Hepatic Lipase Promotes a Loss of Apolipoprotein A-I From Triglyceride-Enriched Human High Density Lipoproteins During Incubation In Vitro

Moira A. Clay, Harvey H. Newnham, and Philip J. Barter

Studies have been performed to investigate a possible mechanism to account for the low concentrations of apolipoprotein A-I (apo A-I) in subjects with hypertriglyceridemia. Incubation of human plasma in vitro with canine hepatic lipase resulted in the hydrolysis of approximately half the triglyceride in the high density lipoproteins (HDLs), but little change in the concentrations of other HDL constituents. However, when the plasma was supplemented with cholesteryl ester transfer protein and very low density lipoproteins to enrich the HDL with triglyceride, hepatic lipase promoted not only a significant reduction in HDL triglyceride acquired by the lipid transfer process but also an enhanced transfer of cholesteryl esters out of the particles. These changes were accompanied by a marked loss of apo A-I from HDL, which was demonstrated independently by ultracentrifugation, size-exclusion chromatography, and gradient gel-immunoblot analysis. The apo A-I lost from HDL was recovered in the “lipoprotein-free” fraction of plasma. The results of these studies indicate that primary reductions in the concentration of HDL core lipids in vitro are accompanied by a secondary loss of apo A-I from HDL. While recognizing the need for caution in any extrapolation from observations made in vitro to what may occur in vivo, these studies are nevertheless consistent with a proposition that the low concentration of apo A-I in subjects with hypertriglyceridemia is secondary to the reduced concentration of HDL core lipids in such subjects. (Arteriosclerosis and Thrombosis 1991;11:415–422)

Remodeling of high density lipoproteins (HDLs) within the plasma compartment is an important determinant of HDL concentration. The concentrations of the lipid constituents in HDL are regulated by the activities of several plasma factors; these include hepatic lipase (HL), the cholesteryl ester transfer protein (CETP), lecithin:cholesterol acyl transferase (LCAT), and lipoprotein lipase (LPL). There is only limited information concerning secondary changes to the concentration of HDL apolipoproteins (apos) that may accompany changes in HDL lipids. Several studies have reported an incorporation of apo A-IV into rat or human HDL in response to the effects of LCAT on the HDL fraction. In addition, we have recently demonstrated that the HL-mediated hydrolysis of triglyceride in rabbit HDL is accompanied by a dissociation of apo A-I from the HDL particles. That study took advantage of the fact that rabbit HDL are naturally enriched with triglyceride as a consequence of the low activity of HL and the high activity of CETP in this species. A logical extension of these findings was to investigate whether a similar phenomenon occurred in a species such as the human, in which the HDLs are not naturally enriched with triglyceride. We now report that incubation of human plasma with HL alone results in a much smaller loss of apo A-I from HDL than was observed previously in studies of rabbit plasma. However, the loss of apo A-I from human HDL was much greater in incubations in which the HDLs were enriched with triglyceride by the action of CETP in the presence of added very low density lipoproteins (VLDLs).

Methods

Plasma Samples

Blood was collected from healthy, normolipemic male subjects (plasma cholesterol 4.1–5.3 mM and triglyceride 0.5–1.1 mM), aged 20–30 years, who had...
Isolation and Assay of Lipases
LPL was purified from bovine milk, and HL was purified from canine postheparin plasma as described previously. Activity of the HL preparations was not inhibited by 1 M NaCl, but when incubated with a specific antibody raised against canine HL, more than 96% of the activity was lost, thus excluding significant contamination by LPL. HL and LPL activities were measured at high and low salt concentrations, respectively, using an artificially labeled triolein emulsion as described by Huttenen et al. Activities are expressed as units per milliliter (1 unit = 1 μmol free fatty acid released/hr).

Isolation and Assay of Cholesteryl Ester Transfer Protein
CETP was partially purified from citrated human plasma. Ammonium sulfate precipitation of proteins, ultracentrifugation to isolate the lipoprotein-free fraction of plasma, hydrophobic interaction chromatography on phenyl-Sepharose CL-4B, and cation-exchange chromatography on CM-52 cellulose was performed as described by Pattanaik et al., except that the plasma proteins were precipitated with ammonium sulfate between 35% and 55% saturation. Fractions containing CETP activity after cation-exchange chromatography were pooled, dialyzed against phosphate-buffered saline (PBS; 0.02 M phosphate buffer [pH 7.4] containing 0.15 M NaCl, 0.01% [wt/vol] Na2 EDTA, and 0.02% [wt/vol] NaN3), and stored at -70°C. Prolonged storage did not affect the activity of the protein. The activity of the CETP preparation was measured as the capacity of the sample to facilitate transfer of [3H]cholesteryl ester from low density lipoproteins (LDLs) to HDLs during an incubation at 37°C. Transfer activity was expressed as units per milliliter of CETP preparation, the number of units being the rate constant k for the transfer of LDL tracer to HDL per 3 hours using the formula of Pattanaik et al. These CETP preparations contained no LCAT activity.

Isolation of Very Low Density Lipoproteins
VLDLs were isolated from the plasma of hypertriglyceridemic subjects (plasma triglyceride 2–5 mM) as the supernatant after ultracentrifugation at a density of 1.006 g/ml at 178,000g for 16 hours.

Incubations and Processing of Samples
Human plasma was either kept at 4°C or supplemented by additions of HL, CETP, or VLDL and incubated at 37°C for 3–4 hours. All incubations contained 100 units heparin/ml incubation mixture to stabilize HL activity and were performed in sealed tubes in a shaking water bath. Incubations were terminated by placing the tubes on ice. After incubation, lipoproteins in the plasma fraction with a density of less than 1.25 g/ml were isolated by ultracentrifugation at 100,000 rpm for 16 hours in a Beckman TL-100 tabletop ultracentrifuge using a Beckman TLA-100.2 rotor, Beckman Instruments, Palo Alto, Calif. The lipoproteins were then subjected to chemical analyses as described below. An aliquot of the d = 1.25 g/ml supernatant was adjusted to d = 1.063 g/ml by drop dialysis for 30 minutes against a solution of KBr (d = 1.063 g/ml). The HDL fraction of 1.063<d<1.25 g/ml was then isolated as the infranatant after ultracentrifugation at 100,000 rpm for 16 hours as described above and subjected to chemical analyses. In some experiments, the incubation mixtures were subjected to either size-exclusion chromatography or gradient gel-immunoblot analysis without prior ultracentrifugation.

Size-Exclusion Chromatography
Lipoproteins were fractionated by size-exclusion chromatography without prior ultracentrifugation as previously described using columns of Superose 6 (preparation grade) and Superose 12 (preparation grade) connected in series (Pharmacia, LKB, Upsala, Sweden). Lipoproteins were eluted with 0.05 M Tris HCl (pH 7.4) containing 0.15 M NaCl at a flow rate of 10 ml/hr. Individual fractions (volume, 0.6 ml) were collected and subsequently analyzed as described below.

Chemical Analyses
All assays were performed on a Cobas-Bio centrifugal analyzer (Roche Diagnostics, Basel, Switzerland). Concentrations of total cholesterol, unesterified cholesterol, triglyceride, and phospholipid were measured using commercial kits as previously described. The concentration of esterified cholesterol was calculated as the difference between the concentrations of total and unesterified cholesterol. Concentrations of apo A-I and apo A-II were measured immunoturbidimetrically using antisera to human apo A-I or apo A-II raised in sheep (Boehringer Mannheim, Mannheim, F.R.G.) as previously described. The assay was standardized using appropriate dilutions of apo calibration serum from Boehringer Mannheim.

Immunoblot Analysis of Gradient Gels
After incubation, samples (without prior ultracentrifugation) were subjected to electrophoresis on 4–30% nondenaturing polyacrylamide gradient gels (Pharmacia). Electrophoresis was performed for 3,000 V-hours. The separated proteins were electrophoretically transferred to Zetabind nylon membranes (pore size, 0.45 μm; AMF Cuno, Melbourne, Australia) at 4°C in 0.025 M Tris, 0.2 M glycine, and 20% (vol/vol) methanol, pH 8.3, at 200 mA for 24 hours. After transfer, the membranes were fixed in PBS containing 0.03% (vol/vol) glutaraldehyde for 1 hour at room temperature. The subsequent procedure for detection of apo A-I on the nylon membranes was essentially as described by Clay et al.
TABLE 1. Incubation of Human Plasma Supplemented With Either No Additions, Added Cholesteryl Ester Transfer Protein, or Added Cholesteryl Ester Transfer Protein Plus Very Low Density Lipoprotein in the Absence or Presence of Hepatic Lipase: Concentration of High Density Lipoprotein

<table>
<thead>
<tr>
<th>Additions</th>
<th>Temperature (°C)</th>
<th>HL added</th>
<th>Concentration (µg/ml)</th>
<th>TG</th>
<th>CE</th>
<th>UC</th>
<th>PL</th>
<th>Apo A-I</th>
<th>Apo A-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>4</td>
<td>—</td>
<td>71 ± 1</td>
<td>71</td>
<td>555</td>
<td>339</td>
<td>74</td>
<td>47</td>
<td>574</td>
</tr>
<tr>
<td>None</td>
<td>37</td>
<td>—</td>
<td>85 ± 7</td>
<td>71</td>
<td>583</td>
<td>332</td>
<td>62</td>
<td>34</td>
<td>546</td>
</tr>
<tr>
<td>CETP</td>
<td>37</td>
<td>+</td>
<td>37 ± 3</td>
<td>34</td>
<td>574</td>
<td>315</td>
<td>62</td>
<td>34</td>
<td>512</td>
</tr>
<tr>
<td>CETP + VLDL</td>
<td>37</td>
<td>—</td>
<td>110 ± 8</td>
<td>85</td>
<td>589</td>
<td>322</td>
<td>63</td>
<td>37</td>
<td>603</td>
</tr>
<tr>
<td>CETP + VLDL</td>
<td>37</td>
<td>+</td>
<td>31 ± 4</td>
<td>47</td>
<td>529</td>
<td>332</td>
<td>57</td>
<td>24</td>
<td>498</td>
</tr>
</tbody>
</table>

Human plasma was either kept at 4°C or supplemented with saline, added cholesteryl ester transfer protein (CETP), or CETP plus very low density lipoprotein (VLDL) and incubated for 3 hours at 37°C in the absence or presence of hepatic lipase (HL). In experiment A (A), final volume of the incubation mixture was 850 µl, which included 300 µl plasma (cholesterol, 4.1 mM; triglyceride [TG], 0.7 mM) supplemented with saline (0.9% wt/vol), 3.6 units CETP, and 1,676 nmoles VLDL TG or 38 units HL. In experiment B (B), final volume of the incubation mixture was 950 µl, which included 280 µl plasma (cholesterol, 5.3 mM; TG, 1.0 mM) supplemented with saline (0.9% wt/vol), 3.4 units CETP, and 1,779 nmoles VLDL TG or 109 units HL. All incubations contained heparin (100 units/ml incubation mixture). After incubation, high density lipoproteins (HDLs) were isolated by ultracentrifugation as the 1.063<d<1.25 g/ml fraction and analyzed as described in "Methods." Concentrations are expressed per milliliter plasma.

Statistical Analyses

All statistical analyses were performed using Student's t test for paired samples to evaluate whether the mean values for incubation in the absence of HL and incubation in the presence of HL were significantly different. The accepted level of significance was taken to be p<0.05.

Results

Effect of Incubation on Concentration of High Density Lipoprotein Constituents: High Density Lipoprotein Isolated by Ultracentrifugation (Tables 1 and 2)

Human plasma containing either no additions, added CETP, or added CETP plus VLDL was incubated for 3 hours at 37°C in the absence or presence of HL. After incubation, the HDLs were isolated ultracentrifugally as the fraction of 1.063<d<1.25 g/ml fraction and analyzed as described in "Methods." Concentrations are expressed per milliliter plasma.

TABLE 2. Incubation of Human Plasma Supplemented With Cholesteryl Ester Transfer Protein Plus Very Low Density Lipoprotein in the Absence or Presence of Hepatic Lipase: Average Concentration of High Density Lipoprotein Constituents From Five Different Subjects

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>HL added</th>
<th>Concentration (µg/ml)</th>
<th>TG</th>
<th>CE</th>
<th>UC</th>
<th>PL</th>
<th>Apo A-I</th>
<th>Apo A-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>—</td>
<td>66±11</td>
<td>467±98</td>
<td>74±24</td>
<td>525±97</td>
<td>513±93</td>
<td>221±22</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>—</td>
<td>240±85</td>
<td>385±76</td>
<td>73±30</td>
<td>510±76</td>
<td>537±75</td>
<td>229±28</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>+</td>
<td>85±24*</td>
<td>221±88*</td>
<td>83±38</td>
<td>561±154</td>
<td>373±126*</td>
<td>201±44</td>
<td></td>
</tr>
</tbody>
</table>

Plasma from five human subjects was supplemented with cholesteryl ester transfer protein (average, 4.3 units/ml incubation mixture) and very low density lipoprotein (VLDL) (average, 1.992 nmol VLDL triglyceride [TG]/ml incubation mixture) and either kept at 4°C or incubated at 37°C for 3 hours in the absence or presence of hepatic lipase (average, 87 units/ml incubation mixture). All incubations contained heparin (100 units/ml incubation mixture). After incubation, high density lipoproteins (HDLs) were isolated by ultracentrifugation as the 1.063<d<1.25 g/ml fraction and analyzed as described in "Methods." Values represent mean±SD of five experiments. Concentrations are expressed per milliliter plasma.

*p<0.01, tp<0.002 (vs. sample incubated in the absence of HL).

CE, cholesteryl ester; UC, unesterified cholesterol; PL, phospholipid; Apo, apolipoprotein.
HDL triglyceride was hydrolyzed in each experiment. There was also a modest reduction in the concentration of HDL phospholipid under these conditions. Coincident with these changes in HDL lipid content in plasma incubated with HL, there was approximately a 10% reduction in the apo A-I concentration of HDL when compared with the sample incubated in the absence of HL.

After incubation of plasma supplemented with CETP but no HL, there were only minor changes to the composition of the HDL compared with plasma incubated in the absence of any additions. When HL was included in the incubation, there was a reduction in the concentration of HDL triglyceride, which was comparable to that in incubations containing HL without CETP. A reduction in the concentration of HDL phospholipid of approximately 15% was also evident under these conditions. In experiment A, these changes were accompanied by a 10% loss of cholesteryl ester, a 25% loss of apo A-I, and a 13% loss of apo A-II from the HDL, although such losses were not apparent in experiment B.

When plasma was supplemented with both CETP and VLDL, there were extensive changes in the concentration of HDL constituents. In the absence of HL, the HDL became markedly enriched with triglyceride and depleted of cholesteryl ester, with relatively minor changes in the other constituents. In the presence of HL, however, most of the acquired HDL triglyceride was lost, and there were further reductions in the concentrations of HDL cholesteryl ester. Under these conditions, there was a small increase in HDL phospholipid in experiment A although there was a 25% loss of phospholipid from the HDL in experiment B. These changes were accompanied by major losses of apo A-I and an approximately 18% loss of apo A-II from the HDL in each experiment. In these incubations of plasma supplemented with VLDL, CETP, and HL, there were reductions of 9% and 25%, respectively, in the concentration of HDL triglyceride in the total mixture of lipoproteins as assayed in the d<1.25 g/ml fraction (results not shown).

To investigate the reproducibility of the changes to the HDL fraction of 1.063<d<1.25 g/ml after incubation with HL, CETP, and VLDL plasma samples from five subjects were supplemented with CETP and VLDL and either kept at 4°C or incubated at 37°C in the absence or presence of HL. There was a large increase in the triglyceride content of the HDL as well as a decrease in HDL cholesteryl ester during incubation in the absence of HL (Table 2). When HL was present, the concentration of HDL triglyceride returned almost to the level of that in the nonincubated sample. In these studies, it was confirmed that the presence of HL was accompanied by a significant enhancement in the loss of cholesteryl esters from the HDL (p<0.01), as well as a major loss (average, 30%) of apo A-I from the HDL (p<0.002). The loss of apo A-II from the HDL in the presence of HL was not statistically significant.

To assess whether the apo A-I that was lost from the HDL fraction had dissociated from the total lipoprotein fraction, the concentration of apo A-I in the plasma fraction of d<1.25 g/ml was also determined in the same incubations described in Table 2. In the absence of HL, there was minimal change in the concentration of apo A-I in the d=1.25 g/ml supernatant; by contrast, in the presence of HL, an average of 29% of the apo A-I was lost from this fraction (p<0.01, n=5).

To determine whether the dissociation of apo A-I was the simple consequence of an accumulation of VLDL lipolytic products in HDL, a study was performed to compare the effects of HL and LPL (Table 3). When plasma supplemented with CETP and VLDL was incubated with HL, there was a concentration-dependent reduction in the levels of both triglyceride and apo A-I in the d=1.25 g/ml supernatant. On the other hand, when plasma supplemented with CETP and VLDL was incubated with LPL, an extensive hydrolysis of triglyceride was not accompanied by a loss of apo A-I from the plasma fraction of d<1.25 g/ml (Table 3). Furthermore, when plasma supplemented with CETP, VLDL, and HL was further supplemented with LPL, the loss of apo A-I from the d=1.25 g/ml supernatant was prevented (Table 3, experiment B).

**Effects of Incubation on Concentration of High Density Lipoprotein Constituents: High Density Lipoprotein Isolated by Size-Exclusion Chromatography (Figure 1)**

To establish that the apo A-I lost from the HDL fraction in the experiments described above was not an artifact of the ultracentrifugal technique, size-exclusion chromatography was used to define the changes in the distribution of cholesteryl ester, triglyceride, and apo A-I. In the representative experiment shown in Figure 1, human plasma was supplemented with CETP and VLDL and either kept at 4°C or incubated at 37°C for 3 hours in the absence or presence of HL. In the incubation containing HL, there was a hydrolysis of 36% of the triglyceride (results not shown). After incubation, aliquots of the incubation mixtures were subjected to size-exclusion chromatography without prior ultracentrifugation; the fractions containing HDL are shown in Figure 1. Incubation in the absence of HL resulted in a loss of approximately 30% of the HDL cholesteryl esters (Figure 1; panel A), which were transferred to VLDL and LDL (results not shown). There was a concomitant enrichment of the HDL with triglyceride (panel B) by comparison with nonincubated samples. Under this condition, there was minimal change in the elution profile of apo A-I (panel C). When HL was included in the incubation, more than 50% of the cholesteryl esters were lost from the HDL (panel D) as a consequence of enhanced transfers to other lipoprotein fractions (results not shown), and there was an almost complete loss of the triglyceride that had been acquired by the HDL fraction (panel E).
Apolipoprotein A-I-containing particles. After incubation with high-molecular-weight protein standards. After incubation, the apo A-I migrated further than that in either lane A or B, indicating a reduction in HDL particle size in the absence of HL. In addition, there were at least two well-defined bands of apo A-I in the small-pore region of the gradient gel. These bands were not evident in either the nonincubated sample or in that incubated in the absence of HL. Since the Stokes’ radii of these bands were less than that of bovine serum albumin, this provided further confirmation that a proportion of the apo A-I had indeed dissociated from the HDL during incubation with HL and was now associated with the “lipoprotein-free” fraction of plasma.

**Discussion**

We have recently reported that the HL-mediated hydrolysis of triglyceride in the naturally triglyceride-enriched HDL in rabbit plasma is accompanied by a loss of as much as 30% of the apo A-I from the HDL. We postulated that the dissociation of apo A-I was secondary to the hydrolysis of HDL triglyceride by HL, which resulted in a reduction in the HDL core volume and created a redundancy of surface constituents that were shed from the particle. The present studies were performed to investigate whether a similar phenomenon occurred in human plasma in which the HDL are not naturally enriched with triglyceride. We have shown that a reduction in the small amount of triglyceride normally present in

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**Table 3. Incubation of Human Plasma Supplemented With Cholesteryl Ester Transfer Protein Plus Very Low Density Lipoprotein: Comparative Effects of Hepatic Lipase and Lipoprotein Lipase**

<table>
<thead>
<tr>
<th>Incubation conditions</th>
<th>Triglyceride (nmol/ml)</th>
<th>Apo A-I (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment A</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Nonincubated sample</td>
<td>2,895</td>
<td>488</td>
</tr>
<tr>
<td>2. No lipase</td>
<td>2,618</td>
<td>454</td>
</tr>
<tr>
<td>3. HL (4 units/ml)</td>
<td>2,208</td>
<td>353</td>
</tr>
<tr>
<td>4. HL (40 units/ml)</td>
<td>1,810</td>
<td>306</td>
</tr>
<tr>
<td>5. LPL (8 units/ml)</td>
<td>1,094</td>
<td>483</td>
</tr>
<tr>
<td>6. LPL (32 units/ml)</td>
<td>651</td>
<td>469</td>
</tr>
<tr>
<td><strong>Experiment B</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Nonincubated sample</td>
<td>2,558</td>
<td>324</td>
</tr>
<tr>
<td>2. No lipase</td>
<td>2,751</td>
<td>339</td>
</tr>
<tr>
<td>3. HL (32 units/ml)</td>
<td>2,218</td>
<td>206</td>
</tr>
<tr>
<td>4. LPL (24 units/ml)</td>
<td>1,401</td>
<td>345</td>
</tr>
<tr>
<td>5. LPL (24 units/ml)+HL (32 units/ml)</td>
<td>971</td>
<td>341</td>
</tr>
</tbody>
</table>

Experiment A: Aliquots (191 µl) of a plasma sample (cholesterol, 5.2 mM; triglyceride, 0.9 mM) were supplemented with very low density lipoprotein (VLDL) (to provide a final triglyceride concentration of 2.9 mM in the incubation mixture), cholesteryl ester transfer protein (CETP) (final concentration, 3.2 units/ml), and heparin (100 units/ml). These mixtures were either (1) stored at 4°C, incubated for 6 hours at 37°C in the absence of further addition, or (3–6) in the presence of hepatic lipase (HL) or lipoprotein lipase (LPL). Final incubation volume was 500 µl. After incubation, d<1.25 g/ml fraction was recovered by ultracentrifugation. Concentrations of triglyceride and apolipoprotein apo A-I in this fraction have been corrected for recovery of cholesterol and expressed per milliliter incubation mixture.

Experiment B: Aliquots (300 µl) of a plasma sample (cholesterol, 4.4 mM; triglyceride, 1.1 mM) were supplemented with VLDL (to provide a final triglyceride concentration of 2.8 mM in the incubation mixture), CETP (final concentration, 4.9 units/ml), and heparin (100 units/ml). These mixtures were either (1) kept at 4°C or incubated in duplicate for 3 hours at 37°C (2) in the absence of lipases, (3) in the presence of HL (32 units/ml), (4) in the presence of LPL (24 units/ml), and (5) in the presence of both LPL (24 units/ml) and HL (32 units/ml). Final incubation volume was 850 µl. After incubation, d<1.25 g/ml fraction was recovered by ultracentrifugation. Concentrations are expressed per milliliter incubation mixture.
human HDL during incubation of plasma with HL is accompanied by only a minor loss of apo A-I from the particles. By contrast, when the plasma was supplemented with CETP and VLDL at concentrations sufficient to enrich the particles with triglyceride, the presence of HL now promoted a major reduction in the apo A-I concentration of the HDL. This result provided further support for the proposition that the loss of apo A-I promoted by HL is related to the hydrolysis of HDL triglyceride.

Two independent techniques were used to exclude the possibility that the loss of apo A-I from human HDL in plasma incubations containing HL, CETP, and VLDL was an artifact of ultracentrifugation. The results obtained by size-exclusion chromatography and gradient gel-immunoblot analysis of samples that had not been subjected to ultracentrifugation were in accord with those obtained by ultracentrifugation and confirmed that incubation of human plasma with added HL, CETP, and VLDL did indeed result in dissociation of apo A-I from the HDL into the lipoprotein-free fraction of plasma. There was also a suggestion of a small loss of apo A-II from HDL during the incubations although this loss was never as extensive as that of apo A-I and was not statistically significant. This result was not unexpected, as it has been demonstrated that apo A-II has a greater affinity for the HDL surface than does apo A-I.

The findings of other studies have suggested that apo A-I may be displaced from either bovine or human HDL by synthetic phospholipid. It is, therefore, conceivable that in incubations containing added VLDL together with HL, lipolysis of the VLDL by HL may have resulted in the transfer of constituents from the VLDL surface to the HDL fraction, causing dissociation or displacement of apo A-I from the HDL. This possibility was all but excluded by finding that an even more extensive hydrolysis of VLDL catalyzed by LPL rather than by HL was not accompanied by a dissociation of apo A-I (Table 3). The additional observation that the dissociation of apo A-I promoted by HL was prevented if the incubation also contained LPL (Table 3, experiment B) is reminiscent of the recent finding that LPL also prevents the HL-mediated reduction in HDL particle size.
ability of LPL to hydrolyze VLDL, the presence of which was shown to be obligatory for HL to promote a significant reduction in the size of HDL during incubation of human plasma. The fact that LPL has the capacity to abolish both the HL-mediated loss of apo A-I and reduction in HDL particle size is consistent with the proposition that the loss of apo A-I from HDL may be secondary to the decrease in particle size. The possibility that it may be a simple consequence of the hydrolysis of HDL phospholipids is excluded not only by the absence of apo A-I loss in incubations, which contain both HL and LPL (Table 3), but also in those containing phospholipase A2.

A recent study has demonstrated that HL, CETP, and VLDL act synergistically during incubation of human plasma in vitro, with evidence that HL enhances the CETP-mediated transfers of cholesteryl esters from HDL to VLDL and LDL. In the studies reported here, the enhancement in the transfer of cholesteryl esters from HDL to other lipoprotein fractions by HL was confirmed. The mechanism of this phenomenon is uncertain although it has been reported elsewhere that lipolytic products, specifically nonesterified fatty acids, enhance the rate of CETP-mediated transfers of cholesteryl esters from HDL to VLDL. Whatever the exact mechanism, the results of the present studies show that HL in the presence of CETP and VLDL promotes not only a major reduction in HDL triglyceride but also a significant loss of cholesteryl esters from triglyceride-enriched human HDL. We suggest that the capacity of HL to deplete HDL of apo A-I is a direct consequence of a reduction in HDL core volume resulting from the loss of cholesteryl esters and triglyceride.

The concentration of apoproteins in HDL is generally regarded as reflecting a balance between their rate of synthesis and their rate of catabolism. However, it has become apparent that plasma factors that regulate the concentration of lipids in HDL may also be important determinants of the concentration of apoproteins in HDL. Previous studies have demonstrated an increase in the level of apo A-IV in rat or human HDL that was secondary to an LCAT-mediated increase in HDL cholesteryl esters. On the other hand, the results of the present study show that a reduction in the core lipid content of human HDL promoted by HL in the presence of CETP and VLDL is accompanied by a significant loss of apo A-I from the particles. The physiological significance of these findings is not addressed by the present studies, and any extrapolation to what may occur in vivo should be made only with the greatest caution. Nevertheless, depending on the metabolic fate of the apo A-I that is lost from the HDL, the changes to the HDL observed during incubation in vitro may provide an explanation for the low levels of apo A-I that accompany the reduced concentrations of HDL cholesterol in subjects with hypertriglyceridemia.

Acknowledgments

We wish to thank O.V. Rajaram for providing the CETP, C.P. Ehnholm for providing the rabbit antihuman apo A-I.

References


**KEY WORDS** • apolipoprotein A-I • high density lipoproteins • hepatic lipase • cholesteryl ester transfer protein • very low density lipoproteins
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density lipoproteins during incubation in vitro.
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Arterioscler Thromb Vasc Biol. 1991;11:415-422
doi: 10.1161/01.ATV.11.2.415
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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