Pigeon aortic smooth muscle cells lack a functional low density lipoprotein receptor pathway

R. Keith Randolph and Richard W. St. Clair

Department of Pathology, Arteriosclerosis Research Center, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27103

Abstract The low density lipoprotein (LDL) receptor pathway was studied in aortic smooth muscle cells from atherosclerosis-susceptible White Carneau pigeons and compared with rhesus monkey cells whose LDL receptor pathway has been previously characterized. Pigeon LDL was bound with high affinity in a saturable manner to both pigeon and monkey aortic smooth muscle cells. The kinetics of binding were different, however. LDL binding to pigeon cells exhibited positive cooperativity at low LDL concentrations and at least two classes of binding sites. The same pigeon LDL bound to monkey cells in a manner consistent with a single class of binding sites. Thus, these differences were a property of the pigeon cells and not the result of differences in the LDL. On the average, pigeon cells bound less than 50% the amount of LDL as monkey cells. Despite the surface binding to pigeon cells, little of the LDL was internalized, whereas pigeon LDL was actively internalized by monkey cells. Consistent with this observation, chloroquine and leupeptin had no effect on accumulation of LDL or on LDL degradation by pigeon cells, and incubation of pigeon cells with LDL produced no increase in cellular cholesteryl ester content. Binding of LDL to pigeon cells also differed from that of monkey cells by being unaffected by pretreatment with the proteolytic enzyme pronase, and by not requiring calcium. Binding was not specific for LDL since acetyl-LDL, and to a lesser degree HDL, were able to compete for LDL degradation of LDL. This apparently occurred on the surface rather than by internalization and degradation within the lysosomes as occurs in mammalian cells. The functional significance of LDL binding to pigeon smooth muscle cells is unclear. The characteristics of binding resemble that of a nonspecific lipoprotein receptor referred to by others as the "lipoprotein receptor" or the "EDTA-insensitive receptor." It is apparent, however, that White Carneau pigeon aortic smooth muscle cells lack a functional LDL receptor pathway and in this way resemble cells from human beings with homozgyous familial hypercholesterolemia or from Watanabe rabbits.


Supplementary key words lipoprotein composition • chlороquine • high density lipoprotein • acetyl LDL • 25-OH cholesterol • lipoprotein-deficient serum

Extrahepatic mammalian cells derive the bulk of their cholesterol from the uptake of low density lipoproteins (LDL) through the LDL receptor pathway (1). The LDL binds to specific high affinity receptors located in specialized areas of the plasma membrane called coated pits. The LDL is rapidly internalized and delivered to lysosomes where the protein is degraded and the cholesteryl esters are hydrolyzed. This results in an increase in cellular cholesterol content which in turn inhibits endogenous cholesterol synthesis, stimulates cholesterol esterification, and down-regulates the synthesis of LDL receptors. Thus, by controlling the number of LDL receptors on the plasma membrane, the cell is able to utilize extracellular cholesterol in a tightly regulated fashion in response to changing cellular cholesterol needs.

Although the magnitude of this regulatory response can vary with cell type, a wide variety of normal mammalian cells has been shown to control LDL receptor activity, endogenous cholesterol synthesis, and cholesterol esterification coordinately in response to fluctuations in cellular cholesterol content (2-8). Changes in cellular cholesterol content resulting in changes in the components of the LDL receptor pathway can be produced in mammalian cells in culture by incubation in a medium containing whole serum, isolated lipoproteins containing apolipoproteins B or E (9-12), or in lipoprotein-free
medium containing a variety of sterols dissolved in ethanol (13, 14).

In a previously published report, we observed a lack of stimulation of cholesterol esterification and little increase in cellular cholesteryl ester content in pigeon arterial smooth muscle cells that were incubated with medium containing whole pigeon serum (15). This occurred despite a substantial increase in the content of cellular free cholesterol. Considered in light of what is known about the regulation of cholesterol metabolism in mammalian cells, these data suggested either that pigeon cells lack a functional LDL receptor pathway or that the regulation of cholesterol metabolism in pigeon cells is fundamentally different from that of mammalian cells. The purpose of the present study was to explore the first of these possibilities by studying LDL metabolism in pigeon aortic smooth muscle cells in culture as compared to monkey aortic smooth muscle cells whose LDL receptor pathway we have previously characterized (16).

The results indicate that pigeon cells bind LDL in a saturable manner but that this binding does not result in internalization of the LDL or in delivery of exogenous cholesterol to the cells. Despite the inability to efficiently internalize LDL, pigeon cells are capable of degrading LDL at rates that exceed that which can be attributed to nonspecific processes.

MATERIALS

Sodium [\( ^{125} \text{I} \)]iodide (17.0 Ci/mg, carrier-free low pH) and [\( 1,2^{-3} \text{H} \)]cholesterol (40 Ci/mmol) were purchased from New England Nuclear Corporation, Boston, MA. The fluorescent probe 3,3',dioctadecyldimethylammonium bromide (DiI) was obtained from Molecular Probes Inc., Plano, TX. Chloroquine, leupeptin, and pronase were purchased from Sigma Chemical Co., St. Louis, MO. All tissue culture supplies were obtained from Flow Laboratories, Rockville, MD.

METHODS

Cell cultures

Pigeon (15) and monkey aortic smooth muscle cells (17) were obtained and cultured by methods that have been previously described. Skin fibroblasts (GM-2000) from a patient with the receptor-negative form of familial hypercholesterolemia were obtained from the Human Genetic Mutant Cell Repository, Camden, NJ. The pigeon cells were grown from aortic explants from young (less than 6 months of age), grain-fed random-bred White Carneau (WC) pigeons. The monkey cells were grown from aortic explants from adult rhesus monkeys (\textit{Macaca mulatta}) fed Monkey Chow (Ralston Purina Co., St. Louis, MO). Stock cultures of pigeon and human cells were maintained in medium consisting of Eagle’s Minimum Essential Medium supplemented with Eagle’s vitamins, fetal bovine serum (FBS) (10% final concentration), glucose (1.5 mg/ml), 25 mM sodium bicarbonate, 200 mM L-glutamine, 100 I.U. penicillin/ml, and 100 mg streptomycin/ml, and will be referred to as medium containing FBS. Cholesterol concentrations in this medium averaged 40 \( \mu \text{g} \) cholesterol/ml. Monkey cells were cultured in identical medium except that the FBS was replaced by calf serum.

Cells from stock cultures were harvested from 75-cm² flasks with 0.05% trypsin-0.2% ethylenediaminetetraacetic acid (EDTA) and plated into 60-mm tissue culture dishes at densities of \( 2.5 \times 10^5 \) cells/dish for monkey and human cells and \( 5.0 \times 10^5 \) cells/dish for pigeon cells. Prior to initiation of experiments, cells were grown to confluence.

Medium containing lipoprotein-deficient serum (LPDS) was prepared by replacing the FBS with the d > 1.21 g/ml fraction of calf serum to give a final protein concentration of 2.5 mg/ml (17). This medium contained less than 1 \( \mu \text{g} \) of cholesterol/ml.

Measurement of cellular free cholesterol and cholesteryl ester content

After incubation with the appropriate medium, cells were harvested from dishes with trypsin-EDTA and washed twice with phosphate-buffered saline (PBS) (16). The cells were suspended in 1.0 ml of deionized water and disrupted by sonication. An aliquot was taken for protein determination (18) and another was extracted for lipids by the method of Bligh and Dyer (19). Free cholesterol and cholesteryl ester mass were quantified as described previously (16) by gas–liquid chromatography using the method of Ishikawa et al. (20).

Lipoprotein isolation, labeling, and characterization

Lipoproteins were isolated from grain-fed WC pigeons and from rhesus monkeys consuming Monkey Chow (Ralston Purina Co.). Both of these diets were essentially cholesterol-free. Blood from fasted animals was collected in tubes containing EDTA at a final concentration of 1 mg/ml and kept at 4°C during subsequent procedures. Low density lipoproteins were separated as described previously using a combination of ultracentrifugation and agarose column chromatography (16). Pigeon high density lipoproteins (HDL) were isolated by adjusting the d > 1.080 g/ml infranatant solution, from which the LDL had been isolated, to 1.21 g/ml with solid KBr and centrifuging for 40 hr at 36,000 rpm in a Beckman SW-40 rotor. The isolated lipoproteins were...
exhaustively dialyzed against 0.9% NaCl and 0.01% EDTA. LDL and HDL migrated on agarose electrophoresis as single bands with beta- and alpha-mobility, respectively. Pigeon LDL, however, migrated only a few millimeters from the point of origin while monkey LDL migrated 10–15 mm from the origin in a manner similar to human LDL.

Pigeon and monkey LDL were labeled with $^{125}$I using the iodine monochloride method as described previously (16). After iodination, the lipoproteins were dialyzed extensively against PBS containing 0.01% EDTA and sterilized by passage through a 0.45-μm filter (Millipore Corp., Bedford, MA). Less than 2% of the radioactivity in the final LDL preparation was soluble in chloroform–methanol 2:1, and greater than 97% of the radioactivity was precipitable in (10%) trichloroacetic acid (TCA). Iodinated LDL preparations had beta-mobility when separated by electrophoresis on agarose, and virtually all radioactivity was localized to the LDL band as determined by radioautography. Specific activities of the $^{125}$I-labeled LDL preparations were typically 300–600 cpm/ng protein.

The chemical composition of pigeon and monkey LDL was determined as previously described (21). Molecular weight of LDL was determined using a $^{125}$I-labeled LDL of known molecular weight as an internal standard (22). Electrophoresis in 12% polyacrylamide gels (PAGE) containing 0.1% sodium dodecyl sulfate was performed on delipidated apolipoproteins (23). Apolipoprotein A-I was isolated from rhesus monkey plasma (23) and used as a standard for PAGE.

Low density lipoproteins were double labeled with $[^3]$H-cholesterol ester and $^{125}$I ($[^3]$H-CE/$^{125}$I-LDL) exactly as described previously (24). Specific activities for $^{125}$I-labeled protein and $[^3]$H-cholesterol ester averaged 38 cpm/ng LDL protein and 12 cpm/ng LDL cholesteryl ester, respectively. The ratio of $^{125}$I to $[^3]$H in the final preparation was 2.5. Low density lipoproteins were labeled with DiI as described by Pitas et al. (25).

**Determination of $^{125}$I-labeled LDL binding, internalization, and degradation**

Cells were grown to confluence in medium containing FBS in 60-mm dishes, washed twice with PBS, and fresh medium containing FBS or LPDS was added. After the indicated period of incubation, the appropriate concentrations of $^{125}$I-labeled LDL, in the presence or absence of a 20-fold excess of homologous unlabeled LDL, were added to the dishes in medium containing FBS or LPDS, and incubated for 5 hr at 4°C or 37°C. Specific binding, internalization, and degradation of $^{125}$I-labeled LDL were determined after subtracting the radioactivity obtained in the presence of a 20-fold excess of unlabeled LDL from that obtained in its absence (26). Total cell associated $^{125}$I-labeled LDL (bound plus internalized) was determined as described by Goldstein and Brown (26) by digesting the washed cells with 1 N NaOH. In some experiments we measured the amount of LDL bound and internalized as heparin-releasable or resistant $^{125}$I-labeled LDL as described by Goldstein et al. (27) or as trypsin-releasable or resistant $^{125}$I-labeled LDL as described by Bierman, Stein, and Stein (7). Cells were washed exhaustively as indicated previously (16) before the above-described methods were applied.

Proteolytic degradation of $^{125}$I-labeled LDL was determined on 1 ml of post-incubation medium by measurement of TCA (10%)-soluble (non-iodide) $^{125}$I radioactivity as described by Goldstein and Brown (26). Results were corrected for TCA-soluble, non-iodide $^{125}$I found in control dishes incubated without cells.

Internalization of $[^3]$H-CE/$^{125}$I-LDL was measured after release of surface-bound material with trypsin. The cell pellet was suspended in 1.0 ml of deionized H$_2$O; 100 μl was used for protein determination and 100 μl was counted for internalized $^{125}$I in the gamma spectrometer, (Tracer model 1885 Autogamma System). Lipids were extracted from the remaining 0.8 ml by the method of Bligh and Dyer (19). An aliquot of the total lipid extract was counted for total internalized $[^3]$H. The remaining lipid extract was dried under a stream of nitrogen and neutral lipids were separated by thin-layer chromatography (TLC) as described previously (21). The free cholesterol and cholesteryl ester bands were visualized with iodine, scraped into scintillation vials, and counted for $[^3]$H radioactivity. With this method, the total lipid and TLC-separated fractions were essentially free of $^{125}$I radioactivity which simplified the $[^3]$H counting, as there was no contamination of $[^3]$H with $^{125}$I.

Deterioration products of $^{125}$I-labeled LDL were characterized exactly as described by Brown and Goldstein (26) using the pooled media from groups of ten dishes for each experimental treatment. Prior to characterization of the degradation products the pooled media were centrifuged to remove cell debris and lyophilized.

All experiments shown in the tables and figures were repeated at least once with similar results.

**RESULTS**

The chemical composition of the pigeon and monkey LDL and pigeon HDL used in these studies is shown in Table 1. Monkey and pigeon LDL were remarkably similar in composition with the exception that pigeon LDL contained somewhat less cholesteryl ester and more triglyceride. The proportion of total mass representing core lipids (cholesteryl esters plus triglycerides) was similar for both species, comprising approximately 45% of total lipoprotein mass.
TABLE 1. Chemical composition of pigeon and monkey LDL and pigeon HDL

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>N°</th>
<th>Molecular Weight ( \times 10^{-6} )</th>
<th>FC ( % )</th>
<th>CE ( % )</th>
<th>TG ( % )</th>
<th>PL ( % )</th>
<th>Prot ( % )</th>
<th>Prot ( % )</th>
<th>PL ( % )</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pigeon</td>
<td>3</td>
<td>3.1 ± 0.4</td>
<td>20.1 ± 1.4</td>
<td>9.8 ± 0.6</td>
<td>30.4 ± 4.9</td>
<td>14.9 ± 7.9</td>
<td>24.8 ± 2.4</td>
<td>0.49 ± 0.04</td>
<td>1.38 ± 0.15</td>
</tr>
<tr>
<td>Monkey</td>
<td>3</td>
<td>2.9 ± 0.2</td>
<td>22.0 ± 0.4</td>
<td>9.0 ± 0.4</td>
<td>37.5 ± 1.0</td>
<td>8.2 ± 1.1</td>
<td>23.3 ± 0.5</td>
<td>0.41 ± 0.03</td>
<td>1.41 ± 0.02</td>
</tr>
<tr>
<td>HDL</td>
<td>1</td>
<td>N.D.</td>
<td>39.4</td>
<td>5.2</td>
<td>17.5</td>
<td>5.1</td>
<td>32.8</td>
<td>0.13</td>
<td>0.40</td>
</tr>
</tbody>
</table>

FC, free cholesterol; CE, cholesteryl ester; TG, triglyceride; PL, phospholipid; TC, total cholesterol.

N° denotes the number of pooled lipoprotein preparations that were characterized.

The chemical composition of pigeon HDL differed somewhat from that of previously published values for rhesus monkey HDL. The percentage of protein in pigeon HDL was lower than for rhesus monkey HDL (28), 39 vs 46%, respectively, while the percentage of phospholipid was higher, 33 vs 27%, respectively. Cholesteryl ester was the predominant component of core lipids in pigeon HDL (approximately 77%) and monkey HDL (approximately 85%).

The apolipoproteins present in pigeon and monkey HDL and LDL are shown in Fig. 1. The major apoprotein of pigeon and monkey LDL was apoB. Of the other apolipoproteins the only consistent difference was the presence in pigeon apoLDL of a band comigrating with monkey apoA-I, and the absence of a band migrating in the position of monkey apoE. ApoA-I was the predominant apoprotein of both pigeon and monkey HDL. The identities of the unlabeled larger molecule weight proteins in pigeon HDL and LDL, that migrated between apoB and apoA-I, are not known.

Since previous studies have shown that pigeon cells fail to increase cholesterol esterification or cholesteryl ester content when incubated with whole serum (15), initial experiments were designed to determine whether isolated pigeon LDL would promote cholesterol accumulation in pigeon and monkey cells (Fig. 2). When incubated with the same medium containing FBS or LPDS, pigeon cells contained less free cholesterol and cholesteryl ester than monkey cells. With increasing concentrations of pigeon LDL there was an increase in the cellular free and esterified cholesterol content of monkey cells similar to previous observations using monkey LDL (21). In contrast, there was no increase in cellular cholesteryl ester content in pigeon cells even though the free cholesterol content increased to a degree similar to that seen in the monkey cells.

Prior to the initiation of studies on the LDL receptor pathway, experiments were carried out to determine the optimal conditions for measurement of LDL binding by pigeon cells. Low density lipoprotein binding was measured by three different methods in cells that were preincubated with medium containing either FBS or LPDS (Table 2). Monkey cells, as do other mammalian cells, increase the number of LDL receptors on their surface when cellular cholesterol concentrations are depleted by incubation with LPDS (2). Pigeon cells, in contrast, bound more LDL when preincubated with medium containing FBS as compared to cells preincubated with medium containing LPDS. This difference was observed in incubations carried out at 4°C and 37°C and occurred regardless of whether binding was measured as total cell-associated \( ^{125} \text{I} \) or as heparin- or trypsin-releasable \( ^{125} \text{I} \). As a result, in subsequent experiments with pigeon cells, \( ^{125} \text{I} \)-labeled LDL was added directly to medium containing FBS and the cells were not preincubated with LPDS.

Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of monkey and WC pigeon apoHDL and apoLDL. B, E, A-I, and C refer to the location of apoproteins B, E, A-I, and C, respectively, from rhesus monkeys. Gels were intentionally overloaded with 100 \( \mu \text{g} \) of protein applied to each gel in order to identify all bands present. MHDL, MLDL, PHDL, PLDL refer to monkey HDL and LDL and pigeon HDL and LDL, respectively.
The binding, internalization, and degradation of $^{125}$I-labeled LDL by pigeon and monkey cells are shown in Table 3. The sum of the $^{125}$I-labeled LDL bound and internalized (NaOH treatment) by monkey smooth muscle cells was more than fourfold greater than for pigeon cells. Heparin removed 25% of the cell-associated $^{125}$I from the pigeon cells and trypsin removed 50%. Under the same conditions, heparin removed 9% of the cell-associated $^{125}$I from monkey cells, and trypsin removed 14%. After removal of surface-bound LDL by trypsin, the amount of cell-associated LDL remaining was only about 10% (119 ng/mg protein) as much in pigeon cells as monkey cells (1188 ng/mg protein). Despite these large differences in the amount of LDL internalized by pigeon and monkey cells, pigeon cells degraded considerable amounts of LDL.

The concentration dependence of LDL binding, internalization, and degradation by pigeon cells is shown in Fig. 3. Binding of $^{125}$I-labeled LDL displayed saturation kinetics that could be separated into "specific" and "nonspecific" components. At low LDL concentrations, binding was predominantly "specific." Saturation occurred at concentrations greater than 50 µg LDL protein/ml, with one-half maximum binding occurring at approximately 15 µg LDL protein/ml. Total binding capacity of pigeon cells was calculated to be 35,000 LDL particles/cell as compared with 80,000 LDL particles/cell for monkey cells (16), and 70,000 LDL particles/cell for human cells (27) under similar conditions. There was again very little $^{125}$I-labeled LDL internalized, and the amount that was measurable did not increase in a concentration-dependent manner, as the binding data would have predicted had LDL binding been coupled with internalization (29). The amount of $^{125}$I-labeled LDL degraded was again surprisingly large compared to the amount of $^{125}$I-labeled LDL internalized by monkey cells.

Table 2. Effect of preincubation with FBS or LPDS on binding of $^{125}$I-labeled LDL by pigeon smooth muscle cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$^{125}$I-labeled LDL Bound/mg Cell Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaOH</td>
<td>507</td>
</tr>
<tr>
<td>FBS</td>
<td>261</td>
</tr>
<tr>
<td>Heparin</td>
<td></td>
</tr>
<tr>
<td>FBS</td>
<td>282</td>
</tr>
<tr>
<td>LPDS</td>
<td>118</td>
</tr>
<tr>
<td>Trypsin</td>
<td></td>
</tr>
<tr>
<td>FBS</td>
<td>313</td>
</tr>
<tr>
<td>LPDS</td>
<td>207</td>
</tr>
</tbody>
</table>

Confluent pigeon smooth muscle cells were preincubated in medium containing 10% FBS or LPDS for 24 hr. Dishes were washed and medium containing FBS or LPDS plus the indicated concentrations of $^{125}$I-labeled pigeon LDL were added to dishes and incubation was continued for 5 hr at 37°C or 4°C. Cells incubated at 37°C were then chilled on melting ice, washed extensively, and binding was determined as indicated. Results are corrected for nonspecific binding as described under Methods and are the average of duplicate dishes. Total cholesterol concentration in cells incubated with FBS or LPDS for 24 hr was 25.0 and 22.5 µg/mg cell protein, respectively. Less than 1 µg of CE was detected under both conditions.

Table 3. Comparison of measures of bound, internalized, and degraded $^{125}$I-labeled LDL in pigeon and monkey smooth muscle cells

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Treatment</th>
<th>Bound</th>
<th>Internalized</th>
<th>Degraded</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pigeon</td>
<td>NaOH</td>
<td>505 ± 14</td>
<td>1169 ± 54</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Heparin</td>
<td>72 ± 12</td>
<td>242 ± 6</td>
<td>1159 ± 84</td>
</tr>
<tr>
<td></td>
<td>Trypsin</td>
<td>148 ± 8</td>
<td>119 ± 9</td>
<td>1183 ± 72</td>
</tr>
<tr>
<td>Monkey</td>
<td>NaOH</td>
<td>1409 ± 226</td>
<td>1807 ± 137</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Heparin</td>
<td>116 ± 11</td>
<td>1258 ± 75</td>
<td>1697 ± 71</td>
</tr>
<tr>
<td></td>
<td>Trypsin</td>
<td>167 ± 11</td>
<td>1188 ± 167</td>
<td>1725 ± 91</td>
</tr>
</tbody>
</table>

Confluent pigeon and monkey smooth muscle cells were preincubated for 24 hr in medium containing 10% FBS and LPDS, respectively. Dishes were washed and experimental medium containing 20 µg of $^{125}$I-labeled pigeon LDL protein/ml was added. After 5 hr incubation at 37°C, binding and internalization were measured by the indicated methods. Results are corrected for nonspecific processes as described under Methods and are the average of triplicate dishes.
Fig. 3. Binding, internalization, and degradation of 125I-labeled LDL by WC pigeon smooth muscle cells. Cells were incubated for 5 hr at 37°C with fresh medium containing FBS plus the indicated concentrations of 125I-labeled pigeon LDL in the presence and absence of a 20-fold excess of unlabeled pigeon LDL. Binding and internalization were measured after removal of surface-bound material with trypsin. Specific binding, internalization, and degradation were calculated as described under Methods. Results are the average of duplicate dishes.

Fig. 4. Comparison of binding 125I-labeled pigeon LDL by monkey and WC pigeon smooth muscle cells. Confluent monkey (panel A) and pigeon cells (panels B, C) were preincubated with medium containing LPDS or FBS, respectively, for 24 hr. Fresh medium containing LPDS (for monkey cells) or FBS (for pigeon cells) plus the indicated concentrations of 125I-labeled pigeon LDL were added and the cells were incubated for 5 hr at 37°C (panel A, B) or 4°C (panel C). Cells were washed extensively as described in the Methods and binding was measured by heparin release (panel A, B) or by NaOH digestion (panel C). The inserts display the Scatchard plot for each experiment. B/F represents the lipoprotein bound (ng protein/dish), divided by the lipoprotein free in the medium (ng/2.0 ml).

suggested some cooperativity of binding of LDL to pigeon cells. When the binding data were analyzed according to the method of Scatchard (31), there was a striking difference between monkey (Fig. 4A) and pigeon...
cells (Fig. 4B and 4C). The complex nature of binding of pigeon LDL to pigeon cells appeared to be a function of the cells themselves and not the LDL, since binding of the same pigeon LDL to monkey cells resulted in a linear Scatchard plot (Fig. 4A).

Unlike the binding of LDL to LDL receptors on human skin fibroblasts, binding of LDL to pigeon smooth muscle cells did not require calcium and was unaffected by pronase treatment (Table 4). The specificity of LDL binding is shown in Fig. 5. Both monkey and pigeon LDL competed effectively with [125I]-labeled pigeon LDL for binding to pigeon

\begin{table}
\centering
\caption{Effect of calcium and pronase on LDL binding to 
porcine and monkey smooth muscle cells} 
\begin{tabular}{|c|c|c|c|c|c|}
\hline
& & & Calcium: & & \\
& Pronase & 4°C & 37°C & & \\
& & - & + & 2 mM & 2 mM & \\
\hline
ng [125I]-labeled LDL/mg cell protein & & & & & \\
Monkey SMC & & & & & \\
Bound & 199 & 69 & 55 & 137 & \\
Cell-associated & 1259 & 303 & & & \\
Degraded & 607 & 108 & 13 & 569 & \\
Pigeon SMC & & & & & \\
Bound & 24 & 59 & 67 & 64 & \\
Cell-associated & 79 & 57 & & & \\
Degraded & 204 & 227 & 30 & 25 & \\
\hline
\end{tabular}
\end{table}

Cells were grown to confluence in medium containing serum. The monkey cells were incubated for an additional 48 hr in medium containing LPDS and the pigeon cells were incubated for 48 hr with fresh medium containing FBS. For the pronase experiment, the cells were incubated for 45 min with 5 µg pronase/ml of HEPES-buffered medium without serum. The medium was discarded and the cells were washed two times with albumin (2 mg/ml PBS) solution and the cells were incubated for 1 hr at 37°C with [125I]-labeled pigeon LDL (10 µg/ml) in LPDS containing HEPES-buffered medium. Surface-bound LDL was released with trypsin, and cell-associated and degraded LDL were determined as described in Methods. For the calcium experiments, bound LDL was determined at 4°C as follows. Cells were preincubated for 50 min at 4°C, washed three times with ice-cold PBS containing 100 µM EDTA, and incubated for 1 hr in HEPES-buffered saline containing 100 µM EDTA, 2.5 mg glucose/ml, 2.5 mg LPDS/ml, and 10 µg/ml of [125I]-labeled pigeon LDL. Cells were washed extensively as described in Methods and surface-bound LDL was released with heparin. Cells remained attached to the dish during the 1-hr incubation. The effect of calcium on LDL degradation was determined at 37°C as follows. At 37°C without Ca²⁺ in the medium, cells released from the dishes within 30-60 min. Thus, in order to compare LDL degradation on cells in the same growth conditions, we first washed cells three times with PBS containing 100 µM EDTA and incubated the cells for 1 hr at 37°C in HEPES-buffered saline containing 50 µM EDTA, 2.5 mg glucose/ml, and 2.5 mg LPDS/ml. The detached cells were transferred to 60-mm petri dishes (not tissue culture dishes) in the same medium. To this was added [125I]-labeled pigeon LDL (final concentration 10 µg/ml) with or without Ca²⁺. After 3 hr incubation at 37°C, the cells were pelleted by centrifugation and an aliquot of the cell-free supernatant fluid was analyzed for [125I]-labeled LDL degradation as described in Methods. During the 3-hr incubation, all cells remained unattached to the dishes. Results are the mean of three dishes for all experiments except the 37°C calcium experiments, in which results are the mean of duplicate dishes.

A similar result was obtained using monkey cells and [125I]-labeled monkey LDL (data not shown). At equivalent protein concentrations, monkey LDL was consistently 10-15% more effective as a competitor for [125I]-labeled LDL binding than was pigeon LDL. Acetyl LDL was nearly as effective a competitor as native LDL, while pigeon HDL competed less effectively, and fibrinogen not at all. In parallel studies with monkey cells, pigeon HDL did not compete for binding with [125I]-labeled monkey LDL (data not shown).

To confirm the apparent lack of high affinity LDL internalization suggested by the data in Table 4 and Fig. 3, cells were incubated with LDL whose protein moiety was labeled with [125I] and whose cholesteryl ester

\begin{figure}
\centering
\caption{Competition of pigeon HDL (a) and LDL (0), monkey LDL (b, o) and acetyl LDL (c), and fibrinogen (w) for binding of [125I]-labeled pigeon LDL by pigeon smooth muscle cells. Cells were 
grown to confluence in medium containing serum. Fresh medium containing FBS was added along with 10 µg of protein of [125I]-labeled pigeon LDL and the indicated concentrations of unlabeled proteins. Monkey LDL (37°C), pigeon LDL, and pigeon HDL were incubated with the cells for 5 hr at 37°C; cells were washed extensively and binding was measured as heparin-releasable [125I]. Monkey LDL (4°C), monkey acetyl LDL, and fibrinogen were incubated with the cells at 4°C for 3 hr; cells were washed extensively and binding was measured as total cell-associated [125I] after digestion of the cells with NaOH. Results are the mean of duplicate cultures at each point and include data from two separate experiments.}
\end{figure}
moiety was labeled with $[^3]$H]cholesteryl oleate (Fig. 6). In monkey cells, with increasing time of incubation, LDL was internalized and reached a plateau by approximately 3 hr. The $[^3]$H]cholesteryl ester was also internalized and rapidly hydrolyzed to $[^3]$H]free cholesterol. Both $^{125}$I-labeled degradation products and $[^3]$H]free cholesterol were lost from the cells and accumulated in the medium with time.

The internalization of $^{125}$I by pigeon cells was less than 20% of that seen in monkey cells. Even this was probably an overestimate since a negligible amount of $^3$H was found in the cells. The small amounts of $^{125}$I found in the cells probably represent uptake of either $^{125}$I-labeled degradation products present in the culture medium or exchange with small amounts of $^{125}$I-labeled phospholipids of the original LDL, rather than uptake of the intact LDL particle. The failure to find $^3$H in the cells was not the result of uptake, hydrolysis, and efflux of $[^3]$H]cholesterol since only trace amounts of $[^3]$H]free cholesterol were found in the culture medium of pigeon cells even after 24 hr in incubation.

In order to determine whether lysosomes were involved in the degradation process, cells were incubated with the $[^3]$H-CE/$^{125}$I-labeled LDL in the presence and absence of chloroquine, a known inhibitor of lysosomal function in both mammalian (32) and avian (33) species. As shown in Table 5, and consistent with previous findings from this laboratory (2) and those of Goldstein, Brunschede, and Brown (32), chloroquine markedly inhibited the degradation of $^{125}$I-labeled LDL protein and the hydrolysis of LDL $[^3]$H]cholesteryl esters in monkey cells. Consistent with a lysosomal site of LDL degradation, there were large accumulations of both $^{125}$I and $[^3]$H-cholesteryl esters in monkey cells incubated with chloroquine. Chloroquine, however, had no effect on either degradation or accumulation of LDL in pigeon cells, suggesting that degradation of LDL by pigeon cells did not occur in the lysosomal compartment. These results further support the conclusion that LDL was not internalized.

In other studies, leupeptin (a thiol-cathepsin inhibitor (34)) was compared with chloroquine for its ability to inhibit LDL degradation by pigeon smooth muscle cells (Table 6). Neither the general lysosomotropic agent
Table 5. Effect of chloroquine on internalization and degradation of $^{3}H$-CE/$^{125}$I-LDL by pigeon and monkey smooth muscle cells

<table>
<thead>
<tr>
<th></th>
<th>$^{125}$I Degraded</th>
<th>$^{3}$H-CE Degraded</th>
<th>$^{125}$I Cell-Associated</th>
<th>$^{3}$H-CE Cell-Associated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monkey cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>841</td>
<td>4,168</td>
<td>1,181</td>
<td>2,350</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>5,518</td>
<td>1,475</td>
<td>11,186</td>
<td>1,000</td>
</tr>
<tr>
<td>Pigeon cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>204</td>
<td>116</td>
<td>58</td>
<td>751</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>220</td>
<td>97</td>
<td>21</td>
<td>791</td>
</tr>
</tbody>
</table>

Cells were grown and preincubated as described in the legend of Table 3. Cells then received medium containing FBS (pigeon cells) or LPDS (monkey cells) plus $^{3}$H-CE/$^{125}$I-LDL at a protein concentration of 20 µg/ml and were incubated for 24 hr at 37°C with and without 50 µM chloroquine. Cell-associated radioactivity was determined after treatment of the cells with trypsin. Degradation and hydrolysis of $^{3}$H-CE/$^{125}$I-LDL were determined as described under Methods. Results are the average of duplicate dishes.

Table 6. Effect of chloroquine and leupeptin on the degradation of LDL by monkey and pigeon smooth muscle cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>Inhibitor</th>
<th>ng $^{125}$I-labeled LDL Degraded/ mg Cell Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pigeon</td>
<td>None</td>
<td>572</td>
</tr>
<tr>
<td></td>
<td>Leupeptin</td>
<td>548</td>
</tr>
<tr>
<td></td>
<td>Chloroquine</td>
<td>560</td>
</tr>
<tr>
<td>Monkey</td>
<td>None</td>
<td>1309</td>
</tr>
<tr>
<td></td>
<td>Leupeptin</td>
<td>906</td>
</tr>
<tr>
<td></td>
<td>Chloroquine</td>
<td>601</td>
</tr>
</tbody>
</table>

Pigeon and monkey smooth muscle cells were grown to confluence in medium containing serum. Monkey cells were preincubated an additional 24 hr in medium containing LPDS. Fresh medium containing FBS (pigeon cells) or LPDS (monkey cells) plus 20 µg $^{125}$I-labeled monkey LDL protein/ml and leupeptin (20 µg/ml) or chloroquine (50 µM) were added and incubation was continued for 3 hr at 37°C. Aliquots of medium were taken for determination of $^{125}$I-labeled LDL degradation. Average cellular protein concentration for dishes of pigeon and monkey cells were 147 and 226 µg protein/dish, respectively. Values are the average of duplicate dishes.

Note: (chloroquine) nor the specific protease inhibitor (leupeptin) were able to affect LDL degradation by pigeon cells. In contrast, chloroquine and leupeptin inhibited LDL degradation by monkey cells, consistent with the results of others using bovine arterial smooth muscle cells (34). These results, together with the results shown in Table 5, indicate that the lysosomes per se were not involved in the degradation of LDL by pigeon cells.

The pattern of fluorescence of cells incubated with Dil-labeled LDL is shown in Fig. 7. Fluorescence was compared in pigeon and monkey smooth muscle cells and in skin fibroblasts from a human patient with the LDL receptor-negative form of familial hypercholesterolemia. In contrast to cells that lack functional LDL receptors (FH cells), pigeon cells exhibited fluorescence that was more abundant and more evenly distributed over the cell surface. Under the same conditions, monkey cells had much more fluorescence, with greatest intensity in the perinuclear region of the cells. There was no such perinuclear concentration of fluorescence in pigeon cells. These results are consistent with the binding and degradation of LDL.
internalization of LDL by monkey cells and the concentration of the fluorescent label in the lysosomes (25), whereas in pigeon cells the pattern of fluorescence is consistent with surface binding, but a lack of internalization.

Cultured bovine endothelial cells have been reported to bind LDL to surface receptors, but to internalize the LDL only when actively proliferating (35). Since our studies were done with confluent cells, it was possible that, like bovine endothelial cells, LDL was internalized only while cells were nonconfluent. To test this possibility we incubated pigeon smooth muscle cells with $^1$H-labeled LDL under conditions where the cells were either confluent or nonconfluent (Table 7). The nonconfluent cells were 60–70% confluent as judged by phase contrast microscopy. This was consistent with the difference in cell protein per dish. Although slightly more LDL was bound, internalized, and degraded in the nonconfluent group there was no evidence to suggest that this was the result of a functional internalization component that was absent in the confluent cells. This conclusion is supported by the observation that there was little change in the proportion of cell-associated and degraded $^1$H-labeled LDL relative to the amount bound in the confluent and nonconfluent cells. As a result, under the conditions of this experiment, pigeon smooth muscle cells appear to lack a functional LDL receptor pathway while actively proliferating as well as while quiescent. Since only one stage of cell growth was studied, we cannot exclude the possibility that during some short period of the cell cycle a functional LDL receptor pathway is expressed. This seems to us unlikely, however.

To test whether LDL binding to pigeon cells could be regulated, pigeon smooth muscle cells were preincubated with LPDS, LDL, cholesterol dissolved in ethanol, or 25-OH cholesterol dissolved in ethanol for up to 24 hr, and the extent of specific binding of $^1$H-labeled LDL was determined. As can be seen from Fig. 8, neither LDL nor cholesterol had any effect on LDL binding, while LPDS reduced LDL binding at the 24-hr time period. Incubation with 25-OH cholesterol, in contrast, resulted in a marked increase in LDL binding. The failure to alter LDL binding by incubation with LDL or cholesterol occurred in spite of an approximate doubling in cellular free cholesterol content (Table 8). Incubation with LPDS for 24 hr did not change the cholesterol content of the cells but, nevertheless, reduced LDL binding by 40%. The response of LDL binding in

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**TABLE 7. Metabolism of $^{125}$I-labeled LDL in confluent and nonconfluent pigeon smooth muscle cells**

<table>
<thead>
<tr>
<th>Protein/Dish</th>
<th>Bound</th>
<th>Cell-Associated</th>
<th>Degraded</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/60-mm dish</td>
<td>$^1$H-labeled LDL/mg cell protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Confluent</td>
<td>500 ± 11</td>
<td>28 ± 6</td>
<td>118 ± 5</td>
</tr>
<tr>
<td>Nonconfluent</td>
<td>222 ± 12</td>
<td>38 ± 3</td>
<td>135 ± 4</td>
</tr>
</tbody>
</table>

White Carneau pigeon smooth muscle cells (BB-I) were plated on day 0 at 0.75 × 10$^6$ or 1.5 × 10$^6$ cells/60-mm dish in medium containing 10% fetal bovine serum. On day 2, the medium was replaced with fresh medium. On day 3, 0.15 mg of $^{125}$I-labeled LDL/ml isolated from cholesterol-fed pigeons, was added and incubated with the cells for 5 hr at 37°C. After washing the cells as described in Methods, the surface-bound LDL was removed with trypsin, and cell-associated and degraded LDL were determined as described. Degradation was corrected for the amount of TCA-soluble noniodide $^{125}$I in no-cell control dishes.

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*Randolph and St. Clair* LDL metabolism in pigeon aortic smooth muscle cells 897
TABLE 8. Effect of LPDS, LDL, and cholesterol on cholesterol mass in pigeon smooth muscle cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>FC (μg/mg cell protein)</th>
<th>CE (μg/mg cell protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBS control</td>
<td>15.4</td>
<td>0.6</td>
</tr>
<tr>
<td>LPDS control</td>
<td>16.0</td>
<td>0.4</td>
</tr>
<tr>
<td>LDL</td>
<td>25.5</td>
<td>0.9</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>33.6</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Cells were grown to confluence in medium containing FBS. The various treatment groups were prepared exactly as described for Fig. 8. After 24 hr of incubation at 37°C, free cholesterol (FC) and cholesteryl ester mass (CE) were determined as described. Results are the mean of duplicate cultures.

Fig. 9. Sephadex G-10 elution profile of 125I-labeled TCA-soluble LDL degradation products from monkey and WC pigeon smooth muscle cells. Prior to the experiment, pigeon and monkey cells were preincubated with medium containing FBS or LPDS, respectively, for 48 hr in order to maximize LDL binding and degradation. At the beginning of the experiment, the medium in each dish was replaced with 2 ml of medium containing LPDS and 10 μg of 125I-labeled pigeon LDL/ml. One group of ten dishes with no cells was incubated for 6 hr at 37°C. Other groups of cells of ten dishes each were incubated for 1 or 6 hr at 37°C. After the appropriate incubation period, medium from each group of ten dishes was collected, pooled, and prepared for chromatography as described under Methods. Retained peaks eluting at exactly the same volume are indicated by the stippling. Recovery of radioactivity after chromatography was approximately 77%. The void volume of the column was determined using blue dextran. The average cell protein for dishes from which the monkey or pigeon medium was obtained was 362 μg and 212 μg/dish, respectively.

Fig. 10. Thin-layer chromatography profile of 125I radioactivity from the most retained fraction (stippled area) from Sephadex G-10 column chromatography shown in Fig. 9. The 125I-labeled TCA-soluble degradation products from the stippled peak from Fig. 9 (6 hr incubation) were pooled and separated by TLC on plastic sheets coated with silica gel or cellulose. An authentic L-tyrosine standard was spotted on sheets with the test material and co-chromatographed. After chromatography (as described under Methods), the sheets were dried, sprayed with ninhydrin, and developed at 90°C for 5 min to localize the L-tryrosine. Thin-layer sheets were cut into 1-cm strips and counted directly for 125I. The cross-hatched spot indicates the location of the L-tryrosine standard. Open symbols (○) represent material from pigeon cells and closed symbols (●) represent material from monkey cells.
and pigeon cells were amino acids or short polypeptides. The material in the peak indicated by the stippling was further characterized by TLC and found to be identical with $^{125}$I-labeled tyrosine (Fig. 10). The other major peak eluted earlier than tyrosine and probably represents a mixture of polypeptide degradation products. Thus, greater than 90% of the TCA-soluble $^{125}$I-labeled LDL degradation products from monkey and pigeon cells were amino acids or short polypeptides.

**DISCUSSION**

Results of this study show that LDL binds to pigeon smooth muscle cells by a saturable and high affinity process ($T_{1/2}$ approximately 15 μg protein/ml), and that a significant proportion of the bound LDL can be released by heparin. These are characteristics of LDL binding to the classical mammalian LDL receptor (1). On the other hand, there are some obvious differences in the characteristics of LDL binding to pigeon cells that distinguish it from the classical LDL receptor pathway. Scatchard analysis showed a complex pattern of binding suggestive of both positive cooperativity and at least two classes of binding sites. When the same pigeon LDL was incubated with monkey cells, Scatchard analysis indicated binding to only a single class of receptors as has been shown previously with monkey LDL (16). As a result, these differences are clearly a property of the cells themselves and are not due to differences in the pigeon LDL.

Low density lipoprotein binding to the classical mammalian LDL receptor has an absolute requirement for calcium (26), is diminished by pretreatment of cells with proteolytic enzymes such as pronase (26), and is highly specific as seen by the failure of certain other lipoproteins, unrelated proteins and chemically modified lipoproteins such as acetyl LDL, to compete for binding (1, 37). In contrast, LDL binding to pigeon smooth muscle cells did not require calcium, was not inhibited by treatment with pronase, and was competed for by acetyl LDL and to a lesser extent by HDL. Competition with HDL could not be explained by the presence of apoE since no apoprotein of the same size as mammalian apoE was seen on PAGE gels. Taken together, these data suggest that LDL binding to pigeon cells is not mediated by the classical LDL receptor. Instead it has many of the properties of a less specific binding site that has been described for lipoprotein binding to hepatic and adrenal cell membranes. Binding of LDL to this site does not require calcium, is not influenced by pronase treatment, and is competed for by a variety of lipoproteins such as LDL, apoE-free HDL, acetyl LDL, and methyl LDL (38-43). A further characteristic of the multiple lipoprotein binding site of liver and adrenal is that LDL, although bound, is not degraded, presumably because it is not internalized (39). Again, this is similar to pigeon cells in which no LDL internalization was demonstrated. It is also possible that the binding of LDL to pigeon cells does not represent binding to a true receptor but rather may be the result of lipid-lipid interactions between lipid domains of the LDL and the plasma membrane, as has been suggested by Fogelman et al. (44) for the uptake of methyl LDL by human monocyte macrophages.

Pigeon cells also differ from mammalian cells in their inability to down-regulate the surface binding of LDL even under conditions in which the cellular cholesterol content is nearly doubled. The mechanism of free cholesterol enrichment in pigeon cells incubated with LDL presumably involves surface transfer of free cholesterol from LDL to the plasma membranes of the cells. A similar enrichment of cellular free cholesterol content can also be observed in FH cells incubated with LDL (24). In normal mammalian cells and in FH cells, the addition of cholesterol in ethanol results in an increase in cellular cholesterol content and the down-regulation of LDL receptor activity on the cell surface (29). This did not occur in pigeon cells, however, suggesting that the site on pigeon cells to which LDL binds is not down-regulated by an increase in cellular cholesterol content similar in magnitude to that shown to down-regulate the LDL receptor on mammalian cells.

LDL binding to pigeon cells can be modulated, however, by LPDS and 25-OH cholesterol. LPDS caused a decrease in LDL binding while 25-OH cholesterol caused an increase. These changes are opposite to those observed in mammalian cells under similar conditions and suggest that LDL binding to pigeon cells does not function to deliver cholesterol to cells. Rather it might be speculated that the site on pigeon cells that binds LDL mediates cellular cholesterol efflux in response to enhancement of the concentration of certain critical intracellular cholesterol pools. Such an hypothesis is consistent with the report by Biesbroek et al. (43) indicating the presence of an HDL receptor on mammalian skin fibroblasts having many of the properties of LDL binding to pigeon cells and whose activity is up-regulated by increases in cholesterol content. Although enrichment of the cells with cholesterol did not alter LDL binding to pigeon cells, 25-OH cholesterol did increase LDL binding in a manner similar to its enhancement of HDL binding to endothelial cells (45) and to fibroblasts and smooth muscle cells (46). It should be emphasized, however, that we have yet to demonstrate a physiological function of lipoprotein binding to pigeon cells.

In spite of the failure of pigeon cells to internalize LDL, there was considerable degradation of LDL. Sev-
eral lines of evidence support the conclusion that this degradation occurred on the cell surface and not within the lysosomes of the cells. Degradation was not susceptible to inhibition by the general lysosomal enzyme inhibitor chloroquine, or the lysosomal cathepsin B inhibitor, leupeptin. Degradation of LDL could not be attributed to secretion of soluble proteases into the culture medium since there was little proteolytic activity detectable in the culture medium in incubations of less than 24 hr. Taken together, these observations lead to the conclusion that degradation occurred on the cell surface and not within the lysosomes of the cells.

Although there was no evidence of lysosomal degradation of LDL in pigeon cells, the products of proteolytic degradation of LDL were remarkably similar to those seen in monkey cells. Similar results have been reported for the LDL degradation products from normal and receptor-negative FH skin fibroblasts (26). This observation, plus the demonstration that chloroquine has a small, but measurable, inhibitory effect on the degradation of LDL by FH cells, has been interpreted by Goldstein and Brown (26) as evidence that the presumed "nonspecific" degradation of LDL occurs in the lysosomes. Since chloroquine had little effect on LDL degradation, it does not appear that a similar "nonspecific" pathway could be responsible for the degradation of LDL by pigeon cells.

A major question raised by these studies is the physiological role in pigeon cells of LDL binding in the absence of internalization. In mammalian cells LDL binds to LDL receptors, is internalized, and ultimately provides cholesterol to the cell for a variety of cellular needs. Low density lipoprotein does not appear to serve this function in pigeon cells, suggesting that this function is served either by another lipoprotein or by endogenous cholesterol synthesis. Previous studies from this laboratory have shown that even whole pigeon serum is ineffective in stimulating cholesterol ester accumulation and cholesterol esterification in pigeon cells (15). This suggests that the other lipoproteins present in whole serum are also ineffective in promoting the influx of extracellular cholesterol. As a result, cellular cholesterol homeostasis in pigeon cells must be regulated at the level of cholesterol synthesis and/or cholesterol efflux.

Since the present studies utilized only cells from the atherosclerosis-susceptible WC pigeon, it is not known whether the atherosclerosis-resistant Show Racer (SR) pigeon also lacks a functional LDL receptor pathway. In a previous study, however, both WC and SR pigeon smooth muscle cells failed to accumulate cholesterol esters or to have cholesterol esterification stimulated when exposed to whole serum (15). This suggests that a similar pattern of LDL metabolism occurs in cells from both WC and SR pigeons. Consequently, it remains to be seen whether the lack of LDL internalization plays a direct role in the susceptibility or resistance of the two breeds of pigeons to atherosclerosis. Nevertheless, the absence of a functional LDL receptor pathway and the marked susceptibility to the development of atherosclerosis gives the WC pigeon a high degree of similarity to humans with familial hypercholesterolemia (1) and to Watanabe rabbits (47).

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