Triglyceride-Rich Lipoproteins Isolated by Selected-Affinity Anti–Apolipoprotein B Immunosorption From Human Atherosclerotic Plaque

Joseph H. Rapp, Anne Lespine, Robert L. Hamilton, Nicholas Colyvas, Amy H. Chaumeton, Judy Tweedie-Hardman, Leila Kotite, Steven T. Kunitake, Richard J. Havel, John P. Kane

Abstract We isolated and characterized immunoreactive apolipoprotein B (apoB)–containing lipoproteins from human atherosclerotic plaque and plasma to determine whether very-low-density lipoprotein (VLDL) can enter and become incorporated into the atherosclerotic lesion and how plaque apoB-containing lipoproteins differ from apoB-containing lipoproteins isolated from plasma. Atherosclerotic plaques were obtained during aortic surgery and processed immediately. Lipoproteins were extracted from minced plaque in a buffered saline solution (extract A). In selected cases a second extraction was done after plaque was incubated with collagenase (extract B). Lipoproteins were then isolated from the extracts by anti-apoB immunosorption and separated into VLDL+intermediate-density lipoprotein (IDL) (d<1.019 g/mL) and low-density lipoprotein (LDL) (1.019<d<1.070 g/mL) fractions by ultracentrifugation. The VLDL+IDL fractions from plaque contained more than one third of the total apoB-associated lipoprotein cholesterol in both extracts A and B. The lipid composition of VLDL+IDL in both extracts was related to that of plasma VLDL+IDL. By electron microscopy mean particle diameters of VLDL+IDL from extracts A and B were 9% and 23%, respectively, greater than VLDL+IDL diameters from plasma. Mean diameters of LDL from extracts A and B were 11% and 31% greater than LDL diameters from plasma. The apoE-apoB ratio of extract A VLDL+IDL was nearly twice that of plasma VLDL+IDL and severalfold higher than that of extract A LDL. Immunoblots of both VLDL+IDL and LDL from extract A demonstrated minimal fragmentation of apoB. These results demonstrate that (1) VLDL, VLDL remnants, or both, can enter human atherosclerotic plaque and become bound to the connective tissue matrix; (2) apoB-containing lipoproteins from plaque differ from plasma lipoproteins in size and apoE content; and (3) there appears to be minimal fragmentation of buffer-extractable apoB in plaque lipoproteins. (Arterioscler Thromb. 1994;14:1767-1774.)

Key Words • atherosclerosis, very-low-density lipoprotein • very-low-density lipoprotein remnants • low-density lipoprotein • apoB

Lipoprotein infiltration into the artery wall is an essential event in the pathogenesis of atherosclerosis, but little is known about the process of lipoprotein infiltration and eventual entrapment in plaque. Even the identity of the lipoproteins involved has not been conclusively determined. It is generally agreed that low-density lipoproteins (LDL) have access to the artery wall, but very-low-density lipoproteins (VLDL) also may infiltrate the artery wall and directly contribute to atherogenesis. VLDL, particularly VLDL remnants, certainly have atherogenic potential. Particles resembling VLDL remnants can be taken up by macrophages to produce foam cells, stimulating endothelial cells to express a monocyte-specific chemotactic factor, and increase monocyte adherence to the endothelium. Furthermore, varying amounts of labeled VLDL apolipoprotein B (apoB) have been found in aortas from human and experimental animals after intravenous administration. Cholesteryl ester–rich lipoproteins in the VLDL+intermediate-density lipoprotein (IDL) density range (d<1.019 g/mL) have been recovered from human aortas, suggesting the presence of a β-VLDL–like lipoprotein. However, triglyceride-rich LDL, typical of the d<1.006 g/mL lipoproteins of plasma have not been recovered from human artery wall.

Although lipoprotein identity is important, determining the modifications that contribute to retention and cellular uptake of lipoproteins within the arterial wall may be even more important to our understanding of atherogenesis. Previous studies of lipoproteins isolated from the artery wall have suggested that the size, density, and even apolipoprotein composition may be altered substantially. Enlargement, possibly caused by fusion of lipoproteins, was observed when rabbit aortas were examined by electron microscopy after intravenous infusion of LDL. Plaque lipoproteins apparently have an increased content of apoE. In addition, several studies have described fragmentation of apoB from LDL isolated from human aortic specimens obtained postmortem consistent with that found in oxidatively modified LDL.
To determine whether triglyceride-rich and cholesterol ester–rich lipoproteins are present in human atherosclerotic plaque and to compare the size and composition of these particles to those in plasma, we isolated apoB-containing lipoproteins both from a buffered saline extract and proteolytic digests of aortic atherosclerotic lesions obtained at surgery and from the patient’s plasma. Our data suggest that VLDL and IDL are present in atherosclerotic lesions. These lipoproteins are larger than those in blood plasma and are enriched in apoE. Minimal fragmentation of apoB in arterial VLDL + IDL and LDL extracted with buffered saline was observed, suggesting that the apoB was not easily oxidized.

**Methods**

**Isolation of Plaque Lipoproteins (Extract A)**

Aortic plaque was removed from 18 individuals (12 men and 6 women) aged 58 to 77 years who were undergoing aortic reconstruction. The specimen sizes and lipoprotein amount extracted were variable, but no single specimen was large enough to be used for all analyses. Plaque material was obtained in the operating room and immediately placed in ice-cold solution containing 7 mmol/L citrate buffer, pH 7.4, in 15 mmol/L NaCl. The following compounds were added to minimize modifications of the lipoproteins ex vivo: EDTA, 3 mmol/L; butylhydroxytoluene, 0.5 mmol/mL; phenylmethylsulfonyl fluoride, 1 mmol/mL; aprotinin, 1.5 mg/mL; benzamidine, 2 mmol/mL; gentamicin sulfate, 0.08 mg/mL; and chloramphenicol, 0.08 mg/mL. Blood and adherent thrombus were removed by blotting with absorbent paper, scrubbing with a small brush, and sharp dissection as necessary. The method for isolating lipoproteins has been described in detail elsewhere.

Briefly, plaque immersed in buffer was minced into pieces 0.5 to 1.0 mm². The resulting gruel was placed in a test tube and turned end over end at 10 rpm in buffer at 4°C. Buffer changes were made at 1, 2, and 14 hours, and extracts were pooled (extract A).

One specimen was acquired from a postmortem sample obtained 10 hours after death. The subject was a 62-year-old man who died after coronary artery bypass surgery. After aortic endarterectomy, the plaque was immediately placed in buffer and processed as above.

The apoB-containing lipoproteins were isolated with a selected-affinity immunosorption column containing pauciclonal sheep anti-human apoB-100 antibodies and eluted by either high or low pH changes at 4°C. Initially, buffer at pH 11.0 was used to release lipoproteins from a column made with antibodies collected over a pH range of 9.5 to 10.8. During these experiments some column deterioration was noted after repeated exposure to high pH buffers. We then used a pH 3.0 buffer to release the apoB-containing lipoproteins from a column made with antibodies collected over a pH range of 5.0 to 3.5. The eluant was immediately buffered in the collecting vial to minimize its exposure to the more extreme pH ranges and further neutralized to pH 7.4 if necessary on complete collection. McConathy and associates have shown by electron microscopy that exposure of plasma LDL to either high or low pH buffers produces no detectable degradation of apoB-100 or alteration in the particles.

With the use of this method, approximately 1 mg of apoB protein was recovered per 10 g of plaque.

**Isolation of Plasma Lipoproteins**

Blood from patients who had fasted overnight was drawn in the preanesthesia area or in the operating room into tubes containing EDTA and promptly centrifuged at 2000 rpm for 20 minutes at 12°C. A portion of this plasma was taken directly for lipoprotein analysis. Five milliliters of plasma was subjected to centrifugation in a 40.3 rotor of a Beckman ultracentrifuge at 36 000 rpm at 12°C for 16 hours, and the supernatant VLDL were obtained by tube slicing. The high-density lipoprotein fraction was separated by precipitation of VLDL and LDL fractions with dextran sulfate/manganese. The concentrations of total cholesterol and triglycerides were measured in these fractions by an automated enzymatic method (Roche Cobas-Mira Chemistry System, Roche Diagnostics). IDL + LDL lipids were estimated by difference.

Plasma for isolation of apoB-containing lipoproteins by selected-affinity immunosorption was mixed with the proteolytic inhibitors and antibiotics listed above and then placed on the immunosorption column and eluted as described above. The recovery of 1 mg apoB-100 in LDL and VLDL + IDL from two normolipidemic individuals was 94% and 82%, respectively.

**Isolation of Lipoproteins From a Digest of Plaque (Extract B)**

After the removal of extract A, rocket immunoelectrophoresis was performed on minced plaque pieces from four samples to determine the completeness of apoB removal. No additional apoB was detected after the third buffer change (16 hours from removal). Plaque was then washed for an additional 24 hours with buffer containing the inhibitor cocktail but no EDTA and centrifuged at 2000 rpm for 20 minutes, and the buffer was decanted. The plaque material was then incubated with phosphate-buffered saline, pH 7.4, containing collagenase for 1.5 hours as described by Smith and associates. For termination of proteolytic activity, EDTA and the other proteolytic enzyme inhibitors and antioxidants were then added, and the plaque material was centrifuged at 2000 rpm and washed once with buffered saline and inhibitors. The collagenase and wash buffers were pooled (extract B) and applied to the anti-apoB selected-affinity immunosorption column and eluted as described above.

**Oxidation of Lipoproteins**

Plasma was obtained from healthy fasting subjects, and LDL (1.019<d<1.063 g/mL) were isolated by sequential ultracentrifugation and dialyzed overnight against 10 mmol/L phosphate-buffered saline, pH 7.4. LDL were then diluted to a concentration of 0.1 mg/mL, and CuSO₄ was added to a concentration of 10 μmol/L. This mixture was incubated at 37°C for either 4 or 24 hours. The reaction was terminated by addition of EDTA to 200 μmol/L and butylhydroxytoluene to 40 μmol/L. LDL were then dialyzed overnight against 4 L of saline and used immediately.

**Identification of ApoB**

ApoB proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis on a gradient of polyacrylamide of 3% to 20%. Western blotting was performed with a standard transfer apparatus (Eida Scientific). The antiserum was sheep anti-human apoB (Balra Corp). Blots were developed with a peroxidase-labeled second antibody (Bio-Rad, Inc) and photographed immediately.
The gels were stained with Coomassie blue R-250. Electron microscope (Siemens Corp) with a condenser aperture of 20,000 and 60,000 at 80 kV in a model 101 with boron trifluoromethane. Free cholesterol and the cholesterol content of cholesteryl esters were also determined by gas liquid chromatography (Hewlett-Packard, model HP 5890, series II) after derivitization with boron trifluoromethane. Protein content was determined by a "micro" method with bicinchoninic acid (Pierce Chemical Co). Lipid compositions of each lipoprotein had been entered into the computer, the particle was assigned a number and reproduced on a Cartesian grid, allowing a review of the congruence generated from perimeter points. The particle size distributions were plotted as histograms. Paired t tests were used to evaluate differences in size between lipoprotein fractions.

### Results

#### Amount of Lipoproteins Extracted From Plaque

The mass of apoB-containing lipoprotein cholesterol extracted from six large plaque specimens and isolated by selected-affinity immunosorption is given in Table 1. Considerable variability was encountered. The mean cholesterol content in the VLDL+IDL fraction was substantial, with VLDL+IDL accounting for 36% and 46% of the lipoprotein cholesterol from extracts A and B, respectively.

#### Particle Size Distribution of Lipoproteins Isolated From Plasma and Atherosclerotic Plaques

The lipoproteins in extract A from plaque were typically round and morphologically indistinguishable from those of plasma. The median diameters of the VLDL+IDL from extract A were consistently larger than those from plasma (Table 2) (327±15 Å in plasma and 357±16 Å in plaque, mean±SD, P<.01). Representative electron microphotographs are shown in Fig 1. The VLDL+IDL fraction of lipoproteins from extract B contained even larger particles with a mean diameter of 404±55 Å (P<.01). Histograms displaying the distribution of particle diameters in the VLDL+IDL fractions from plasma, plaque extract A, and extract B are shown in Fig 2. These distributions had a skewness of 1.0 to 1.5 and kurtosis of 4.5 to 6.6.

LDL from plaque extract A also were larger than those from plasma (Table 2). Mean diameters were 235±13 Å for plasma LDL and 261±26 Å for LDL from plaque extract A (P<.01). The LDL-like lipoproteins recovered in extract B were larger still, with a mean

### Table 1. Recovery of Lipoprotein Cholesterol From Plaque

<table>
<thead>
<tr>
<th>Patient</th>
<th>VLDL+IDL</th>
<th>LDL</th>
<th>VLDL+IDL</th>
<th>LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>ND</td>
<td>192</td>
<td>227</td>
<td>17</td>
<td>16</td>
</tr>
<tr>
<td>BR</td>
<td>217</td>
<td>258</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RE</td>
<td>115</td>
<td>261</td>
<td></td>
<td></td>
</tr>
<tr>
<td>JS</td>
<td>91</td>
<td>279</td>
<td>120</td>
<td>221</td>
</tr>
<tr>
<td>MN</td>
<td>275</td>
<td>160</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SC</td>
<td>41</td>
<td>502</td>
<td>78</td>
<td>10</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>155±88</td>
<td>281±116</td>
<td>72±52</td>
<td>83±120</td>
</tr>
</tbody>
</table>

VLDL indicates very-low-density lipoprotein; IDL, intermediate-density lipoprotein; and LDL, low-density lipoprotein. Data are expressed as micrograms cholesterol extracted from 10 g plaque.

### Table 2. Ratio of Apolipoprotein E to Apolipoprotein B in Lipoprotein Fractions

<table>
<thead>
<tr>
<th>Patient</th>
<th>Plasma</th>
<th>Extract A</th>
<th>Plasma</th>
<th>Extract A</th>
</tr>
</thead>
<tbody>
<tr>
<td>FF</td>
<td>0.058</td>
<td>0.076</td>
<td>0.005</td>
<td>0.008</td>
</tr>
<tr>
<td>GF</td>
<td>0.057</td>
<td>0.164</td>
<td>0.034</td>
<td>0.012</td>
</tr>
<tr>
<td>AP</td>
<td>0.072</td>
<td>0.082</td>
<td>0.013</td>
<td>0.018</td>
</tr>
<tr>
<td>BH</td>
<td>0.069</td>
<td>0.120</td>
<td>0.004</td>
<td>0.016</td>
</tr>
<tr>
<td>TH</td>
<td>0.043</td>
<td>0.066</td>
<td>0.003</td>
<td>...</td>
</tr>
<tr>
<td>GS</td>
<td>0.014</td>
<td>0.061</td>
<td>0.020</td>
<td>0.047</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>0.052±0.021</td>
<td>0.094±0.041*</td>
<td>0.013±0.012</td>
<td>0.020±0.015</td>
</tr>
</tbody>
</table>

Definitions are as in Table 1.

*Significantly different from plasma particles, P<.05.
diameter of 307±27 Å (P<.01). Representative electron photomicrographs are shown in Fig 1 and histograms in Fig 3. These distributions had a skewness of 0.8 to 1.3 and kurtosis of 4.8 to 8.6.

The calculated volume of extract A VLDL+IDL (19.1×10^7 Å^3) was 30% larger than VLDL+IDL from plasma (14.6×10^7 Å^3), whereas the volume of extract B VLDL+IDL (27.6×10^7 Å^3) was 45% larger than VLDL+IDL from extract A and nearly twice the volume of VLDL+IDL from plasma. The volume of extract A LDL (7.5×10^7 Å^3) was 39% larger than LDL from plasma (5.4×10^7 Å^3), and the volume of extract B LDL (12.1×10^7 Å^3) was 60% larger than LDL from extract A and more than twofold larger than LDL from plasma.

To determine whether collagenase treatment alone could produce lipoprotein fusion, we isolated plasma VLDL+IDL and LDL and exposed these particles to collagenase for 1.5 hours and reisolated them by ultracentrifugation. Electron photomicrographs of plasma VLDL+IDL and LDL showed no evidence of fusion (data not shown).

**ApoE in Plaque and Plasma**

Mass ratios of apoE to apoB in the lipoproteins from plasma and extract A from six subjects are given in Table 2. Although there were wide variations among samples in both fractions, the mean ratio of apoE to apoB in the plaque VLDL+IDL fractions was almost
twice that of plasma. As expected, there was substantially less apoE in the plaque LDL fractions. The calculated molar ratio of apoE to apoB in plaque VLDL+IDL was approximately 1.5:1, whereas that of plaque LDL was 1:4.

**ApoB in Plasma and Plaque Lipoproteins**

We found that it was critical to isolate the lipoproteins from the plaque extract A as soon as possible, as the apoB in these lipoproteins broke down with storage at 4°C even in buffer A containing the cocktail of inhibitors. Immunoblots of rapidly processed plaque VLDL+IDL and LDL fractions showed very little evidence of apoB-100 breakdown compared with that seen in plasma VLDL+IDL and LDL fractions from the same patient (Fig 4). The extent of apoB breakdown in these samples was comparable to that of centrifugally isolated LDL oxidized with Cu^{2+} for 4 hours, but the peptide array included smaller fragments in the plaque samples. Cleavage of apoB in LDL exposed to Cu^{2+} for 24 hours was substantially greater than that seen in any of our samples.

In immunoblots from extract B, discrete peptide fragments of apoB were seen, but no apoB-100 band could be detected (data not shown). Exposure of plasma LDL to collagenase had similar effects.

Immunoblots of buffer-extracted apoB from an atherosclerotic plaque (extract A) obtained at autopsy showed substantial breakdown of apoB in both the VLDL+IDL and LDL fractions (Fig 5).

**Immunoelectrophoresis of Plasma and Plaque Lipoproteins**

Lipoproteins from extract A of three patients were subjected to electrophoresis before centrifugation (Fig 6).
Triglycerides generally constitute only about 1% of atherosclerotic plaque lipid, but in the two patients with hypertriglyceridemia, triglyceride-rich lipoproteins accounted for an appreciable portion of the extractable lipoproteins in plaque. This discrepancy may reflect intralesional hydrolysis, possibly by lipoprotein lipase, shown to be secreted by macrophages in tissue culture. Recently, lipoprotein lipase message has been demonstrated in some but not all macrophages and smooth muscle cells located within the human artery wall. Macrophages also possess neutral lipase activity, which could facilitate the known cholesteryl ester accumulation resulting from macrophage VLDL and VLDL remnant uptake.

Using ultracentrifugation alone, other investigators have recovered only cholesteryl ester-rich lipoproteins in the VLDL density range from aortic samples. As part of their isolation procedure, Yla-Herttuala et al discarded lipoproteins that floated after ultracentrifugation at 100 000g for 30 minutes, possibly excluding triglyceride-rich lipoproteins from the final analysis. Their d<1.012 g/mL fraction also contained apoE, but the amount was not compared with that of the other density fractions. Hollander et al did not use this preliminary ultracentrifugation, but their arterial LDL and VLDL were "markedly heterogeneous," including large particles with pitted and cratered surfaces that may have contained cholesteryl ester-rich plaque lipids. Such obviously abnormal particles were not seen in our preparations or reported by Yla-Herttuala et al. As suggested in a previous report, we found a distinct increase in lipoprotein size when lipoproteins from the arterial wall were compared with those from plasma. Both VLDL+IDL and LDL from the initial saline extract of plaque (plaque extract A) were significantly larger than the respective lipoproteins from plasma. Lipoproteins released after exposure of plaque to collagenase (extract B) were even larger than those from plaque extract A, suggesting that size could be an important determinant of lipoprotein entrapment in the artery wall. The process by which these lipoproteins could enlarge is not apparent. Exposure of plasma VLDL and LDL to collagenase did not change their appearance on electron microscopy, making it unlikely that the increased size seen in extract B was an artifact of isolation or proteolysis. The lipoproteins in extracts A and B could enlarge by acquiring lipid from the atherosclerotic lesion, but plaque lipoproteins of comparable size have been found in a saline extract of nonlesioned aortic tissues. Selective trapping of larger subfractions of VLDL and LDL or, alternatively, a preferential uptake of smaller lipoproteins by cellular elements in

6. The apoB-containing lipoproteins from plaque uniformly had pre-beta mobility.

**Lipoprotein Compositions**

Lipoprotein composition data from four patients are shown in Table 3. The most notable observation was the triglyceride content of patients JS and SC, who had elevated plasma VLDL triglycerides as shown in their plasma profiles (Table 4). In these hypertriglyceridemic patients, triglycerides were the predominant lipid component of the VLDL+IDL fraction from plaque. Interestingly, the plaque LDL fractions from this patient were also relatively triglyceride rich. The relative amounts of free and esterified cholesterol in plaque and plasma lipoproteins were similar.

**Discussion**

We have used an immunosorption column to isolate lipoproteins containing apoB from extracts of atherosclerotic plaques obtained as surgical endarterectomy specimens. More than one third of the lipoprotein cholesterol was separated by ultracentrifugation at a density lower than that of LDL (ie, <1.019 g/mL). These lipoproteins resembled plasma VLDL and IDL in size and shape, and their composition varied with the patient's plasma lipid profile. Plaque VLDL+IDL fractions from two hypertriglyceridemic patients were triglyceride rich, whereas in two normotriglyceridemic patients the plaque VLDL+IDL fractions contained predominantly cholesteryl esters. Triglyceride-rich lipoproteins were found in both isotonic buffer extractions and extracts obtained after collagenase digestion, suggesting that some triglyceride-rich VLDL are bound to the connective tissue matrix of the atherosclerotic plaque. Whether they contained primarily triglycerides or cholesteryl esters, plaque VLDL+IDL had substantially more apoE than plasma VLDL+IDL. The increased apoE could reflect either the presence of B-VLDL-like particles or acquisition of apoE from tissue macrophages.

Triglycerides generally constitute only about 1% of atherosclerotic plaque lipid, but in the two patients with hypertriglyceridemia, triglyceride-rich lipoproteins accounted for an appreciable portion of the extractable lipoproteins in plaque. This discrepancy may reflect intralesional hydrolysis, possibly by lipoprotein lipase, shown to be secreted by macrophages in tissue culture. Recently, lipoprotein lipase message has been demonstrated in some but not all macrophages and smooth muscle cells located within the human artery wall. Macrophages also possess neutral lipase activity, which could facilitate the known cholesteryl ester accumulation resulting from macrophage VLDL and VLDL remnant uptake.

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**Fig 5.** Western immunoblots show apolipoprotein (APO) B in plaque lipoproteins from a cadaveric aorta extract A. Lane 1, Very-low-density lipoprotein+intermediate-density lipoprotein; lane 2, low-density lipoprotein. The aorta was processed 10 hours after death.

**Fig 6.** Immunoelectrophoresis shows plaque and plasma lipoproteins. Lane 1, Plasma; lanes 2 and 6, control low-density lipoprotein; lanes 3 through 5, whole extract A from three plaques subjected to electrophoresis on 1% agarose. A polyclonal antibody against human apolipoprotein A-I was also placed in the topmost well.
plaque is plausible, but mechanisms for such phenomena have not been described. Plasma lipoproteins could also fuse to yield larger particles. After a bolus infusion of human LDL into rabbits, particles as large as 1000 Å in diameter have been seen in aortic tissue.\textsuperscript{14} Large lipoproteins with density similar to LDL have been isolated from inflammatory lymph, but those particles were not spherical, as observed by electron microscopy.\textsuperscript{41} Conceivably, an inflammatory milieu promotes clumping and eventual fusion of these particles as proposed by Hoff and O'Neil.\textsuperscript{18}

The alteration in size seen in the plaque lipoproteins raises questions regarding the relation between the density of the lipoproteins in plaque and those in plasma. If particles enlarge by accepting additional lipid, there would be a reduction in particle density. However, if particles fuse, it is not clear whether the molar ratios of lipid and protein would change. ApoB on these particles identifies their plasma origin. Therefore, when plaque VLDL+IDL are triglyceride rich, as in our two hypertriglyceridemic patients (Table 3), we can be reasonably confident that they contain VLDL+IDL as defined in plasma. The origin of plaque cholesteryl ester–rich "VLDL+IDL" is less clear. They may represent mainly β-VLDL and IDL from plasma, but delineation of their origin requires information about the mechanism by which plaque lipoproteins enlarge.

We examined the apoB in each fraction by Western blotting to determine the extent of apoB fragmentation as an indicator of lipoprotein modification. ApoB-100 in both VLDL+IDL and LDL from extract A had undergone some fragmentation. Breakdown of apoB by collagenase precluded examination of this question in extract B. The extent of apoB fragmentation in the lipoproteins of extract A appeared comparable to that seen after a 4-hour oxidation of plasma LDL. We saw no evidence of more extensively degraded lipoproteins in these preparations. It is possible that we failed to isolate more extensively degraded lipoproteins, although at least some plasma LDL oxidized for 24 hours is recognized by our selected-affinity immunosorption system. Immunoblots of VLDL+IDL and LDL apoB from extract A of an aorta processed 10 hours postmortem demonstrated extensive fragmentation, consistent with previous reports of LDL removed from cadaveric aortas.\textsuperscript{15–18} As cathepsins and other proteolytic enzymes may be activated in the postmortem state, some degradation may occur in the hours that elapse between death and aortic removal. We also observed that if plaque lipoproteins from surgical specimens were stored in the extract buffer, fragmentation of apoB continued despite the addition of proteolytic and oxidative inhibitors to the buffer. This proteolysis ceased after lipoprotein isolation but shows how easily artificial degradation can occur. Although not all cadaveric tissues may promote proteolysis to the degree seen in the specimen we examined, these data indicate that observations made on lipoproteins from cadaveric aor-

### Table 3. Composition of Lipoprotein Fractions

<table>
<thead>
<tr>
<th>Fraction and Patient</th>
<th>Plasma</th>
<th>Extract A</th>
<th>Extract B</th>
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<tbody>
<tr>
<td></td>
<td>FC/TC</td>
<td>TC/TG</td>
<td>FC/TC</td>
</tr>
<tr>
<td>VLDL+IDL</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>ND</td>
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</tr>
<tr>
<td>BR</td>
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<td>1.66</td>
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<td>JS</td>
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<td>0.42</td>
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<td>LDL</td>
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<td></td>
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<tr>
<td>ND</td>
<td>0.40</td>
<td>3.70</td>
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<td>0.28</td>
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<tr>
<td>JS</td>
<td>0.29</td>
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<td>SC</td>
<td>0.28</td>
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FC indicates free cholesterol; TC, total cholesterol; TG, triglyceride; and as in Table 1. Data are expressed as molar ratios of lipid and protein.

### Table 4. Plasma Lipoprotein-Lipid Concentrations

<table>
<thead>
<tr>
<th>Patient</th>
<th>Chol</th>
<th>TG</th>
<th>Chol</th>
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<tbody>
<tr>
<td></td>
<td>Total</td>
<td>VLDL</td>
<td>IDL+LDL</td>
<td>HDL</td>
<td></td>
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<td>42</td>
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</tbody>
</table>

Definitions are as in Table 1. HDL indicates high-density lipoprotein; Chol, cholesterol; and TG, triglycerides. The compositions of these patients' plaque lipoproteins are shown in Table 3. Values are expressed as milligrams per deciliter.
References


Triglyceride-rich lipoproteins isolated by selected-affinity anti-apolipoprotein B immunosorption from human atherosclerotic plaque.


Arterioscler Thromb Vasc Biol. 1994;14:1767-1774
doi: 10.1161/01.ATV.14.11.1767

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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