Fresh plasma from control (C) and hypercholesterolemic (HC) cynomolgus monkeys was analyzed by agarose electrophoresis-immunoblotting with antibody to cynomolgus monkey apolipoprotein (apo) A-I. Two bands were evident on the autoradiogram: an α-migrating band (high density lipoprotein) and a β-migrating band that comigrated exactly with cynomolgus monkey low density lipoprotein (LDL). The presence of β-migrating apo A-I in the plasma of these monkeys was confirmed by Geon-Pevikon preparative electrophoresis, crossed immunoelectrophoresis, and isotope dilution studies in which radiolabeled apo A-I was found to equilibrate also with α and β-migrating pools of apo A-I in the plasma. Subfractionation of C and HC plasma by agarose column chromatography (Bio-Gel A-0.5M and A-15M) followed by agarose electrophoresis-immunoblotting indicated that the β-migrating apo A-I in C was relatively homogeneous and eluted with proteins of Mr ~50 kD [apo A-I(50 kD)], whereas two β-migrating fractions were identified in HC, one that eluted with the 50-kD proteins, and the other that eluted in the LDL Mr range [apo A-I(LDL)]. The apo A-I(LDL) was precipitated by antibody to cynomolgus monkey apo B. The apo A-I(50 kD) accounted for 5±1% (mean±SD) of the plasma apo A-I in C plasma, and 15±7% in HC plasma. No apo A-I(LDL) was detected in C plasma, but that fraction accounted for 9±7% of the apo A-I in HC plasma. These data establish the presence of multiple pools of apo A-I in the cynomolgus monkey, which must be taken into consideration in any comprehensive model of apo A-I metabolism in this species. (Arteriosclerosis 9:470-478, July/August 1989)

Several years ago, Schonfeld et al. reported that significant quantities of plasma apolipoprotein (apo) A-I were associated with particles of molecular weight 50 k Daltons (kD). Subsequent studies, in which that pool has been characterized by other methods, have shown that those particles migrate pre-β during agarose electrophoresis, and have confirmed Schonfeld's original observation that the size of that apo A-I fraction can vary substantially in different disease states. Cynomolgus monkey apo A-I is a 28-kD apolipoprotein with an amino acid sequence almost identical to that of the human. As in the human, the majority of the plasma apo A-I is associated with high density lipoprotein (HDL) in fasted cynomolgus monkeys and migrates with the α lipoproteins during agarose electrophoresis. We report here on studies in which we identified and partially characterized β-migrating pools of apo A-I in the plasma from normal and hypercholesterolemic monkeys and showed that the severity of the hypercholesterolemia has a marked effect on the size and distribution of the β-migrating apo A-I.

Methods

Animals and Diets

Adult male and female cynomolgus monkeys obtained from the Upjohn colony were used for these studies. The animals had consumed only monkey chow (Ralston Purina Company, St. Louis, MO) prior to the initiation of these studies and had otherwise unremarkable medical histories. Some of the monkeys were fed a previously described high-cholesterol diet rich in saturated fat (P/S ratio=0.36) and containing added crystalline cholesterol.

Apoprotein Quantification

The blood for lipoprotein isolation or immunoassay was collected in tubes containing ethylenediaminetetraacetic acid (EDTA) (1 mg/ml) and sodium azide (1 mg/ml). When serum was required, the EDTA was added after the serum was obtained. The samples were kept at 4°C until use. The electroimmunoassay (EIA) was performed exactly as described previously. Ultracentrifugation of the samples was avoided when possible; however, in those instances when it was necessary, it was performed essentially as described previously. The lower limit of detection of that assay was 0.07 mg/dl. Ultracentrifugation of the samples was avoided when possible; however, in those instances when it was necessary, it was performed essentially as described previously. Agarose column chromatography with Bio-Gel A-0.5M and A-15M (BioRad, Richmond, CA) was also carried out as described previously.

Abbreviations: LDL = apo B-containing lipoproteins that migrate β during agarose electrophoresis and elute from a 4% agarose column with lipoproteins of d=1.006 to 1.063 g/ml; HDL = apo A-I-containing lipoproteins that migrate α during agarose electrophoresis; Apo A-I(LDL) = apo A-I that elutes from a 4% agarose column with LDL and is precipitated with antibody to apo B; Apo A-I(HDL) = apo A-I that is associated with HDL as defined above; Apo A-I(50 kD) = apo A-I that migrates β during agarose electrophoresis, is not precipitated from plasma with antibody to apo B, and elutes from 10% agarose columns with 50-kD proteins.
Agarose Electrophoresis Immunoblotting

Agarose electrophoresis was performed with a Multiphor horizontal electrophoresis unit (Pharmacia LKB Biotecnology Incorporated, Piscatway, NJ) essentially as described by Noble,12 except that the gel was 0.5% agarose, and Tris-tricine buffer (pH 8.6) was used. Cynomolgus monkey lipoproteins migrate slightly faster in this system than do their human counterparts. The terms "α-" and "β-migrating," as used here, refer to the points at which cynomolgus monkey HDL and low density lipoprotein (LDL), respectively, migrate in this system.

In some studies, the redistribution of radiolabeled, free apo A-I among the α- and β-migrating lipoproteins was followed. Once the electrophoresis was complete, those apo agelsones were cut into strips ranging from the origin through the albumin zone, and the radioactivity in each strip was measured by gamma counting. Recovery of the radioactivity applied to the gel averaged 95±5%.

Transfer of proteins from the agarose gel to nitrocellulose paper was accomplished by pressure blotting. A 4×8-inch sheet of nitrocellulose paper (0.45-μm pore size; Schleicher & Schuell, Keene, NH) was wetted with distilled water and was laid atop the agarose gel, care being taken to ensure that no air bubbles formed between the gel and the paper. The nitrocellulose paper was then covered with three 4×8-inch sheets of #470 filter paper (Whatman, St. Louis, MO), and Carnation nonfat dry milk (5%) was added. The weight was left in place for at least 3 hours, but usually overnight, after which the nitrocellulose paper was removed and dried in a 50 M Tris-HCl buffer (pH 8.0) containing CaCl₂ (2 mM), NaCl (80 mM), Nonidet P-40 (0.2%; BDH Chemicals Limited, Poole, England), anti-foam A concentrate (0.01%; Sigma Chemical Company, St. Louis, MO), and Carnation nonfat dry milk (5%) to saturate the remaining protein binding sites. (This buffer will hereafter be referred to as the blocking buffer.) That buffer was then swirled for 1 hour at room temperature in fresh blocking buffer containing 3%. The radiolabeled antibody was then swirled for 1 hour at room temperature in fresh blocking buffer containing 125I-labeled donkey antirabbit IgG (Amersham, Arlington Heights, IL) for 3 hours at room temperature. The radiolabeled antibody was diluted with blocking buffer so that the concentration of the radioactivity was 600 000 cpm/ml. The nitrocellulose paper was then washed three times with swirling by fresh buffer devoid of antiserum for 15 minutes each time. It was then washed in fresh blocking buffer containing 125I-labeled donkey antirabbit IgG (Amersham, Arlington Heights, IL) for 3 hours at room temperature. The radiolabeled antibody was diluted with blocking buffer so that the concentration of the radioactivity was 600 000 cpm/ml. The nitrocellulose paper was then washed three times with swirling by fresh buffer devoid of antiserum for 15 minutes each time. It was then washed in fresh blocking buffer containing 125I-labeled donkey antirabbit IgG (Amersham, Arlington Heights, IL) for 3 hours at room temperature. The radiolabeled antibody was diluted with blocking buffer so that the concentration of the radioactivity was 600 000 cpm/ml. The nitrocellulose paper was then washed three times with swirling by fresh buffer devoid of antiserum for 15 minutes each time. It was then washed in fresh blocking buffer containing 125I-labeled donkey antirabbit IgG (Amersham, Arlington Heights, IL) for 3 hours at room temperature. The radiolabeled antibody was diluted with blocking buffer so that the concentration of the radioactivity was 600 000 cpm/ml. The nitrocellulose paper was then washed three times with swirling by fresh buffer devoid of antiserum for 15 minutes each time. It was then washed in fresh blocking buffer containing 125I-labeled donkey antirabbit IgG (Amersham, Arlington Heights, IL) for 3 hours at room temperature. The radiolabeled antibody was diluted with blocking buffer so that the concentration of the radioactivity was 600 000 cpm/ml. The nitrocellulose paper was then washed three times with swirling by fresh buffer devoid of antiserum for 15 minutes each time. It was then washed in fresh blocking buffer containing 125I-labeled donkey antirabbit IgG (Amersham, Arlington Heights, IL) for 3 hours at room temperature. The radiolabeled antibody was diluted with blocking buffer so that the concentration of the radioactivity was 600 000 cpm/ml. The nitrocellulose paper was then washed three times with sw
Figure 1. Apolipoprotein (apo) A-I distribution in plasma as measured by immunoblotting after agarose electrophoresis. Plasma from severely hypercholesterolemic (lane A), moderately hypercholesterolemic (lane B), and control monkeys (lane C) was analyzed by the agarose electrophoresis-immunoblotting method using antibody to cynomolgus monkey apo A-I. There was a distinct β-migrating band of activity in each case. The β-migrating band comigrated exactly with cynomolgus monkey low density lipoprotein.

Figure 2. Agarose electrophoresis of serum fractions purified by Geon-Pevikon preparative electrophoresis. Radiolabeled apo-lipoprotein (apo) A-I was added to the serum and used to follow apo A-I distribution after electrophoresis. The block was separated into five zones: Zone 1 (not shown) ranged from the origin to just in front of the β-migrating proteins. Zone 2 consisted of the entire β-band and was subdivided into 2A (the slower-migrating β-fraction) and 2B (the faster-migrating β-fraction). Zones 3 and 4 consisted of the pre-β- and α-migrating proteins.

Figure 3. Electroimmunoassay of apolipoprotein (apo) A-I in protein fractions isolated by Geon-Pevikon electrophoresis. Aliquots of each of the fractions shown were analyzed by electroimmunoassay. Note the distinct rocket present in Zone 2A, confirming that β-migrating apo A-I was present in the serum of these monkeys.

Geon-Pevikon Preparative Electrophoresis

Geon-Pevikon preparative electrophoresis was performed essentially as described by Mahley and Weisgraber. Approximately 10 ml of fresh plasma was loaded onto the Geon-Pevikon block, and the proteins were electrophoresed for 18 hours. The block was then subdivided into five zones: the origin (Zone 1), the β-migrating fraction (Zone 2), and the pre-β- and α-migrating fractions (Zones 3 and 4). The β-migrating zone was further subdivided into Zone 2A (that portion closest to the origin) and Zone 2B (the β-migrating fraction farthest from the origin). Previous experience with this method had shown that the leading edge of the β zone sometimes had small amounts of proteins that migrated α during agarose electrophoresis. Since the purpose of this particular study was to show immunochemically that there was β-migrating apo A-I in the plasma of these animals, it was important that no α-lipoprotein contamination be present. Subsequent evaluation of Zone 2A by agarose electrophoresis showed that that zone was, in fact, devoid of β-migrating proteins.

Calculations

The total plasma apo A-I concentration (mg/dl) was determined as described in the immunoassay procedure above. The apo A-I(LDL) concentration was determined as the difference in apo A-I concentration of the plasma before and after immunoprecipitation of apo B-containing lipoproteins. To determine the apo A-I((50 kD) concentration, the plasma was analyzed by X-EIA before and after immunoprecipitation of apo B-containing lipoproteins, and the ratio of the A-I((50 kD): A-I(LDL) pools was determined. The absolute mass of the A-I((50 kD) was then taken as the product of that ratio and the absolute mass of A-I(LDL) in plasma as determined by EIA after immunoprecipitation of apo B-containing lipoproteins. This approach allowed us to quantify the apo A-I((50 kD) and apo A-I((50 kD) fractions without having to make a direct comparison of X-EIA and EIA rockets.

Results

When the apo A-I distribution among the plasma lipoproteins of the cynomolgus monkey was analyzed by agarose electrophoresis immunoblotting, the antibody identified two distinct apo A-I-containing fractions (Figure 1): one that migrated with the α lipoproteins (HDL); and a
second that migrated with the \( \beta \) lipoproteins. The \( \beta \)-migrating fraction was often faint in control monkeys (Figure 1C), but was prominent in hypercholesterolemic monkeys and appeared to increase in intensity as the hypercholesterolemia progressed (Figures 1A and 1B).

To examine the possibility that the \( \beta \)-migrating radioactivity represented some type of artifactual or nonspecific binding of the antibody, fresh plasma from a hypercholesterolemic monkey was fractionated by Geon-Pevikon preparative electrophoresis, the \( \alpha \) and \( \beta \)-migrating zones were cut from the block, and the apo A-I content of each fraction was measured by EIA. The results of those studies are shown in Figures 2 and 3. Zone 2A was found to be completely free of any \( \alpha \)-migrating proteins, yet a clear rocket was obtained from that fraction, confirming that apo A-I was, in fact, present in the \( \beta \)-lipoprotein fraction.

Previous studies had suggested that some apo A-I was associated with LDL in hypercholesterolemic monkeys. To determine if that accounted for the \( \beta \)-migrating apo A-I evident in Figure 1, the apo A-I distribution between apo B-containing lipoproteins and those devoid of apo B was determined by immunoprecipitation by using anti-cynomolgus monkey apo B and antihuman apo A-I. Those studies showed that 9±7\% (mean±SD, \( N=8 \)) of the plasma apo A-I in hypercholesterolemic monkeys was precipitated by anti-apo B; and 25±15\% (\( N=5 \)) of the apo B was precipitated by anti-apo A-I. Thus, a significant portion of the \( \beta \)-migrating apo A-I appears to be associated with apo B-containing lipoproteins in hypercholesterolemic monkeys. No apo B-associated apo A-I was detected in the control monkeys.

The apo A-I distribution in control and hypercholesterolemic monkeys was also evaluated by molecular sieve chromatography. The fractions so obtained were analyzed by EIA and agarose electrophoresis-immunoblotting. The results of those studies are shown in Figures 4 to 7. Figure 4 shows a comparison of the apo A-I distribution when plasma from a control and a hypercholesterolemic monkey was fractionated on 4% agarose columns. Those data indicate that the hypercholesterolemic monkey plasma did, in fact, have a significant percentage of the total apo A-I elute with apo B-containing lipoproteins. They
also showed that a substantial fraction of the apo A-I in the hypercholesterolemic monkey plasma eluted with or after albumin, suggesting that, in general, it was associated with smaller particles than in the control monkey.

When those same column fractions were analyzed by agarose electrophoresis-immunoblotting (Figure 5), two β-migrating fractions were evident in hypercholesterolemic plasma: one that eluted from the column with the apo B-containing lipoproteins, and one that eluted with or slightly before albumin (in the region that HDL would be expected to elute). A low molecular weight, β-migrating pool of apo A-I was also evident in the column fractions from control monkeys (data not shown); however, there was no detectable LDL-associated apo A-I in the control monkeys whether measured by EIA or immunoblotting of the column fractions. Therefore, the β-migrating fraction evident in control monkey plasma (Figure 1C) can be completely accounted for as the low molecular weight entity.

To estimate the molecular weight of the β-migrating apo A-I fraction eluting from the 4% agarose column between 360 and 480 ml, (Figure 5) hypercholesterolemic plasma was passed through a 10% agarose column. The LDL-associated apo A-I eluted in the void volume. The distribution of the remaining apo A-I is shown in Figure 6. Note that a significant fraction of that apo A-I eluted with or after albumin. Figure 7 shows the electrophoretic mobility of the various apo A-I-containing fractions as measured by agarose electrophoresis-immunoblotting. The mean molecular weight of that β-migrating fraction was estimated to be approximately 50 kD by using molecular weight standards passed over the same column and was not different when control and hypercholesterolemic monkeys were compared.

When the same column fractions shown in Figure 6 were transferred to nitrocellulose paper and probed with anti-cynomolgus monkey apo A-II, no β-migrating radioactivity was detected on the autoradiogram. Thus, those lipoproteins were devoid of apo A-II.

The density distribution of the various apo A-I-containing fractions was also evaluated; however, when the d<1.225 g/ml fraction from a given monkey’s plasma was passed through the 4% agarose column, the elution profiles of both the apo A-I and apo B were different than when the same animal’s plasma was passed through the column. In every instance, when the d<1.225 g/ml fraction was passed through the column, the elution profiles of both apo B and apo A-I were significantly shifted to the left (toward the void volume) of those obtained when plasma was passed over the column. In addition, the concentration of the β-migrating apo A-I in the “lipoprotein deficient plasma” (d>1.225 g/ml) was 140% to 150% of that in the uncentrifuged plasma, suggesting that β-migrating apo A-I could be created by the centrifugation process (that may represent free apo A-I dislodged from the lipoproteins). Thus, ultracentrifugation caused a severe distortion of the apo A-I profile in these hypercholesterolemic monkeys. Nonetheless, certain information regarding the density distribution of these apo A-I-containing fractions was obtained. First, density gradient ultracentrifugation of the plasma indicated that the distribution of the apo A-I(LDL) was the same as that of the apo B. Therefore, the apo A-I(LDL) are probably not a distinct subfraction of the LDL, but were evenly dispersed throughout that density frac-
Table 1. Apolipoprotein A-I Distribution among Plasma Pools in Control and Cholesterol-fed Monkeys

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Total</th>
<th>Alpha</th>
<th>Beta</th>
<th>LDL</th>
<th>50 kD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>147</td>
<td>139</td>
<td>8</td>
<td>ND*</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>(33)</td>
<td>(31)</td>
<td>(2)</td>
<td></td>
<td></td>
<td>(2)</td>
</tr>
<tr>
<td>Cholesterol-fed</td>
<td>5</td>
<td>71</td>
<td>52</td>
<td>19</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>(31)</td>
<td>(22)</td>
<td>(8)</td>
<td>(3)</td>
<td>(5)</td>
<td></td>
</tr>
</tbody>
</table>

The values are given as mg/dl, means (SD). The values of the cholesterol-fed group were significantly different (p<0.05) from those of the control group for every column except that headed 50 kD, for which p=0.15.

*Not detected.

LDL = low density lipoprotein.

A-I(50 kD) are small lipoproteins, and not dimers of free apo A-I.

With the existence of β-migrating apo A-I particles established, the question arose as to what portion of the total apo A-I they represented. Figure 8 shows the results obtained when plasma from a single monkey was analyzed by X-EIA before and at several times after he began to consume a high-cholesterol diet. Note that the β-migrating fraction increased substantially as the monkey became hypercholesterolemic. This supports the perception acquired from viewing the immunoblots (Figure 1).

Table 1 compares the mean apo A-I levels and distribution among the various fractions between control and hypercholesterolemic monkeys. Those data indicate that the total β-migrating apo A-I increases about twofold in the hypercholesterolemic monkeys and that the majority of that increase is due to the increase in apo A-I(LDL). The apo A-I(50 kD) may also increase slightly, but that change was not statistically significant. However, because in the hypercholesterolemic monkeys the β-migrating fraction increased while the total plasma apo A-I levels decreased, the β-migrating fraction became a substantial percentage of the total, averaging 27% but ranging as high as 50%.

Thus, these β-migrating pools are not a minor component in the hypercholesterolemic monkeys.

Apo A-I is thought to be in a dynamic equilibrium among the lipoproteins, and that interchange is thought to take place rapidly. To determine if the various apo A-I pools identified in the hypercholesterolemic monkeys were, in fact, in equilibrium, free apo A-I was radiolabeled and mixed with fresh plasma at 37°C for 1 hour, and the LDL and HDL were isolated by agarose (4%) column chromatography followed by ultracentrifugation. Each of those fractions (free apo A-I, LDL, or HDL, now containing radiolabeled apo A-I) was then added to fresh plasma from control and hypercholesterolemic monkeys, and the distribution of the labeled apo A-I among the lipoproteins measured after 1 hour at 37°C in the absence of LCAT inhibitors. Those data indicated that no matter which apo A-I-containing fraction was added to the plasma, radioactivity from that fraction could be detected in all of the other fractions identified to date.

Figure 9 shows the curve describing the movement of label into α-migrating particles when 125I-labeled, free apo A-I was added to plasma from control and hypercholesterolemic monkeys. Note that in both control and
Figure 9. Equilibration of free apolipoprotein (apo) A-I with α-lipoproteins in vitro. Purified cynomolgus monkey apo A-I was radiolabeled and added to control (○) and hypercholesterolemic (●) monkey plasma and was incubated at 37°C for the times indicated. The fraction (%) of the dose recovered in the α-lipoproteins (mean and range, n=2) was determined by agarose electrophoresis of that plasma. For the two hypercholesterolemic monkeys, 88% and 84% of the apo A-I mass migrated α; for the control monkeys, 94% and 95% migrated α. In every instance, the remainder of the mass and radioactivity migrated β.

hypercholesterolemic plasma, a plateau was reached within 20 minutes, and that the fraction of that isotope moving into the α-migrating fraction was greater than 95% in the control animals, but only 80% to 85% in the hypercholesterolemic monkeys. X-EIA indicated that β-migrating apo A-I mass averaged 5% of the total plasma apo A-I in the control monkeys and 16% of the total apo A-I mass in the hypercholesterolemic plasma. Thus, the isotope distribution approached that of the mass within 20 minutes in vitro.

We also followed redistribution of the isotope in vivo, after radiolabeled, free apo A-I was administered intravenously to control and hypercholesterolemic cynomolgus monkeys. The data for two hypercholesterolemic monkeys are shown in Figure 10, and indicate that 4 to 8 hours was required for complete equilibration of the radiolabeled apo A-I. However, once equilibrium was reached, the isotope distribution between the α- and β-migrating apo A-I fractions stayed relatively constant through at least 96 hours, and the isotope and mass distribution were similar (Figure 11).

Discussion

During a series of studies that were designed to measure apo A-I turnover in cynomolgus monkeys, we consistently observed β-migrating radioactivity in the plasma of both control and hypercholesterolemic monkeys for extended periods after 125I-labeled apo A-I had been administered intravenously. Since purified cynomolgus monkey apo A-I migrates with the β-lipoproteins during agarose electrophoresis, it was not clear whether the β-migrating radioactivity that we observed in the plasma...
samples was some of the purified apo A-I that had been injected (which for some reason was not taken up completely by the HDL), or whether it was administered radioactivity that was tracing an endogenous pool of \( \beta \)-migrating apo A-I. The studies described here were undertaken to determine if such a pool existed in the cynomolgus monkey, and if so, to characterize its size, lipoprotein distribution, and response to a diet-induced hypercholesterolemia.

A \( \beta \)-migrating pool of apo A-I was identified in the plasma of both control and hypercholesterolemic monkeys by immunoblotting after agarose electrophoresis of plasma, by EIA of \( \beta \)-proteins separated from plasma by Geon-Pevikon electrophoresis, and by crossed immuno-electrophoresis of fresh plasma. In addition, we repeated the original studies (in which radiolabeled apo A-I was injected intravenously) and confirmed that a significant fraction of the plasma radioactivity migrated \( \beta \) both in vivo and in vitro. Thus, the existence of an endogenous pool of \( \beta \)-migrating apo A-I was demonstrated both immunologically and by isotope dilution.

The question then arose as to the identity of this plasma pool, that is, was it an endogenous pool of free apo A-I or some type of lipoprotein? These studies indicated that there are at least two forms of \( \beta \)-migrating apo A-I in the cynomolgus monkey. One form is associated with 50-kD particles and is present in both control and hypercholesterolemic monkeys; a second is associated with apo B-containing particles and was detected only in hypercholesterolemic monkeys.

It is not clear at this point whether all of the 50-kD particles are small lipoproteins, or if some are free apo A-I dimers. Apoproteins are known to aggregate in solution,\(^{17,18}\) and purified cynomolgus monkey apo A-I (dissolved in PBS) behaved exactly like the 50-kD entities in their mobility during agarose electrophoresis and their elution from Biogel A-0.5M columns. However, some apo A-I(50 kD) appeared to float during ultracentrifugation, and we did detect trace amounts of phospholipid and cholesterol in column fractions that appeared by immunoblotting to contain only \( \beta \)-migrating particles and that eluted from the columns with or after albumin. Thus, the apo A-I(50 kD) probably contain some lipid. In that regard, it is noteworthy that what may be an analogous apo A-I fraction has been identified in human plasma,\(^{1-6}\) and the human entity apparently does contain lipid;\(^{2,8}\) however, the human analogue of the monkey apo A-I(50 kD) appears to be somewhat larger (approximately 71 kD), and the difference in size in the human and monkey entities may be due to differences in their lipid content.

The monkey apo A-I(50 kD), like its human counterpart, contains no apo A-II.

The other \( \beta \)-migrating entity was clearly associated with LDL, in that it eluted from the 4% agarose column with LDL and was precipitated from solution with antibody to cynomolgus monkey apo B. The levels of the apo A-I(LDL) in hypercholesterolemic plasma varied substantially among the monkeys, from being nondetectable to accounting for as much as 20% of the total apo A-I. Interestingly, not all of the LDL in the hypercholesterolemic monkeys contained apo A-I, that is, essentially complete precipitation of the plasma apo A-I only removed an average of 25% of the apo B (the apo B concentration in those monkeys ranged from 150 to 250 mg/dl; the LDL cholesterol ranged from 450 to 700 mg/dl). Therefore, 75% of the LDL were apparently devoid of apo A-I in those monkeys. However, when we evaluated the density distribution of the apo A-I(LDL), we found it to be essentially the same as that of the apo B. Thus, we could not identify a distinct subfraction of the LDL that was rich in apo A-I as has been reported for apo E and apo A-II.\(^{11,19}\)

The function, if any, of the apo A-I in these diet-induced LDL is unknown. The particles with which it is associated may be chylomicron core remnants waiting to be cleared by the liver or, as suggested previously, hepatic LDL onto which apo A-I has adsorbed. We established that free apo A-I could associate with the LDL in hypercholesterolemic plasma by adding radiolabeled purified apo A-I to that plasma in vitro and recovering radioactivity in the LDL isolated by both column chromatography and ultracentrifugation. Furthermore, it should be noted that the HDL that are present in these animals are small, lipid-poor, apo A-I-rich particles that may be incapable of absorbing additional apo A-I. Thus, as newly synthesized apo A-I enters the plasma of hypercholesterolemic monkeys, it may move into the 50-kD pool or associate with the LDL, but it apparently does not progress through the HDL3-HDL2-HDL\(_2\) cascade as it does in some other cholesterol-fed species.\(^{20,21}\)

Given the existence of these various pools of apo A-I, the question arose as to whether they were, in fact, in equilibrium, and what potential effect they might have on the interpretation of apo A-I turnover studies. Zech et al.\(^{22}\) for example, proposed a model for apo A-I turnover in humans that contained two plasma pools of apo A-I that were not in equilibrium; that is, isotopic apo A-I in plasma pool 1 did not exchange with that in plasma pool 2. Our data with radiolabeled apo A-I as tracer indicated that apo A-I does exchange between the various pools in vitro, for when radiolabeled apo A-I was introduced into any one pool, it was subsequently detected in the others. Furthermore, when radiolabeled free apo A-I was administered intravenously to hypercholesterolemic monkeys, the distribution of the label approached that of the mass within a few hours, and remained constant for 72 hours thereafter, suggesting that the various apo A-I-containing pools were in equilibrium.

However, it is noteworthy that the specific activity of the apo A-I(HDL) pool was usually higher than that of the other pools (Figure 11); that is, although the isotope could be detected in each of the apo A-I-containing fractions, its affinity for the HDL appeared to be higher than that for the LDL and 50-kD fractions. Because of the variability of the methods, we could not measure the specific activity of the individual apo A-I pools with enough precision to show that those differences were statistically significant; nor are we confident that there are not subtle differences in the way the immunosay quantifies "free" apo A-I as compared to lipoprotein-associated apo A-I. Therefore, the slight differences in distribution of the isotope and mass may be an artifact of the methods. Nonetheless, the possibility that "nonexchangeable" apo A-I exists in these primates is
intriguing and bears further investigation, but unless such a pool can be clearly demonstrated to exist by biochemical methods and also shown to be a significant metabolic entity, one cannot assess that measurements of total plasma apo A-I specific activity over time do, in fact, give reliable estimates of apo A-I turnover in this animal model.

Acknowledgments

The authors acknowledge the excellent technical assistance of Phil Roehm and Robert Forbes and thank Jeanne Obreiter for her help with preparation of the manuscript.

References


Index Terms: apo A-I • β-migrating apo A-I • high density lipoproteins • cynomolgus monkeys • pre-β-migrating HDL
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doi: 10.1161/01.ATV.9.4.470

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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