Role of LDL Receptors in the In Vitro Uptake and Degradation of LDL in the Media of Rabbit Thoracic Aorta

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The possible role of plasma low-density protein (LDL) receptors in the uptake and degradation of LDL in the whole arterial wall was investigated by comparison of the in vitro uptake of $^{125}$I-native LDL (nLDL) and $^{3}H$-methylated LDL (mLDL) by the media of deendothelialized rabbit thoracic aorta excised at in vivo length and pressurized to 70 mm Hg, taking the advantage that mLDL is not recognized by the LDL receptor. The distribution of the relative concentrations of nLDL ($C_n$) and mLDL ($C_m$) across the wall was obtained using a serial frozen sectioning technique. The aorta was incubated under three different conditions for varying periods of incubation in order to analyze separately the processes of binding, binding-internalization, and degradation. At 39°C, in which binding-internalization and degradation occurred, $C_n$ was significantly higher than $C_m$ at each position across the media. The mean medial $C_n/C_m$ ratio was 1.36±0.15 ($n=5$) after 1 hour of incubation, and decreased to 1.23±0.22 ($n=7$) after 2 hours of incubation and to 1.13±0.11 ($n=5$) after 4 hours of incubation. At 4°C, in which internalization and degradation were blocked, the $C_n/C_m$ ratio reflected the surface nLDL binding alone; the $C_n/C_m$ ratio was 1.47±0.20 ($n=5$) after 4 hours of incubation, higher than the value obtained at 39°C. To investigate whether degradation of nLDL occurred after receptor binding, the interstitial LDL was washed out by an LDL-free solution after 2-hour incubation at 39°C. After 30 minutes of washout, the $C_n/C_m$ ratio decreased to 1.06±0.20 ($n=5$) in the inner media and was unchanged in the outer media. After 1 hour of washout, the ratio declined to 0.57±0.18 ($n=7$) in the inner part of the media and increased progressively to 1 at the media-adventitia boundary. The $C_n/C_m$ ratio, at 0.67±0.12 ($n=5$), was practically constant throughout the media after 2 hours of washout. The nLDL degradation rate across the media was obtained from the comparison of nLDL and mLDL before and after the washout. A steep decreasing gradient in nLDL degradation rate was observed from the luminal to the external surface. The mean medial nLDL degradation rate value was 9.6±4.5 µl/cm² wet tissue/hr. We concluded that functional LDL receptors participate in the uptake and degradation of LDL in the whole aorta. (Circulation Research 1989;64:957–966)

Fatty streaks have been recognized as the first early lesions occurring in atherosclerosis. Cholesterol in these lesions is known to derive from plasma low-density lipoproteins (LDLs). However, the mechanisms responsible for intimal accumulation of lipids are not completely elucidated.

Transport of atherogenic substances across the vascular wall is believed to play a major role in initiation of intimal lipid deposits. Like most of the plasma macromolecules, LDLs pass through the vascular endothelium by a vesicular pathway and are then transported by diffusion/convection across the media. As a result of the high resistance offered by this layer, LDL accumulation may develop in the subendothelial space. Several investigators have examined the transport of LDL across the vascular wall but were not concerned with the effect of the LDL receptor, which is present on the endothelial cell and smooth muscle cell membranes. Only a few studies have dealt with the role of this receptor in LDL transport. In situ and in vivo studies showed that LDLs cross the endothelium in a receptor-independent pathway, but the role of LDL receptor in the transport across the media is still controversial. Whereas the short-term (30- to 60-minute) in vivo uptake of LDL...
by the media of rabbit thoracic aorta seems not to be influenced by LDL receptors, 20 4 hours after injection of native LDL (nLDL) and methylated LDL (mLDL), a modified form of LDL not recognized by the LDL receptor. 21 nLDL was taken up at a higher rate than mLDL. 22 In these in vivo studies, the much greater decrease in plasma nLDL as compared with mLDL and the possible nLDL degradation by the medial smooth muscle cells 23 warranted further investigation.

For examination of the importance of the LDL receptors of medial smooth muscle cells in the uptake and degradation of LDL, in vitro experiments were performed in deendothelialized rabbit thoracic aorta that was excised at in vivo length and pressurized to 70 mm Hg. The uptake of nLDL and mLDL was studied under three experimental conditions: 1) incubation of the aorta at 39° C, in which both uptake and degradation occurred; 2) incubation at 4° C, which allowed cell surface binding but inhibited LDL internalization and degradation 24-25; and 3) incubation at 39° C in the presence of tracers, followed by an LDL-free washout for estimation of the contribution of LDL degradation to the LDL clearance from the wall. Under all these conditions, varying incubation periods were used for analysis of the kinetics of the processes into the tissue. After incubation, the distribution of the relative concentrations of nLDL and mLDL across the arterial wall was determined by means of a serial frozen sectioning technique.

Our results showed that in normal conditions, at 39° C, the relative concentration of nLDL was higher than that of mLDL. This was accounted for by a specific binding of nLDL, as demonstrated in the 4° C experiments, that was incompletely compensated by the larger degradation of nLDL as compared with mLDL, as shown by the washout experiments. A preliminary report of this work has been published. 26

Materials and Methods

LDL Preparation and Labeling

LDL (1.025<d<1.050 g/ml) was isolated from fresh human plasma by sequential ultracentrifugation. 27 Reductive methylation was performed as described by Weisgraber et al. 21 More than 85% of the lysine residues were routinely methylated as estimated with the colorimetric assay based on the reaction of 2,4,6-trinitrobenzene-1-sulfonic acid with primary amines. 28 The nLDL was labeled with 125I and the mLDL with 131I (Amersham France, 91944 Les Ulis) by use of the iodine monochloride method of McFarlane 29 as modified by Bilheimer et al. 30 Free iodine was removed by passage of the solution through a SEPHADEX G-50 column and extensive dialysis at 4° C against 0.15 M NaCl containing 30 mg/l gentamicin sulfate and 100 mg/l EDTA. After dialysis, more than 99±0.5% of the radioactivity was precipitable by 10% trichloroacetic acid (TCA) (final concentration). On 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. 31 Specific activity ranged from 350 to 650 cpm/ng of protein.

Artery Preparation

New Zealand white rabbits (2-2.5 kg) were anesthetized with sodium pentobarbital (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 ligated. The proximal and distal ends of the aorta were then ligated. During the procedure saline solution was continually applied to the outer surface of the aorta to prevent drying.

For the study of LDL uptake into the media, the endothelium was stripped from the aorta by use of a balloon catheter. A 16-gauge cannula was inserted into the distal end of the thoracic aorta and connected to a reservoir that was 80 cm above the rabbit and filled with Krebs' solution containing 4% bovine serum albumin (pH 7.4). A second cannula, connected to a closed tap, was then inserted pointing distally into the midregion of the aorta and tied in place. The two cannulas were clamped into an adjustable rig that held the vessel at its normal in vivo length and prevented shortening as the vessel was excised and immersed in a 10-ml bath of Krebs' solution containing 50 mg/l gentamicin sulfate. The upper part of the thoracic aorta was then cannulated, excised, and transferred to the incubation bath in a similar manner. The vessel was flushed at physiological transmural pressure with Krebs' solution containing 4% albumin and 0.03% Evans blue. The presence of protein in flushing and luminal solutions served to maintain a normal osmotic pressure gradient across the vessel wall. The presence of dye allowed detection of leaks and assessment of the uniformity of endothelium removal. The luminal solution contained both radioactive nLDL and mLDL at the same concentration of about 0.15 mg/ml. The aorta was incubated under transmural pressure of 70 mm Hg.

It has been shown in earlier studies in which the methods used for vessel excision and deendothelialization were similar to those employed in the present work that after removal of the endothelium, an intact internal elastic lamella and a normal underlying tissue were maintained 32 and the viability of the smooth muscle cells was preserved. 8,33

The experiments were performed under different conditions. Seventeen arteries were incubated at 39° C in the presence of radiolabeled nLDL and mLDL: five arteries were incubated for 1 hour, seven for 2 hours, and five for 4 hours. In a series of
12 experiments, the aorta was incubated at 4°C to inhibit internalization and degradation of LDL: seven arteries were incubated for 2 hours and five for 4 hours. In a series of 17 experiments, arteries were incubated with tracers at 39°C for 2 hours, followed by further incubation with a tracer-free solution to wash out unbound LDL from the media: five arteries were washed for 30 minutes, seven for 1 hour, and five for 2 hours.

To ensure that the difference in concentration observed between nLDL and mLDL was actually due to LDL-receptor-related processes, two series of experiments were carried out. In one series, suramin, an inhibitor of LDL binding, was used at a concentration of 50×10^{-3} M to compete with nLDL. An arterial segment of one rabbit was incubated with suramin for 1 hour at 39°C; the luminal surface was then exposed to the tracers for 2 hours in the presence of suramin. An arterial segment of another rabbit was incubated under the same conditions and then washed out with an LDL-free solution containing suramin for 1 hour. The second arterial segment from each rabbit was used as a control. Each control segment was incubated under the same conditions as the other segment from the same animal except for the absence of suramin. In the second series of experiments, the cell membranes were broken in such a way that the cell receptor cycle was no longer effective. Four relaxed arterial segments (unstretched and unpressurized) were dipped into liquid nitrogen for 1 minute and then into Krebs' solution for 1 minute. The procedure was repeated three times. The four arterial segments were then incubated with labeled nLDL and mLDL for 2 hours at 39°C; two segments were analyzed, and the other two segments were transferred into a tracer-free solution to be washed out in a further 2-hour incubation.

**LDL Estimation in the Arterial Wall**

After incubation, the aorta was cut from the cannulas, opened axially, and divided into four segments of roughly equal area. The segments were laid on a lightly greased microscope slide and quickly frozen in a cryostat at -20°C for prevention of further diffusion or degradation of LDL. The edges of the segments were trimmed to remove overhanging material, and their surface area was measured (about 0.4 cm²). En face serial sections 20 μm thick were cut through the whole thickness of the wall. The boundary between the media and the adventitia was noted by an alteration in the appearance of the section and was located between 160 and 180 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.

For precipitation of the protein-bound label, 500 μl of 20% TCA was added and the mixture was centrifuged at 2,500g for 15 minutes. Half of the supernatant (500 μl) was carefully removed. 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. In a control series of experiments 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. The TCA-soluble radioactivity was similar to that in the unhomogenized tissue sections.

123I and 131I radioactivities were assayed simultaneously in each test tube with a double-counting procedure on a gamma counter for 3 minutes (Kontron GAMMAmatic, Basel, Switzerland). Spillover of 131I into the 123I channel was corrected by the channel ratio method. Decay rates of 123I were determined by subsequent counting. TCA-precipitable radioactivity was obtained by subtraction of the radioactivity of the 500-μl supernatant from that of the remaining 500 μl of the mixture. Tissue counts ranged from 100 to 1,000 cpm (after subtraction of background, which was about 20 cpm in 123I and 5 cpm in 127I).

Relative tissue concentrations of both TCA-precipitable nLDL (Cn) and mLDL (Cm) 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, Cn and Cm values permitted calculation of the ratio of the relative concentration of nLDL to mLDL (Cn/Cm). The Cn, Cm, and Cn/Cm values for the various sections cut from a single segment were plotted against their distance from the luminal surface to the external surface. For the sake of normalization, distances for each segment were divided by the medial thickness. Average concentration profiles were constructed by averaging the values at equal intervals across the wall. From these values mean medial Cn, Cm, and Cn/Cm values were calculated. The first section whose thickness was not accurately known was excluded in the calculation of the mean medial Cn and Cm values, but not in the calculation of the mean medial Cn/Cm value because its ratio was independent of the thickness of the section.

**Statistical Methods**

A two-way analysis of variance was constructed on the data of Cn, Cm, and Cn/Cm to test the effects of the incubation time and the location across the wall. When the variations were statistically significant (p < 0.05), multiple comparisons were performed using the Bonferroni method. Comparisons between the Cn and Cm values were performed using a paired t test. The data are reported with standard deviations.

**Results**

The concentration profiles of TCA-nonprecipitable tissue radioactivity in 123I and 131I were relatively flat across the wall. The ratio of TCA-
soluble radioactivity to total tissue radioactivity ranged from 2% to 20% in the 39°C and 4°C experiments and from 1% to 3% after washout.

Label Uptake Studies at 39°C

The average transmural profiles of \( C_n \) and \( C_m \) obtained after 1-, 2-, and 4-hour incubation at 39°C and the corresponding transmural profiles of the relative concentrations ratio, \( C_n/C_m \), are shown in Figures 1 and 2.

After the 1-hour incubation, the \( C_n \) and \( C_m \) profiles showed a slight concentration gradient from the internal side to the external side of the media except for the first value, which was underestimated (Figure 1a). At each location across the media, the \( C_n \) values were significantly higher than the \( C_m \) values \((p<0.01)\). In the adventitia, they were not significantly different. The mean medial \( C_n \) and \( C_m \) values were 0.029±0.034 and 0.020±0.021 \((n=5),\)
respectively. The C_{m}/C_{m} profile rose from the inner media, then decreased in the outer media (Figure 2a). The mean medial ratio, 1.36±0.15, was significantly higher than 1 (p<0.01).

After the 2-hour incubation, the relative concentration profiles showed a concentration gradient in the first half of the media except for the first value, which was underestimated, and were relatively flat in the rest of the layer (Figure 1b). The C_{n} and C_{m} values were not significantly different in the first two sections of the media, but the C_{n} values were significantly higher than the C_{m} values in the rest of the media (p<0.05). The adventitial C_{n} and C_{m} values were not significantly different. The mean medial C_{n} and C_{m} values were 0.032±0.009 and 0.026±0.005 (n=7), respectively. The mean medial C_{n}/C_{m} ratio, 1.23±0.22, was significantly higher than 1 (p<0.05), whereas the mean adventitial ratio was not different from 1 (1.01±0.15). However, the ratios obtained in the first two sections of the media were not significantly different from 1 (Figure 2b).

After the 4-hour incubation, the C_{n} and C_{m} profiles were relatively flat over the media and rose in the adventitial layer (Figure 1c). The C_{n} values were not significantly different from the C_{m} values in the inner media but became higher in the outer part of the layer. The mean medial C_{n} and C_{m} values were 0.032±0.013 and 0.028±0.010 (n=5), respectively. The C_{n}/C_{m} value averaged on the first half of the media was not significantly different from 1 (1.06±0.15), whereas that averaged over the outer half of the media became 1.20±0.06, significantly higher than 1 (p<0.01). This value was similar in the adventitia (Figure 2c).

The analysis of variance showed that the C_{m} values at 2 hours and 4 hours were significantly higher than those at 1 hour, but no significant difference was observed between the values at 2 hours and 4 hours. Conversely, no significant differences in C_{n} values were found between 1, 2, and 4 hours. The C_{n}/C_{m} ratio was significantly higher after 1 hour of incubation than after 2 and 4 hours, and was significantly higher after 2 hours than after 4 hours.

**Label Uptake Studies at 4°C**

At 4°C, internalization and degradation of LDL were inhibited. However, binding of nLDL to its specific receptor was maintained. The average transmural profiles of C_{n}, C_{m}, and C_{n}/C_{m} obtained after 2 and 4 hours of incubation are shown in Figures 3 and 4.

After the 2-hour incubation, the relative concentration of nLDL and mLDL decreased from the inner media, then increased in the outer media (Figure 2a). The mean medial ratio, 1.36±0.15, was significantly higher than 1 (p<0.01). The adventitial C_{n} and C_{m} values were not significantly different in the first two sections of the media, but the C_{n} values were significantly higher than the C_{m} values in the rest of the media (p<0.05). The C_{n}/C_{m} value averaged on the first half of the media became 1.20±0.06, significantly higher than 1 (p<0.05), whereas the mean adventitial ratio was not different from 1 (1.01±0.15). However, the ratios obtained in the first two sections of the media were not significantly different from 1 (Figure 2b).

After the 4-hour incubation, the C_{n} and C_{m} profiles were relatively flat over the media and rose in the adventitia (Figure 1c). Furthermore, at each location across the media, the C_{n} values were much higher than the C_{m} values. The mean medial C_{n} and C_{m} values were 0.044±0.016 and 0.032±0.014 (n=5), respectively. The C_{n}/C_{m} profile was relatively flat across the media and showed a slight decrease in the outer media and in the adventitia (Figure 2b). The mean medial C_{n}/C_{m} value was 1.47±0.20 (n=5), significantly higher than 1 (p<0.01).

The C_{n} and C_{m} values increased significantly between the 2- and 4-hour incubations (p<0.01), as did the C_{n}/C_{m} ratio values.

**Label Uptake Studies in the Washout Experiments**

The average transmural profiles of C_{n} and C_{m} obtained after an incubation period of 2 hours at...
39 °C with labeled nLDL and mLDL and after incubation followed by a 30-minute, 1-hour, and 2-hour washout with LDL-free solution are shown in Figure 5. The corresponding average transmural profiles of the Cn/Cm ratio are shown in Figure 6.

After the 30-minute washout, the Cn and Cm values (Figure 5b) fell significantly (p<0.01) as compared with those obtained before washout (39 °C, 2-hour experiments) (Figure 5a). This decrease was greater in Cn than in Cm and occurred only in the inner media. In the outer media, Cn and Cm were not significantly different from the Cn and Cm values obtained before washout. The average value of Cn and Cm calculated from the four first sections of the media (Figure 6b) The Cn/Cm values were 1.06 ± 0.20 in the first half of the media and 1.36 ± 0.31 in the outer media (n=5). The adventitial values were similar to those obtained after the 30-minute washout. How-ever, the Cn fell much more and became lower than the Cm values. The mean medial Cn and Cm values were 0.0080 ± 0.0035 and 0.011 ± 0.003 (n=7), respectively. The Cn/Cm profile was practically flat with a mean medial ratio of 0.67 ± 0.12 (n=5), significantly lower than 1 (p<0.01) (Figure 6d).

The analysis of variance showed that the Cn and Cm values did not vary significantly between the 1-hour and 2-hour washouts.

Specific nLDL Degradation

The specific nLDL degradation (receptor-mediated) was calculated across the wall from the data obtained in the 2-hour incubation at 39 °C (no washout) and those obtained in the washout experiments. The total removal of nLDL from the wall during washout was due to both nonspecific processes and specific degradation and was calculated as the difference between the Cn obtained before washout and that obtained after washout. On the other hand, mLDL was removed by the nonspecific processes only (nonspecific removal); removal was calculated as the difference between the Cm obtained before washout and that obtained after washout. Specific nLDL degradation, calculated as the difference between the total removal and the nonspecific removal, was observed only in the inner media after 30 minutes of washout, throughout the media between 30 minutes and 1 hour, and only in the outer media between 1 hour and 2 hours of washout. The transmural distribution of the total degradation activity was then obtained as the sum of the degradation activities calculated in each section during these three periods of washout. The total nLDL specific degradation activity (in microliters luminal solution per cubic centimeter wet tissue) decreased quasilinearly from 12.8 ± 6.8 in the inner media to 7.4 ± 2.5 in the outer media and became 4.1 ± 3.6 in the adventitia (Figure 7).

Label Uptake Studies in Arteries With Suramin and in Arteries With Broken Cell Membranes

In the arterial segment incubated with suramin for 2 hours at 39 °C, the Cn/Cm ratio was constant over the media and was practically equal to 1 (0.97). In the absence of suramin, the Cn/Cm profile obtained in the other arterial segment from the same animal increased from 1.12 in the inner media to 1.35 in the outer media. In the artery exposed to tracers and washed out, the Cn/Cm ratio over the media was constant to 0.91 in presence of suramin, whereas that obtained in the absence of suramin was about.
0.40 in the first half of the media and increased to 1 in the outer media.

In the two relaxed arterial segments that were dipped in liquid nitrogen to fracture the cell membranes and incubated at 39°C for 2 hours with labeled nLDL and mLDL, the mean medial C/Cm ratio was 1.00±0.08. In the two other segments with broken cell membranes that were washed out in a tracer-free solution for 2 hours, the mean medial C/Cm ratio was 0.89±0.09. The C/Cm ratios obtained before and after washout were not significantly different, nor were these ratios different from 1.

**Discussion**

Understanding of the mechanisms of interaction between LDL and its specific receptor has been derived from studies performed in cells in culture.24-37,38 However, these mechanisms and their possible influence on LDL transport in the whole arterial wall are far less well documented and understood. Hitherto the processes of receptor-mediated uptake and degradation of LDL in arteries have been studied in vivo by comparing either the fate of nLDL and LDL labeled with tyramine-cellobiose (TC-LDL) or the fate of nLDL and mLDL.

Taking advantage of the fact that the degradation products of TC-LDL remain trapped in the cell while those of unmodified LDL freely diffuse out of the wall, Carew et al23 calculated LDL degradation as the difference between TC-LDL and nLDL present in the wall (TC-LDL degradation products). These authors showed that in the rabbit aorta, 24 hours after simultaneous injection of the tracers, the amount of TC-LDL degradation products trapped by the media represented 60% of the total TC-LDL degradation products retained by the whole aorta. This finding demonstrated the presence in the media of functional LDL receptors capable of degrading LDL in vivo. However, the TC-LDL method does not permit assessment of the role of binding-internalization in the LDL uptake by the wall. This was accomplished by use of LDL modified by methylation. Wiklund et al20 observed no difference in the relative concentrations of the tracers in the media of the rabbit thoracic aorta 30 or 60 minutes after intravascular injection of nLDL and mLDL. This could be interpreted as a lack of influence of medial LDL receptors in the short-term uptake of LDL, possibly because of the different lag times between cell exposure to nLDL and receptor binding (half-time is about 30 minutes37) and between binding and internalization (half-time is about 10 minutes38). Furthermore, it has been shown that the in vivo LDL influx rate in the rabbit thoracic aorta after 30 minutes is very low, and that the LDL uptake by the media is not yet at steady state.14
FIGURE 6. Average profiles of relative concentrations ratio of $^{125}$I-native LDL to $^{131}$I-methylated LDL (Cn/Cm) as a function of normalized distance from lumen (1 represents media-adventitia boundary) in aortas incubated at 39°C for 2 hours (Panel a) and at 39°C for 2 hours followed by a 30-minute (Panel b), 1 hour (Panel c), or 2-hour (Panel d) washout with LDL-free solution. Panel a is the same as Figure 2b. The Cn/Cm ratio values were obtained from the activity in $^{125}$I and $^{131}$I determined simultaneously in each section. Bars represent standard error of mean.

Therefore, it seems that short-term in vivo experiments were probably not suitable for demonstration of any role of LDL receptors in the uptake of LDL even though such processes exist, as suggested by the degradation of LDL observed at 24 hours. In fact, Srinivasan et al reported that 4 hours after in vivo injection of nLDL and mLDL, the concentration of nLDL in the rabbit aortic media was about 45% higher than that of mLDL.

The in vitro preparation used in the present work allowed performance of experiments that were unrealizable in vivo, permitting a separate analysis of the processes of binding and degradation. Indeed, in both in vivo experiments and our in vitro experiments at 39°C, use of nLDL and mLDL did not permit distinction between the processes of binding—internalization and degradation. In the present studies, a value of the Cn/Cm ratio higher than 1 indicated that binding and internalization occurred but did not rule out the possibility that some of the nLDL taken up by the smooth muscle cells had been degraded. The two other experimental conditions (4°C washout) permitted evaluation of the relative importance of binding—degradation. The experiments carried out at 4°C to prevent internalization and degradation of LDL allowed assessment of the surface nLDL-binding process alone. Those performed at 39°C with an LDL-free intraluminal solution after incubation with tracers permitted calculation of LDL degradation by comparison of the Cn and Cm values obtained before and after washout.

The results obtained in arteries with suramin and in arteries with broken cell membranes strongly supported the assumption that the differences in
concentration between nLDL and mLDL were due to the effect of the cell membrane receptors.

Deendothelialized vessels were used because the influx rate of LDL was substantially increased and, thus, the steady state was more rapidly obtained. The ratio of nLDL to mLDL to which the media was exposed was not altered by the absence of endothelium, since it has been shown that the transport of LDL across the endothelium is receptor independent. An additional advantage of the in vitro study was that the luminal concentrations of both nLDL and mLDL remained constant during the incubation periods; thus, the problem of the varying decrease in concentration of plasma tracers that occurs in vivo was overcome.

In the 39° C experiments, LDL entering the media was distributed to both specific and nonspecific binding sites. After specific binding, nLDL was internalized, whereas mLDL could not follow a receptor-mediated endocytosis. Differences in relative concentrations of nLDL and mLDL in the media were due both to differences in surface binding and to differences in internalization. The Cm values increased significantly between 1 and 2 hours of incubation but did not vary between 2 and 4 hours, indicating that a steady state in Cm was achieved after 2 hours. Conversely, the Cn values, which were always higher than the Cm values, did not change significantly with duration of incubation.

The significant difference between Cn and Cm observed after 1 hour of incubation was certainly due to the presence of surface-receptor-bound and internalized nLDL. However, because the time lag for binding is about 30 minutes and that for internalization about 10 minutes, the pool of internalized nLDL was expected to be low after this period of incubation. The lack of change in Cn with time of incubation probably reflected the existence of an nLDL-specific degradation process that was initiated after 1 hour. Indeed, if no degradation of nLDL occurred, the change in Cn should be either parallel to the change in Cm or higher due to specific binding. The absence of change in Cn might be accounted for by the balance between the amount of nLDL entering the wall and the amount taken up by the cells' receptors and degraded. This finding is in agreement with the theoretical work by Truskey et al., which showed that metabolic phenomena must be included in models to predict the experimental results obtained by Bratzler et al. on the distribution of LDL across the aortic media.

Because of the longer exposure of the inner media to nLDL, the nLDL degradation was expected to begin earlier in the inner media than in the rest of the media. This effect might explain the greater decrease of the Cn/Cm ratio observed in the inner media than in the outer media. However, the gradient in the Cn/Cm ratio profiles might also be caused by the difference in the functional activity of smooth muscle cells across the media, with the inner cells being more active for degradation than the outer cells, as suggested by the washout experiments.

After 4 hours of incubation at 4° C, the Cn values were considerably greater than those at 39° C, whereas the Cm values were similar to those at 39° C. The Cn/Cm ratio was about 1.5, substantially higher than that obtained at 39° C. This seemed to indicate that in the absence of degradation and even though the capacity of binding was decreased at 4° C, more than 30% of the nLDL uptake was due to the specific receptors.

In the washout experiments, it may be assumed that LDL was removed from the wall in four different ways, three affecting both nLDL and mLDL and one involving only nLDL: 1) The filtration of the tracer-free solution washed out the unbound interstitial nLDL and mLDL; 2) as a result of the decrease in the interstitial LDL concentration, some of the nLDLs and mLDLs were removed from their binding sites; 3) nLDL and mLDL could be degraded via the nonspecific pathway; and 4) nLDL was degraded via the receptor pathway.

The nLDL and mLDL concentrations decreased progressively with the duration of the washout periods. This decrease was observed first in the inner media and then in the outer media as the front of washout moved toward the adventitia. In each section, the most dramatic decrease of Cn and Cm was obtained after 1 hour of washout. The time course of the Cn/Cm profile during the washout period (Figure 6) suggests that as the front of washout attained a layer, nLDL was removed from the wall faster than mLDL. The Cn/Cm ratio reached a value of about 0.6 after 1 hour of washout in the inner media. This value was practically unchanged and was observed throughout the media after 2 hours of washout. This seems to indicate that all the nLDL available for specific degradation had been degraded after 1 hour of washout.

The observed change of the Cn/Cm ratio from a value higher than 1 before washout to about 0.6 after washout might have resulted from different mechanisms. The nLDL bound to its receptor before washout was internalized and degraded. This process tended to make the Cn/Cm ratio equal to unity, but could not account for the observed value of 0.6. Some of the interstitial nLDLs were likely to be bound, internalized, and degraded during the washout period. In that way, as long as the interstitial LDLs were not totally washed out from the media, the Cn/Cm ratio decreased and became lower than 1. Between 1 and 2 hours of washout, the mLDL concentration no longer changed significantly, suggesting that all free diffusible mLDL was removed from the wall. The remaining mLDL in the media was possibly distributed into two compartments, one consisting of mLDL irreversibly bound to extracellular matrix components and the other consisting of mLDL nonspecifically bound to cells. The ability of nLDL to bind nonspecifically to extracellular matrix components and to cells was likely
to be the same as that of nLDL. Yet, it is possible that a larger number of nonspecific cellular sites were occupied by mLDLs than by nLDLs, yielding the larger C_m than C_n observed after the washout period. To account for this phenomenon, it may be assumed that as long as interstitial nLDLs and mLDLs were available, as many nonspecific sites were occupied by nLDL as by mLDL. However, once the available pool of nLDL and mLDL had been washed out or degraded, nLDL previously nonspecifically bound to cells was likely to be taken up by the receptor, internalized, and degraded as a result of the permanent receptor recycling process. It follows that most of the nLDL remaining in the wall was probably nonspecifically bound to extracellular matrix.

The nLDL-specific degradation activity represented the total amount of nLDL degraded between 0 and 2 hours of washout. The greater values observed in the inner media than in the outer media might reflect a difference in the ability of the smooth muscle cells to degrade LDL. It also might be possible that degradation was not completely achieved in the outer layers. However, after the 2-hour period of washout, C_n was already very low in the outer media and was actually similar to that in the inner media. Nevertheless, even though all nLDL remaining in the outer media after the 2-hour washout had to be degraded, the value of nLDL-specific degradation would have been still lower than that in the inner media.

The mean medial total degradation rate calculated in the present work was 9.6±4.5 μl/cm³ wet tissue/hr. Carew et al23 found that the in vivo degradation rate in the media was about 5.3×10⁻⁶% of the plasma pool per gram aortic wet weight per day. Assuming a plasma volume of 135 ml in the rabbit,20 the LDL degradation rate would be 0.3 μl/g tissue/hr, about 30-fold lower than that in our study. However, the in vivo concentration of LDL in the media of the rabbit thoracic aorta is about 10-fold lower (3 μl/cm³ wet tissue) than that of the present study. Thus, it appears that if our value of degradation activity is expressed as a function of the medial nLDL concentration, it is of the same order as that reported by Carew et al.23

We concluded from the present study that functional LDL receptors participate in the uptake and degradation of LDL by the media of deendothelialized rabbit thoracic aorta. Our model permitted calculation of the nLDL-specific degradation across the media. We found a functional difference in the degradation activity throughout the media, with the inner cells being more active than the outer cells in the degradation of LDL.

The ability of LDL receptors to bind and degrade LDL may prevent any focal accumulation of LDL within the arterial wall that would contribute to the development of atherosclerosis. Further experiments in intact vessels, when compared with the present results obtained in deendothelialized arteriies, should permit assessment of whether endothelial cells in the whole organ are capable of inducing modifications of LDL, as has been found by use of endothelial cells in culture, and study of the effects of such modification on LDL transport and degradation.

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