This suggested that the discs were rapidly remodeled in vivo to generate physiological preβ HDLs. This is compatible with the results of our preliminary electron microscopic examinations of plasma HDLs (d = 1.063 to 1.21 g/mL), which showed only occasional discoidal particles after infusion (results not shown). It is also supported by the results of our Superdex HP-SEC separations, which showed that the concentration of the smallest apoA1-containing particles increased during the infusions. As described under Methods, one of us (M.N.N.) has shown that the composition, physical properties, electrophoretic mobility, and metabolic behavior of these particles are similar to those of the preβ1 HDLs (lipid-poor apoA1).3,6,37–39 The infused disc preparation contained no such particles (Figure 1). Although it cannot be completely excluded, it seems unlikely that LCAT played a major role in this process, as the rise in the concentration of small preβ HDLs preceded that in plasma HDL CE concentration (Figures 3, 6, and 8). Our incubation experiments in vitro showed that the generation of small preβ HDLs by the discs was not dependent on the liver or other organs or on active lipolysis; nor did it require erythrocytes. One possibility is that it resulted from fusion of the discs with endogenous spheroidal α-HDLs, in a process similar to that observed by others when reconstituted discoidal lipoproteins or PL liposomes were incubated with isolated plasma HDLs in vitro. The decline in small preβ HDLs and rise in large α-HDLs which occurred after infusion in vivo can be attributed to the conversion of the former to the latter through the action of LCAT.6,38,39 Some small preβ HDLs may have been metabolized by the kidney or have moved into the extravascular space.

One of us (M.N.N.) has shown that the small HDLs isolated by Superdex 200/75 HP-SEC (fractions 41 to 55) contain no PL that is detectable by our enzymatic assay. Based on the known sensitivity of this assay, these smallest particles must each contain <5 molecules of PL. Thus, some of them may be lipid-free apoA1, which is well known to have preβ mobility (eg, Figure 1). Thus, we are unable to determine from our results whether the disc infusions produced lipid-poor apoA1-containing particles (preβ1 HDLs)
directly, or induced the release of lipid-free apoA1, as has been reported from studies in vitro. Such lipid-free apoA1 presumably would then have associated with cell membrane PLs to form preβ HDLs. Because these and similar apoA1/PC discs have been shown to promote the release of cholesterol from cultured cells, it is likely that circulating discs removed UC from any cells that were directly exposed to them. However, as the discs were evidently short-lived and were rapidly remodeled in the circulation, this probably applied only to those tissues (eg, liver, spleen) that have an open endothelial architecture through which the discs could have readily passed. In most peripheral tissues, however, the principal acceptors of cell-derived UC are more likely to have been the small preβ HDLs, which on charge and size considerations would have crossed normal capillary endothelium more readily than the discs.

This is the first study in which the effects of reconstituted discoidal HDLs on lipoprotein subclasses have been studied in normal humans. When Carlson infused recombinant pro-apoA1/PC discs into 4 men (3 of whom had plasma TG >5 mmol/L), HDL cholesterol increased on average by 0.14 mmol/L. However, interpretation of this result was complicated because plasma TG also decreased by 1.35 mmol/L. Kuivenhoven et al reported that IV apoA1/PC discs increased plasma total cholesterol in 3 subjects with Tangier disease. We have previously reported that IV infusion of lipid-free apoA1 failed to increase HDL cholesterol in humans. However, because the infusions acutely raised plasma TG, no conclusions could be drawn from those data about the role of lipid-free apoA1 in RCT. We and others have shown that IV Intralipid (which contains both TG/PL particles and PL liposomes) increased plasma total UC in humans, but reduced HDL CE, and had no effect on plasma total CE. Neary et al reported that Intralipid also reduced plasma preβ apoA1 concentration in humans. Koizumi et al found that apo-HDL/PC complexes increased HDL cholesterol and reduced non-HDL cholesterol in rabbits, whereas PL liposomes (Lipostabil) decreased both. In the same species Rodrigueza et al showed that large unilamellar PL liposomes increased VLDL UC and LDL UC, but had no effect on plasma total CE. Small PL liposomes raised HDL CE, but their major effect was on LDL UC. Thus, Intralipid and PL liposomes probably increase the nonspecific diffusional transfer of UC from cell membranes to plasma, but are unlikely to mimic the effects of nascent HDLs.

In summary, our findings suggest that infusion of apoA1/PC discs increased the mobilization of UC from tissues to

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**Figure 9.** Changes in the concentrations of UC, CE, and total cholesterol in whole plasma, HDL, and non-HDL lipoproteins during incubation of whole EDTA-blood (1 mg/mL) at 37°C in vitro, in the absence of discs (○; apoA1 conc, 100 mg/dL) and in the presence of discs at apoA1 concentrations of 12.5 mg/dL (△) and 25 mg/dL (□). Results are the differences from the preincubation values (Time 0) in the absence of discs. HDL and non-HDL lipoproteins were separated by PEG. Results from one representative experiment.
plasma HDLs in humans. This probably involved 2 independent processes: (1) nonspecific transfer of UC to the discs from those cells (e.g., in liver and spleen) which, on account of a fenestrated local endothelium, were directly exposed to the discs; and (2) stimulation of the specific apoA1 dependent pathway in other peripheral tissues, secondary to the generation of small lipid-poor preβ1 HDLs (either directly or after the release of lipid-free apoA1) and

Figure 10. Concentrations of apoA1 in size subclasses of lipoproteins, separated by HP-SEC of plasma through Superdex 200 and 75 in series, during incubation of whole EDTA-blood (1 mg/dL) at 37°C in vitro in the absence (control) or presence of discs (50 mg/dL). The Time 0 results in the presence of discs were obtained by centrifuging blood (4°C, 0.5 minutes, 12,000 g) 1 minute after addition of discs, and then immediately injecting an aliquot of plasma into the column. The apoA1 profile of the infused discs (shaded region) is shown for comparison. Results from 1 representative experiment.

Figure 11. Preβ- and α-migrating apoA1-containing particles in plasma during incubation of whole EDTA-blood (1 mg/mL) at 37°C in vitro in the absence (control) or presence of discs. Same experiment as in Figure 9. Crossed immunoelectrophoresis was performed as described under Methods.
their transfer across capillary endothelia into the extravascular space. Esterification of tissue-derived UC by LCAT led to the conversion of the discs and preβ HDLs to CE-rich α-HDLs, and to the transfer of some CE to apoB-containing particles. Thus, nascent HDL secretion may have a significant impact on RCT in humans. More work will be needed to fully understand the mechanism(s) of preβ, HDL production, to identify the tissues affected, and to determine the extent to which the mobilized cholesterol is eliminated via the liver.

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References


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