Aortic Permeability to LDL as a Predictor of Aortic Cholesterol Accumulation in Cholesterol-Fed Rabbits

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The aim of this study was to investigate the possibility that the permeability characteristics of the arterial wall are related to the development of atherosclerosis. The in vivo regional variation of aortic permeability to iodinated human low density lipoprotein (LDL) in normal rabbits was compared with the regional variation in aortic cholesterol accumulation in cholesterol-fed rabbits. Aortas were divided into the aortic arch, thoracic aorta, and abdominal aorta, and each of these three parts was further subdivided into four segments of similar size. The permeability to LDL was 40±7 nl • cm⁻² • hr⁻¹ (mean±SEM, n=11) in the most proximal segment of the aortic arch and decreased throughout the length of the aorta to 3±1 nl • cm⁻² • hr⁻¹ in the most caudal segment of the abdominal aorta. In such normal rabbits the aortic cholesterol content was similar in all 12 arterial segments at 0.08 ±0.005 µmol/cm² (mean±SEM, n=3x12). Aortic cholesterol accumulation was determined in other rabbits with an average plasma cholesterol level of 32±1 mmol/l for 96 days; the cholesterol content in the most proximal segment of the aortic arch was 2.7±0.5 µmol/cm² (mean±SEM, n=11) and decreased with increasing distance from the heart to 0.17±0.03 µmol/cm² in the most caudal segment of the abdominal aorta. Linear regression analysis showed a close positive association between the permeability to LDL of a given aortic segment and the cholesterol accumulation in that same aortic segment after cholesterol feeding (r²=0.96, p<0.001). These results suggest that the aortic permeability to LDL is an important predictor for the development of atherosclerosis in cholesterol-fed rabbits.

Key Words • aortic permeability • atherosclerosis • cholesterol-fed rabbits • LDLs

It is well established that atherosclerotic lesions are characterized by cholesterol accumulation, an increase in intercellular matrix, and cell proliferation. Several lines of evidence from both rabbit and human studies support the conclusion that the cholesterol in atherosclerotic lesions is derived from plasma lipoproteins.1-3 In humans as well as rabbits, there is considerable variation in the extent and severity of atherosclerotic lesions between different arterial sites, even though all arterial surfaces within a given individual or rabbit have been exposed to the same plasma cholesterol level.4-9 This suggests that regional variation in lipoprotein–arterial wall interactions is important for the development of atherosclerosis. Such differences between lesion-resistant and lesion-prone arterial sites could be caused by differences in arterial lipoprotein influx, retention, degradation, or efflux. Differences in arterial lipoprotein influx between arterial sites would be reflected in differences in aortic permeability between these sites.

The present study considers the hypothesis that the aortic permeability to human low density lipoprotein (LDL) is a predictor of aortic cholesterol accumulation in cholesterol-fed rabbits and thereby is important for the initiation of fatty streak lesions; aortic permeability to human LDL in vivo at different arterial sites in normal rabbits is compared with aortic cholesterol accumulation at the same aortic sites in cholesterol-fed rabbits.

Methods

Animals

Twenty-seven white male rabbits of the Danish Country Strain (Statens Serum Institut, Copenhagen, Denmark) weighing 3.1–3.9 kg were used. Sixteen rabbits were fed essentially cholesterol-free rabbit chow ad libitum (Altromin 2113, Lage, FRG) and were designated as normal rabbits. The remaining 11 cholesterol-fed rabbits were fed 88–90 g rabbit chow daily supplemented with 0–2 g cholesterol (USP cholesterol, Sigma, Copenhagen, Denmark) and 10 g corn oil (Oleum maidaes BP80, Mecobenzon, Copenhagen, Denmark); cholesterol was dissolved in heated corn oil and subsequently mixed with the rabbit chow. The experimental protocols were in accordance with Danish regulations for experiments on animals.
Isolation and Labeling of LDL

To measure aortic permeability to similar LDL particles in all rabbits, LDL was always obtained from the same normocholesterolemic human male donor. LDL was prepared from freshly drawn blood containing Na2EDTA (2 mg/ml), chloramphenicol (40 μg/ml), gentamicin sulfate (0.1 mg/ml), e-amino-n-caproic acid (2.6 mg/ml), benzamidine (10 μg/ml), and apro tinin (10 kallikrein units per milliliter) (all from Sigma).

Sequential ultracentrifugation at 4°C was performed at solvent densities of 1.019 g/ml and 1.063 g/ml in a Beckmann 50.3 Ti rotor for 2.16×10⁸ g×min (average). An additional ultracentrifugation step was performed at a solvent density of 1.063 g/ml to remove contaminating particles of d>1.063 g/ml, mainly albumin. The protein concentration in the isolated LDL fraction was estimated from the absorbance at 220 nm, as described by Zilversmit and Shea. 10

For iodination of LDL, 11,12 the LDL solution (300–600 μl, 5 mg protein) was mixed with glycine buffer (1 mol/l, 0.4 ml) and 110–370 MBq 131I (Amersham, Birkerød, Denmark) before iodine monochloride (14–48 nmol/mg protein) was added. Unbound iodine was removed with a PD-10 column (Sephadex G-25M, Pharmacia, Copenhagen, Denmark) followed by dialysis overnight at 4°C against phosphate-buffered saline (pH 7.3) or saline (0.9% NaCl). To minimize possible self-irradiation damage mainly from 131I, 100 mg rabbit albumin (Sigma) was added after the PD-10 column procedure and before dialysis.

Iodination efficiency was 29±8% for 125I and 23±9% for 131I. The radioactivity in the injected doses was 98±0.3% precipitable with trichloroacetic acid (TCA), and 6±1% of the radioactivity was extractable with chloroform/methanol (1:1, vol/vol); labeled LDL in a volume of 10 μl was added to 100 μl unlabeled plasma, mixed with 1 ml methanol and 1 ml chloroform, and then extracted for 20 minutes before centrifugation and determination of radioactivity in the precipitate and supernatant. Iodinated LDL was used for permeability measurements within 24 hours of labeling.

For the labeling of LDL with [3H]cholesteryl ester, 4.2 ml plasma was passed through a 0.22-μm filter (Millex GS Millipore S.A., Molsheim, France) into a rubber-sterile glass vial. [3H]Cholesterol (150 MBq of [1α,2α(n)-3H]cholesterol, Amersham) dissolved in 50 μl ethanol was injected into the vial. After incubation at 37°C for 60 hours, LDL was isolated by sequential ultracentrifugation and dialyzed overnight. All labeled preparations were filtered through 0.22-μm or 0.45-μm filters (Millex GS Millipore S.A.) before injection.

Protocol for Aortic Permeability Measurements

The regional variation in aortic permeability to LDL was determined in 11 normal rabbits; 131I-LDL ("permeability tracer"; 1.5–2 ml, 2.5×10⁶±1.2×10⁶ cpm) was injected into the left lateral ear vein followed by 2 ml saline. Blood samples were drawn from the right lateral ear vein into tubes containing Na2EDTA at 10, 20, 40, 60, 120, 178, 182, and 188 minutes (mean values), and the aorta was removed at 60, 120, 178, 182, and 188 minutes after the injection of the permeability tracer. To allow the determination of plasma contamination of arterial tissue, 125I-LDL ("contamination tracer"; 1.3–2 ml, 5.8×10⁶±1.9×10⁶ cpm) was injected 20 minutes before removal of the aorta. In two of the 11 rabbits the isotopes were reversed. The volume of distribution, calculated as injected dose divided by the concentration of the radiolabel in plasma 10 minutes after injection (TCA-precipitable radioactivities) and the body weight of the rabbit, was 30±3 ml/kg for both the permeability tracer and the contamination tracer. The plasma concentration of TCA-precipitable permeability tracer decreased to 64±9% of the 10-minute value during the experimental period of 3.2 hours. The percentage of radiolabel in plasma in the LDL fraction (1.019 g/ml<d<1.063 g/ml) was 93±2%, 92±2%, and 90±2% after 15, 60, and 188 minutes, respectively.

Protocol for Cholesterol Feeding

The regional variation in aortic cholesterol content after 96 days of cholesterol feeding was determined in 11 rabbits. To reduce the plasma cholesterol–induced variation in aortic cholesterol accumulation between rabbits, a similar mean plasma cholesterol concentration during the cholesterol feeding period in the rabbits was attempted; the amount of cholesterol fed to each rabbit (on average, 1.1±0.1 g cholesterol per day) was adjusted on the basis of weekly determinations of plasma cholesterol concentration. 14 For comparison, three normal rabbits were also used for aortic cholesterol determinations.

Aortic Tissues

At the termination of the permeability and cholesterol-feeding experiments, the rabbits were killed by intravenous injections of a 5% pentobarbital solution (50–200 mg/kg body wt). Through a needle inserted into the left ventricle of the heart, the systemic circulation was perfused for 10–15 minutes at a pressure of approximately 100 cm H2O, with approximately 1 l (rabbits used for permeability measurements) or 250 ml (rabbits used for determination of aortic cholesterol) of saline (4°C). The aorta was excised and carefully freed of adventitia. Thereafter it was kept at 4°C, normally for 30–60 minutes and maximally for 2 hours, before it was opened longitudinally, rinsed with saline, and fixed with pins on a cork board. The area was outlined on graph paper, and the aorta was divided into the aortic arch (from the heart to the first intercostal branches), the thoracic aorta (to the celiac orifice), and the abdominal aorta (to the terminal bifurcation). Each of these three aortic parts was then subdivided into four consecutive segments of similar size. The intima–inner media was stripped from the outer media in each of the 12 aortic segments. The total procedure lasted approximately 30 minutes, and immediately thereafter in the permeability experiments, TCA precipitation was performed.

The average thickness of the intima–inner media and outer media was calculated from the areas and the wet weights of the tissues by assuming a tissue density of 1 g/cm². In the normal rabbits used for permeability measurements, the thickness of the intima–inner media of the aortic arch, the thoracic aorta, and the abdominal aorta was 502±25, 361±11, and 339±13 μm, respectively (n=11); the corresponding values for the outer media were 393±24, 242±9, and 279±11 μm, respectively.
Analyses
In the permeability measurements, TCA-precipitable radioactivity was used for the calculations; aortic tissues, plasma samples (10 μl), and diluted doses (0.1 μl of dose) had 100 μl unlabeled plasma added, and the protein was precipitated at 4°C with TCA at a final concentration of 15% wt/vol. After mixing and centrifugation, total radioactivity as well as radioactivity in an aliquot of the supernatant was determined. TCA-precipitable radioactivity was calculated by subtraction of TCA-soluble radioactivity from the amount of total radioactivity. The amount of radioactivity in plasma precipitable with TCA decreased from 98.9±0.3% at 10 minutes after injection to 94.4±1.3% at 188 minutes after injection. In the aortic intima-inner media and outer media, 47.2±1.8% and 63.5±1.8%, respectively, of the total permeability tracer radioactivity was precipitable with TCA. Radioassays for 125I and 131I were performed by using aSelektronik double-channel gamma counter (Copenhagen, Denmark).

For the determination of [3H]cholesterol ester radioactivity in the sink assumption experiments, the TCA precipitates of aortic tissues and plasma samples were minced, and lipids were extracted for 24 hours with chloroform/methanol (1:1, vol/vol); the precipitate was then centrifuged and washed twice with chloroform/methanol (1:1, vol/vol).15 Esterified cholesterol in the combined extracts and washes was isolated by thin-layer chromatography, and the amount of H was determined by liquid scintillation counting in a TRICARP 2000 counter (Packard, Greve, Denmark) with Instafluor (Packard) as the scintillation liquid. No 125I or 131I contaminated the cholesteryl ester fractions isolated by thin-layer chromatography, as detected by the gamma counter.

Cholesterol in the aortas of normal and cholesterol-fed rabbits was extracted with chloroform/methanol (1:1, vol/vol) and determined by the Liebermann-Burchard method after saponification as described earlier.15 Plasma and lipoprotein cholesterol concentrations were measured with an enzymatic kit (Boehringer Mannheim, Mannheim, FRG).

Calculations
TCA-precipitable radioactivity in aortic tissue and whole plasma was used in the calculations. Aortic permeability was calculated by the "sink" method, in which the aortic radioactivity is divided by the area under the plasma radioactivity curve. Previous studies of rabbits suggest that LDL enters the media from both the luminal and adventitial sides. Consequently, only the radioactivity in the intima-inner media was used in the permeability calculations. The injection of two differently labeled LDL preparations 3 hours and 20 minutes, respectively, before removal of the aorta made it possible to correct for plasma contamination of each arterial segment by solving a set of linear equations with two unknowns, permeability (P, in nanoliters per square centimeter per hour) and plasma contamination of arterial tissue (C, in nanoliters per square centimeter):

\[ A(t) = (P \times c_{\text{avg}}(t) + C \times c_{\text{end}}(t)) \]

in which \( A \) is the aortic radioactivity (in counts per minute per square centimeter), \( c_{\text{avg}} \) is the mean plasma radioactivity concentration (in counts per minute per nanoliter), \( c_{\text{end}} \) is the plasma radioactivity concentration at the end of the experiment (in counts per minute per nanoliter).

The calculation of the permeability and plasma contamination of arterial tissue with the use of Equations 1 and 2 assumes that the loss of radioactivity from the arterial tissue during the experimental period of 3 hours is negligible compared with the amount of labeled LDL entering the arterial tissue during that period. To investigate the validity of this sink assumption, two different calculations were performed for two rabbits, each of which was injected with [125I]-LDL, [131I]-LDL, and [3H]cholesterol ester-labeled LDL at 3 hours, 1.5 hours, and 20 minutes before removal of the aorta.

First, the permeability to 131I-LDL based on an exposure time of 3 hours was compared directly with the permeability to 125I-LDL based on an exposure time of 1.5 hours; corrections for plasma contamination of arterial tissues were performed by using [3H]cholesterol ester-labeled LDL as the contamination tracer in Equations 1 and 2.

Second, the permeability was calculated by a method that takes the loss of labeled LDL from the aorta during the experimental period into account. This method assumes that the kinetics of newly entered LDL in the aortic intima-inner media can be described by an open one-compartment model and can be expressed by the equations:

\[ \frac{dA_{131I}}{dt} = \left[ P \times c_{131I}(t) \right] - \left[ K \times A_{131I}(t) \right] \]

\[ \frac{dA_{125I}}{dt} = \left[ P \times c_{125I}(t) \right] - \left[ K \times A_{125I}(t) \right] \]

with two unknowns, permeability (P, in nanoliters per square centimeter per hour) and the fractional loss of newly entered LDL from the artery (K, in hours⁻¹). A(t) is the aortic radioactivity (in counts per minute per square centimeter) and c(t) the concentration of labeled LDL in plasma at time t (in counts per minute per nanoliter). (Plasma radioactivity curves were fitted to two double-exponential functions in rabbit A and to a three- and four-degree polynomial in rabbit B.) Iteration procedures for the solution of Equations 3 and 4 (as described in detail by Schwenke and Zilversmit) were performed on an XT personal computer using the spreadsheet program QUATTRO (Borland International). Aortic [3H]cholesterol ester was used to correct arterial 131I and 125I content for plasma contamination. The plasma contamination was derived from Equations 1 and 2, using 1.5 hours as the LDL influx time.

Statistical Analysis
Values are given as mean±SEM.

Results
Regional Variation in Aortic Permeability to LDL
In normal rabbits with plasma cholesterol and LDL cholesterol concentrations of 0.8±0.1 mmol/l and 0.2±0.0 mmol/l, respectively, there was a continuous
decrease in aortic permeability to LDL with increasing distance from the heart; the permeation of iodinated LDL into the intima-inner media in the most caudal segment of the abdominal aorta was only about 1/14th of that in the most proximal segment of the aortic arch (Figure 1). When the permeability was calculated on the basis of labeled LDL that had permeated into both the intima-inner media and the outer media, a similar but less steep decrease in permeability with increasing distance from the heart was found (Table 1).

The plasma contamination of the arterial intima-inner media varied greatly between rabbits as well as between aortic segments (Table 1) and was, on average for all aortic segments, 6 nl/cm². This value is in accordance with previous findings. In the aortic outer media the plasma contamination was 30–40 nl/cm² intimal surface.

In the aortic arch the amount of labeled LDL that accumulated in the intima-inner media, expressed as the aortic radioactivity divided by the time-averaged concentration of radioactivity in plasma, increased almost linearly for 3 hours in the two rabbits examined (Figure 2). During the same time period, the curves for accumulation of labeled LDL in the intima-inner media of the thoracic aorta and of the abdominal aorta were curvilinear with a concavity toward the x axis.

**Figure 1.** Plot showing variation of aortic permeability to low density lipoprotein (LDL) between 12 consecutive segments of aortas from normal rabbits. Values are mean±SEM.

**Figure 2.** Line plots showing accumulation of labeled low density lipoprotein (LDL) in intima-inner media as a function of time in two normal rabbits (A and B).

**Table 1. Regional Variation in Aortic Permeability to LDL and in Amount of Plasma Contamination in Aortic Tissues of Normal Rabbits**

<table>
<thead>
<tr>
<th>Aortic segment</th>
<th>Intima-inner media (nl·cm⁻²·hr⁻¹)</th>
<th>Intima-inner + outer media (nl·cm⁻²·hr⁻¹)</th>
<th>Intima-inner media (nl/cm²)</th>
<th>Outer media (nl/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arch 1</td>
<td>39.8±7.3</td>
<td>53.5±8.5</td>
<td>2.6±4.2</td>
<td>37.8±8.0</td>
</tr>
<tr>
<td>Arch 2</td>
<td>39.9±10.2</td>
<td>56.2±11.3</td>
<td>8.1±4.8</td>
<td>41.4±7.5</td>
</tr>
<tr>
<td>Arch 3</td>
<td>29.1±7.5</td>
<td>33.8±18.1</td>
<td>0.8±2.8</td>
<td>34.3±9.3</td>
</tr>
<tr>
<td>Arch 4</td>
<td>15.1±4.1</td>
<td>19.7±4.2</td>
<td>4.3±1.8</td>
<td>26.5±5.1</td>
</tr>
<tr>
<td>Mean</td>
<td>32.1±6.6</td>
<td>42.3±7.2</td>
<td>3.8±3.1</td>
<td>35.7±6.2</td>
</tr>
<tr>
<td>Thoracic 1</td>
<td>11.7±3.0</td>
<td>17.6±3.1</td>
<td>4.1±1.3</td>
<td>31.5±0.4</td>
</tr>
<tr>
<td>Thoracic 2</td>
<td>8.6±2.3</td>
<td>12.7±2.4</td>
<td>4.9±1.1</td>
<td>32.4±5.4</td>
</tr>
<tr>
<td>Thoracic 3</td>
<td>6.4±1.6</td>
<td>9.4±2.1</td>
<td>3.1±2.3</td>
<td>30.9±6.3</td>
</tr>
<tr>
<td>Thoracic 4</td>
<td>7.4±2.5</td>
<td>12.3±3.0</td>
<td>5.1±1.3</td>
<td>34.3±7.4</td>
</tr>
<tr>
<td>Mean</td>
<td>8.5±2.0</td>
<td>13.1±2.3</td>
<td>4.0±1.3</td>
<td>31.5±5.0</td>
</tr>
<tr>
<td>Abdominal 1</td>
<td>7.4±1.9</td>
<td>16.8±2.4</td>
<td>9.1±1.7</td>
<td>44.3±7.2</td>
</tr>
<tr>
<td>Abdominal 2</td>
<td>3.4±0.7</td>
<td>11.4±1.7</td>
<td>9.5±1.7</td>
<td>38.4±7.9</td>
</tr>
<tr>
<td>Abdominal 3</td>
<td>2.3±0.4</td>
<td>8.0±1.1</td>
<td>7.2±1.2</td>
<td>35.4±4.6</td>
</tr>
<tr>
<td>Abdominal 4</td>
<td>2.8±1.1</td>
<td>9.7±1.3</td>
<td>12.9±4.3</td>
<td>46.3±9.1</td>
</tr>
<tr>
<td>Mean</td>
<td>4.2±0.8</td>
<td>11.8±1.1</td>
<td>9.4±1.7</td>
<td>40.2±6.2</td>
</tr>
</tbody>
</table>

LDL, low density lipoprotein. Permeability and contaminating plasma were calculated using Equations 1 and 2 and are expressed as mean±SEM.
FIGURE 3. Plot showing variation in cholesterol content in the intima–inner media between 12 consecutive segments of aortas from cholesterol-fed and normocholesterolemic rabbits. Values are mean ± SEM.

indicates that some of the radioactivity had left the tissue between 1.5 and 3 hours after intravenous injection. Thus, the permeability value calculated by using the sink assumption at 3 hours to some degree underestimated the “true” value for the thoracic and abdominal aortas. The true permeability values are the slopes at time zero for the relations shown in Figure 2. Using two different methods of calculation (see “Calculations” section), we found that the underestimation of the permeability values after the 3-hour exposure time was, on average for the two rabbits, 7% and 8% for the aortic arch, 33% and 58% for the thoracic aorta, and 43% and 69% for the abdominal aorta.

Regional Variation in Aortic Cholesterol Accumulation in Cholesterol-Fed Rabbits

In rabbits fed cholesterol for 96 days (average plasma cholesterol level of 32 ± 1 mmol/l), macroscopically visible aortic fatty streak lesions were evident in 10 of 11 rabbits. The cholesterol content in the intima–inner media decreased with increasing distance from the heart; the cholesterol content in the most caudal segment of the abdominal aorta was about 1/14th of that in the segment of the aortic arch nearest the heart (Figure 3). The regional variation in aortic cholesterol content after cholesterol feeding was very similar to the regional variation in aortic permeability to LDL before cholesterol feeding (compare Figures 1 and 3), with both curves having larger values in the area of the celiac axis, the superior mesenteric artery, and the renal arteries (thoracic segment No. 4 and abdominal segments No. 1 and 2) compared with adjacent aortic segments.

Linear regression analysis of aortic permeability to LDL of a given aortic segment as a predictor of cholesterol accumulation in that same segment after cholesterol feeding demonstrated a close association between the two variables ($r^2 = 0.96, p < 0.001$; Figure 4).

The cholesterol content in the intima–inner media of normal rabbits with a plasma cholesterol level of 0.3 ± 0.0 mmol/l (n = 3) was similar in the 12 aortic segments and markedly lower than that in cholesterol-fed rabbits (Figure 3).

Discussion

Regional Variation in Aortic Permeability to LDL

The present demonstration in normocholesterolemic, nonatherosclerotic rabbits (normal rabbits) that aortic permeability to LDL decreases with increasing distance from the heart is in accordance with previous findings in dogs. In minipigs, aortic permeability to cholesteryl ester–labeled lipoproteins showed a similar trend, as did the aortic distribution of the protein-binding Evans blue dye in dogs and pigs. In a recent series of three very extensive papers on the possible factors influencing the initiation of fatty streak lesions in rabbits, Schwenke and Carew demonstrated a fourfold increase in LDL permeability in lesion-susceptible branch sites of the abdominal aorta of normal rabbits compared with lesion-resistant nonbranch sites, but they did not find permeability differences between the lesion-susceptible aortic arch and the lesion-resistant thoracic aorta. However, in a recent preliminary report from the same laboratory, the permeability to LDL in the abdominal aorta without branches was only 25% of that in the aortic arch and 73% of that in the thoracic aorta.
When examining the factors that influence fatty streak and atherosclerosis development, it seems reasonable to examine only the amount of plasma lipoprotein that enters the intima, the anatomic site of fatty streaks, and not to include lipoproteins in the outer media, since most of these lipoproteins presumably enter the arterial wall via the vasa vasorum. In the present study, only the radioactivity in the intima-inner media was used in the permeability calculations, and appropriate corrections for plasma contamination were performed; the permeability to LDL in the most distal aortic segment was then only 7% of that in the segment nearest the heart compared with 18% and 38% if the calculation of permeability was based on the radioactivity in the entire thickness of the arterial wall, with and without corrections for plasma contamination, respectively. With all three calculations, LDL permeability decreased with increasing distance from the heart.

The shorter the exposure time, the larger the contribution of radioactivity in contaminating plasma in the aortic surface to the total amount of radioactivity found in the intima-inner media layer. This point is most evident in the abdominal aorta, where even after 3 hours' exposure the contamination was of the same magnitude as the amount of LDL that had permeated into the intima-inner media. The major concern with using relatively long exposure times is that the sink assumption (i.e., that loss of labeled LDL from the intima during the exposure time is minimal compared with the influx of labeled LDL) is not fulfilled, whereby the aortic permeability value may be underestimated. The extent of underestimation in this study was evaluated from data obtained from two rabbits by two different mathematical approaches (see "Methods" and "Results" sections). Since this estimate of underestimation is based on data from only two rabbits, it should be taken with some caution. However, the data from the two rabbits are suggestive of a larger loss of labeled LDL from the arterial intima-inner media in the abdominal aorta compared with the aortic arch during the 3-hour influx period. This observation could be related to differences in intimal thickness or to differences in the binding of LDL to extracellular matrix components between different arterial sites and has previously been examined and thoroughly discussed by Schwenke and Carew. When the average of the two estimates was used to correct the results obtained in the 11 rabbits that were used for permeability measurements, the average permeability to LDL was increased from 32.1 to 34.7 nl cm⁻² hr⁻¹ in the aortic arch, from 8.5 to 16.1 nl cm⁻² hr⁻¹ in the thoracic aorta, and from 4.2 to 10.5 nl cm⁻² hr⁻¹ in the abdominal aorta. Thus, even when the estimated underestimation of permeability values is taken into account, the aortic permeability to LDL decreased with increasing distance from the heart. We have also used the estimates of underestimation for the whole aortic arch, thoracic aorta, and abdominal aorta to correct permeability values for each of the four segments within each of the three large aortic parts and then rerun the linear regression analysis of Figure 4; the $r^2$ then was 0.93 ($p<0.001$).

The underestimation of the permeability to LDL leads to an overestimation of the plasma contamination calculated by Equations 1 and 2; this overestimation is most pronounced in the abdominal aorta. If it is assumed that the true permeability in the abdominal aorta is the corrected value of 10.5 nl cm⁻² hr⁻¹ instead of the original calculated value of 4.2 nl cm⁻² hr⁻¹, then the plasma contamination would only decrease from the original calculated value of 9.4 nl/cm². This decrease in plasma contamination would increase the LDL permeability in the abdominal aorta from 4.2 to only 4.8 nl cm⁻² hr⁻¹. The slightly higher plasma contamination in the abdominal aorta compared with the aortic arch may have been included in the intima-inner media in the abdominal aorta. Alternatively, a relatively larger part of the outer media with higher contamination (Table 1) may have been included in the intima-inner media in the abdominal aorta compared with the aortic arch and the thoracic aorta.

Little is known about the mechanism for variations in aortic permeability to macromolecules between different arterial sites, but factors like blood flow patterns and endothelial cell turnover and death may be important. The effect of blood flow patterns on the endothelial morphology and on increased permeability in areas of disorganized flow has long been recognized. Disturbances in blood flow at the level of the large arteries for the intestines could therefore possibly contribute to the finding of an increased permeability to LDL at these sites, as reported in the present and a previous study. It is possible that complex flow patterns behind the aortic valves in the aortic arch may also help explain the high permeability values in this aortic region. Experimental endothelial injury with a balloon catheter increased the influx of lipoproteins into the arterial wall, and in the rat aorta, 37% of arterial foci with high permeability to Evans blue-labeled albumin were associated with endothelial cell death. In the normal rabbit thoracic aorta, endothelial cell death is predominant in the lesion-prone sites.

### Regional Variation in Aortic Cholesterol Accumulation in Cholesterol-Fed Rabbits

The decrease in aortic cholesterol content with increasing distance from the heart is not limited to cholesterol-fed rabbits clamped at an average plasma cholesterol concentration of about 30 mmol/l as in the present study; a similar distribution was also observed in four additional cholesterol-fed rabbits with mean plasma cholesterol concentrations during 3 months of 5, 20, 25, and 54 mmol/l, respectively (data not shown). Furthermore, in rabbits with genetic hyperlipidemia, like those from the St. Thomas's Hospital strain and Watanabe hereditable hyperlipidemic rabbits, with average plasma cholesterol levels in those studies of about 8 and 20 mmol/l, respectively, and plasma lipoprotein patterns resembling those of hypercholesterolemic humans, the development of atherosclerotic lesions was likewise more pronounced in the aortic arch compared with more distal parts of the aorta.

### Aortic Permeability as a Predictor of Atherosclerosis

The present demonstration of a strong, linear, positive association ($r^2=0.96$) between LDL permeability of a given aortic site in rabbits before cholesterol feeding and the cholesterol accumulation in that same aortic segment after 3 months of cholesterol feeding suggests...
that in a given rabbit, the permeability characteristics of a given arterial site are an important determinant for the rate of fatty streak development in that particular site.

The chain of events from increased arterial permeability to LDL to the development of atherosclerosis could be as follows. The arterial influx of a lipoprotein (nanomoles per square centimeter per hour) is the product of the arterial wall permeability (nanometers per square centimeter per hour) and the plasma concentration of that lipoprotein (nanomoles per nanoliter). Therefore, at a given plasma concentration of lipoproteins, a higher lipoprotein influx occurs at sites with high permeability than at sites with low permeability. Such increased amounts of LDL in the aortic intima may increase the amount of LDL that is oxidized, and thereby more LDL would be susceptible to uptake and degradation by macrophages, leading to fatty streak development. There is evidence that the degradation of LDL is increased at sites susceptible to the development of fatty streak lesions. In light of these simple mechanistic considerations it seems likely that rabbits with a high overall arterial permeability to LDL will develop more atherosclerosis with cholesterol feeding than will rabbits with a low overall LDL permeability. To address this question directly, LDL permeability before cholesterol feeding and aortic cholesterol accumulation after cholesterol feeding should be measured in the same rabbits.

Whether increased arterial permeability in humans is also a predictor of increased propensity to develop atherosclerosis can be debated. In the cholesterol-fed rabbit with very high plasma cholesterol levels (32 mmol/l in our study), the arterial wall is literally bombarded with lipoproteins, and under such circumstances it is possible that influx and thereby permeability characteristics are more important than is either degradation or efflux of lipoproteins. In humans, on the contrary, who have much lower plasma cholesterol levels (e.g., 5–7 mmol/l), it is possible that the capacity for efflux and degradation of lipoproteins may also be an important determining factor for development of atherosclerosis. Furthermore, in humans an increased arterial wall permeability would mean an increase in both high density lipoprotein and LDL influx and possibly also intermediate density lipoprotein and very low density lipoprotein influx. High density lipoprotein is thought to mediate reverse cholesterol transport and could potentially remove cholesterol from the arterial wall. Therefore, it can be speculated that if the ratio of high density lipoprotein to LDL in plasma is high, a high arterial permeability may be of less importance than if the ratio is low. In the cholesterol-fed rabbit, high density lipoprotein carries only a small percentage of the total plasma cholesterol.

In conclusion, the present data suggest that high arterial permeability at certain aortic sites in rabbits is closely related to the initiation of fatty streak development at these particular sites.

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