Accumulation of $^{125}$I-Tyramine Cellobiose–Labeled Low Density Lipoprotein Is Greater in the Atherosclerosis-Susceptible Region of White Carneau Pigeon Aorta and Further Enhanced Once Atherosclerotic Lesions Develop

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Previous studies have suggested that greater arterial concentrations of undegraded low density lipoprotein (LDL) and/or greater arterial rates of LDL degradation may play role(s) in determining regional differences in arterial susceptibility to atherosclerosis in rabbits (Schwenke and Carew, Arteriosclerosis 1989;9:895–918). The White Carneau (WC) pigeon is also useful for investigating potential mechanism(s) that might account for regional variation in arterial susceptibility to atherosclerosis because atherosclerosis develops predictably in the aorta at the level of the celiac bifurcation (atherosclerosis-susceptible celiac site). In this study we sought to determine whether the $^{125}$I-tyramine cellobiose ($^{125}$I-TC) content 1 day after injecting $^{125}$I-TC-LDL ($^{125}$I-TC-LDL accumulation) would be greater in the celiac site in arteries of WC pigeons and whether $^{125}$I-TC-LDL accumulation would be exaggerated by cholesterol feeding. Because $^{125}$I-TC remains trapped in cells after cellular degradation, arterial sites that either degrade LDL at higher rates or contain higher concentrations of undegraded LDL or both will demonstrate greater $^{125}$I-TC-LDL accumulation. Young WC pigeons were studied while consuming a cholesterol-free diet and after consuming a cholesterol-containing diet for 1, 2, 4, and 8 weeks. In pigeons fed a cholesterol-free diet, $^{125}$I-TC-LDL accumulation in the celiac site was equivalent to 0.24±0.02 $\mu$g LDL cholesterol/cm$^2$ aortic surface/day compared with only 0.14±0.02 $\mu$g LDL cholesterol/cm$^2$/day for the adjacent aorta, which is resistant to atherosclerosis (atherosclerosis-resistant site) (p<0.025). In atherosclerotic lesions excised from the celiac site, $^{125}$I-TC-LDL accumulation was equivalent to 21±10 $\mu$g LDL cholesterol/cm$^2$/aortic surface/day compared with 0.66±0.17 $\mu$g LDL cholesterol/cm$^2$/day for the adjacent atherosclerosis-resistant site (p<0.001). During cholesterol feeding, $^{125}$I-TC-LDL cholesterol accumulation in the celiac site as a whole increased 30-fold compared with a fivefold increase in plasma LDL cholesterol. In comparison, $^{125}$I-TC-LDL cholesterol accumulation in the adjacent atherosclerosis-resistant arterial site increased at the same rate as the plasma LDL cholesterol, while $^{125}$I-TC-LDL cholesterol accumulation in two other relatively atherosclerosis-resistant arterial sites that we studied increased relatively little during cholesterol feeding. The results of this study suggest that differences in arterial $^{125}$I-TC-LDL accumulation, both those present in normal animals and those induced by cholesterol feeding, may contribute to the characteristic regional variation in arterial susceptibility to atherosclerosis in WC pigeons. Additional studies will be needed to determine whether this difference reflects differences in arterial permeability to LDL, retention of undegraded LDL within the arterial extracellular matrix, or the cellular metabolism of LDL. (Arteriosclerosis and Thrombosis 1992;12:446–460)

KEY WORDS • pigeons • atherosclerosis • low density lipoproteins • atherosclerosis susceptibility • cholesterol feeding • arterial wall metabolism • atherogenesis mechanisms

The clinical sequelae of atherosclerosis, coronary heart disease, stroke, and peripheral vascular disease, are the leading causes of morbidity and mortality in the United States.1 Evidence from several sources indicates that elevated levels of cholesterol in plasma, particularly that in the low density lipoprotein (LDL) fraction, are causally related to atherosclerosis.2-4 Because most of the cholesterol in atherosclerotic lesions is derived from plasma lipoproteins,5-7 it seems likely that increased concentrations of LDL cholesterol in plasma promote atherosclerosis by directly or indirectly increasing delivery of cholesterol to cells within the arterial wall. Consideration of all factors known to influence atherogenesis, including the LDL cholesterol concentra-
tion, can only explain part of the risk of atherosclerosis in human beings. In addition, in humans and experimental animals, atherosclerosis develops preferentially at certain susceptible arterial sites, whereas other adjacent sites are resistant to atherosclerosis. These observations suggest that other yet-to-be-identified “risk factors” acting at the level of the arterial wall may determine the regional differences in susceptibility to atherosclerosis within an individual and may also be responsible for some of the unexplained variability in the extent of atherosclerosis among different individuals. Differences in hemodynamics may play a role in the regional variation in the development of atherosclerosis. However, it seems equally likely that localized alteration(s) in the interaction of lipoproteins with the arterial wall (permeability, cellular metabolism, and/or retention within the arterial wall) may also play a role in the preferential development of atherosclerosis at characteristic sites.

Previous studies of rabbits showed that arterial concentrations of undegraded LDL and rates of LDL degradation were enhanced in atherosclerosis-susceptible aortic sites and that these parameters, most notably the arterial concentration of undegraded LDL, were enhanced during 16 days of cholesterol feeding. The localization of these changes to the most atherosclerosis-susceptible aortic sites and the fact that these changes were observed before the appearance of fatty streak lesions or large accumulations of foam cells within the intima suggest that such changes are an early manifestation of the pathogenesis of atherosclerosis in the rabbit.

The White Carneau (WC) pigeon is another well-characterized model of human atherosclerosis. This species develops atherosclerosis that shares several features of the human disease, including accumulation of cholesterol esters, macrophages, and smooth muscle cells, and, unique among animal models of atherosclerosis, the development of complicated plaques including mineralization, ulceration, and thrombosis.

In WC pigeons, atherosclerosis develops spontaneously in the aorta near the site of bifurcation of the celiac artery (celiac site), while the immediately proximal part of the aorta is highly resistant to atherosclerosis. Cholesterol feeding accelerates the development of atherosclerosis at the susceptible celiac site.

In this study we considered whether the total arterial 214T-tyramine cellulose (TC) content, reflecting the sum of arterial undegraded LDL and products of arterial LDL degradation (214T-TC-LDL accumulation), might be enhanced at the atherosclerosis-susceptible celiac site of WC pigeons and thus suggest potential mechanism(s) to account for the characteristic development of atherosclerosis at this site. In this report we show that 214T-TC-LDL accumulation is greater in the celiac site than in the adjacent atherosclerosis-resistant site in the same pigeons. 214T-TC-LDL accumulation was further increased in the celiac site when atherosclerotic lesions were present after cholesterol feeding.

Methods

Animals and Diets

Fifteen random-bred WC pigeons were obtained from our breeding colony, where they were fed a cholesterol-free grain diet. Young birds (3.5–7 months of age) were chosen for this study so that little if any spontaneous atherosclerosis would be present. The birds were divided into five groups of three each so that the average age of the pigeons in each group was similar. One group was retained on the grain diet as a control. The other four groups were fed a grain diet containing 0.5% (wt/wt) cholesterol and 10% (wt/wt) lard beginning at appropriate intervals so that all cholesterol-fed birds could be studied during the same 3-day interval and after the four groups of pigeons had consumed the atherogenic diet for 1, 2, 4, and 8 weeks, respectively. The control pigeons were studied during this same 3-day interval.

Plasma Lipids and Lipoproteins

Plasma and lipoprotein lipids were determined in 4–5 ml blood samples collected just before the pigeons were killed. The blood samples, which were collected from an alar vein, were immediately mixed with Na2EDTA at a final concentration of 2.7 mM. Plasma was obtained after low-speed centrifugation. The concentrations of cholesterol and triglyceride in plasma were measured by enzymatic methods. Very low density lipoproteins (VLDLs) were isolated from the tops of the tubes after ultracentrifuging 1.5-ml plasma samples at a density of 1.006 g/ml for 4.38×10^8 g•min at 4°C in an SW 55 rotor. High density lipoproteins (HDLs) were obtained from the d>1.006 g/ml fraction after precipitating LDL with heparin–manganese. The cholesterol concentrations in VLDL, the d>1.006 g/ml fraction, and HDL were corrected for recovery of cholesterol from ultracentrifugation, which averaged 94.9±1.4% (mean±SEM, n=14). The LDL cholesterol was calculated as the difference between the d>1.006 g/ml cholesterol and the HDL cholesterol.

Isolation and Labeling of Low Density Lipoprotein for Reinjection Studies

LDLs for labeling and reinjection were isolated from pooled plasma obtained from donor WC pigeons fed the atherogenic diet. The mean plasma cholesterol concentration of these plasma donors was 33.0 mM (SEM=3.6, n=11). Blood collected from these donor birds was immediately mixed with Na2EDTA and the lecithin: cholesterol acyltransferase inhibitor 5,5′-dithio-bis(2-nitrobenzoic acid) at final concentrations of 2.7 mM and 1.0 mM, respectively, and placed on ice. The VLDL fraction was removed by ultracentrifugation at d=1.006 g/ml for 2.5×10^8 g•min at 4°C in an SW 40 rotor. The infranatant was adjusted to d=1.080 g/ml with solid KBr and layered with a NaCl/KBr solution of density 1.063 g/ml containing 2.7 mM EDTA. After ultracentrifuging for 3.9×10^8 g•min at 4°C in an SW 40 rotor, the 1.006<d<1.063 g/ml fraction, which we denote here as LDL, was isolated from the top of the tubes using a tube slicer. The isolated LDL was dialyzed against five changes of 500 volumes of 0.15 M NaCl, 2 mM EDTA, pH 7.4 (buffer A). After dialysis, protein content was determined by the method of Lowry et al, with bovine serum albumin as a standard, and incorporated extraction with chloroform to eliminate turbidity due to lipids. Three aliquots of LDL protein (8 mg each) were separately labeled with 125I (0.6 mCi/mg protein)
using 1,3,4,6-tetracloro-3α,6α-diphenylglycouril (Iodogen) as described. Doubly labeled LDL preparation was separated and coupled to 125I-TC. TC was labeled with Iodogen (0.1 mCi 125I/nmol TC), and the 125I-TC was covalently linked to the 125I-LDL (6.2 nmol TC/mg LDL protein) after activating the 125I-TC with cyanuric chloride. Afterward, this 125I-LDL (doubly labeled LDL) was dialyzed overnight against 500 volumes of buffer A.

**Depletion of Non-Apolipoprotein B Radiolabel From Doubly Labeled Low Density Lipoprotein by Exchange With High Density Lipoprotein**

To deplete doubly labeled LDL of radiolabel present in small apoproteins and in lipid, each doubly labeled LDL preparation was separately incubated with the d>1.063 g/ml plasma fraction of WC pigeons fed the atherogenic diet as described in detail in the footnote to Table 1. After incubation, the mixtures were transferred to separate ultracentrifuge tubes, and solid KBr was added to increase the density to 1.080 g/ml. Each mixture was overlaid with a 1.063 g/ml density solution containing 2.7 mM EDTA, and doubly labeled LDL was resolated from the tops of the tubes after ultracentrifugation in 3.4×10^9 g·min at 4°C in an SW 40 rotor. The resolated doubly labeled LDL was dialyzed against two changes of 600 volumes of buffer A and sterilized by passing it through a 0.45-μm filter before injection into the pigeons.

**Characterization of Doubly Labeled Low Density Lipoprotein**

Both before and after incubation with the d>1.063 g/ml plasma fraction, doubly labeled LDLs were characterized by agarose gel electrophoresis,[@ref14] by precipitation with 10% (vol/vol, final concentration) trichloroacetic acid (TCA), and extraction with chloroform/methanol. The integrity of apoprotein B (apo B) and the distribution of radioactivity between apo B and other apoproteins was evaluated by electrophoresis of delipidated LDL on 4-12% gradient polyacrylamide gels under denaturing conditions.[@ref29]

**Animal Studies**

To determine the arterial degradation rate and the intra-arterial concentration of unprocessed LDL, the pigeons were injected intravenously with doubly labeled LDL (9.07±0.20×10^9 cpm 125I and 1.35±0.06×10^9 cpm 131I/kg body wt). To avoid potential confounding of comparisons among data for different times of cholesterol feeding by differences in LDL preparations, each of the three doubly labeled LDL preparations was given to one untreated pigeon and one pigeon fed cholesterol for each of the four different intervals. One of the pigeons fed cholesterol for 2 weeks died shortly after injection of doubly labeled LDL. Thus, data for 14 pigeons are reported here. To follow the decline of radioactivity in plasma, 0.5-mL blood samples were collected at 2, 10, 25, 45, and 80 minutes, at 2, 4, 8, and 12-15 hours, and in some cases at 20 hours after injecting doubly labeled LDL. Just before terminating the metabolic study at 25-29 hours after injecting doubly labeled LDL, a 4-5-mL blood sample was collected for measurement of radioactivity and cholesterol content of plasma and lipoprotein fractions. Immediately afterward, the birds were anticoagulated with heparin (400 IU) and anesthetized with the inhalant methoxyflu-
proximal atherosclerosis-resistant parts of the arterial tree. P₁ and P₂ were analyzed separately. Each sample was weighed and cut into the most distal 8-9 mm containing the atherosclerosis-resistant site R (Figure 1). When presented, areas of macroscopic atherosclerotic lesions were dissected from within each of the four arterial segments and analyzed separately. Each sample was weighed and its perimeter outlined on paper. The surface areas of all arterial samples were determined by planimetry facilitated by a Hipad Digitizer (Houston Instruments, Houston) and software on an Apple IIe computer. Surface areas of each arterial sample were determined at least three times. Coefficients of variation for replicate measurements were typically less than 3%.

To determine if the more luminal portion of pigeon artery degrades LDL at a higher rate than the more abluminal portion, as has been reported for the thoracic aorta of normal rabbits, atherosclerosis-free areas of the proximal atherosclerosis-resistant artery (P₁ and P₂, Figure 1) were divided with forceps into inner and outer layers that were then weighed. The inner layers represented 45.2±1.0% and 39.7±1.4% (mean±SEM, n=14) of the weight (and probably also the thickness) of the atherosclerosis-free portions of segments P₁ and P₂, respectively. Susceptible and resistant areas of the distal thoracic aorta were too thin to permit their separation into inner and outer layers in this manner.

Radioassay

Total and TCA-soluble $^{125}\text{I}-\text{TC}$ and $^{131}\text{I}$ radioactivity in plasma, lipoprotein fractions, and slices from sodium dodecyl sulfate (SDS)–polyacrylamide gels and $^{125}\text{I}-\text{TC}$ and $^{131}\text{I}$ radioactivity in arterial samples after fixation were determined simultaneously in a two-channel gamma counter equipped with a 7.6-cm crystal (Gamma 5500B, Beckman Instruments, Inc., Norcross, Ga.). Radioactivity in all samples was corrected for background radioactivity, overlap of the energy spectra of $^{125}\text{I}$ and $^{131}\text{I}$, and isotopic decay. Aliquots of plasma and lipoprotein fractions and slices from SDS-polyacrylamide gels were counted for sufficient time so that the standard deviation of the observed net counting rate was ≤1%. Arterial samples were counted for 40 minutes. Standard deviations of observed net counting rates for $^{125}\text{I}$ in arterial samples were typically ≤1%. Arterial $^{131}\text{I}$ data will not be reported here (see below). The procedures for radioiodination, use, and disposal of labeled LDLs were approved by the Bowman Gray School of Medicine Office of Health Protection.

Arterial Cholesterol Content

After radioassay arterial samples were extracted with 2:1 (vol/vol) chloroform/methanol, and the resulting extracts were washed with water. [3H]cholesterol (99% pure by thin-layer chromatography [TLC] on silica gel 60 [EM Science, Cherry Hill, N.J.] with development in 70:30:1 [vol/vol/vol] hexane/diethyl ether/acetic acid) was added as an internal standard to correct for procedural losses. The total cholesterol content was determined after saponification of an aliquot of the lipid extract. Nonesterified and esterified cholesterol were separated from another aliquot of the lipid extract by TLC as described above. Cholesterol present in the nonesterified form was determined and corrected for recovery from TLC. The esterified cholesterol content of each arterial sample was calculated as the difference between the measured total and nonesterified cholesterol contents. For a few samples the measured value for nonesterified cholesterol content was slightly greater than that for the total cholesterol content (esterified...
choleresterol not detectable). For these samples the esterified cholesterol content was considered to be zero.

**Arterial Accumulation of $^{125}$I-Tyramine Cellobiose-Low Density Lipoprotein**

In these studies we set out to independently determine the arterial content of undegraded LDL (using directly incorporated $^{125}$I) and the sum of the arterial content of undegraded LDL and products of arterial LDL degradation (using the $^{125}$I-TC label). Those data would have then allowed calculation of both arterial concentrations of undegraded LDL and arterial rates of LDL degradation. However, for reasons described in the "Results" section, we report here only data for the total arterial $^{125}$I-TC content after fixation. We denote the total arterial $^{125}$I-TC content after fixation as arterial $^{125}$I-TC-LDL accumulation. The arterial $^{125}$I-TC-LDL accumulation was first expressed in terms of equivalent volumes of plasma (microliters plasma per gram or square centimeter artery per day). This provides a measure of the sum of the arterial undegraded LDL and products of arterial LDL degradation that is independent of the plasma LDL concentration. This was calculated by dividing the $^{125}$I-TC present in each arterial sample after fixation (counts per minute per gram or square centimeter) by the area under the curve for the decline of protein-bound $^{125}$I-TC in plasma after injecting $^{125}$I $^{125}$I-TC-LDL (counts per minute per microliter · day). Protein-bound radioactivity in plasma was calculated by subtracting the radioactivity soluble in 10% (vol/vol, final concentration) TCA from the total plasma radioactivity. The area under each of these plasma $^{125}$I-TC radioactivity curves was determined by integrating a biexponential equation fitted to the protein-bound $^{125}$I-TC radioactivity in the plasma from each bird using the Simulation Analysis and Modeling (SAAM) program.

The arterial $^{125}$I-TC accumulation was converted to amounts of LDL cholesterol by multiplying the $^{125}$I-TC accumulation by arterial samples of individual birds, expressed in plasma equivalents, by the concentration of LDL cholesterol in their own plasma.

**Whole-Body Metabolism of Low Density Lipoprotein**

The fractional catabolic rate (FCR) of LDL by the whole body, a measure of whole-body LDL metabolism, was calculated from coefficients and exponents determined by the biexponential equation fitted to protein-bound $^{125}$I-TC radioactivity in the plasma from each bird (see above).

**Statistical Analysis**

Comparisons of $^{125}$I-TC accumulation and cholesterol concentrations between arterial sites were made either with paired $t$ tests (two arterial sites) or analysis of variance (ANOVA) with multiple measures on the arterial site (three or more arterial sites). The Scheffé criterion was used for unplanned comparisons between the atherosclerosis-susceptible celiac site and the proximal atherosclerosis-resistant artery (P, and P2). For the ANOVAs comparing the three atherosclerosis-resistant arterial sites (R, P, P2, Figure 1), the two degrees of freedom for the arterial site were partitioned into a comparison of the distal atherosclerosis-resistant site with the proximal atherosclerosis-resistant arterial sites (R versus P, and P combined) and a comparison of the two proximal atherosclerosis-resistant sites (P versus P2). Data for each group of birds were considered alone to investigate differences between these arterial sites under normal conditions and at each time of cholesterol feeding.

To investigate the effect of cholesterol feeding, data for all birds were considered together by ANOVA (a single arterial site) or ANOVA with multiple measures (two or more arterial sites) and included the grouping factor, time of cholesterol feeding (0 [control], 1, 2, 4, 8 weeks) or diet (cholesterol free versus cholesterol containing).

Regression analyses were used to consider whether the characteristics of arterial sites changed in consistent manners during cholesterol feeding and whether corresponding characteristics changed at different rates in adjacent arterial sites. The effect of cholesterol feeding on plasma lipids, lipoproteins, and whole-body LDL catabolism was evaluated using linear regression. ANOVAs and regression analyses were done with BMDP statistical programs (BMDP Statistical Software, Inc., Los Angeles). When coefficients of variation of measured or calculated values were proportional to individual mean values, ANOVAs and $t$ tests were performed on the logarithms of data or logarithms of (data + 1) for measurements with small values (arterial esterified cholesterol concentration). A probability value of less than 0.05 was considered significant.

**Results**

**Labeled Low Density Lipoprotein**

As shown in Table 1, the methods used for labeling LDL resulted in significant incorporation of radiiodine into LDL lipid and proteins other than apo B. Exchanging the doubly labeled LDL against a source of HDL removed about 53% of the $^{125}$I associated with lipid and that comigrating with apo A-I and about 35% of the $^{125}$I-TC label associated with lipid and that comigrating with apo A-I. About 20% of the $^{125}$I and $^{125}$I-TC labels comigrating with apoproteins of less than 14 kd were also removed by the exchange. The label comigrating with an unidentified apoprotein of apparent molecular weight 45 kd and a protein of about 66 kd remained with the doubly labeled LDL.

In these pigeons as in others described previously, more than 90% of the injected LDL was cleared from plasma in 1 day. Despite prior exchange against HDL, some of the $^{131}$I label on the injected LDL was present on small apoproteins and lipids, which not only exchange between lipoprotein fractions but also are cleared from plasma more slowly than apo B. Thus, it was not surprising to find that other lipoproteins (principally the HDL fraction; Table 1) contained significant portions of the $^{131}$I label remaining in plasma 1 day after injection when the arterial samples were analyzed. Because albumin (an example of a small protein) equilibrates more rapidly with the artery than do lipoproteins and the concentration of albumin in the artery of normal animals has been estimated to be two to 10 times that of LDL, it seems likely that the presence of a significant portion of the protein-bound $^{131}$I radioactivity in the HDL-plasma protein fraction would result in significant overestimation of the arterial.
Low Density Lipoprotein Catabolism

Plasma Lipids and Lipoproteins and Whole-Body Low Density Lipoprotein Catabolism

Table 2. Plasma Lipids and Lipoproteins and Fractional Catabolic Rate of Low Density Lipoprotein*

<table>
<thead>
<tr>
<th>Duration of cholesterol feeding</th>
<th>Plasma</th>
<th>VLDL</th>
<th>LDL</th>
<th>HDL</th>
<th>Triglyceride</th>
<th>FCR of LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Control)</td>
<td>6.0±0.6</td>
<td>0.31±0.10</td>
<td>2.90±0.23</td>
<td>2.82±0.49</td>
<td>1.46±0.14</td>
<td>0.188±0.017</td>
</tr>
<tr>
<td>1 Week</td>
<td>15.1±0.7</td>
<td>3.28±1.01</td>
<td>7.01±0.28</td>
<td>4.78±0.62</td>
<td>1.71±0.34</td>
<td>0.149±0.016</td>
</tr>
<tr>
<td>2 Weeks</td>
<td>17.1±0.8</td>
<td>4.58±0.70</td>
<td>8.54±0.44</td>
<td>3.96±0.36</td>
<td>1.27±0.09</td>
<td>0.085±0.033</td>
</tr>
<tr>
<td>4 Weeks</td>
<td>27.3±3.5</td>
<td>11.3±5.1</td>
<td>11.6±1.8</td>
<td>4.32±0.47</td>
<td>1.38±0.78</td>
<td>0.095±0.010</td>
</tr>
<tr>
<td>8 Weeks</td>
<td>33.7±6.8</td>
<td>13.9±4.3</td>
<td>15.5±3.0</td>
<td>4.27±0.21</td>
<td>2.75±0.98</td>
<td>0.093±0.006</td>
</tr>
</tbody>
</table>

VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; FCR, fractional catabolic rate.
*Values are mean±SEM. Lipid and lipoprotein concentrations are presented in mM, whereas LDL FCRs are presented in pools per hour, n=3 birds at each time period except for the birds fed cholesterol for 2 weeks, for which n=2. To obtain cholesterol and triglyceride concentrations in milligrams per deciliter, multiply values in mM by 38.7 and 88.5, respectively. The concentration of cholesterol in plasma, VLDL, and LDL increased progressively with length of cholesterol feeding, p<0.005 (regression analyses). Neither HDL cholesterol nor plasma triglyceride concentrations were significantly affected by cholesterol feeding. FCR of LDL decreased during cholesterol feeding, p<0.02 (regression analysis).

Comparison of Atherosclerosis-Susceptible and Atherosclerosis-Resistant Arteries

Figure 2 shows 125I-TC accumulation by atherosclerosis-susceptible and atherosclerosis-resistant arterial sites in terms of equivalent volumes of plasma. In normal pigeons (0 weeks of cholesterol feeding), 125I-TC-LDL accumulation in the atherosclerosis-susceptible celiac site was about 80% greater per unit aortic surface area than in the adjacent atherosclerosis-resistant site of the same birds (0.215±0.005 versus 0.121±0.018 μl plasma/cm² aortic surface/day, mean±SEM, p<0.05; Figure 2A). 125I-TC-LDL accumulation also tended to be higher in the susceptible site when expressed per unit fixed weight (Figure 2B). Surprisingly, when expressed per unit surface area, 125I-TC-LDL accumulation in the proximal atherosclerosis-resistant artery (P₁ and P₂) of normal birds was 3.3 times that of the atherosclerosis-susceptible celiac site (S) of the same birds (p<0.005).

Regression analysis indicated a significant increasing trend for 125I-TC-LDL accumulation by the atherosclerosis-susceptible celiac site during cholesterol feeding both per unit surface area and per unit fixed weight (for each, p<0.01). As shown below (Table 4), this increase was due to the development of atherosclerotic lesions in the susceptible site. In contrast, 125I-TC-LDL accumulation (in plasma equivalents) by the adjacent atherosclerosis-resistant site (R) was not influenced by cholesterol feeding. Surprisingly, expressed as plasma equivalents, the 125I-TC-LDL accumulation by the two proximal atherosclerosis-resistant sites (P₁ and P₂) was decreased about 50% by cholesterol feeding (p<0.05 or better; Figure 2) with no difference among birds fed cholesterol for different times.

As indicated in Table 2, the cholesterol carried by the LDL in a unit volume of plasma increased about fivefold during cholesterol feeding. Figure 3 shows arterial 125I-TC-LDL accumulation expressed as amounts of LDL cholesterol for normal birds and those fed cholesterol. Per unit surface area (Figure 3A), 125I-TC-LDL accumulation by the atherosclerosis-susceptible celiac

content of undegraded LDL. In addition, about 23% of the 125I radioactivity remaining in plasma at the time that the arteries were analyzed was accounted for by labeled lipids. These labeled lipids could potentially exchange with arterial membrane lipids,44 causing the arterial 125I radioactivity to further overestimate the arterial concentration of undegraded LDL. Thus, we do not believe that it would be valid to estimate arterial concentrations of undegraded LDL from the arterial 125I data. In fact, for two of the 14 birds the estimate of undegraded LDL provided by the 125I label exceeded the estimate for undegraded LDL plus products of LDL degradation provided by the 125I-TC label.

At the end of the metabolic study when only 10% of the injected 125I-TC remained in the plasma, about 25% of the 125I-TC label was not present in LDL compared with about 45% for 125I. However, for the following reasons we believe that the arterial content of 125I-TC should reflect primarily undegraded LDL and products of arterial LDL degradation. First, the 125I-TC representing products of lipoprotein degradation remains trapped within lysosomes after cellular degradation. Second, relatively more of the arterial 125I-TC content would have entered the artery early after injection when the specific activity of LDL in plasma was high and relatively more of the 125I-TC label was present in the LDL fraction. Third, most of the 125I-TC label lost from LDL was transferred to VLDL, which at least in humans47 and rabbits,48,49 enters the artery less rapidly than LDL. Thus, whereas the arterial content of 125I-TC after fixation, denoted here as arterial 125I-TC-LDL accumulation, should reflect primarily undegraded LDL and products of LDL degradation, the portion that represents undegraded LDL and products of arterial LDL degradation must await further study.

Plasma Lipids and Lipoproteins and Whole-Body Low Density Lipoprotein Catabolism

Table 2 shows plasma lipids and lipoproteins just before euthanasia for the controls and the birds fed cholesterol for 1–8 weeks. The concentration of cholesterol in plasma and within the VLDL and LDL fractions increased progressively with the duration of cholesterol feeding (for each, p<0.005). The increase in the VLDL fraction reflects the appearance of cholesterol-enriched β-migrating VLDL.48 The HDL cholesterol concentration was unaffected by cholesterol feeding. Neither the concentration of triglyceride in plasma nor the distribution of triglyceride among lipoprotein fractions (not shown) was affected by cholesterol feeding. The whole-body FCR of LDL decreased with cholesterol feeding (p<0.02), reaching a plateau at about 50% of the normal value by 2 weeks of cholesterol feeding.

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per square centimeter in proximal (P1+P2) and distal (R), site S (data per square centimeter and per gram, p<0.01 for site Pi, p<0.001; for site P2, p<0.05; per gram for site P, rithms, Scheffe criterion). During cholesterol feeding 125 I-TC-LDL accumulation increased most markedly in the atherosclerosis-susceptible celiac site (per square centimeter, p<0.005) (t transformed to logarithms). Difference between proximal (P1+P2) and distal (R) atherosclerosis-resistant arteries of birds for 125 I-TC-LDL accumulation per square centimeter and per gram, p<0.01 and p<0.02, respectively (analysis of variance [ANOVA] on data transformed to logarithms). Difference between 125 I-TC-LDL accumulation per square centimeter in proximal (P1+P2) and distal (R), atherosclerosis-resistant arteries of birds fed cholesterol for 1 week and 4 weeks, p<0.02 for each (ANOVA on data transformed to logarithms). Difference between proximal atherosclerosis-resistant artery (P1+P2) and atherosclerosis-susceptible celiac site S of normal birds for 125 I-TC-LDL accumulation per square centimeter, p<0.005 (t test on data transformed to logarithms, Scheffé criterion). During cholesterol feeding 125 I-TC-LDL accumulation showed a significant increasing trend in site S (data per square centimeter and per gram, p<0.01 for each by regression analyses). Normal vs. all cholesterol-fed birds for 125 I-TC-LDL accumulation per square centimeter for site P1, p<0.001; for site P2, p<0.05; per gram for site P1, p<0.0001; for site P2, p<0.025 (ANOVA).

site of normal birds represented 0.24±0.02 µg LDL cholesterol/day compared with only 0.14±0.02 µg LDL cholesterol/day by the adjacent atherosclerosis-resistant site (R) (p<0.025). During cholesterol feeding, 125 I-TC-LDL accumulation most markedly in the atherosclerosis-susceptible celiac site (per square centimeter and per gram; for each, p<0.005). 125 I-TC-LDL accumulation also increased in the adjacent atherosclerosis-resistant site (R) (per square centimeter and per gram; for each, p<0.005). 125 I-TC-LDL accumulation in the two proximal atherosclerosis-resistant sites P1 and P2 also showed small increasing trends (p<0.05), but these rates of increase were much lower than the rate of increase of LDL cholesterol in plasma (Table 2).

Arterial Esterified and Nonesterified Cholesterol

The extent of atherosclerosis was assessed chemically by determining the arterial esterified and nonesterified cholesterol concentration. As shown in Figure 4A, the esterified cholesterol concentration of the atherosclerosis-susceptible celiac site increased during cholesterol feeding (p<0.005), with most of the increase occurring between weeks 2 and 8. The esterified cholesterol concentration of the adjacent atherosclerosis-resistant site (R) and the two proximal atherosclerosis-resistant arterial sites (P1 and P2) showed smaller increasing trends during cholesterol feeding (site R, p<0.02; site P1, p<0.001; site P2, p<0.05). Esterified cholesterol concentrations were elevated in all arterial sites by 4 weeks of cholesterol feeding but were increased most in the susceptible site. After 8 weeks of cholesterol feeding, the esterified cholesterol concentration of the atherosclerosis-susceptible celiac site was eight times that of the adjacent atherosclerosis-resistant site (R) and 27 times as great as the atherosclerosis-susceptible celiac site of untreated WC pigeons.

The nonesterified cholesterol concentration (Figure 4B) of the atherosclerosis-susceptible celiac site increased during cholesterol feeding (p<0.001) but did so more slowly than the esterified cholesterol concentration, only reaching twice the normal value by 8 weeks. In the adjacent atherosclerosis-resistant site (R) the nonesterified cholesterol concentration increased significantly (p<0.005) during cholesterol feeding but at a much lower rate than that in the susceptible celiac site (p<0.005).

To determine the extent to which any changes in arterial cholesterol concentration could be attributed to the presence of atherosclerotic lesions, macroscopic raised atherosclerotic lesions, when present, were dissected from within each arterial site and analyzed separately. Table 3 shows data for the concentration of esterified and nonesterified cholesterol of atherosclerotic lesions and the adjacent macroscopically normal parts of the atherosclerosis-susceptible celiac site and for the atherosclerosis-resistant site (R). A single small raised lesion was detected in the atherosclerosis-susceptible celiac site of one bird fed cholesterol for only 1 week. Single larger lesions were present in this arterial site in three of three and two of three birds studied after 4 and 8 weeks of cholesterol feeding, respectively. Lesions were not detected in atherosclerosis-resistant site R, although single small lesions were found in proximal atherosclerosis-resistant sites P1 and P2 of one of three birds studied at each of 4 and 8 weeks of cholesterol feeding.

When atherosclerotic lesions within the atherosclerosis-susceptible celiac site were compared with the adjacent macroscopically normal part of the same site without regard to the length of cholesterol feeding, the lesions were found to contain 13.4±4.5 mg esterified cholesterol/g fixed wt compared with 0.92±0.26 mg esterified cholesterol/g for areas without lesions.
FIGURE 3. Bar graphs of \(^{125}\text{I}-\)tyramine cellobiose-low density lipoprotein (TC-LDL) accumulation in arterial sites of White Carneau pigeons. \(^{125}\text{I}-\)TC-LDL accumulation expressed as amounts of LDL cholesterol are shown per unit of arterial surface area (panel A) and per gram fixed weight (panel B) per day. Numbers of birds and identity of bars are the same as in Figure 2. \(^{125}\text{I}-\)TC-LDL accumulation per square centimeter, site S vs. site R of normal birds, \(p<0.025\) (paired t test). Difference between proximal (P1+P2) and distal (R) atherosclerosis-resistant artery of normal birds for \(^{125}\text{I}-\)TC-LDL accumulation per square centimeter, per gram, \(p<0.02\); for birds fed cholesterol for 1 week, \(^{125}\text{I}-\)TC-LDL accumulation per square centimeter, \(p<0.02\); for birds fed cholesterol for 4 weeks, \(^{125}\text{I}-\)TC-LDL accumulation per square centimeter, \(p<0.05\); for sites P1 and P2 of birds fed cholesterol for 4 weeks, \(^{125}\text{I}-\)TC-LDL accumulation per square centimeter, \(p<0.05\) (all by analysis of variance on data transformed to logarithms). Difference between proximal atherosclerosis-resistant artery (P1+P2) and atherosclerosis-susceptible celiac site S of normal birds, \(p<0.005\) for \(^{125}\text{I}-\)TC-LDL accumulation per square centimeter (1 t test on data transformed to logarithms, Scheffé criterion). Increasing trends for \(^{125}\text{I}-\)TC-LDL accumulation during cholesterol feeding (all regression analyses) for site S, per square centimeter and per gram, \(p<0.005\); for site R per square centimeter and per gram, each \(p<0.005\); for site P1 per square centimeter, \(p<0.05\); for site P2 per square centimeter and per gram, each \(p<0.05\). Difference in rate of increase in \(^{125}\text{I}-\)TC-LDL accumulation during cholesterol feeding between site S and site R per square centimeter and per gram, each \(p<0.005\).

These lesions also contained more nonesterified cholesterol per gram of artery than did the macroscopically normal part of the same arterial site (7.83±0.46 versus 2.74±0.17 mg/g fixed wt, \(p<0.001\)). The macroscopically normal areas of the atherosclerosis-susceptible celiac site surrounding lesions contained more esterified cholesterol than did the adjacent atherosclerosis-resistant site (R) (0.92±0.26 versus 0.59±0.27 mg esterified cholesterol/g, \(p<0.01\)). Although not statistically significant, the esterified cholesterol concentration of the atherosclerosis-susceptible celiac site tended to be higher than that of the atherosclerosis-resistant site R even for birds without lesions. Nonesterified cholesterol contents of the macroscopically normal atherosclerosis-susceptible site S also tended to be higher than those of the resistant site R regardless of the presence of atherosclerotic lesions in the susceptible site, but the difference was only statistically significant for the birds without atherosclerotic lesions.

FIGURE 4. Bar graphs showing cholesterol concentrations in arterial sites of normal and cholesterol-fed White Carneau pigeons. Panel A: Esterified cholesterol. Panel B: Nonesterified cholesterol. Numbers of birds and identity of bars are the same as in Figures 2 and 3 except that \(n=2\) for site R at 1 week of cholesterol feeding (one sample lost). Influence of length of cholesterol feeding on esterified cholesterol concentration for site S, \(p<0.005\); for site R, \(p<0.02\); for site P1, \(p<0.001\); for site P2, \(p<0.05\); nonesterified cholesterol for site S, \(p<0.001\); for site R, \(p<0.005\); for site P1, \(p=0.05\). Trend for difference between site S and site R during cholesterol feeding for esterified and nonesterified cholesterol, each \(p<0.005\) (all regression analyses).

To consider whether any changes in the arterial \(^{125}\text{I}-\)TC-LDL accumulation during cholesterol feeding could be attributed to the development of atherosclerotic lesions, raised atherosclerotic lesions were separated from macroscopically normal parts of each arterial site (the same areas for which cholesterol concentrations are presented in Table 3) before radioassay. Data for \(^{125}\text{I}-\)TC-LDL accumulation are presented as equivalent volumes of plasma (Table 4) and as amounts of LDL cholesterol (Table 5). For the six birds with lesions...
### Table 3. Cholesterol Concentrations of Atherosclerotic Lesions and Macroscopically Normal* Distal Aorta

<table>
<thead>
<tr>
<th>Length of cholesterol feeding (weeks)</th>
<th>Atherosclerosis-susceptible celiac site (S)</th>
<th>Atherosclerosis-resistant site (R) (normal)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Esterified cholesterol</td>
<td>Nonesterified cholesterol</td>
</tr>
<tr>
<td>0</td>
<td>0.243±0.107</td>
<td>0.014±0.014</td>
</tr>
<tr>
<td>1</td>
<td>20.0</td>
<td>4.81±0.193</td>
</tr>
<tr>
<td></td>
<td>(1/3)</td>
<td>(3/3)</td>
</tr>
<tr>
<td>2</td>
<td>22.9±8.5</td>
<td>Δ</td>
</tr>
<tr>
<td>4</td>
<td>7.83±0.46</td>
<td>0.916±0.263</td>
</tr>
<tr>
<td></td>
<td>(6/14)</td>
<td>(6/14)</td>
</tr>
<tr>
<td>All without lesions†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All with lesions‡</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are in milligrams of cholesterol per gram fixed weight.

*"Normal," macroscopically normal portions of these arterial sites. For normal areas of each arterial site, values are mean±SEM for three birds at each of 0, 1, 4, and 8 weeks of cholesterol feeding and two birds at 2 weeks of cholesterol feeding. The number of birds from which atherosclerotic lesions (lesions) were identified and analyzed separately are indicated in parentheses.

†All birds without lesions in the atherosclerosis-susceptible site, regardless of the diet fed.

‡All birds with lesions in the atherosclerosis-susceptible site, irrespective of the length of cholesterol feeding.

§n=2, one sample lost.

\*p<0.025, \#p<0.005 vs. adjacent normal artery, paired t test on data transformed to logarithms.

**p<0.01, §§p<0.001 vs. adjacent atherosclerosis-resistant site R, paired t test.

Influence of length of cholesterol feeding (regression analyses) for normal part of the atherosclerosis-susceptible celiac site (site S): esterified cholesterol, \( p<0.001 \); nonesterified cholesterol, \( p<0.001 \); for the atherosclerosis-resistant site R: esterified cholesterol, \( p<0.02 \); nonesterified cholesterol, \( p<0.005 \); for the difference between trends in the normal part of the atherosclerosis-susceptible celiac site and atherosclerosis-resistant site R: nonesterified cholesterol, \( p<0.05 \).

Overall difference between macroscopically normal atherosclerosis-susceptible site S and atherosclerosis-resistant site R: esterified cholesterol, \( p<0.005 \) (analysis of variance on data transformed to logarithms); nonesterified cholesterol, \( p<0.005 \) (analysis of variance on data transformed to logarithms).

in the atherosclerosis-susceptible celiac site, \( ^{125}I-TC-LDL \) accumulation in excised lesions was equivalent to 3.1±1.1 μl plasma/cm²/day compared with only 0.309±0.090 μl plasma/cm²/day in the macroscopically normal part of the celiac site of the same birds (\( p<0.005 \)). Qualitatively similar results were obtained when \( ^{125}I-TC-LDL \) accumulation was expressed per unit fixed weight; \( ^{125}I-TC-LDL \) accumulation by the lesions in the atherosclerosis-susceptible celiac site represented 21±10 μg LDL cholesterol/cm² aortic surface compared with 1.6±0.5 μg LDL cholesterol/cm² for the adjacent macroscopically normal part of the same site and 0.66±0.17 μg LDL cholesterol/cm² for the adjacent atherosclerosis-resistant site R (\( p<0.005 \) and \( p<0.001 \), respectively; Table 5). Per unit weight, the results were qualitatively similar.

For birds without atherosclerotic lesions, \( ^{125}I-TC-LDL \) accumulation in the atherosclerosis-susceptible celiac site was 76% greater than in the adjacent atherosclerosis-resistant site R of the same birds when expressed per unit surface area (0.183±0.023 versus 0.104±0.015 μl plasma/cm²/day, \( p<0.005 \), \( n=8 \)) and 38% greater when expressed per unit fixed weight (7.6±0.8 versus 5.5±0.8 μl plasma/g/day, \( p<0.02 \); Table 4). The percentage increases in these amounts of \( ^{125}I-TC-LDL \) accumulation in the atherosclerosis-suscepti-
TABLE 4. $^{125}$I-Tyramine Cellobiose-Low Density Lipoprotein Accumulation in Atherosclerotic and Macroscopically Normal Distal Aorta Expressed as Plasma Equivalents*

<table>
<thead>
<tr>
<th>Length of cholesterol feeding (weeks)</th>
<th>Atherosclerosis-susceptible celiac site (S)</th>
<th>Atherosclerosis-resistant site (R) (normal)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lesion</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>Microliters plasma/cm² surface area/day</td>
<td>Microliters plasma/g fixed wt/day</td>
</tr>
<tr>
<td>0</td>
<td>...</td>
<td>0.215±0.005§</td>
</tr>
<tr>
<td></td>
<td>1.77 (1/3)</td>
<td>0.194±0.102</td>
</tr>
<tr>
<td>2</td>
<td>...</td>
<td>0.246±0.013</td>
</tr>
<tr>
<td>4</td>
<td>2.08±0.53¶</td>
<td>0.183±0.047#</td>
</tr>
<tr>
<td></td>
<td>(3/3)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>5.9±2.8</td>
<td>0.350±0.180</td>
</tr>
<tr>
<td>All without lesions†</td>
<td>...</td>
<td>0.183±0.023¶</td>
</tr>
<tr>
<td></td>
<td>(8/14)</td>
<td>(8/14)</td>
</tr>
<tr>
<td>All with lesions‡</td>
<td>3.1±1.1**††</td>
<td>0.309±0.090¶</td>
</tr>
<tr>
<td></td>
<td>(6/14)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>...</td>
<td>8.7±0.8</td>
</tr>
<tr>
<td></td>
<td>56.4 (1/3)</td>
<td>7.2±2.8</td>
</tr>
<tr>
<td>2</td>
<td>...</td>
<td>9.8±0.02</td>
</tr>
<tr>
<td>4</td>
<td>57.7±21.8</td>
<td>7.2±1.6‡‡</td>
</tr>
<tr>
<td></td>
<td>(3/3)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>135±51</td>
<td>13.7±6.5</td>
</tr>
<tr>
<td>All without lesions†</td>
<td>...</td>
<td>7.6±0.8§§</td>
</tr>
<tr>
<td></td>
<td>(8/14)</td>
<td>(8/14)</td>
</tr>
<tr>
<td>All with lesions‡</td>
<td>83.2±23.2**††</td>
<td>11.7±3.2‡‡</td>
</tr>
<tr>
<td></td>
<td>(6/14)</td>
<td></td>
</tr>
</tbody>
</table>

*††As in Table 3.
§p<0.05, §§p<0.02 compared with atherosclerosis-resistant site R, paired t test.
¶p<0.05, **p<0.005 compared with normal area of the atherosclerosis-susceptible celiac site, paired t test on log-transformed data.
##p<0.005, ††p<0.001, †††p<0.005 compared with atherosclerosis-resistant site R, paired t test on log-transformed data.

Overall difference between macroscopically normal atherosclerosis-susceptible site S and atherosclerosis-resistant site R, microliters per square centimeter, $p<0.02$; microliters per gram, $p<0.025$ (analysis of variance).

Able celiac site compared with the atherosclerosis-resistant site R for these birds without lesions were identical to the percent enhancement of $^{125}$I-TC-LDL accumulation in the atherosclerosis-susceptible celiac site of birds not fed cholesterol. The $^{125}$I-TC-LDL accumulation in plasma equivalents either per unit surface area or per unit fixed weight was not influenced by the length of cholesterol feeding in the macroscopically normal part of the atherosclerosis-susceptible celiac site or atherosclerosis-resistant site R (Table 4). However, these amounts of $^{125}$I-TC-LDL accumulation represented progressively larger amounts of LDL cholesterol during cholesterol feeding (Table 5) due to the increase in concentration of LDL cholesterol per unit volume of plasma.

$^{125}$I-Tyramine Cellobiose-Low Density Lipoprotein Accumulation by Inner and Outer Layers of Proximal Atherosclerosis-Resistant Artery

A previous study of rabbits has suggested that after a 1-day metabolic study, the intima of the thoracic aorta accounted for a disproportionately large fraction of arterial LDL degradation. To investigate whether $^{125}$I-TC-LDL accumulation might be greater in the inner part (intima plus some media) of the arteries of WC pigeons, the macroscopically normal part of the proximal atherosclerosis-resistant artery (P1 and P2) was separated into inner and outer layers. Figure 5 shows the $^{125}$I-TC-LDL accumulation (plasma equivalents, Figure 5A; amounts of LDL cholesterol, Figure 5B) for inner and outer layers of macroscopically normal parts of the two proximal atherosclerosis-resistant arterial sites (P1 and P2). Data for inner and outer layers do not include the tiny atherosclerotic lesions found in each of these sites for one bird at 4 and 8 weeks of cholesterol feeding. The data shown above (Figures 2–4) for sites P1 and P2 did include these tiny lesions. For untreated birds, $^{125}$I-TC-LDL accumulation was 146% and 118% greater for inner arterial layers of sites P1 and P2 than for the outer layers of these sites (19.4±2.8 versus 7.9±0.9 and 22.6±4.7 versus 10.4±1.4 μl plasma/g/day, respectively, each $p<0.025$). $^{125}$I-TC-LDL accumulation expressed as equivalent volumes of plasma was decreased 69% and 65% in inner layers of sites P1 and P2 of cholesterol-fed birds compared with birds not fed...
TABLE 5. **I-Tyramine Cellobiose-Low Density Lipoprotein Accumulation in Atherosclerotic and Macroscopically Normal Distal Aorta Expressed as Amounts of Low Density Lipoprotein Cholesterol**

<table>
<thead>
<tr>
<th>Length of cholesterol feeding (weeks)</th>
<th>Atherosclerosis-susceptible celiac site (S)</th>
<th>Atherosclerosis-resistant site (R) (normal)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lesion</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>Micrograms LDL cholesterol/cm² surface area/day</td>
<td>Micrograms LDL cholesterol/g fixed wt/day</td>
</tr>
<tr>
<td>0</td>
<td>...</td>
<td>0.24±0.02§</td>
</tr>
<tr>
<td>1</td>
<td>4.9</td>
<td>0.53±0.28</td>
</tr>
<tr>
<td>2</td>
<td>...</td>
<td>0.81±0.003</td>
</tr>
<tr>
<td>4</td>
<td>9.7±3.0%††</td>
<td>0.84±0.31††</td>
</tr>
<tr>
<td>8</td>
<td>45±26</td>
<td>2.1±1.1</td>
</tr>
<tr>
<td>All without lesions†</td>
<td>...</td>
<td>0.43±0.09#</td>
</tr>
<tr>
<td>All with lesions‡</td>
<td>21±10**†††</td>
<td>1.6±0.5§††</td>
</tr>
<tr>
<td></td>
<td>(6/14)</td>
<td>(6/14)</td>
</tr>
</tbody>
</table>

**I-DL, low density lipoprotein.**

**II As in Table 3.**

§p<0.025, §§p<0.05 compared with atherosclerosis-resistant site R, paired t test.

†p<0.05, **p<0.005 compared with normal area of the atherosclerosis-susceptible celiac site, paired t test on data transformed to logarithms.

†‡p<0.005, ††‡p<0.001, †††p<0.05 compared with atherosclerosis-resistant site R, paired t test on data transformed to logarithms.

Time trends for **I-Tyramine cellobiose-LDL accumulation during cholesterol feeding (all regression analyses): for normal part of the atherosclerosis-susceptible celiac site S, data per square centimeter, p<0.02; per gram, p<0.01; for atherosclerosis-resistant site R, data per square centimeter and per gram, each p<0.005; difference between normal part of the atherosclerosis-susceptible celiac site and atherosclerosis-resistant site R, per square centimeter and per gram, each p<0.05.

Overall difference between normal part of the atherosclerosis-susceptible site S and atherosclerosis-resistant site R, micrograms per square centimeter, p<0.0005; micrograms per gram, p<0.005 (analysis of variance on logarithms of data).

Discussion

In this study we asked whether the arterial content of **I-TC 1 day after injecting **I-TC-LDL (**I-TC-LDL accumulation) would be greater in the atherosclerosis-susceptible celiac site of WC pigeons before development of atherosclerosis and whether arterial **I-TC-LDL accumulation would be exaggerated by cholesterol feeding. For birds not fed cholesterol, the **I-TC-LDL accumulation per unit arterial surface area was greater in the atherosclerosis-susceptible celiac site compared with the adjacent atherosclerosis-resistant site (R). If we assume that all of the arterial content of **I-TC reflects products of arterial LDL degradation, the rate of LDL degradation in the celiac site of these normal pigeons (calculated as described in References 14 and 15, except using the total arterial **I-TC content after fixation) would be 1.21±0.08×10⁻⁵ of the plasma LDL pool/cm²/day, about three times the value that Schwenke and Carew 14,15 have reported for the corresponding atherosclerosis-susceptible site of rabbit aorta (branch orifice regions of the abdominal aorta, 0.37±0.07 and 0.47±0.11, each ×10⁻⁵ of the plasma LDL pool/cm²/day, from References 14 and 15, respectively). However, because the rate of whole-body catabolism of LDL in normal pigeons (4.5±0.4 pools/day, this study; 4.9±0.2 pools/day, Reference 39) is about three times that reported for normal rabbits (1.6 pools/day; References...
14 and 15), this suggests that arteries of normal rabbits and normal WC pigeons each account for the same fraction of whole-body LDL metabolism. Our value of $^{125}$I-TC-LDL accumulation (per unit surface area) was about 80% greater for the atherosclerosis-susceptible celiac site of normal WC pigeons compared with the adjacent atherosclerosis-resistant site R, in good agreement with the 60% greater rate of LDL degradation per unit surface area at branch orifices of abdominal aortas of rabbits compared with the adjacent atherosclerosis-resistant aorta.14

In normal pigeons $^{125}$I-TC-LDL accumulation in the arterial sites closest to the heart (sites P1 and P2) was two to three times as great as that in the susceptible celiac site of the distal aorta. In comparison, the aortic arch of normal rabbits degraded LDL at a rate twice that of the abdominal branch orifice regions.14 The difference, however, is that the aortic arch of rabbits is highly susceptible to atherosclerosis,11 whereas these proximal sites in the pigeon artery are relatively resistant to atherosclerosis (References 16–19; Figure 4). A possible explanation for this apparent discrepancy may be that there is a difference in the part of the arterial $^{125}$I-TC content that reflects undegraded LDL and that represents products of arterial LDL degradation at the proximal sites compared with the susceptible site. Alternatively, changes in arterial rates of LDL degradation or concentrations of undegraded LDL may occur during cholesterol feeding that protect the proximal arterial sites from the development of atherosclerosis.

During the first 16 days of cholesterol feeding when arteries remained macroscopically normal, fractional rates of LDL degradation decreased in all arterial sites in the rabbit except the highly atherosclerosis-susceptible abdominal branch orifices. However, the decreases in fractional rates of arterial LDL degradation were similar to or less than the decrease in the whole-body FCR of LDL.15 In the pigeon, $^{125}$I-TC-LDL accumulation expressed in terms of volumes of plasma did not change significantly during cholesterol feeding in either the macroscopically normal atherosclerosis-susceptible celiac site or the adjacent atherosclerosis-resistant site (R) and increased in the celiac site as a whole because of the development of atherosclerotic lesions, even though the whole-body FCR of LDL was suppressed by cholesterol feeding. However, by 1 week of cholesterol feeding, $^{125}$I-TC-LDL accumulation (microliters plasma per gram or square centimeter per day) in the two proximal atherosclerosis-resistant sites (P1 and P2) was decreased by 50%, 2.5 times the decrease in the whole-body FCR of LDL at that time. After feeding rabbits cholesterol for 16 days, the fractional rate of LDL degradation (per unit weight) in the atherosclerosis-susceptible aortic arch, although decreased compared with that in untreated rabbits, was 2.5 times as great as that in the adjacent atherosclerosis-resistant descending thoracic aorta and 1.5 times as great as that in the atherosclerosis-susceptible abdominal branch orifices.15 In contrast, after feeding WC pigeons cholesterol for 1 week, $^{125}$I-TC-LDL accumulation (per unit fixed weight) (Figure 2B) in the proximal arterial sites (P1 and P2) had decreased so that they were no greater than in the atherosclerosis-susceptible celiac site. Furthermore, by 4 weeks of cholesterol feeding, $^{125}$I-TC-LDL accumulation in the atherosclerosis-susceptible celiac site of pigeons was about twice that of the other arterial sites that we studied (see Figure 2B). Thus, it seems that inherent differences in $^{125}$I-TC-LDL accumulation, reflecting the combination of arterial rates of LDL degradation and the arterial concentration of undegraded LDL, among arterial sites within normal WC pigeons may play a role in regional variation in susceptibility to atherosclerosis just as rates of LDL degradation and concentrations of undegraded LDL appear to do in normal rabbits. The ability of arterial sites to regulate metabolism of LDL (i.e., decrease fractional rates of LDL degradation) may play a more significant role in determining regional susceptibility to atherosclerosis in WC pigeons than in rabbits.

Separate analysis of atherosclerotic lesions and macroscopically normal artery allowed us to consider whether the marked increase in $^{125}$I-TC-LDL accumulation at the atherosclerosis-susceptible celiac site during cholesterol feeding (Figures 2 and 3) could be
completely attributed to the presence of macroscopically evident atherosclerotic lesions. $^{125}$I-TC-LDL accumulation in atherosclerotic lesions was much greater than in the macroscopically normal part of the same arterial site. Furthermore, the greater $^{125}$I-TC-LDL accumulation in the atherosclerotic lesions contributed substantially to the greater $^{125}$I-TC-LDL accumulation during cholesterol feeding in the atherosclerosis-susceptible celiac site as a whole. Nonetheless, after 8 weeks of cholesterol feeding, $^{125}$I-TC-LDL accumulation expressed as amounts of LDL cholesterol increased about eightfold and fivefold in the macroscopically normal areas of the atherosclerosis-susceptible celiac site and the adjacent atherosclerosis-resistant site R, respectively. This compares with as much as a 170-fold increase in atherosclerotic lesions themselves. However, it should be pointed out that the increase in the LDL cholesterol represented by the $^{125}$I-TC-LDL accumulation in the atherosclerosis-resistant site during cholesterol feeding was completely accounted for by the increase in the LDL cholesterol concentration in the plasma, as $^{125}$I-TC-LDL accumulation in this site was not altered by cholesterol feeding when expressed as volumes of plasma equivalents decreased more in the inner layers than in the outer layers. Because amounts of LDL cholesterol must subsequently be lost because of efflux processes.

It was of interest to consider how the cumulative delivery of cholesterol to arterial cells via estimated cellular degradation of LDL, calculated as described in References 14 and 15 but using the total arterial $^{125}$I-TC-LDL content, would compare with the observed increase in arterial cholesterol concentration during cholesterol feeding. We first estimated the cumulative degradation of LDL by the four arterial sites in normal animals during their average lifetimes of 7.5 months, assuming that arterial rates of LDL degradation were constant over that interval at the values calculated from the total arterial $^{125}$I-TC-LDL content (S, 11.3; R, 8.0; P1, 16.7; P2, 18.8; all in micrograms LDL cholesterol per gram per day). For the atherosclerosis-susceptible celiac site and atherosclerosis-resistant site R, the estimated cumulative degradation of LDL accounted for 112% and 89% of the observed arterial cholesterol concentration, respectively. For the two proximal atherosclerosis-resistant sites (P1 and P2), the estimated cumulative degradation of LDL accounted for 229% (P1) and 263% (P2) of the observed arterial cholesterol concentration. These calculations suggest that cellular uptake and metabolism of LDL alone may be sufficient to supply the cholesterol content of arterial sites in normal WC pigeons. Furthermore, these calculations suggest that significant efflux of cholesterol may occur from the proximal arterial sites (P1 and P2) of normal WC pigeons. This could suggest an important independent role for cholesterol efflux in mediating susceptibility and resistance to atherosclerosis of specific arterial sites.

Assuming that the rate of LDL degradation increased in a linear fashion between the rates estimated for normal birds and those estimated in the same way for each time of cholesterol feeding, it was possible to compute estimated cumulative degradation of LDL by the arterial sites during each interval of cholesterol feeding. By subtracting the cumulative rate of LDL degradation estimated to have occurred over these same intervals if the birds had not been fed cholesterol, it was possible to estimate the incremental cumulative LDL degradation associated with cholesterol feeding and compare this increment with the observed increment in arterial cholesterol concentration (Figure 4 and Table 3). The estimated cumulative incremental LDL degradation could account for 47% and 82% of the increment in cholesterol concentration of atherosclerotic lesions found at the susceptible celiac site after 2 and 8 weeks of cholesterol feeding, respectively. For the macroscopically lesion-free part of the susceptible celiac site, the estimated cumulative incremental LDL degradation could account for 55% and 89% of the observed increase in cholesterol concentration after 2 and 8 weeks of cholesterol feeding, respectively. The estimated cumulative incremental LDL degradation accounted for 85±6% (mean±SEM, n=6) of the cholesterol increment observed in the three atherosclerosis-resistant arterial sites (R, P1, and P2) after 4–8 weeks of cholesterol feeding. On the other hand, the estimated incremental cumulative LDL degradation could account for only 2.2% of the incremental cholesterol concentration of the single small lesion found in the atherosclerosis-susceptible celiac site of one of the birds fed cholesterol for 1 week, suggesting that that lesion had begun developing spontaneously before the onset of cholesterol feeding. These comparisons suggest that cumulative LDL degradation may be nearly sufficient to provide the increase in arterial cholesterol concentration observed during cholesterol feeding. It is likely, however, that other lipoproteins such as β-VLDL also contribute cholesterol to the arterial wall. If this is added to the amount estimated to be delivered by LDL, then it would seem that some of this lipoprotein-derived cholesterol must subsequently be lost because of efflux processes.

In this study we have reported values for arterial $^{125}$I-TC-LDL accumulation, which we assume primarily reflects products of arterial LDL degradation, both per unit surface area and per unit weight. This is so because although all cells within an arterial site potentially degrade LDL (thus the reason for expressing $^{125}$I-TC-LDL accumulation per unit weight), data presented by Carew et al would suggest that after a 24-hour experiment, the intima of the thoracic aorta of rabbits accounted for a disproportionately large fraction of the arterial LDL degradation (providing a rationale for expressing the arterial $^{125}$I-TC-LDL accumulation per unit surface area). Consistent with those reports, we found that in WC pigeons not fed cholesterol, $^{125}$I-TC-LDL accumulation in the inner layers of the two proximal arterial sites (P1 and P2) was more than twice as great (per unit weight) as that in the corresponding outer layers. The difference between the inner and outer layers was abolished by 1 week of cholesterol feeding when the $^{125}$I-TC-LDL accumulation expressed as plasma equivalents decreased more in the inner layers than in the outer layers. Because amounts of LDL cholesterol represented by the $^{125}$I-TC-LDL accumulation were influenced relatively little during cholesterol feeding in these atherosclerosis-resistant sites, it is
tempting to speculate that the decrease in $^{125}$I-TC-LDL accumulation when expressed as amounts of plasma reflects saturation of specific processes for degradation of LDL or its binding to the extracellular matrix at these arterial sites.

In the course of these studies, we calculated the whole-body FCR of LDL and determined that it decreased to a plateau value that was 50% of normal by 2 weeks of cholesterol feeding. This would appear to contradict an earlier result obtained in this laboratory that cholesterol feeding did not downregulate clearance of LDL in WC pigeons. However, another study showed that whereas cholesterol feeding had almost no influence on clearance of LDL isolated from normocholesterolemic donor pigeons, clearance of LDL isolated from hypercholesterolemic donor pigeons was decreased 50% in cholesterol-fed recipient pigeons, in excellent agreement with the results of this study.

In summary, we found differences in $^{125}$I-TC-LDL accumulation among arterial sites of normal and cholesterol-fed WC pigeons. The $^{125}$I-TC-LDL accumulation was greater in the atherosclerosis-susceptible celiac site than in the adjacent atherosclerosis-resistant site before the development of atherosclerosis. However, differences in $^{125}$I-TC-LDL accumulation among arterial sites of normal pigeons did not always predict susceptibility to atherosclerosis. In normal pigeons two relatively atherosclerosis-resistant arterial sites close to the heart accumulated the most $^{125}$I-TC-LDL. However, these arterial sites showed a striking ability to regulate $^{125}$I-TC-LDL accumulation to levels that represented nearly constant amounts of LDL cholesterol despite marked increases in plasma LDL cholesterol during cholesterol feeding. This was in contrast to the macroscopically normal atherosclerosis-susceptible celiac site and the adjacent atherosclerosis-resistant site, where the increase in $^{125}$I-TC-LDL accumulation paralleled the increase in plasma LDL cholesterol during cholesterol feeding, and atherosclerotic lesions in the susceptible site, where $^{125}$I-TC-LDL accumulation increased much more than the increase in plasma LDL cholesterol.

Thus, arterial sites resistant to development of atherosclerosis in WC pigeons differ from the susceptible site by either accumulating less $^{125}$I-TC-LDL or having the capacity to limit increases in $^{125}$I-TC-LDL accumulation during exposure to hypercholesterolemia. These observations provide a potential mechanistic explanation for the preferential development of atherosclerosis in WC pigeons.

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