Effect of Transmural Pressure on Low Density Lipoprotein and Albumin Transport and Distribution Across the Intact Arterial Wall

Patrick A. Curmi, Lucienne Juan, and Alain Tedgui

To investigate the effect of hyperpressure on the transport of low density lipoprotein (LDL) and albumin in the arterial wall, we measured in vitro the uptake of both iodine-131-labeled LDL and iodine-125-labeled albumin in intact rabbit thoracic aorta, held at in vivo length and pressurized to 70 or 160 mm Hg. Arteries were incubated for 2 hours (n=8) at 70 mm Hg, and for 5 minutes (n=4), 30 minutes (n=4), 1 hour (n=5), and 2 hours (n=5) at 160 mm Hg. The transmural distribution of the relative concentrations of LDL (C_{LDL}) and albumin (C_{ alb}) across the wall was determined by using a serial frozen sectioning technique. At 70 mm Hg, the mean medial C_{LDL} and C_{ alb} values were 0.0018±0.0007 and 0.0039±0.0013, respectively. At 160 mm Hg, C_{LDL} and C_{ alb} were markedly increased. The distribution of labeled albumin was almost uniform across the media and reached a steady state after 30 minutes, whereas labeled LDL accumulated in the first inner layers, a steady state being achieved after 1 hour. The 1-hour values of C_{LDL} in the first and second luminal sections (0.24±0.03 and 0.13±0.05, respectively) were much higher than those of C_{ alb}, the C_{LDL}/C_{ alb} ratios being 4.12±0.94 and 2.34±0.42 (p<0.01), respectively. In the subsequent sections, the C_{LDL} decreased markedly and became much lower than the C_{ alb}, the C_{LDL}/C_{ alb} ratio averaging 0.2 in the two-thirds outer media. To investigate whether LDL was trapped at high pressure in the inner layers, vessels were exposed to a tracer-free intraluminal solution for 30 minutes, after a 30-minute incubation with tracers. After washout, albumin was almost totally removed from the wall, while the C_{LDL} were practically unchanged. Compaction of the media induced by high distending stresses applied to the vessel might have hindered the efflux of LDL, whereas albumin moved freely through the wall. Synergy between increased endothelial permeability and compaction of the media together with enhanced pressure-driven convection might account for the marked increase in LDL concentration observed in the inner wall at high pressure. (Circulation Research 1990;66:1692–1702)

Recent progress in biology of the arterial wall has given a framework for the understanding of the formation of atherosclerotic lesions. In particular, foam cells appear to be the result of low density lipoprotein (LDL) oxidation and of its uptake by the scavenger receptors of monocytes-macrophages that have migrated into the intima. However, early accumulation of plasma LDL in the subendothelial space seems to be required to trigger this process. In this respect, the full knowledge of the mechanisms of transport and distribution of plasma macromolecules in the arterial wall might contribute to the understanding of atherosclerosis. Hemodynamic factors have long been recognized as initiating, or at least potentiating, factors in atherosclerosis. In particular, hypertension is a well-known risk factor, especially when associated with hypercholesterolemia. However, the actual mechanism by which hypertension contributes to atherogenesis has not been established.

Several studies have investigated the effects of pressure on the transport of macromolecules. Most of those used albumin as a standard for plasma macromolecules and reported an increase of the transport of the protein with increasing pressure. However, LDL, an atherogenic plasma molecule, may behave quite differently than albumin, as pointed out by Smith and Staples, who found that in postmortem human aortas, concentration was proportional to the size of the macromolecule in the intima and inversely proportional in the media. Therefore, it seems that information is necessary not only about...
total concentration but also about its regional distribution across the arterial wall. To the best of our knowledge, the only study reporting the effects of pressure higher than 100 mm Hg on the LDL transport in the intact arterial wall was that of Bretherton et al., but they did not address the issue of the wall distribution of LDL. Therefore, the aim of the present study was to investigate the effect of transmural pressure on the uptake and distribution of both 131I-human LDL and 125I-albumin in freshly excised rabbit thoracic aorta, at both low pressure (70 mm Hg) and high pressure (160 mm Hg).

Materials and Methods

Tracers

LDL (1.025<d<1.050 g/ml) was isolated from fresh human plasma by sequential ultracentrifugation, LDL and serum bovine albumin (fraction V, Sigma Chemical, St. Louis, Missouri) were labeled with 131I and 125I (Amersham France, Les Ulis), respectively, by using the iodine monochloride method of McFarlane as modified by Bilheimer et al. Free iodine was removed by passing the solution through a Sephadex G-50 column and by extensive dialysis at 4°C against 0.15 M NaCl containing 50 mg/l gentamicin sulfate and 100 mg/l EDTA. After dialysis, more than 99.2±0.4% of the 131I radioactivity and 99.6±0.2% of the 125I radioactivity was precipitable by 10% trichloroacetic acid (TCA) (final concentration). During agarose electrophoresis, LDL migrated as a single band. Less than 8% of the total radioactivity was lipid bound. The concentration of lipoproteins was determined by the Lowry method. Specific activity ranged from 300 to 500 cpm/ng of albumin and from 350 to 650 cpm/ng of protein for LDL.

Artery Preparation

Male New Zealand White rabbits (2–2.5 kg) were anesthetized with pentobarbital sodium (30 mg/kg i.v.). The trachea was intubated, and the animals were mechanically ventilated. The thorax was opened by midline section of the sternum, and the aorta was exposed between the heart and the diaphragm by lateral displacement of the lungs. The ventral surface of the vessel was dissected free from its bed, and the first eight pairs of intercostal arteries were cauterized 2–3 mm from the aorta. During the whole operative procedure, saline solution was continually applied to the outer surface of the aorta to prevent drying. The distal end of the thoracic aorta was ligated, and a 16-gauge cannula was inserted retrograde proximal to the ligature and connected to a reservoir 80 cm above the animal. The reservoir contained oxygenated Tyrode’s solution with 4% bovine serum albumin at pH 7.4. The animal was then killed with an overdose of pentobarbital, and the aorta was flushed with Tyrode’s solution from the reservoir. The proximal end of the aorta was then ligated just below the arch and a second cannula, connected to a second pressurizing reservoir filled with the same solution, inserted anterograde, and tied in place. In this way, pressure was continuously maintained within the artery preventing collapse of the aorta. A ligature was tied around the mid region of the aorta, and a cannula was inserted distal to this ligature. The lower part of the aorta was excised while held at its in vivo length by clamping the two lower cannulas into an adjustable rig. The intercostal arteries of the excised arterial segment were then carefully ligated close to the wall to avoid leakage. The vessel, connected to a pressurization chamber, was then immersed in a 10-ml bath of Tyrode’s solution containing 50 mg/l gentamicin sulfate (pH 7.4) that was oxygenated with 95% O2-5% CO2 and maintained at 39°C. The upper part of the thoracic aorta was then cannulated, excised, and transferred to the incubation bath in a similar manner as the lower part. The vessels were then flushed at physiological transmural pressure with Tyrode’s solution containing 4% albumin and 0.03% Evans blue. The presence of protein in flushing and luminal solutions served to maintain the molecular sieving properties of the endothelium and to keep a normal osmotic pressure gradient across the vessel wall. The presence of dye allowed us to detect leaks and to test the integrity of the endothelium at the end of the experiment. The luminal solution contained both radioactive 131I-LDL at a concentration of 0.15 mg/ml and 125I-albumin. The intraluminal pressure was established at 70 or 160 mm Hg.

It has been shown in earlier studies in which the methods used for vessel excision were similar to those used in the present work that by examination under scanning and transmission electron microscopes, the ultrastructural integrity of the vessel wall was preserved, as well as a normal viability of the smooth muscle cells as assessed by the histoenzymic determination of phosphorylase activity.

The experiments were performed under different conditions. 1) Thirteen arteries were incubated for 2 hours in the presence of radiolabeled LDL and albumin. Eight of these arteries were pressurized at 70 mm Hg and five at 160 mm Hg. 2) To determine the kinetics of the transport processes at 160 mm Hg, four arteries were incubated for 5 minutes, four for 30 minutes, and five for 1 hour. 3) To evaluate the possibility that LDL was trapped in the media, two aortas were incubated at 160 mm Hg for 30 minutes, after which the luminal solution was changed to a tracer-free solution for an additional 30-minute incubation.

Estimation of Tracers in the Wall

After incubation, the aorta was cut from the cannulas, opened axially, and divided into four segments of roughly equal area. Presence of blue dye on the luminal surface was checked, and stained parts were discarded. At 70 mm Hg, the endothelial surface was usually found white except for the regions near cannulas, which were trimmed away. At 160 mm Hg, the endothelial surface was usually found with a
uniform pale blue staining except for the regions near cannulas, which were also trimmed away. The segments were laid on a lightly greased microscope slide and quickly frozen in a cryostat at -20°C to prevent further diffusion or degradation of albumin and LDL. The edges of the segments were trimmed to remove overhanging material, and their surface area was measured (range, 0.4–0.7 cm²). En face serial sections, 20 µm thick, were cut parallel to the luminal surface through the whole thickness of the wall from the intima to the adventitia. The boundary between the media and the adventitia was noted by an alteration in the appearance of the section and was found between 140 and 160 µm from the luminal surface. The volume of each tissue section was calculated from its thickness and surface area. The sections were placed in precooled test tubes for radioassay containing 500 µl of 1% albumin solution.

To precipitate the protein-bound label, 1.25 ml of 16% TCA was then added, and the mixture was centrifuged at 2,500g for 15 minutes. The supernatant was then carefully discarded. The same procedure was followed with triplicate 20-µl aliquots of the labeled intraluminal solution obtained at the beginning and at the end of the incubation period. To ensure that no TCA-soluble radioactivity was trapped in the 20-µm-thick tissue sections, homogenization of the tissue sections was performed before the TCA precipitation in a control series of experiments. The TCA-soluble radioactivity was similar to that found in the unhomogenized tissue sections.

125I and 131I radioactivities were assayed simultaneously in each test tube with a double counting procedure for 3 minutes on a gamma counter (Kontron GAMMAmatic, Basel, Switzerland). Spillover of 131I into the 125I channel was corrected by the channel ratio method. Values were corrected for radioactivity decay of 125I during the counting period. Tissue counts ranged from 100 to 5,000 cpm (after subtraction of background, which was about 20 cpm in 125I and 0-2 cpm in 131I).

Relative tissue concentrations of both TCA-precipitable LDL (C_{LDL}) and albumin (C_{album}) were calculated for each section as the counts per minute per unit volume of wet tissue divided by the counts per minute per unit volume of intraluminal solution. In each section, the C_{LDL} and C_{album} values permitted us to calculate the ratio of LDL to albumin content (C_{LDL}/C_{album}). The C_{LDL}, C_{album} and C_{LDL}/C_{album} values for the various sections cut from a single segment were plotted against their distance from the luminal surface to the external surface. Distances for each segment were normalized by dividing by the medial thickness. Average concentration profiles were constructed by averaging the values at equal intervals across the wall. From these values, a mean medial C_{LDL}, C_{album} and C_{LDL}/C_{album} value was calculated.

Staining of the Endothelial Cell Junctions by Silver Nitrate

En face preparation of endothelium was performed with the technique described by Gabaldon. Aortic segments prepared as described above were pressurized at 70 or 160 mm Hg for 2 hours and then fixed and stained under pressure. The procedure was as follows: the luminal surface was exposed for 10 minutes to fixative (1% paraformaldehyde–2% glutaraldehyde–6 mM NaCl in 0.1 M phosphate buffer, pH 7.4), washed for 1 minute with 8.9% sucrose in 20 mM HEPES buffer, pH 7.4, perfused successively for 1 minute with 0.05% AgNO₃ in washing solution (pH 7.4), 1 minute with washing solution, and then 2 minutes with fixative. Postfixation was performed for 20 hours in 1% paraformaldehyde–2% glutaraldehyde–6 mM NaCl in 0.1 M cacodylate buffer, pH 7.4, in room light. After postfixation, the intima-media was separated from the adventitia by the method of Wolinsky and Daly and laid on a glass slide with a drop of fixative, which was then covered with a coverslip, endothelium facing upward, to be photographed.

Statistical Methods

A two-way analysis of variance has been constructed on the data of C_{LDL}, C_{album} and C_{LDL}/C_{album} to test the effects of the incubation time and the location across the wall. When the variations were found statistically significant (p<0.05), multiple comparisons were performed by using Bonferroni’s method. Comparisons between the C_{LDL} and C_{album} values were performed using a paired t test. The data are reported with standard deviations.

Results

Staining of the Endothelial Cell Junctions by Silver Nitrate

Luminal surfaces stained with silver nitrate obtained in aortas pressurized at 70 and 160 mm Hg are shown in Figure 1. No endothelial cell leak or denudation was noted under these two conditions in the areas kept for analysis (white areas at 70 mm Hg, light blue areas at 160 mm Hg).

Albumin and LDL 2-Hour Uptake at 70 and 160 mm Hg

In arteries incubated for 2 hours at 70 mm Hg (Figure 2), the C_{album} values slightly decreased from about 0.0052±0.0027 in the inner media to a minimum of 0.0029±0.0013. The C_{LDL} values were significantly lower than the corresponding C_{album} at each position across the media (p<0.01), varying from 0.0044±0.0031 to 0.0007±0.0003. After 2 hours of incubation at 160 mm Hg (Figures 3a and 3b), both C_{LDL} and C_{album} values were significantly increased across the wall (p<0.01). However, their transmural profiles showed striking differences. That of C_{album} was practically flat, whereas that of C_{LDL} showed a steep gradient decreasing from the luminal side to the outside. The C_{album} values were increased almost uniformly across the media, being about 10-fold higher than those found at 70 mm Hg, whereas the C_{LDL} values were increased 44-fold in the first layer and about 10-fold in the outer media.
At low pressure, the $C_{\text{LDL}}/C_{\text{alb}}$ ratio values in the media (Figure 4) were always lower than 1, showing a gradient from the luminal side to the media-adventitia boundary. The ratio values decreased from $0.8\pm0.2$ to $0.19\pm0.07$. At high pressure, they became markedly higher than 1 in the first and second sections of the media, practically equal to 1 in the third section, and remained significantly lower in the rest of the media ($p<0.01$). The $C_{\text{LDL}}/C_{\text{alb}}$ ratio values were $4.08\pm1.49$ in the first section ($p<0.01$, as compared with 1), $2.74\pm1.23$ in the second ($p<0.05$), and ranged from 0.18 to 0.38 in the outer media. The latter values were not significantly different from those found at 70 mm Hg.

**Time Course of Albumin and LDL Uptake at 160 mm Hg**

The kinetics of the albumin uptake showed that after 5 minutes of incubation at 160 mm Hg (Figure 5a), the albumin relative concentrations decreased from $0.013\pm0.002$ near the lumen to $0.003\pm0.001$ in the mid media. LDL was found only in the first two sections of the media and in the adventitia, $C_{\text{LDL}}$ being $0.0092\pm0.0013$ in the first section and $0.0034\pm0.0013$ in the second.

After 30 minutes at 160 mm Hg, a steady state appeared to have been reached for albumin. The albumin relative concentration values obtained at 1 and 2 hours were not significantly different from those found at 30 minutes even though they were slightly lower. The mean medial $C_{\text{alb}}$ values were equal to $0.062\pm0.016$, $0.056\pm0.028$, and $0.048\pm0.011$ at 30 minutes, 1 hour, and 2 hours, respectively.

The kinetics of the LDL uptake was markedly different (Figure 5b). After 30 minutes, an important increase of the LDL relative concentrations was observed in the first three sections of the media.
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FIGURE 2. Average profiles of relative concentrations of 125I-albumin (■) and 131I-low density lipoprotein (LDL) (□) as a function of normalized distance from the lumen (1.0 represents the media-adventitia boundary) obtained from aortas incubated for 2 hours at 70 mm Hg. The number of arteries (n) is given in parentheses. Bars represent SEM.

(p<0.01). In the rest of the wall, low C_{LDL} values were observed. Between 30 minutes and 1 hour, the C_{LDL} values continued to increase in the first two sections to 0.24±0.03 and 0.13±0.05, respectively, and did not change significantly in the subsequent sections. After 2 hours, the C_{LDL} values were not significantly different from those obtained at 1 hour.

The transmural distributions of the C_{LDL}/C_{alb} ratios obtained at 160 mm Hg after various periods of incubation are shown in Figure 6. After 5 minutes, the relative concentrations of LDL were significantly lower than those of albumin throughout the media (p<0.01). After 30 minutes, the C_{LDL}/C_{alb} ratio became significantly higher than 1 in the first section (2.13±0.67, p<0.05) and increased further after 1 hour, reaching 4.12±0.94 in the first section and 2.34±0.42 in the second (p<0.01). In the subsequent sections of the media, the ratio showed no significant differences with time.

Washout Experiments

In the washout experiments (Figure 7), albumin relative concentration fell markedly after the 30-minute exposition of the artery to a tracer-free solution. The mean medial C_{alb} value became 0.0051±0.0004 after washout, whereas it was 0.063±0.016 at the end of the 30-minute incubation in the presence of tracers. On the contrary, the C_{LDL} values were practi-

FIGURE 3. Average profiles of relative concentrations of 125I-albumin (panel a) and 131I-low density lipoprotein (LDL) (panel b) as a function of normalized distance from the lumen (1.0 represents the media-adventitia boundary) obtained from aortas incubated for 2 hours at 70 mm Hg (□) and 160 mm Hg (■). Curves at 70 mm Hg are the same as in Figure 2. The numbers of arteries (n) are given in parentheses. Bars represent SEM.
physically unchanged after washout; only the value in the first section decreased slightly from 0.12±0.03 to 0.073±0.015 \((p<0.05)\), and no significant difference was found in the subsequent sections.

**Discussion**

Most of the earlier in vitro studies concerned with the effect of high pressure on the macromolecular arterial transport were performed in deendothelialized arteries\(^8\)\textsuperscript{,}\textsuperscript{,}\textsuperscript{13}-\textsuperscript{14} or in arteries excised in such a way that it is likely that the endothelium was not preserved.\(^6\)\textsuperscript{,}\textsuperscript{10} However, because it has been shown that in the absence of endothelium the media can undergo expansion at high pressure whereas medial compaction is observed when endothelium is preserved,\(^15\) results obtained from studies performed in deendothelialized vessels are expected to differ substantially from those obtained in studies with intact endothelium. In in vivo studies, the different interventions used to acutely increase blood pressure such as section of the carotid sinus depressor nerves or the vagi\(^2\) or the perfusion of vasoactive substances\(^12\) might have had direct effects on the permeability of the endothelium and media\(^27\)-\textsuperscript{29} in addition to those due to pressure. On the other hand, the structural changes of endothelium and media associated with long-term hypertension make experimental models of chronic hypertension, such as that used by Bretherton et al\(^17\) for studying LDL transport, unsuitable for investigation of the effect of hyperpressure on the arterial mass transport in the normal wall. Moreover, in vivo studies, the plasma decrease of tracers complicates the interpretation of concentration of the tracer found within the wall.

The in vitro preparation used in the present work allowed us to perform experiments in rabbit thoracic aortas with undamaged endothelium, as demonstrated by the functional and morphological controls (Figure 1). It permitted us to adjust the pressure level

**FIGURE 5.** Average profiles of relative concentrations of \textsuperscript{125}I-albumin (panel a) and \textsuperscript{131}I-low density lipoprotein (LDL) (panel b) as a function of normalized distance from the lumen (1.0 represents the media-adventitia boundary) obtained from aortas incubated for 5 minutes (○), 30 minutes (●), 1 hour (■), and 2 hours (■) at 160 mm Hg. The numbers of arteries (n) are given in parentheses. Bars represent SEM.

**FIGURE 6.** Average profiles of the relative concentration ratio of \textsuperscript{131}I-low density lipoprotein (LDL) to \textsuperscript{125}I-albumin (C\textsubscript{LDL}/C\textsubscript{album}) as a function of normalized distance from the lumen (1.0 represents the media-adventitia boundary) obtained from aortas incubated for 5 minutes (○), 30 minutes (●), 1 hour (■), and 2 hours (■) at 160 mm Hg. The C\textsubscript{LDL}/C\textsubscript{album} ratio values were obtained by using the activity in \textsuperscript{131}I and \textsuperscript{125}I determined simultaneously in each section. The numbers of arteries (n) are given in parentheses. Bars represent SEM.
without the use of vasoactive substances, to overcome the problem of the varying plasma decrease of LDL and albumin, and to carry out experiments, such as washout, unrealizable in vivo, but important for elucidation of the transport mechanisms. The transmural distribution of LDL and albumin relative concentrations was determined by serially sectioning the aortic tissue parallel to the intima surface at 20-μm intervals.20–33 Albumin has been the most widely used macromolecule in studying arterial transport processes. The effects of high transmural pressure on the albumin transport in the arterial wall are well documented.5–15 All studies reported an increase in albumin uptake by the wall with increasing pressure that might result from an increase in endothelial permeability,11 pressure-driven convection,14,15 or pressure-induced stretching of the wall.6,8 Transport of LDL in normal conditions has also been reported.30 However, the effects of pressure on the LDL transport in the arterial wall has been the subject of very few investigations. Fry et al14 carried out experiments in minipig aorta at 0 and 100 mm Hg, but this was performed in deendothelialized arteries. Bretherton et al17 reported the effects of hypertension on the in vivo uptake of LDL by the aorta of chronically hypertensive rabbits, with diastolic blood pressure averaging only 97 mm Hg. It thus appears that, so far, no study dealing with LDL transport in the normal wall has been performed at pressure largely exceeding 100 mm Hg. Furthermore, it seems that the simultaneous uptake of LDL and albumin by the arterial wall has not yet been reported.

**LDL and Albumin Uptake at Low Pressure**

In arteries pressurized for 2 hours at 70 mm Hg, the relative concentration of albumin was found to be higher than that of LDL. This might be accounted for by the higher permeability of the endothelium to albumin than to LDL. Bratzler et al30,32 reported an in vivo value of the endothelial permeability to albumin that was about two times higher than that to LDL (0.4×10⁻⁷ versus 0.2×10⁻⁷ cm/sec). The distribution volume for LDL in the media might also be expected to be lower than that for albumin, because of steric exclusion of the macromolecules.15 Moreover, LDL might have been internalized and degraded by the medial smooth muscle cells.31 The mean medial albumin and LDL relative concentration values found in the present study (0.0039 and 0.0018, respectively) are in good agreement with those previously reported in intact rabbit aorta (Table 1). The C_LDL value of 0.085 reported by Virag et al37 was probably overestimated due to the presence of adventitia in the tissue samples.

**LDL and Albumin Uptake at High Pressure**

When the transmural pressure was increased to 160 mm Hg for 2 hours, both albumin and LDL relative concentrations were increased. The mean medial albumin concentration value (0.048) is in agreement with those found by Auvert et al9 in situ preparation of endothelialized rabbit thoracic aorta (Table 2), and by Tedgui and Lever15 in an in vitro study in which the aortic tissue was exposed to albumin from both luminal and adventitial aspects. Feig et al12 reported a much lower value than ours. However, in that study, mean arterial blood pressure was increased to about 150 mm Hg for less than 5 minutes, then fell to 115 mm Hg for the following 15–20 minutes. The mean medial LDL concentration value found in our study (0.052) is markedly higher than that reported by Bretherton et al17 (Table 2). However, in their study, the level of the arterial

**FIGURE 7.** Average profiles of relative concentration of 125I-albumin (panel a) and 125I–low density lipoprotein (LDL) (panel b) as a function of normalized distance from the lumen (1.0 represents the media-adventitia boundary), obtained from aortas incubated at 160 mm Hg for 30 minutes (the same as in figure 5a and 5b) (□) and at 160 mm Hg for 30 minutes followed by 30-minute washout of the wall with a tracer-free solution (■). The numbers of arteries (n) are given in parentheses. Bars represent SEM.
pressure (diastolic pressure, 97 mm Hg) was much lower than in our study.

The albumin concentration profile became flat under high pressure, and the time course of the albumin uptake showed that a steady state was achieved within 30 minutes. The kinetics of the LDL uptake showed a marked increase in LDL concentration localized in the inner layers of the arterial wall, the \( \text{C}_{\text{LDL}} \) values being about four times higher than the \( \text{C}_{\text{ab}} \) values after 1 hour in the first luminal section, whereas the \( \text{C}_{\text{LDL}} \) values in the outer two thirds of the media remained markedly lower than the corresponding \( \text{C}_{\text{ab}} \). A steady state seems to have been reached after 1 hour.

The increase in albumin and LDL relative concentrations might be due to the following.

1) An increased macromolecular influx into the wall resulting from higher permeability of the endothelium at high pressure. As noted by Bratzler et al.,\(^{32}\) estimates of the permeability of the endothelium to albumin and LDL can be calculated from the normalized mass transfer rate \( J_s/C_1 \) at early times, where \( C_1 \) is the intraluminal concentration and the mass transfer rate, \( J_s \), is calculated as \( C_1 \cdot V/A \cdot T \), where \( C_1 \) is the mean tissue concentration, \( V \) is the volume of tissue, \( A \) is the surface area, and \( T \) is the duration of the experiment. \( V/A \) represents the thickness of the stretched wall. Assuming a medial thickness of 100 \( \mu \)m at 160 mm Hg\(^{15}\) and using the 5-minute mean medial \( C_{\text{ab}} \) and \( \text{C}_{\text{LDL}} \) values at 160 mm Hg, the permeability of the endothelium was estimated to be \( 2 \times 10^{-7} \) cm/sec and \( 0.8 \times 10^{-7} \) cm/sec for albumin and LDL, respectively. These values are about fivefold and fourfold higher than those reported by Bratzler et al in their in vivo study of albumin\(^{32}\) and LDL\(^{30}\) uptake in the rabbit thoracic aorta at normal arterial pressure.

2) The building of a concentration polarization of tracers at the interface between the lumen and the wall resulting from ultrafiltration of LDL and albu-

### Table 1. Mean Relative Tissue Concentrations Found at Normal Pressure Across the Rabbit Endothelialized Thoracic Aorta

<table>
<thead>
<tr>
<th>Authors</th>
<th>Model</th>
<th>Time after injection</th>
<th>( C_1/C_{p_0} )</th>
</tr>
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<tbody>
<tr>
<td>Duncan et al(^{34})</td>
<td>In vivo</td>
<td>6 hr</td>
<td>0.014</td>
</tr>
<tr>
<td>Bratzler et al(^{32})</td>
<td>In vivo</td>
<td>30 min</td>
<td>0.005</td>
</tr>
<tr>
<td>Feig et al(^{12})</td>
<td>In vivo</td>
<td>30 min</td>
<td>0.0038</td>
</tr>
<tr>
<td>Ramirez et al(^{18})</td>
<td>In vivo</td>
<td>30 min</td>
<td>0.0052</td>
</tr>
<tr>
<td>Caro et al(^{36})</td>
<td>In vivo</td>
<td>3 hr</td>
<td>0.009</td>
</tr>
<tr>
<td>Tedgui and Lever(^{15})</td>
<td>In vitro</td>
<td>1.5 hr</td>
<td>0.0056</td>
</tr>
<tr>
<td>Tedgui et al(^{29})</td>
<td>In vitro</td>
<td>1.5 hr</td>
<td>0.0045</td>
</tr>
<tr>
<td>Virag et al(^{37})</td>
<td>In vitro</td>
<td>2 hr</td>
<td>---</td>
</tr>
<tr>
<td>Bratzler et al(^{30})</td>
<td>In vivo</td>
<td>4 hr</td>
<td>0.0055 (Inner layers)</td>
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<tr>
<td>Bretherton et al(^{17})</td>
<td>In vivo</td>
<td>6 hr</td>
<td>0.0059</td>
</tr>
<tr>
<td>This study</td>
<td>In vitro</td>
<td>2 hr</td>
<td>0.0039 0.0018</td>
</tr>
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</table>

Values reported are relative tissue concentrations, \( C_1/C_{p_0} \), where \( C_1 \) is the tissue concentration and \( C_{p_0} \) is the initial plasma concentration in in vivo studies and the concentration of the intraluminal solution in in situ and in vitro studies. LDL, low density lipoprotein.

### Table 2. Mean Relative Tissue Concentrations Found at High Pressure Across the Rabbit Endothelialized Thoracic Aorta

<table>
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<tr>
<th>Authors</th>
<th>Model</th>
<th>( P ) (mm Hg)</th>
<th>Time after injection</th>
<th>( C_1/C_{p_0} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bratzler et al(^{32})</td>
<td>Perinephritis</td>
<td>97 (Diastolic)</td>
<td>10 min</td>
<td>0.05 (Inner layers)</td>
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<tr>
<td>Auvert et al(^{9})</td>
<td>In situ</td>
<td>180</td>
<td>10 min</td>
<td>0.03 (Outer media)</td>
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<td>Feig et al(^{12})</td>
<td>Angiotensin II</td>
<td>120 (Mean)</td>
<td>30 min</td>
<td>0.0038</td>
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<tr>
<td>Tedgui and Lever(^{15})</td>
<td>In vitro</td>
<td>180</td>
<td>90 min</td>
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<tr>
<td>This study</td>
<td>In vitro</td>
<td>160</td>
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<td></td>
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</tbody>
</table>

Values reported are relative tissue concentrations, \( C_1/C_{p_0} \), where \( C_1 \) is the tissue concentration and \( C_{p_0} \) is the initial plasma concentration in in vivo studies and the concentration of the intraluminal solution in in situ and in vitro studies. LDL, low density lipoprotein.

*Measurement for inner wall. This was the same correction as by Bratzler et al.\(^{32}\)
†Albumin was also present in the incubation bath.
min through the endothelium, causing an increase in the actual macromolecular concentration to which the artery was exposed. Fry et al. showed in vitro, in deendothelialized minipig aorta pressurized to 100 mm Hg, that the uptake of albumin and LDL was reduced by 40% and 63%, respectively, by stirring the solution. This demonstrated the existence of a concentration polarization at the lumen-wall interface. This effect might partly explain the increase in albumin and LDL uptake found in the present study. However, if our values of mean medial C_{\text{ alb}} and C_{\text{ LDL }} are corrected by the factors found by Fry, they became 0.037 and 0.019 for C_{\text{ alb}} and C_{\text{ LDL }} respectively, but still remained higher than those found at low pressure. However, we cannot rule out the possibility that the effect of concentration polarization was more important in endothelialized arteries. Yet, it is unlikely that this would be sufficient to account for the present findings, since in washout experiments, after removing the tracers from the lumen, the LDL concentrations did not significantly decrease.

3) An increase in water filtration through the wall with increasing transmural pressure. In an aortic preparation similar to that used in the present work, it has been reported that the filtration flow rate rises from 2.8×10^{-6} cm/sec at 70 mm Hg to about 4.4×10^{-6} cm/sec at 180 mm Hg. This contributes to increase the convective flux of macromolecules, which has been shown to play an important role in the transport of albumin through the arterial wall. The enhanced albumin and LDL uptake may have been a direct consequence of increased convection through the media. Albumin was possibly forced by pressure-driven convection into interstitial spaces that were not accessible at 70 mm Hg by simple diffusion and low pressure-driven convection. The uniform distribution of albumin across the media at high pressure strongly suggests that the albumin transport at 160 mm Hg was mainly convective.

Transmural Distribution of LDL and Albumin Concentrations

The different reasons mentioned above can explain the overall increase in albumin and LDL concentrations observed at high pressure but do not suffice to account for the striking differences between the transmural distributions of albumin and LDL. The time course of the LDL uptake indicates that LDL accumulates in the inner media while penetration into the subsequent layers was impeded. The higher concentration of LDL, as compared with that of albumin, obtained at the luminal side, might be accounted for by a higher permeability of the endothelium to LDL than to albumin. However, we found that even though the permeabilities of LDL and albumin across the endothelium were enhanced with increasing pressure, the permeability to LDL remained two times lower than that to albumin, which is in agreement with the finding of Bratzler et al. at low pressure.

Tedgui and Lever\textsuperscript{15} found that the water content and the extracellular space in endothelialized aorta decreased at elevated pressure and that the arterial wall can undergo compaction when the artery is pressurized. Such a compaction might have made the interstitial matrix less permeable to macromolecules. However, it is thought that the effect of matrix compaction may have impeded the passage of LDL across the wall more than that of albumin, because the diameter of LDL is about three times higher than that of albumin (about 220 versus 70 Å). Indeed, the time course of the transmural distribution of LDL at high pressure (Figure 5b) suggests that LDL was forced by pressure-driven convection into a porous material, poorly permeable to the lipoprotein, resulting in its accumulation in the first layers. Conversely, albumin, which was certainly also forced by pressure-driven convection, was uniformly distributed across the media, because it moved freely from the luminal to the external side. Therefore, albumin did not accumulate focally in the media. The possibility that the compacted media impeded LDL egress from the wall was further supported by our experiments of washout. After a 30-minute incubation in the presence of tracer, the intraluminal solution was changed to a tracer-free solution for an additional 30-minute incubation. Albumin was almost totally removed from the wall, while LDL concentration was unchanged, except in the first section (Figure 7), in which LDL concentration slightly decreased, possibly due to convection from this layer to the next. The absence of decrease in LDL concentration supports the idea that LDL was trapped in the inner layers. The comparison of the present findings with our previous results of LDL and albumin uptake in deendothelialized rabbit aortas pressurized to 70 mm Hg further supports the view that LDL accumulates in the inner layers as a result of the synergy between convection and molecular sieving of LDL by the media. We found earlier that at 70 mm Hg, after removal of the endothelium, the LDL distribution across the media was almost uniform with \( C_{\text{ LDL }} \) values of about 0.03\textsuperscript{31} that is, about six times lower than the present high-pressure \( C_{\text{ LDL }} \) in the inner layers, but three times higher than the present \( C_{\text{ LDL }} \) values in the outer media. Furthermore, after washout LDL was removed from the wall, which was not the case in the present study. On the other hand, at 70 mm Hg in deendothelialized aortas, albumin concentrations were also uniform across the media, being about 0.08\textsuperscript{29} that is, about 60% higher than those found in the present work at 160 mm Hg in intact vessels, indicating that the endothelium still provided a high barrier to macromolecular influx into the wall at high pressure.

The present findings are consistent with the views developed by Fry\textsuperscript{40} in a mathematical model of LDL transport across a multiple layered vessel wall. This author proposed that a slight increase in endothelial permeability together with an elevated pressure-driven convection lead to very high values of intersti-
tial chemical activity in the intima due to the presence of the internal elastic lamina acting as a molecular sieve, as well as to the presence of the endothelium providing a significant barrier to retrograde diffusive flux. In our experiments at high pressure, the endothelial permeability was indeed increased, possibly as a result of pressure-induced wall distension, and our washout experiments did prove that retrograde flux of LDL did not occur. However, we cannot conclude whether this was due to the barrier offered by the endothelium or solely due to the convective forces opposite to the retrograde diffusive flux. Moreover, the present experiments suggest that the entire media behaves as a molecular sieve for LDL and not only the internal elastic lamina. Because the intima is very thin in rabbit aorta (less than 5 μm), the serial sectioning technique that we used did not allow us to determine the actual intimal concentration, because the first 20-μm-thick section contained both intima and part of the inner media. Therefore, the LDL concentration in the intima might be expected to be higher than that obtained in the first section. By simply extrapolating the 1- or 2-hour curves of C12LDI transmural distribution (Figure 5b), we estimate the intimal relative concentration of LDL to be about 0.35–0.4.

Our findings are also consistent with the work of Smith and Staples,16 who have reported that in postmortem human aorta, the LDL concentration appears to be higher than that of albumin in the intima and lower in the media.

In conclusion, our results showed that, at high pressure, the macromolecular concentration appeared to be proportional to the size of the macromolecule in the inner layers of the intima-media and remained inversely proportional in the outer media. High levels of transmural pressure were responsible for a much increase in concentration of LDL in the inner layers of the arterial wall associated with a much lower increase in the subsequent layers. A strong synergy among increased endothelial permeability, increased LDL convection, and compaction of the media at high pressure might be responsible for this. The present results might account for one of the deleterious effects of hypertension in atherosclerosis.

References


Key Words: hypertension • albumin • LDL • uptake • aorta
Effect of transmural pressure on low density lipoprotein and albumin transport and distribution across the intact arterial wall.
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