experiments that the negative inotropic effects of NIC in the denervated heart are mediated through stimulation of the intrinsic cardiac nerves.

References


Quantification of Apolipoprotein B in Grossly Normal Human Aorta

HENRY F. HOFF, CAROL L. HEIDEMAN, JOHN W. GAUBATZ, ANTONIO M. GOTTO, JR., ETHEL E. ERIKSON, AND RICHARD L. JACKSON

SUMMARY A quantitative electroimmunoassay (EIA) assay was developed for apolipoprotein B (apoB), the major apoprotein of human plasma low density lipoproteins (LDL) and very low density lipoproteins (VLDL). Specificity, sensitivity (30-200 ng) and reproducibility (11%) were established. We used this system to determine the amount of buffer-soluble apoB in supernatant fractions from homogenates of the intima from grossly normal human aortas. Assays of whole tissue minces yielded only one-third of the apoB in supernatant fractions. Intimal homogenates contained 0.34-18.45 µg of apoB/mg of tissue, dry weight (mean 5.42 ± 3.95 SD). We also found that no apoB was detected in the homogenates of adjacent tunica media. Furthermore, most of the intimal apoB was found in the LDL density (d) fraction (d 1.006-1.063) after differential ultracentrifugation, while the VLDL (d < 1.006) fraction contained only negligible amounts of apoB. By electron microscopy, it was determined that the LDL density fraction contained particles 200-250 A in diameter which were similar in size to plasma LDL. These results suggest that grossly normal aortas contain significant quantities of intact LDL.

NUMEROUS clinical and epidemiological studies have shown a positive correlation between atherosclerosis leading to coronary heart disease and elevated levels of plasma cholesterol.1-4 The hypercholesterolemia is largely due to an increase in plasma low density lipoproteins (LDL).7-8 Several studies have also suggested a correlation between both coronary and cerebral artery disease and endogenous hypertriglyceridemia due to an increase in plasma very low density lipoproteins (VLDL).1-4,6,8-12 The major protein of LDL and VLDL, apolipoprotein B (apoB), has been identified by immunohistochemical techniques in atherosclerotic arteries.19-20 By use of these techniques this laboratory recently found more widespread localization of apoB in lesions from subjects with type II hyperlipoproteinaemia than from normolipemics.16 Qualitative limitations in the immunofluorescence procedures, however, precluded a direct quantitative assessment. Therefore, in the present report we describe a quantitative immunologi-
Methods

PREPARATION OF LIPOPROTEINS, apoB, AND ANTISERA

LDL were isolated from the plasma of normolipemic donors by sequential ultracentrifugation in KBr (d 1.020-1.050 g/ml). The initial ultracentrifugations were performed in a 60 Ti rotor at 55,000 rpm at 8°C for 18 hours. The LDL were routinely subjected to recentrifugation at d 1.050 g/ml in an SW 50.1 rotor. VLDL from normolipemic donors were isolated at plasma density (d < 1.006). The isolated LDL and VLDL were dialyzed extensively against 0.9% NaCl, 0.01% ethylenediaminetetraacetic acid (EDTA), 0.001% NaN3, pH 7.4. Lipid-free LDL and VLDL were prepared by delipidation at 4°C with diethyl ether-ethanol (3:1). The apoB was purified by fractionation of lipid-free LDL on Sephadex G-150 as described previously. Antisera against apoB were raised in goats and were partially purified by affinity chromatography using LDL-Sepharose. The antisera gave precipitin lines of complete identity against LDL and VLDL, but did not react with human serum albumin, high density lipoproteins (HDL) and its major apoproteins, apoA-I or apoA-II, or the apoC proteins, either on double gel diffusion plates or in the electroimmunodiffusion (EID) system described below.

ELECTROIMMUNODIFFUSION OR "ROCKET" TECHNIQUE

A modification of the EID or Laurell "rocket" technique22 was employed using a Gelman water-cooled electrophoresis cell that permitted 20 duplicate samples to run at one time; 5-μl quantities of dilutions of an LDL standard or unknown were dispensed into the wells. All electrophoresis runs were performed at 15°C at 4 V/cm in a 0.03 M Na barbital (Veronal) buffer, pH 8.6. It was empirically determined that precipitin peaks reached maximum heights by 5.5 hours. The amount of anti-apoB added to molten agarose (1:300 dilution of the full-strength antisem) was such that the following criteria were met: precipitin peaks reached maximum height; they were at least 0.5 cm high at the lower end of the LDL standard curve (30 ng) and they did not demonstrate antigen excess at the upper end of the curve (200 ng). The concentration of apoB in the standard LDL sample was determined by amino acid analyses.

The intra-run variability of the EID system was calculated on 40 sets of duplicate measurements. The coefficient of reliability (r) was found to be 0.995, giving an error of 1%. Inter-run variability or test-retest reliability, as determined on 20 sets on successive days, gave a coefficient of reliability of 0.943, or an error of 11%. All apoB values were recorded as micrograms per milligram of tissue, dry weight. The dry weight was determined by weighing to obtain a mean dry weight because small dry weights of individual minces precluded accurate measurements. Peak heights for these six minces were measured to obtain a mean dry weight because small dry weights of individual minces precluded accurate measurements. Peak heights for these six minces were measured to obtain a mean apoB value. The other set of six minces was pooled and homogenized, and the supernatant fractions were measured by EID to determine whether any apoB remained in the tissue after electrophoresis.

TISSUE SAMPLES

Samples of grossly normal human aorta from the descending thoracic aorta to the iliac bifurcation were obtained at autopsy within 12 hours after death from 40 subjects between the ages of 12 and 80 years. Twenty-three subjects were trauma victims (gunshot wounds and car accidents), seven died from coronary heart disease, and ten from miscellaneous causes (drug overdose, brain tumor, pulmonary embolism, ruptured aneurysm, or renal failure). After rinsing in saline twice for 10 seconds each at 4°C, the intimal surface (9-25 cm2) was stripped from the underlining tunica media at a cleavage plane close to the first fragmented elastic membrane. Although intimal tissue was taken primarily from the descending thoracic aorta, in some cases, particularly those from younger individuals with very thin intimas, part of the abdominal aorta was also used.

EXTRACTION PROCEDURE

From preliminary studies, it was determined that the following conditions for extraction yielded the optimal amount of apoB: 1 g, wet weight, or aortic intimal mince was added to 10 ml of a standard buffer [0.13 M tromethamine (Tris)-HCl, 0.01% EDTA buffer, pH 7.4]. The amount of apoB extracted was not increased by utilizing 1 M NaCl. Aortic minces in standard buffer were homogenized for 30 seconds at 4°C in a Polytron homogenizer (Brinkmann) equipped with a microprobe operated at full power. Following a 30-minute incubation at 4°C, the debris was sedimented by slow speed centrifugation and the supernatant fraction was assayed for apoB by the EID system.

WHOLE TISSUE MINCES

Minces of aortic intima from individual cases were divided into two portions. One portion was homogenized as described above and the supernatant fractions were assayed for apoB by EID. The other portion was further subdivided into 12 minces and each was placed directly into a well of the EID system. Following electrophoresis, minces from six of the wells were pooled, lyophilized, and weighed to obtain a mean dry weight because small dry weights of individual minces precluded accurate measurements. Peak heights for these six minces were measured to obtain a mean apoB value. The other set of six minces was pooled and homogenized, and the supernatant fractions were measured by EID to determine whether any apoB remained in the tissue after electrophoresis.

RADIOLABELING OF LDL

125I-LDL was prepared by the iodine monochloride method of MacFarland, as modified by Bilheimer et al. Carrier-free Na125I (Amersham/Searle), with a specific activity of 10 mCi/mmol was used. The efficiency of iodination was 20%, the percent of soluble counts in 20% trichloroacetic acid (TCA) was 2-5%, and the percent of counts extractable by chloroform-methanol (2:1) was 4%. The 125I-LDL gave precipitin lines of complete identity to unlabeled LDL.
DIFFERENTIAL ULTRACENTRIFUGATION OF SUPERNATANT FRACTIONS

Supernatant fractions of aortic intimal homogenates were subjected to differential ultracentrifugation to obtain LDL (d 1.006-1.063) and VLDL (d < 1.006) density fractions as previously reported. Briefly, the procedure consisted of dialyzing the supernatant fraction against 0.9% sodium chloride (d 1.006), centrifuging at the solvent density in an SW 50.1 rotor at 50,000 rpm at 8°C for 18 hours, and removing the top 1 ml (VLDL fraction). The LDL (d 1.006-1.063) fraction was isolated by adjusting the previous infranatant fraction to d 1.063 with KBr and ultracentrifuging under the same conditions.

LIGHT MICROSCOPY

Specimens adjacent to areas used for homogenization were either snap-frozen prior to cryostat sectioning or fixed in formalin prior to dehydration and embedding in paraffin. Cryostat sections were subjected to the immunofluorescence procedure to localize apoB, while serial sections were stained with oil red O-hematoxylin to localize neutral lipids and nuclei, respectively. Paraffin-embedded sections of tissue blocks adjacent to those used for cryostat sections were stained with hematoxylin and eosin and with Movat’s pentachrome stain to assess the general morphology of the sections. Pellets of aortic homogenates were also subjected to the immunofluorescence procedure to localize apoB. A Leitz Orthoplan photomicroscope was used for bright field and fluorescence microscopy. For the latter, an HBO 200 mercury light source, a UG-1 primary filter, a K-430 secondary filter, and dark field condenser were used.

ELECTRON MICROSCOPY

The ultrastructural morphology of the density fraction in which d 1.006-1.063 was observed by electron microscopy following negative staining with phosphotungstic acid.

Results

QUANTIFICATION OF apoB

A representative electroimmunodiffusion gel for standard LDL and buffer extracts of aortic intimal homogenates is shown in Figure 1. By plotting peak height vs. apoB content in a 5-μl sample, a linear curve was obtained (Fig. 2). The maximal sensitivity of the assay was in the range of 30-200 ng of apoB. Since apoB is also a major apoprotein of VLDL, it was important to determine whether the EID technique accurately measured its content of apoB. When peak heights obtained by EID for the native LDL and VLDL preparations were plotted against their respective apoB content, values for both fell on the same linear curve, suggesting that the EID system measured the apoB content of LDL and VLDL identically. To determine their

Figure 1  Typical pattern of precipitin peaks formed during electroimmunodiffusion (EID) of dilutions of a standard low density lipoprotein (LDL) and supernatant fractions of arterial tissue. The amount of apolipoprotein B (apoB) (ng) measured in each dilution of LDL were: wells 1-7 = 227, 148, 111, 87, 74, 56, and 45, respectively; wells 8-13 contained 5 μl of supernatant fractions from different intimal homogenates. The details for the EID system are described in the text. Gels were stained with Coomassie brilliant blue.
APOLIPOPROTEIN B IN NORMAL AORTA/Hoff et al.

Figure 2

Standard curve for plasma low density lipoprotein (LDL) and very low density lipoprotein (VLDL); height of precipitin peaks vs. amount of apolipoprotein B (apoB) in a 5-μl sample. The apoB content of LDL was determined by amino acid analyses. The amount of apoB in the VLDL preparation was determined after chromatography of lipid-free VLDL on Sephadex G-150 as described in the text.

EXTRACTION OF LDL FROM HOMOGENATES

To aid in the assessment of the efficiency of extracting apoB from tissue, 125I-LDL (2.7-5.4 μg of apoB) were added to an aortic intimal homogenate and incubated for 30 minutes at 4°C; the apoB was then extracted as described in the Methods. As shown in Table 1, 83% of 125I-LDL was recovered in the supernatant fraction of the first extraction; 11% was recovered in the second extract. Since 2% of the counts was bound to the surface of the tubes (Table 1), as shown in the control experiment without homogenate, only 4% of the added 125I-LDL was associated with the pellet. The addition of increasing amounts of unlabeled LDL (Table 1) to the homogenate did not appreciably change the percentage of 125I-LDL extracted from the homogenate.

To determine whether the efficiency of extracting apoB was dependent on the concentration of added apoB, a study was made in which increasing amounts of exogenous LDL (12.5-63 ng of apoB) were added to 1-ml samples of aortic intimal homogenates and allowed to incubate for 30 minutes at 4°C. A 5-μl sample of each supernatant fraction was dispensed into wells of the EID system. Figure 4 shows a plot of apoB (μg) of the homogenate plus exogenous LDL as measured by EID vs. apoB (μg) of exogenous LDL as determined by amino acid analyses. The homogenate alone contained 51 μg of LDL. Using multiple regression analysis,27 the experimental points fell on a regression line ($r^2 = 0.9842$). The coefficient of determination ($r^2$) was obtained, giving an error of 4%.

BREAKDOWN OF apoB DURING EXTRACTION

Table 1 Binding of 125I-Labeled Low Density Lipoproteins (LDL) to Homogenates of Normal Human Aortic Intima

<table>
<thead>
<tr>
<th>125I-LDL (μg apoB)</th>
<th>LDL (μg apoB)</th>
<th>Tissue</th>
<th>% 125I-LDL in 1st extraction</th>
<th>% 125I-LDL in 2nd extraction</th>
<th>% 125I-LDL in pellet</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.7</td>
<td>-</td>
<td>-</td>
<td>87.2</td>
<td>10.8</td>
<td>2.0</td>
</tr>
<tr>
<td>2.7</td>
<td>+</td>
<td>+</td>
<td>82.1</td>
<td>12.0</td>
<td>5.9</td>
</tr>
<tr>
<td>3.4</td>
<td>-</td>
<td>+</td>
<td>82.8</td>
<td>10.7</td>
<td>6.5</td>
</tr>
<tr>
<td>5.4</td>
<td>-</td>
<td>+</td>
<td>82.9</td>
<td>10.9</td>
<td>6.2</td>
</tr>
<tr>
<td>2.7</td>
<td>27.5</td>
<td>+</td>
<td>83.0</td>
<td>11.2</td>
<td>5.8</td>
</tr>
<tr>
<td>2.7</td>
<td>55.0</td>
<td>+</td>
<td>83.3</td>
<td>10.7</td>
<td>6.0</td>
</tr>
<tr>
<td>2.7</td>
<td>137.0</td>
<td>+</td>
<td>82.3</td>
<td>11.4</td>
<td>6.3</td>
</tr>
<tr>
<td>2.7</td>
<td>275.0</td>
<td>+</td>
<td>83.2</td>
<td>10.4</td>
<td>6.4</td>
</tr>
<tr>
<td>2.7</td>
<td>550.0</td>
<td>+</td>
<td>82.9</td>
<td>10.9</td>
<td>6.2</td>
</tr>
<tr>
<td>2.7</td>
<td>1380.0</td>
<td>+</td>
<td>82.1</td>
<td>10.3</td>
<td>7.6</td>
</tr>
</tbody>
</table>

125I-LDL [6.2 × 10⁷ cpm/mg of apolipoprotein B (apoB)] or unlabeled LDL were added to a homogenate (20 mg, dry weight) of normal aortic intima and the final volume was adjusted to 1.0 ml with standard buffer; the tube without homogenate contained 10 mg of bovine serum albumin. After incubation for 30 minutes at 4°C, the homogenates were centrifuged (10 minutes at 5,000 rpm) and the 125I-LDL in the supernatant fraction was determined. The 2% bound 125I-LDL in the absence of homogenates represents nonspecific binding to the tube. The values represent an average of duplicate analyses. The same results were obtained at 23°C. Plus signs and minus signs signify presence or absence, respectively, of tissue.
formed. In the first study, the solubility of $^{125}$I-LDL in 20% TCA as a function of homogenization was determined. In the second, the immunological reactivity of a homogenate to which unlabeled LDL were added was assayed by the EID system.

$^{125}$I-LDL (645 ng) was added to the following: (1) standard buffer containing 1% bovine serum albumin (BSA); (2) an aortic mince prior to homogenization; (3) an aortic mince following homogenization. Samples 1 and 2 were subsequently homogenized and the supernatant fractions were obtained after centrifugation as described in Methods. The percentage of counts in the TCA-soluble fraction of each supernatant fraction was then determined. As shown in Table 2, the percentage of TCA-soluble counts of $^{125}$I-LDL plus albumin was the same before and after homogenization. In the presence of aortic intimal minces, the percentage increased to 7.4% without homogenization and to 8.7% with homogenization (Table 2). Similar results were obtained when employing homogenates of the adjacent aortic tunica media which were previously shown both histochemically and quantitatively not to contain measurable amounts of LDL and VLDL. These data show that the maximal breakdown of $^{125}$I-LDL from homogenization with tissue was less than 7%.

The results of adding unlabeled LDL to aortic tissue and measuring the apoB content in 5 μl by EID are shown in Table 3. LDL (32 μg of apoB) were added to minces either before or after homogenization. By EID, there was a 12.6% loss in immunological reactivity due to homogenization of the LDL alone in 1% BSA. In the absence of
TABLE 2 Breakdown of I25I-Labeled Low Density Lipoproteins (LDL) during Homogenization

<table>
<thead>
<tr>
<th>Sample</th>
<th>% TCA-soluble I25I-LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL added before homogenization</td>
<td>LDL added after homogenization</td>
</tr>
<tr>
<td>125I-LDL + BSA</td>
<td>2.4</td>
</tr>
<tr>
<td>125I-LDL + intima</td>
<td>8.7</td>
</tr>
<tr>
<td>125I-LDL + media</td>
<td>9.1</td>
</tr>
</tbody>
</table>

125I-LDL [6.2 × 10^7 cpm/mg of apolipoprotein B (apoB)] were added to intima which was previously homogenized and to intima which was subsequently homogenized (20 mg, dry weight, in both) and the final volume was adjusted to 1.0 ml with standard buffer. The samples were incubated for 30 minutes at 4°C, centrifuged (10 minutes at 5,000 rpm), and the supernatant fractions were decanted. The tubes were incubated at 4°C for 10 minutes, centrifuged, and the radioactivity in the supernatant fraction was determined. Corrections were made for quenching of 125I by TCA. The numbers shown in the table represent the average of duplicate analyses. The same results were obtained at 23°C.

TABLE 3 Immunological Breakdown of Low Density Lipoproteins (LDL) during Homogenization

<table>
<thead>
<tr>
<th>Sample</th>
<th>apoB, total (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LDL added before homogenization</td>
</tr>
<tr>
<td>LDL + bovine serum albumin</td>
<td>28</td>
</tr>
<tr>
<td>Intima</td>
<td>56</td>
</tr>
<tr>
<td>LDL + intima</td>
<td>84 (88)</td>
</tr>
<tr>
<td>Media</td>
<td>0</td>
</tr>
<tr>
<td>LDL + media</td>
<td>27 (32)</td>
</tr>
</tbody>
</table>

LDL (32 μg) were added both to tissue minces which were previously homogenized and to tissue minces which were subsequently homogenized. The samples were incubated at 4°C for 10 minutes, centrifuged (10 minutes at 5,000 rpm), and the apolipoprotein B (apoB) in the supernatant fractions was determined by electroimmunodiffusion (EID). The numbers in parentheses represent theoretical values. The same results were obtained at 23°C.

exogenous LDL, the intimah homogenate contained 56 μg of apoB. When 32 μg of apoB were added to the arterial intimal mince either before or after homogenization, the total apoB measured by EID was 84 and 81.6 μg of apoB, respectively (Table 3). Addition of 32 μg of apoB to an arterial medial mince either before or after homogenization resulted in a slight reduction of apoB in the mixture. Since the arterial media does not contain measurable amounts of apoB, this result would suggest a slight breakdown of apoB by some factors in the medial extract. Overall, these results suggest a loss of only a few percent of apoB during the combined steps of homogenization and extraction. The slight loss of immunological reactivity of apoB when LDL was homogenized in the presence of 1% BSA suggests that arterial intima offers greater protection to LDL against denaturation during homogenization than does BSA.

LONG-TERM BREAKDOWN OF apoB

To assess whether the LDL standards or the homogenates were broken down over longer time intervals, TCA-soluble counts of 125I-LDL added to an aortic intimal homogenate were determined. An initial increase in TCA solubility of 3% remained constant over a 24-hour period. Although there was no decrease in immunological reactivity of the LDL standard with time, there was a gradual reduction in apoB values in intimal homogenates over a 20-day period (Fig. 5). However, if the extracts were measured within a few days following homogenization, the values were reliable.

apoB CONTENT OF WHOLE TISSUE MINCES AND HOMOGENATES

Recent studies on the accumulation of LDL in human aortas utilized whole minces of intima and the EID or "rocket" technique. We therefore compared the apoB content of supernatant fractions of homogenates and of adjacent whole minces. The results of studies on aortas from six individuals are presented in Table 4. A mean of about 3 times more apoB was found in homogenates than in minces. Immunofluorescence techniques localizing apoB demonstrated only a small reduction in positive fluorescence in minces which had been run in EID relative to those which had not. This was in contrast to a nearly complete reduction of positive fluorescence in buffer-extracted pellets of intimal homogenates. Supernatant fractions of minces homogenized after EID showed small peaks, but these were just below the threshold of the assay (<30 ng).

![Figure 5](http://circres.ahajournals.org/)

**Figure 5** The effect of time on the immunological reactivity of standard low density lipoprotein (LDL) (A) and aortic homogenates (B). The apolipoprotein B (apoB) was measured as described in the legends to Tables 2 and 3.

TABLE 4 Apolipoprotein (apoB) Content in Minces and Homogenates of Normal Aortic Intima

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Homogenate apoB (μg/mg tissue, dry wt)</th>
<th>Mince apoB (μg/mg tissue, dry wt)</th>
<th>Ratio, homogenate/mince</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.06</td>
<td>1.79</td>
<td>2.83</td>
</tr>
<tr>
<td>2</td>
<td>4.65</td>
<td>1.65</td>
<td>2.82</td>
</tr>
<tr>
<td>3</td>
<td>4.09</td>
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<td>1.66</td>
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<td>3.69</td>
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<td>3.57</td>
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<tr>
<td>6</td>
<td>6.97</td>
<td>1.21</td>
<td>5.76</td>
</tr>
<tr>
<td>Mean</td>
<td>5.57</td>
<td>1.39</td>
<td>3.29</td>
</tr>
</tbody>
</table>

The amount of buffer-soluble apoB was determined in both homogenates and minces of aortic intima from six subjects. Samples of whole minces as well as 5 μg of supernatant fractions of homogenates were placed in wells of the electroimmunodiffusion (EID) system and their apoB content was determined. Mince dry weights were determined following EID. Homogenate dry weights were measured from a 1-ml sample taken before EID.
CHARACTERIZATION OF LIPOPROTEINS FROM AORTIC INTIMAL EXTRACTS

The apoB-containing lipoproteins from supernatant fractions of aortic intimal extracts were isolated by differential ultracentrifugation into the LDL (d 1.006-1.063) and VLDL (d < 1.006) density ranges. When the apoB content in both isolated density fractions was measured by EID in six separate intimal extracts from grossly normal aortas, approximately 95% of the total apoB content was found in the LDL density range (d 1.006-1.063). Only negligible amounts of apoB were found in the VLDL density range (d < 1.006). Electron microscopy of negatively stained preparations of the LDL density fraction showed spherical particles with diameters of 200-250 Å (Fig. 6A), consistent with the size of plasma LDL (Fig. 6B). Furthermore, lines of complete identity against anti-apoB were obtained between the material in the LDL density range and plasma LDL.

apoB CONTENT IN AORTIC HOMOGENATES

The apoB content in supernatant fractions of intimal homogenates from 40 separate human aortas was measured by EID. Supernatant fractions were diluted 1:1 with standard buffer to fall within the standard curve. The apoB values ranged from 0.34 to 18.45 μg/mg of tissue, dry weight, with a mean of 5.42 ± 3.95 sd. When plotted against age (Fig. 7), most apoB values ranged between 0.2 and 7 μg/mg of tissue, dry weight, except for a group of six subjects, between ages 40 and 55 years, with values greater than 10 μg/mg of tissue, dry weight.

Homogenates of tunica media from grossly uninvolved thoracic aortas consistently failed to demonstrate measurable quantities of apoB. The importance of using intimal preparations was verified by the fact that intima-media preparations gave apoB values appreciably lower than adjacent intimal preparations.

Discussion

In the present paper, we have described a quantitative, specific assay for apoB, the major apoprotein of LDL and VLDL. By use of this procedure the buffer-soluble apoB content in the intima of the grossly normal human aorta was determined and shown to range in value between 0.34

![Figure 6](http://circres.ahajournals.org/)

**FIGURE 6** Electron micrograph of particles in a negatively stained preparation of the low density lipoprotein (LDL) (d 1.006-1.063) density fraction of supernatant material from aortic homogenates (A) and plasma LDL (B). Note the presence of particles in the same size range as LDL. Both micrographs 144,000×.

![Figure 7](http://circres.ahajournals.org/)

**FIGURE 7** Apolipoprotein B (apoB) (μg/mg of tissue, dry weight) of aortic intima vs. age of subject. The apoB content was determined as described in the text.
and 18.45 μg/mg of tissue, dry weight. A technique previously reported by Smith and Slater used whole minces of human aortic intima which were inserted directly into the gel of the EID system. However, as shown in our present study, minces gave apoB values which were 3 times lower than those of homogenates. Smith and Slater expressed their values in terms of LDL-cholesterol. When converted into LDL-apoB, their values from minces were about 1/5 of the values recorded in our present study using homogenates.

Another procedure recently reported attempts to extract apoB in LDL and VLDL by pressing the tissue fluid from the aorta and precipitating the lipoproteins with heparin. Although apoB from both extracellular and intracellular compartments would be removed, one could envisage that an appreciable fraction would still adhere to insoluble components of the arterial wall.

Evidence from our present study suggesting that the material extracted from aortic intimal tissue resembles plasma LDL was obtained by differential ultracentrifugation studies. The LDL (d 1.006-1.063) density fraction contained measurable quantities of apoB and gave precipitation lines of complete identity with plasma LDL using antisera. The fraction in which d 1.006-1.063 also contained particles similar in size to LDL (200-250 Å). While these data suggest that the aorta contains native plasma LDL, more specific characterization of this fraction, as well as the VLDL density fraction, is still needed. We have previously demonstrated the presence of particles in the LDL and VLDL size range in saline extracts of human arteries with atherosclerotic involvement. Sections of adjacent areas showed the presence of spheres in this size range surrounded by reaction product following an immunoperoxidase procedure to localize apoB on the ultrastructural level.

Saline extraction in conjunction with immunoelectrophoresis or differential ultracentrifugation identified LDL and VLDL in human aortas usually associated with glycosaminoglycans. Specifically, chondroitin sulfate C and hyaluronic acid. It has been suggested that glycosaminoglycans play a role in the pathogenesis of atherosclerosis by retaining lipoproteins in the arterial wall. Our earlier studies have demonstrated the presence of both glycosaminoglycans and lipoproteins in the aortic intima. The glycosaminoglycan that complexes to LDL and VLDL in arterial extracts does not appear to affect the electrophoretic mobility of the lipoproteins, and, therefore, quantitative immunoassay, since these complexes are dissociated in the electric field at pH 8.6.

Although most aortic intimal apoB values were below 8 μg/mg of tissue, dry weight, a small group had values above 10 μg/mg. Whether these inordinately high values are related to changes in the arterial wall of 40-55-year-old individuals, or to such factors as hyperlipoproteinemia and hypertension, is unclear and still needs to be explored. A possible correlation between arterial and plasma apoB is presently being investigated.

Acknowledgments

We acknowledge the help of the staff of the Harris County Medical Examiners for assisting in the procurement of arterial specimens, and we are indebted to Debbie Mason, who prepared the manuscript for publication.

References

Reflex Changes in Hindlimb and Renal Vascular Resistance in Response to Distention of the Isolated Pulmonary Arteries of the Dog

JOHN R. LEDSOME AND WAI-ON KAN

SUMMARY We describe a preparation that uses a constant flow, right heart bypass for perfusion of an isolated pouch of the main pulmonary arteries at controlled pressures, and show that increments in pressure in the pulmonary arterial pouch are accompanied by increases in systemic vascular resistance and in hindlimb vascular resistance. These changes are demonstrated over the whole range of 5-120 cm H2O pressure in the pulmonary arterial pouch. In contrast there are no significant changes in renal vascular resistance or heart rate. We find that changing the temperature of the perfusate in the pulmonary arterial pouch from 37°C to 30°C is associated with a decrease in systemic vascular resistance. Furthermore, the effects of raising the pulmonary arterial pouch pressure and of cooling are abolished by cervical vagotomy. These findings suggest that there is a tonic reflex vasocostrictor tone generated by the activity of receptors lying in or close to the walls of the pulmonary artery. These findings also suggest that the differential effects on systemic vascular resistance and renal resistance may provide one mechanism by which changes in blood volume may lead to appropriate changes in renal solute excretion.

THE HISTOLOGICAL appearances of the different types of sensory nerve ending which exist in the walls of the pulmonary artery and its main branches have been reviewed in detail. That at least some of these sensory nerve endings may be regarded as pressoreceptors has been clearly demonstrated in both the dog and the cat. Electrophysiological techniques have shown that afferent fibers from sensory endings in the pulmonary arteries may be found in the vagus nerves as myelinated or unmyelinated fibers or may be found in the sympathetic system. Attempts to demonstrate the physiological response to stimulation of the pulmonary arterial baroreceptors have produced variable results and have left the physiological significance of the responses in doubt.

The present investigation was designed to study the reflex effect on systemic arterial pressure, hindlimb vascular resistance, renal vascular resistance, and heart rate, of distention of the main pulmonary artery and its branches with controlled increments in pressure. The aim was to determine whether reproducible reflex cardiovascular responses could be observed by using a more controlled stimulus and method of assessing responses than has been used previously.

Methods

The general plan of the preparation to be described was to create a right atrium to left pulmonary artery bypass through which the left lung was perfused at constant blood flow. This provided for adequate gas exchange and also ensured a constant output from the left ventricle in the steady state. Creation of the bypass then made it possible to establish a pulmonary arterial pouch consisting of the main pulmonary artery, most of the left pulmonary artery, and the right pulmonary artery and its major branches. This pulmonary arterial pouch was perfused with either venous blood or saline at different controlled pressures. Reflex responses were observed in heart rate, mean arterial pressure (systemic vascular resistance), and hindlimb vascular resistance by using an isolated, constant flow, perfused hindlimb preparation, or in renal vascular resistance by using an isolated, constant pressure or constant flow, perfused kidney preparation.

Mongrel dogs of 20-35 kg were injected with morphine sulfate (0.5 mg/kg, sc). One hour later under local anesthesia (mepivacaine hydrochloride, 1%) a polyethylene...
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