Metabolic evidence for sequestration of low-density lipoprotein in abdominal aorta of normal rabbits

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Schwenke, Dawn C. Metabolic evidence for sequestration of low-density lipoprotein in abdominal aorta of normal rabbits. Am J Physiol Heart Circ Physiol 279: H1128–H1140, 2000.—In rabbits, atherosclerosis develops preferentially at branch sites compared with the adjacent uniform aorta. This study investigated the hypothesis that low-density lipoprotein (LDL) is “sequestered” (present in a form that exchanges slowly with plasma LDL) in the aortas of normal rabbits and that more LDL is sequestered at branch sites. Thus, 35 normal rabbits were injected with LDL labeled with 125I-labeled tyramine cellulose (125I-TC) to trace both undegraded LDL and aortic LDL degradation products. For 25 rabbits, LDL was also labeled with 131I to trace undegraded LDL alone. The time-dependent aortic 125I-TC and 131I accumulation was determined from 0.6 to 120 h after injection. Compartmental modeling provided metabolic evidence for sequestration of LDL at the branch (P < 0.01) and uniform (P < 0.005) abdominal aorta. Concentrations of sequestered LDL were 109 ± 28% higher (P < 0.0005) for branch sites. LDL mean residence time was 23.5 ± 3.1 h for branch sites, 7.6 ± 3.5 h longer (P < 0.05) than for the uniform abdominal aorta. Enhanced retention of higher concentrations of sequestered LDL at branch sites could account for the increased susceptibility of these aortic sites to atherosclerosis.

MATERIALS AND METHODS
Rabbits. These studies used sexually mature, young female New Zealand White rabbits from Robinson Services...
injection. To determine the sum of aortic undegraded LDL with 125I-TC, 131I-LDL (5.2 ± 0.71–0.55 pmol/mg protein) with cyanuric chloride (7, 28, 32, 33). For two experiments, LDL (10.8–11.2 mg protein) was coupled to 125I-TC only (125I-TC-LDL; 7.1–7.4 nmol TC and 0.89–0.92 mCi/mg protein) (7, 28, 32, 33). Labeled LDLs were dialyzed for 35–43 h with five to six changes of 1,000–2,000 volumes of buffer A (32, 33, 36, 37). Labeled LDLs were sterilized by filtration (35) before injection to 5 to 6 days after isolation of LDL. Specific activities were 648 ± 138 (n = 5) cpm/ng for 125I and 126 ± 28 (n = 3) cpm/ng for 131I. Polycrylamide gel electrophoresis (22, 39, 40) of delipidated (13) LDL showed only 2.42 ± 0.1% (n = 5) and 2.44 ± 0.12% (n = 3) of 125I-TC and 131I labels to be associated with apoprotein E, as in earlier studies with LDL isolated from normal rabbits (32, 33, 35–37). Agarose gel electrophoresis (25) was performed on a few LDL preparations. For these and other similar preparations (32, 33, 35–37), 95% of 125I-TC and 131I comigrated with unlabeled LDL. Only 1.4 ± 0.2% (n = 5) and 2.1 ± 0.6% (n = 3) of 125I-TC and 131I, respectively, were soluble in 10% trichloroacetic acid (TCA) (39, 40), whereas 6.9 ± 1.1% (n = 5) and 3.5 ± 0.5% (n = 3) of these labels could be extracted with chloroform/methanol (13). Iodination, use, and disposal of labeled lipoproteins followed procedures recommended by the Wake Forest University School of Medicine Office of Environmental Health and Safety.

Metabolic studies. The time-dependent aortic accumulation of undegraded LDL and aortic LDL degradation products was determined up to 120 h after injection of radiolabeled LDL in 33 normal rabbits. Twenty-five rabbits were injected with 125I-TC, 131I-LDL (5.2 ± 0.5–0.1 × 10^8 125I cpm/kg and 12 ± 0.1 × 10^8 131I cpm/kg). Blood samples were collected after 5 min and at increasing intervals after injection (33, 35, 36) until perfusion (below) of 1,000 IU heparin (32, 33, 35), euthanized with pentobarbital sodium (100 mg/kg body wt), and perfused with 1 liter of 0.15 M sodium phosphate buffer, pH 7.3 (33, 37). The arterial system was fixed in situ by perfusing with half-strength Karnovsky’s fixative for 10 min (32, 33, 37). All procedures with animals were approved by the Wake Forest University School of Medicine Animal Care and Use Committee.

Aortic sampling. After fixation in situ, the aorta was removed (32, 33). Fixation was continued overnight in half-strength Karnovsky’s fixative (35, 36) to preserve the TC label present on the undegraded LDL and aortic LDL degradation products (7). The thoracic and abdominal aortas were separated (31). After adventitial tissue was removed from the abdominal aorta, this aortic segment was opened longitudinally, pinned flat, and photographed. Samples of the abdominal aorta distal to orifices of the celiac, superior mesenteric, and right and left renal arteries (branch sites) were collected (31). The remaining atherosclerosis-resistant uniform abdominal aorta were photographed again. Fixed aortic samples were weighed and counted for radioactivity.

Lipids and lipoproteins. Blood samples collected just before euthanization were immediately mixed with disodium EDTA (final concentration 2.7 mmol/l). Very-low-density lipoprotein plus intermediate-density lipoprotein, LDL, and high-density lipoprotein were isolated from these plasma samples at d < 1.020, 1.020 < d < 1.060, and d > 1.060 g/ml (16, 33). Plasma lipoproteins were also separated by electrophoresis (25, 36, 37). Plasma cholesterol concentrations were determined (23) in the Centers for Disease Control-standardized lipid analytical laboratory of the Wake Forest University School of Medicine. LDL cholesterol concentrations were determined as described (36).

Radioassay. Total and TCA-soluble 125I and 131I radioactivity in the aortic samples, plasma, and lipoprotein fractions were determined in a well-type gamma counter with a 3-inch crystal (Cobra II autogamma, Packard) as described (32, 33).

Total body LDL fractional catabolic rate. The total body fractional catabolic rate (FCR) of LDL was calculated for each rabbit euthanized ≥6 h after injection as described (33, 35, 36). The aggregate FCR for LDL for all 25 rabbits injected with 125I-TC, 131I-LDL was determined similarly from combined plasma data for these rabbits.

LDL transport into intima media. Intima-media permeability to LDL (µl plasma·h⁻¹·g aorta⁻¹) was calculated as described (32, 33, 37) from aortic data from rabbits perfused 0.66 ± 0.03 or 1.26 ± 0.01 h after injection of 125I-TC-LDL. Intima-media permeability to LDL was also calculated by multiplying the fractional rate constant for transfer of LDL from plasma into the aorta (determined from fits of the models to aortic data, below) by the mean plasma volume of rabbits in this study. The mean plasma volume of the rabbits (108.5 ± 2.4 ml, 42.2 ± 0.8 ml/kg, n = 31) was determined by dilution of the injected doses.

Determination of rate constants for transport of LDL into and out of aorta and metabolism of LDL by aorta. Fractional rate constants defining the aortic transport and metabolism of LDL were determined by fitting compartmental models to aortic data. These analyses were done with pooled aortic data from all 33 rabbits using the simulation, analysis, and modeling program (SAAM 3.1.0) (5). For rabbits given LDL labeled with 131I and 125I-TC, aortic data used in these analyses were data for the time-dependent aortic accumulation of undegraded LDL (traced by protein-bound 131I) and the time-

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dependent aortic accumulation of products of aortic LDL degradation (determined by subtracting protein-bound aortic 131I from aortic total 125I-TC, each expressed in terms of a fraction of the injected doses) (8, 33, 35, 36). For rabbits given LDL labeled only with 125I-TC, total aortic 125I-TC, representing the sum of aortic undegraded LDL and aortic LDL degradation products, was used in this analysis. Fractional rate constants defining the transfer of LDL into and out of the aorta and between aortic compartments and the metabolism of LDL within the aorta were assumed identical for all rabbits. However, because data for the time-dependent specific activity of radiolabeled LDL in plasma of individual rabbits were collected, and because there were small differences among rabbits, 125I-TC, 131I-LDL (or 125I-TC-LDL) was considered to enter the aortas of the rabbits at the time-dependent specific activity of 125I-TC, 131I-LDL (or 125I-TC-LDL) in plasma of those individual rabbits. In these analyses, aortic data were weighted by the inverse of the standard deviation for aortic data for replicate rabbits studied at the same time after injection. For this purpose, aortic data from the single rabbit studied 30 h after injection were assumed to have standard deviations equal to the average for corresponding aortic data of rabbits studied 6–48 h after injection.

The compartmental models that were used were designed to obtain kinetic evidence that would either support or refute the hypothesis that some LDL within aorta is sequestered (present in a form that does not readily exchange with plasma LDL). Aortic data were first fitted to a model (Fig. 1) in which aortic LDL was considered to be homogeneous. In this model, LDL was assumed to enter the aortic LDL pool of undegraded LDL (pool 1, model 1A, Fig. 1, left) from plasma at a constant fractional rate (k15), where kij represents the fractional rate constant for transfer into compartment i from compartment j (kij) are shown.

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A model with LDL present in both metabolically active (freely exchanging with plasma LDL and undergoing degradation by aortic cells, pool 1, “metabolically active pathway”) and sequestered (pool 2, “sequestered pathway”) forms (Fig. 1, middle) was considered next. Originally, all possible pathways for the entrance and exit of LDL and the transfer of LDL between metabolically active and sequestered compartments were considered. By sequentially eliminating different pathways and combinations of pathways and testing (see below) the ability of these simpler models to fit aortic data, I determined the minimum number of pathways needed to fit aortic data.

Evidence suggests that 125I-TC degradation products leak out of cells (28) and aortas in vivo (37). Thus all models included a pathway (arrow exiting pool 4, Deg Prod, Fig. 1) representing this leakage. For the results described below, the fractional rate constant for leakage of 125I-TC degradation products from the aorta ($k_{04}$) was fixed at 0.005 h$^{-1}$, a conservative value consistent with earlier studies (28, 37). This was done for two reasons. First, values for this fractional rate constant estimated from the data for the time dependence of the aortic accumulation of LDL degradation products for both the abdominal branch sites and uniform abdominal aorta (0.0067 ± 0.0017 and 0.0043 ± 0.0013 h$^{-1}$, respectively) did not differ significantly from 0.005 h$^{-1}$. Second, estimating this fractional rate constant from the present aortic data did not improve the ability to fit the aortic data (not shown).

The two-pool model including one pool of sequestered LDL did not adequately fit the aortic data, and the nature of the deviation appeared consistent with the presence of LDL in more than two forms. Thus another model including metabolically active LDL (pool 1) and two forms of sequestered LDL (pools 2 and 3) was considered (Fig. 1, right). Because the data limited the number of fractional rate constants that could be defined, this final model did not include fractional rate constants for transfer among the three forms of aortic undegraded LDL.

The terminal slope of the time dependence of aortic undegraded LDL should reflect the rate of loss from the aortic pool from which LDL is most slowly removed. Thus both the two-pool and three-pool models were also fitted with the fractional rate constant for loss from pool 2 (sequestered LDL) ($k_{02}$) fixed at the values for the terminal slopes (means ± SE) of the time dependence of aortic undegraded LDL. For each of the branch sites and the uniform abdominal aorta, the terminal slopes were determined by fitting monoexponential equations to aortic undegraded LDL from 15.85 to 120 h after injection. For the two-pool models, allowing $k_{02}$ to adjust to best fit the data enhanced the fit of the model to the aortic data for undegraded LDL. In comparison, for the three-pool models, allowing $k_{02}$ to adjust did not enhance the ability of the model to fit the aortic data. However, fixing $k_{02}$ at the value of the terminal slope, determined as described above, reduced the mean correlation among fractional rate constants from 0.66 to 0.13 and allowed a more precise estimation of the other rate constants (data not shown).

**Undegraded LDL concentrations.** Aortic undegraded LDL concentrations were calculated from fractional rate constants determined by the fitting of kinetic models to the aortic data (5). These calculations also used data for the plasma volume (above) and mean plasma LDL cholesterol concentrations (below) for these rabbits. Concentrations of aortic undegraded LDL were expressed both in terms of absolute amounts of LDL cholesterol and as percentages of the plasma LDL cholesterol concentration (33, 35, 36).

**LDL mean residence time within aortas.** LDL mean residence times within the aortas were calculated for the kinetic models using the fractional rate constants determined by the fitting of these models to aortic data (5, 37). LDL mean residence times were also determined by an independent method, stochastic analysis (41). This method makes no assumptions about the metabolic or transport processes that LDL undergoes within the aorta. The only assumption required to calculate LDL mean residence times by this method is that LDL enters the aorta at a constant rate at the mean specific activity of plasma LDL (41). LDL mean residence times determined by this method were calculated as the area under the curve of the time dependence of aortic undegraded LDL (traced by protein-bound $^{131}$I) from injection until infinite time divided by cumulative delivery of $^{131}$I-LDL from plasma to the aorta at infinite time (41). The area under the curve of the time dependence of aortic undegraded LDL from 0 to 15.85 h was calculated by the trapezoidal rule. The area under the aortic curve of undegraded LDL from 15.85 h to infinity was calculated by integrating (from 15.85 h to infinity) a monoexponential equation fitted to the aortic data from 15.85 to 120 h after injection. Cumulative delivery of $^{131}$I-LDL from the plasma to the aorta was determined by multiplying aortic permeability to LDL (determined from the 0.66 h period of uptake, above) by the area under the time-dependent $^{131}$I-LDL in plasma. The area under the time-dependent $^{131}$I-LDL in the plasma was calculated by integrating a biexponential equation fitted to composite data for clearance of $^{131}$I from plasma of all rabbits injected with $^{125}$I-TC,$^{131}$I-LDL (Fig. 2).

**Statistical methods.** After analysis with the SAAM program, residual sums of squares from the fitting of the models to aortic data were compared using the $F$ statistic (1). This provides a statistical basis for choice of one model over another (at $P < 0.05$). Data for branch sites and the uniform abdominal aorta were fitted in the same SAAM analysis to facilitate estimation of the differences between the corresponding parameters for these aortic sites for each model. Differences between the corresponding parameters determined for branch sites and the uniform abdominal aorta were evaluated by paired $t$-tests (43). All values are presented with standard errors.
RESULTS

Lipids and lipoproteins. In these normal rabbits, plasma cholesterol averaged 1.25 ± 0.06 mmol/l, LDL cholesterol was 0.39 ± 0.02 mmol/l, and the LDL cholesterol pool size was 39 ± 3 μmol.

Whole body LDL metabolism. For the 21 rabbits killed ≥6 h after injection, the FCR of LDL calculated from 125I-TC was 0.075 ± 0.010 pools/h, whereas that calculated from 131I was 0.086 ± 0.010 pools/h. The ratio of the FCR calculated from 131I to that calculated from 125I was 1.04 ± 0.06, not significantly different from unity and similar to earlier results (33, 36). This suggests that the concentration of EDTA used here and previously (33, 36) was sufficient to prevent the oxidation and subsequent rapid plasma clearance of LDL that occurred when 131I-LDL was dialyzed with much lower concentrations of EDTA (20). The FCR for LDL, determined from composite data for plasma clearance of 131I for all rabbits given 125I-TC, 131I-LDL, was 0.0878 ± 0.0002 pools/h (Fig. 2).

At the end of the metabolic studies, 0.52 ± 0.06% (n = 32) and 2.2 ± 0.3% (n = 24) of 125I and 131I, respectively, were in the d < 1.020 g/ml plasma fraction, whereas 5.6 ± 1.0% (n = 16) and 6.3 ± 1.4% (n = 8) of 125I and 131I, respectively, showed α migration on electrophoresis in agarose. Thus nearly all of the 125I and 131I would have entered the aorta on LDL, a necessary condition for this study.

Aortic accumulation of undegraded LDL and LDL degradation products. Figure 3 shows the time dependence of aortic undegraded LDL traced by protein-bound 131I (Fig. 3A), LDL degradation products (difference between 125I-TC and 131I-labels, Fig. 3B), and aortic undegraded LDL plus the aortic LDL degradation products traced by total 125I-TC (Fig. 3C). Compared with the uniform abdominal aorta, branch sites accumulated more undegraded LDL (ratio 2.31 ± 0.15, P < 0.00002), more LDL degradation products (ratio 1.73 ± 0.15, P < 0.00005), and more total 125I-TC (ratio 1.85 ± 0.09, P < 0.00002).

Comparison of ability of models with one, two, and three compartments of aortic undegraded LDL to fit aortic data. Table 1 compares the goodness of fit of models with one, two, and three pools of aortic undegraded LDL to the aortic data for undegraded LDL and the LDL degradation products. As shown here, for the two-pool model, eliminating pathways of transfer between metabolically active and sequestered LDL (model 2B) did not significantly alter the residual sums of squares (P > 0.25). Eliminating other pathways of transport or metabolism of LDL reduced the ability of the two-pool model to fit aortic data (data not shown). Because residual sums of squares for models both with (model 2A, Table 1) and without (model 2B, Table 1) pathways of transfer between the metabolically active and sequestered LDL are indistinguishable, the simpler reduced model (model 2B) is the best statistically justifiable two-pool model. For the three-pool models, residual sums of squares for the model including all transport pathways (model 3A, Fig. 1, right) were not significantly different (P > 0.25) from those for the reduced model lacking the pathway for efflux from metabolically active LDL (k(t1)) and with the fractional rate constant for efflux from one pool of sequestered LDL (k(02)) fixed at the value for the terminal slope of the time dependence of aortic undegraded LDL (model 3B). However, eliminating other transport or metabolic pathways impaired the ability of model 3B to fit the data for aortic undegraded LDL. Thus model 3B is the best statistically justified three-pool model for aortic undegraded LDL.

Figure 4 shows the observed data for undegraded LDL alone for abdominal branch sites (Fig. 4A) and the
Residual sums of squares (shown after multiplication by 10^4) were computed from the least squares fit of the models to the aortic data, weighting each aortic datum by the standard deviation of replicate animals studied at that time point. Sums of squares accounted for by each reduced model shown were not significantly different (P > 0.25) from those accounted for by the corresponding full model. For all models, and for both aortic sites, k_{i2} (k_{i2}) from the fractional rate constant for transfer into compartment i from compartment j was fixed at 0.005 h^{-1}, a conservative value consistent with earlier studies (28, 37) and current data. Model 1A, 1 pool (full), see Fig. 1. left; models 2A, 2 pool (full), and 2B, 2 pool (reduced), see Fig. 1, middle; and models 3A, 3 pool (full), and 3B, 3 pool (reduced), see Fig. 1, right. For model 2B, there was no k_{12}, and no k_{23}. For model 3B, there was no k_{i2}; k_{i2} fixed at the values for the terminal slopes for data for aortic undegraded low density lipoprotein (LDL) as described in MATERIALS AND METHODS (1.05 ± 0.33 × 10^{-2} and 1.15 ± 0.25 × 10^{-2}, respectively, for the branch and uniform abdominal aorta). *P < 0.005, †P < 0.05 for comparison with model 1A. ‡P < 0.025 for comparison with model 2B. §P < 0.01 for comparison with model 1A.

Table 1. Comparison of the goodness of fit of full and reduced one-, two-, and three-pool models of aortic undegraded LDL to data for undegraded LDL and LDL degradation products in abdominal aorta

<table>
<thead>
<tr>
<th>Model</th>
<th>Undegraded LDL</th>
<th>LDL degradation products</th>
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<tbody>
<tr>
<td></td>
<td>Branch Uniform</td>
<td>Branch Uniform</td>
</tr>
<tr>
<td>1A</td>
<td>5.491 9.072</td>
<td>17.59 18.90</td>
</tr>
<tr>
<td>2A</td>
<td>4.219 3.769</td>
<td>8.537 13.12</td>
</tr>
<tr>
<td>2B</td>
<td>4.173 3.810†</td>
<td>8.318§ 12.94†</td>
</tr>
<tr>
<td>3A</td>
<td>2.851 2.819</td>
<td>8.364 13.03</td>
</tr>
<tr>
<td>3B</td>
<td>2.933‡§</td>
<td>8.324* 12.99</td>
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</tbody>
</table>

Residual sums of squares (shown after multiplication by 10^4) were computed from the least squares fit of the models to the aortic data, weighting each aortic datum by the standard deviation of replicate animals studied at that time point. Sums of squares accounted for by each reduced model shown were not significantly different (P > 0.25) from those accounted for by the corresponding full model. For all models, and for both aortic sites, k_{i2} (k_{i2}) from the fractional rate constant for transfer into compartment i from compartment j was fixed at 0.005 h^{-1}, a conservative value consistent with earlier studies (28, 37) and current data. Model 1A, 1 pool (full), see Fig. 1. left; models 2A, 2 pool (full), and 2B, 2 pool (reduced), see Fig. 1, middle; and models 3A, 3 pool (full), and 3B, 3 pool (reduced), see Fig. 1, right. For model 2B, there was no k_{12}, and no k_{23}. For model 3B, there was no k_{i2}; k_{i2} fixed at the values for the terminal slopes for data for aortic undegraded low density lipoprotein (LDL) as described in MATERIALS AND METHODS (1.05 ± 0.33 × 10^{-2} and 1.15 ± 0.25 × 10^{-2}, respectively, for the branch and uniform abdominal aorta). *P < 0.005, †P < 0.05 for comparison with model 1A. ‡P < 0.025 for comparison with model 2B. §P < 0.01 for comparison with model 1A.

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uniform abdominal aorta (Fig. 4B). As shown here, the data for aortic undegraded LDL for both the branch and uniform abdominal aorta were poorly fitted by both the model with one compartment of undegraded LDL (model 1A, Table 1) and the best model with two compartments of undegraded LDL (model 2B, Table 1). In comparison, the best three-pool model (model 3B) fit the aortic data for undegraded LDL relatively closely throughout the 120-h interval after injection. For branch sites, residual sums of squares for undegraded LDL were reduced 47% (P < 0.01) for model 3B compared with model 1A and 30% (P < 0.025) for model 3B compared with model 2B. For the uniform abdominal aorta, residual sums of squares for undegraded LDL were reduced 69% (P < 0.005) for model 3B compared with model 1A. Model 3B fit the aortic data for undegraded LDL better than model 2B for the uniform abdominal aorta (26% reduction in sums of squares, P < 0.025), although the difference between these models was less than that for the abdominal branch sites.

Figure 4 shows the observed data for the aortic LDL degradation products alone for the abdominal branch sites (Fig. 4C) and the uniform abdominal aorta (Fig. 4D). Model 1A consistently underestimated the aortic data for the LDL degradation products during the first 40 h (uniform abdominal aorta) to 48 h (abdominal branch sites) after injection. Residual sums of squares for the degradation products were reduced 53% (P < 0.005) and 32% (P < 0.05) for the branch and uniform abdominal aorta, respectively, for model 2B compared with model 1A. However, unlike abdominal branch sites, for the uniform abdominal aorta even model 3B consistently underestimated LDL degradation products during the first 40 h after injection. For the uniform abdominal aorta, the fit to data for the aortic products of LDL degradation during the first 2 to 40 h after injection could only be further improved by increasing the rate of transfer of LDL into the aorta to values that were incompatible with the aortic data collected at 0.66 and 1.26 h after injection (data not shown). There was little difference between the ability of models 2B and 3B to fit the aortic data for the LDL degradation products for either the abdominal branch sites or the uniform abdominal aorta. This can be explained by the fact that the metabolically active and sequestered pathways are separate for both the two-pool and three-pool models (Fig. 1).

Figure 4 also presents data for the observed values for the sum of the undegraded LDL and LDL degradation products for abdominal branch sites (Fig. 4E) and the uniform abdominal aorta (Fig. 4F). All models closely fit these aortic data up to 1.26 h after injection (the only such data fitted in the model), indicating that fractional rates of entry of LDL into the aorta are likely to be accurately determined. In comparison, data between 2.3 and 30 h (uniform abdominal aorta) or 40 h (abdominal branch sites) after injection were consistently underestimated by the sum of individual fitted values for undegraded LDL (Fig. 4, A and B) and the aortic LDL degradation products (Fig. 4, C and D). For both aortic sites, there was relatively little difference among the models for the comparison of the sum of fitted values for undegraded LDL and LDL degradation products with the observed sum during the entire 120 h after injection.

Fractional rate constants for LDL transport and metabolism in abdominal aorta. Table 2 presents fractional rate constants determined from the fits to aortic data for the models that are shown in Fig. 4. Fractional rate constants for influx into the metabolically active pool as determined by all models were greater for abdominal branch sites. This difference was significant for model 1A and for model 3B, which fitted aortic data best. The fractional rate constant for efflux of LDL from the metabolically active pool was significantly greater for branch sites compared with the uniform abdominal aorta only for model 1A. No other fractional rate constants differed significantly between the abdominal branch sites and the uniform abdominal aorta for any of the models.
Fig. 4. Observed and fitted values for the time dependence of aortic undegraded LDL and aortic LDL degradation products for branch (left) and uniform (right) abdominal aorta. Data (n = 33 normal rabbits) from Fig. 3 are plotted together with values calculated from fits of 3 models. A and B: protein-bound 131I representing undegraded LDL. C and D: difference between 125I-TC and 131I representing aortic LDL degradation products. E and F: total 125I-TC representing undegraded LDL plus aortic LDL degradation products. All aortic data are presented as %injected dose/g aorta. ● Data that were fitted to the compartmental models during the 120-h interval after injection (A–D) and up to 1.26 h after injection (E and F). ○, Observed values for the sum of undegraded LDL and LDL degradation products not fitted to the models but compared with the sum of fitted values for undegraded LDL and LDL degradation products. All data are means ± SE of observed values (n = 2–4 rabbits, except for 30 h when n = 1). In some cases, error bars do not extend beyond the boundaries of symbols. Thin broken lines, fits with a model with 1 pool of aortic undegraded LDL (model 1A). Dotted lines, best model with 2 pools of aortic undegraded LDL (model 2B). Thick dashed lines, fits with the best model with 3 pools of aortic undegraded LDL (model 3B). For abdominal branch sites, residual sums of squares for undegraded LDL were reduced 47% (P < 0.01) with model 3B vs. model 1A and 30% (P < 0.0025) for model 3B vs. model 2B. For uniform abdominal aorta, residual sums of squares for undegraded LDL were reduced 69% (P < 0.005) with model 3B vs. model 1A and 26% (P < 0.0025) for model 3B vs. model 2B. This provides evidence for the presence of aortic undegraded LDL in at least three metabolically distinct forms within both branch and uniform abdominal aorta. For branch sites, residual sums of squares for degradation products were reduced 53% (P < 0.005) for model 2B vs. model 1A. Similarly, for uniform abdominal aorta, residual sums of squares for degradation products were reduced 32% (P < 0.05) for model 2B vs. model 1A. For both aortic sites, there was no significant additional reduction in sums of squares for degradation products for model 3B vs. model 2B.

Thus total aortic undegraded LDL concentrations calculated by models 2B and 3B were 117% and 109% greater, respectively, for abdominal branch sites (P < 0.0002 and P < 0.00005, respectively; Fig. 6C). For each aortic site, rank order for concentrations of sequestered aortic undegraded LDL were three pool > two pool, whereas rank order for concentrations of total aortic undegraded LDL were three pool > two pool > one pool. Concentrations of metabolically active LDL did not differ between the aortic sites for models with two or three compartments of aortic undegraded LDL. However, concentrations of metabolically active LDL were increased 143% (P < 0.00002) for model 1A, where metabolically active aortic undegraded LDL constituted all of the aortic undegraded LDL (Fig. 6B).

Louder mean residence time within abdominal aorta. Figure 7 shows LDL mean residence times within branch sites and the uniform abdominal aorta. Mean residence times as determined by the different methods differed by up to a factor of two, with rank-order

Undegraded LDL concentrations in abdominal aorta. Figure 6 shows aortic undegraded LDL concentrations determined from fractional rate constants (Table 2) characterizing the fits of the models to aortic data shown in Fig. 4. Aortic concentrations of sequestered undegraded LDL differed between the aortic sites for both the two-pool and three-pool models. For models 2B and 3B, concentrations of sequestered aortic undegraded LDL averaged 124% and 109% higher, respectively (P < 0.0002 and P < 0.0001, respectively) for branch sites (Fig. 6A). Sequestered LDL, as determined by models 2B and 3B, accounted for most (>87% and >98%, respectively) of the aortic undegraded LDL. Thus total aortic undegraded LDL concentrations calculated by models 2B and 3B were 117% and 109% greater, respectively, for abdominal branch sites (P < 0.0002 and P < 0.00005, respectively; Fig. 6C). For each aortic site, rank order for concentrations of sequestered aortic undegraded LDL were three pool > two pool, whereas rank order for concentrations of total aortic undegraded LDL were three pool > two pool > one pool. Concentrations of metabolically active LDL did not differ between the aortic sites for models with two or three compartments of aortic undegraded LDL. However, concentrations of metabolically active LDL were increased 143% (P < 0.00002) for model 1A, where metabolically active aortic undegraded LDL constituted all of the aortic undegraded LDL (Fig. 6B).

LDL mean residence time within abdominal aorta. Figure 7 shows LDL mean residence times within branch sites and the uniform abdominal aorta. Mean residence times as determined by the different methods differed by up to a factor of two, with rank-order
Table 2. Fractional rate constants for LDL transport and metabolism for abdominal aorta of normal rabbits determined from compartmental models

<table>
<thead>
<tr>
<th>Model</th>
<th>Aortic Site</th>
<th>k_{15}</th>
<th>k_{25}</th>
<th>k_{35}</th>
<th>k_{01}</th>
<th>k_{02}</th>
<th>k_{03}</th>
<th>k_{41}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>Branch</td>
<td>2.79</td>
<td>(0.093)</td>
<td>0.060*</td>
<td>(0.17)</td>
<td>0.013</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Uniform</td>
<td>1.71</td>
<td>(0.048)</td>
<td>0.093</td>
<td>(0.094)</td>
<td>0.023</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2B</td>
<td>Branch</td>
<td>1.80</td>
<td>(0.37)</td>
<td>0.70</td>
<td>(1.1)</td>
<td>0.021</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Uniform</td>
<td>1.60</td>
<td>(0.21)</td>
<td>0.84</td>
<td>(0.20)</td>
<td>0.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3B</td>
<td>Branch</td>
<td>0.641‡</td>
<td>(0.034)</td>
<td>F</td>
<td>(0.56)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Uniform</td>
<td>0.346</td>
<td>(0.021)</td>
<td>0.16</td>
<td>(0.26)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fractional rate constants were determined from data from 33 rabbits. Fractional rate constants are presented in pools/h except for k_{15}, k_{25}, and k_{35}, which are presented as 10^5 × pools/h. Fractional standard deviations are shown in parenthesis. For all models, and for both aortic sites, k_{64} was fixed at 0.005 h^{-1}, a conservative value consistent with earlier studies (28, 37) and current data. *P < 0.0005, †P < 0.02, and ‡P < 0.00002 for comparison with corresponding fractional rate constant for uniform abdominal aorta. F, data fixed at the values determined from the least squares fit to the terminal slope of data for aortic undegraded LDL as described in MATERIALS AND METHODS (1.05 ± 0.32 h and 1.15 ± 0.27 h, respectively, for the branch and uniform abdominal aorta).

Absolute rates of LDL degradation. Figure 8 shows absolute rates (nmol LDL cholesterol·h^{-1}·g aorta^{-1}) of entry, degradation, and efflux of LDL for the abdominal aorta determined by the compartmental models. Consistent with similar values for fractional rates for total transport of LDL into the aorta (k_{15} + k_{25} + k_{35}), absolute transport of LDL into a given aortic site as determined by all compartmental models was similar (Fig. 8A). Absolute transport of LDL into abdominal branch sites as determined by models 1A and 3B were 63% (P < 0.0005) and 42% (P < 0.025) greater, respectively, than for the uniform abdominal aorta. Importantly, absolute rates of degradation of LDL were remarkably similar for all compartmental models and 86–88% (P < 0.00002) greater for branch sites than the uniform abdominal aorta (Fig. 8B), even though fractional rate constants for LDL degradation differed among the models (Table 2). Absolute rates of LDL efflux as determined by the different compartmental models were also quite similar for a given aortic site (Fig. 8C). Both the three-pool and two-pool models indicated no difference in absolute rates of efflux between the branch and uniform abdominal aorta. All compartmental models indicated that most of the LDL entering each aortic site was lost by efflux (compare Fig. 8, A and C).

DISCUSSION

The goal of this study was to obtain metabolic evidence that would either support or refute the hypothesis that some undegraded LDL within the aorta of normal rabbits is sequestered in a form that does not readily exchange with plasma LDL. I also considered whether more LDL might be sequestered in the atherosclerosis-susceptible abdominal aorta compared with atherosclerosis-resistant abdominal aorta and whether LDL mean residence time would be increased at these sites. To accomplish these aims, techniques that allowed tracing aortic undegraded LDL and aortic LDL degradation products were combined with a mathematical analysis. This approach allowed sequen-
tial investigation of a model that assumed all aortic undegraded LDL was homogeneous, followed by models that included aortic undegraded LDL in both metabolically active and sequestered forms.

The principle findings of the study were as follows. For the first time, it was possible to show metabolic evidence that for normal rabbits, some LDL in the abdominal aorta is sequestered in a form that is not available for cellular degradation and that exchanges slowly with plasma LDL (Table 1, Fig. 4). Second, most undegraded LDL within each aortic site was sequestered (Fig. 6). Third, undegraded LDL concentrations calculated by all compartmental models were consistently larger for branch sites (Fig. 6). Fourth, LDL mean residence times within the aorta calculated by compartmental models both with and without sequestered LDL were consistently larger for branch sites than the uniform abdominal aorta (Fig. 7). The stochastic method, which is independent of any assumptions regarding transport or metabolism of LDL within the aorta, also indicated a significantly longer mean residence time for LDL in the abdominal branch sites (Fig. 7).

Influence of the nature of the compartmental model considered on calculated parameters of aortic LDL transport and metabolism. For each aortic site, both arterial permeability to LDL (Fig. 5) and mass transport of LDL into the aorta (Fig. 8) were similar irrespective of the compartmental model that was considered. However, as one might expect due to differences in the structure of the compartmental models (Fig. 1), individual fractional rate constants for LDL entry into the various aortic pools, and for transport and metabolism of LDL within the aortic pools, differed among the models (Table 2).

As one might expect, masses of LDL in the metabolically active pool differed between models with (models 2A, 2B, 3A, and 3B) and without (model 1A) sequestered LDL (Fig. 6B). However, masses of metabolically active and sequestered LDL agreed rather well among the models including sequestered LDL. Whereas the mass of metabolically active LDL (Fig. 6B) and the fractional rate constant for metabolism of such LDL (Table 2) could not be precisely determined individually for models including sequestered LDL, the absolute rate of aortic LDL degradation (Fig. 8B) could be very precisely estimated for all models and was identical for all compartmental models that were consid-
Evidence for sequestration of LDL within the aorta. If the hypothesis that some aortic LDL is sequestered is correct, one would expect to find 1) evidence for two or more aortic pools of undegraded LDL and 2) that the fractional rate constant for total loss of LDL via the


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sequestered pathway might be less than that via the metabolically active pathway. Sequestered LDL could be readily detected within both the branch sites and the uniform abdominal aorta (Table 1, Fig. 4). As shown, this study provides metabolic evidence that LDL is present within both the branch sites and the uniform abdominal aorta in at least three forms, of which two are sequestered. For both aortic sites, and for both model 2B and 3B, fractional rate constants for LDL total loss via (each of) the sequestered pathway(s) \( k_{02}, k_{03} \) were only 1/6 to 1/100 that for total loss via the metabolically active pathway (model 2B, \( k_{01} + k_{41} \), model 3B, \( k_{41} \); Fig. 1, Table 2).

No previous study has provided metabolic evidence for sequestration of LDL within the aorta of normal rabbits. However, consistent with the present results, detailed electron microscopic studies demonstrated accumulation of lipoproteins in the aortas of normal rabbits only a few hours after injection of a bolus of LDL (24). Also, another study provided evidence consistent with sequestration of LDL in injury-induced atherosclerotic lesions in normallipidemic rabbits (26).

**Interpretation and relevance of sequestered LDL.**

Previous studies (2, 9) showed that arterial proteoglycans can bind LDL in vitro. Atherosclerotic lesions both contain increased total concentrations of proteoglycans (42, 45, 52) and higher concentrations of proteoglycans with high affinity for LDL (48). Even in macroscopically normal human arteries, the affinity of arterial glycosaminoglycans for LDL correlated with susceptibility to atherosclerosis (6). The binding properties of proteoglycans from branch sites and the uniform abdominal aorta of normal rabbits remain to be determined. However, preliminary data indicate that characteristics of proteoglycans may differ between branch sites and the uniform abdominal aorta and that concentrations of proteoglycans may be increased in the abdominal branch sites of normal rabbits (38). My working hypothesis, which will need to be tested by further experiments, is that increased concentrations of proteoglycans with increased affinity for LDL account for sequestration of increased concentrations of undegraded LDL and prolonged residence of undegraded LDL within the branch sites.

Previous studies have reported that association of LDL with arterial extracellular matrix components increases metabolism of that LDL by macrophages (18, 49, 50), the characteristic cells of atherosclerotic lesions (4). In addition, the association of LDL with the arterial extracellular matrix and prolonged intra-arterial LDL retention could promote intra-arterial oxidation of LDL and further enhance macrophage uptake of LDL and cholesterol accumulation (17). In these studies, sequestered LDL was found to be metabolically inactive [no need for a pathway from either pool 2 or 3 to pool 4 (Deg Prod, Fig. 1, middle and right)], possibly due to the lack of macrophages in the aorta of normal rabbits. However, one could hypothesize that after onset of a hypercholesterolemic stimulus and recruitment of macrophages into the intima, some LDL sequestered within the aorta may be degraded by macrophages, either with or without prior oxidation, contributing to development of atherosclerotic lesions selectively at susceptible aortic sites.

**Aortic LDL retention.** A previous study (37) used the same one-pool model as this paper (model 1A, Fig. 1, left) to calculate LDL mean residence times for branch sites and the uniform abdominal aorta. Mean residence times reported in that study for normal rabbits were \(~3\) h (37). A later study that used a stochastic method to calculate LDL mean residence times for normal rabbits reported values of 9.28 ± 0.57 and 7.09 ± 0.49 h for the branch and uniform abdominal aorta, respectively (46). LDL mean residence times determined in both of those studies (37, 46) are shorter than those determined in the present study (Fig. 7). Also, in contrast to the present results, neither of the earlier studies found LDL mean residence time to be prolonged in the branch sites of normal rabbits (37, 46).

Several factors may have contributed to the difference between the results of the current and the previous (37, 46) studies. First, in the current study, aortic accumulation of undegraded LDL and aortic LDL degradation products was determined at 11 intervals from 0.6 to 120 h after injection. In contrast, the earliest study considered only two time points (1 and 24 h for normal rabbits) (37), whereas the more recent study reported by Tozer and Carew (46) considered only six intervals between 0.5 and 72 h. This is important because if the mean residence time of LDL within the aorta was calculated by the stochastic method from the current data for the six time points corresponding to those used by Tozer and Carew (46), resulting values for mean residence time were 24.2 ± 5.0 and 17.8 ± 5.4 h, respectively, for the branch and uniform abdominal aorta. These values were shorter and less precisely determined than those determined from all 11 time points (Fig. 7) and did not differ between the branch and uniform abdominal aorta. In addition, Tozer and Carew (46) only collected data for aortic undegraded LDL. This is important because in this study, LDL mean residence times could be more precisely estimated when data both for undegraded LDL alone and for aortic LDL degradation products were considered as was done with the mathematical models. This is because use of data for aortic LDL degradation products allows for precise estimation of total degradation of aortic undegraded LDL (Fig. 8B) and facilitated estimation of fractional rate constants for the compartmental models. Thus it seems reasonable that the present values for LDL mean residence times, determined from more complete data for aortic undegraded LDL and data for aortic LDL degradation products, are likely to be more accurate than the previously reported (37, 46) values.

**Limitations of these studies.** In these studies, the metabolic heterogeneity of aortic LDL and the lack of participation of some of the aortic LDL pools in aortic LDL degradation were considered to reflect extracellular sequestration of LDL. However, one could propose that either one or both of the metabolic pathways denoted as sequestered pathway(s) might reflect retro-
endocytosis. However, a previous study (15) with cultured cells suggests that the fractional rate constant for retroendocytosis of LDL would be greater than 1.0 h\(^{-1}\), considerably greater than the fractional rate constants for efflux of LDL from sequestered pools of both model 2B and 3B (\(k_{02}\) and \(k_{03}\) Table 2). Thus it is unlikely that LDL in sequestered pools reflects that destined to be exocytosed.

As described above, these studies provide metabolic evidence supporting the idea that LDL within both atherosclerosis-susceptible abdominal aorta and atherosclerosis-resistant abdominal aorta of normal rabbits is sequestered in association with one or several components of the extracellular matrix. I speculate that the two metabolically distinct pools of sequestered LDL in model 3B reflect LDL bound to different components of the extracellular matrix. However, other biochemical studies will be needed to establish whether or not that is correct and to identify the nature of the extracellular matrix components that might account for retention of LDL within the aorta.

In summary, the results of this study provide, for the first time, metabolic evidence for the presence of undegraded LDL in the abdominal aorta in a form that is sequestered so that it is not available for degradation and effluxes slowly from the aorta. This sequestration increases the concentrations and mean residence times of LDL within the aorta. The focal exaggeration of these processes at the atherosclerosis-susceptible abdominal aorta branch sites could have atherogenic consequences that may help explain the characteristic development of atherosclerosis at these aortic sites.

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REFERENCES

6. Cardoso LEM and Mourao PAS. Glycosaminoglycan fractions from human arteries presenting diverse susceptibilities to atherosclerosis have different binding affinities to plasma LDL. *Atherosclerosis* 14: 115–124, 1994.


