Comparison of Arterial Intimal Clearances of LDL from Diabetic and Nondiabetic Cholesterol-fed Rabbits

Differences in Intimal Clearance Explained by Size Differences

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Arterial intimal clearances of low density lipoproteins (LDL) from diabetic cholesterol-fed rabbits (D-LDL) and LDL from nondiabetic cholesterol-fed rabbits (N-LDL) were compared. In six experiments, D-LDL and N-LDL were isolated from a diabetic and a nondiabetic rabbit, were iodinated with 125I and 131I, respectively, were mixed, and were reinjected into the same two rabbits as well as into a normal rabbit. Fractional catabolic rates for D-LDL and N-LDL in normal rabbits were 0.065 and 0.074 h⁻¹ (p<0.05), respectively. For five of the six pairs of LDL, the D-LDL was smaller than N-LDL as determined by gel filtration. The arterial permeability to N-LDL, when normalized for differences in arterial cholesterol content, did not appear to differ between diabetic and nondiabetic rabbits. The relative arterial intimal clearance (D-LDL/N-LDL) in arteries from diabetic and nondiabetic rabbits was inversely related to the relative molecular weight (D-LDL/N-LDL). For example, when the molecular weight of D-LDL was as low as 60% of that of N-LDL (i.e., the diameter of D-LDL was reduced 16%), the intimal clearance of D-LDL was 40% larger than that of N-LDL. When, on the other hand, molecular weights and diameters of the two LDL were similar, the intimal clearance was also quite similar. These results suggest that arterial intimal clearance of LDL from diabetic and nondiabetic cholesterol-fed rabbits is comparable unless the two types of LDL have a different size. (Arteriosclerosis 9:175-183, March/April 1989)

Humans with diabetes mellitus in Western societies develop more atherosclerosis-related morbidity and mortality than do nondiabetic humans.1,2 Although a higher frequency of other cardiovascular risk factors in human diabetics may explain part of the increased risk for atherosclerosis-related morbidity and mortality in diabetics,3 diabetes, per se, is an independent cardiovascular risk factor.

A possible cause for diabetes being an independent cardiovascular risk factor may be that the diabetic state causes alterations in low density lipoproteins (LDL); LDL from diabetics may be taken up by arterial tissue faster than LDL from nondiabetics. Certainly, diabetes is known to cause alterations in LDL; for example, LDL apolipoprotein B is glycosylated,4-6 and LDL from diabetics have altered in vivo behavior in plasma, that is, altered fractional catabolic rate (FCR), as compared to LDL from nondiabetics.7

The present paper compares arterial intimal clearance of LDL from diabetic and nondiabetic cholesterol-fed rabbits. To assess the degree of alterations of LDL from diabetic rabbits, glycosylation of LDL apolipoprotein B and LDL FCR were determined and compared to those of LDL from nondiabetic rabbits. Since it is known that the size of lipoproteins affects arterial intimal clearance,8,10 sizes of the various LDL used for arterial intimal clearance measurements were determined.

Methods

Animals

Twenty-six female New Zealand White rabbits (Becken Research Animal Farm, Sanborn, NV) weighing 2.0 to 3.9 kg were used as follows: six diabetic and six nondiabetic cholesterol-fed rabbits for influx experiments, three nondiabetic cholesterol-fed rabbits for isotope equivalency studies, and 11 normal rabbits for FCR determinations. The cholesterol-fed rabbits were fed daily 92 g Purina Rabbit Laboratory Chow (Ralston Purina Company, St. Louis, MO) supplemented with 0.5 g cholesterol (ICN Biochemicals, Cleveland, OH) and 7.5 g com oil (Mazola) for 5 to 15 weeks before the influx study (cholesterol was dissolved in corn oil at 120°C and subsequently mixed with chow). To avoid weight loss, the diabetic rabbits were fed an additional 50 g chow daily. The 11 normal rabbits were fed 100 g chow daily. All experimental protocols were in accordance with Cornell University guidelines. Unless noted otherwise, blood samples were adjusted to

D-LDL = LDL from diabetic cholesterol-fed rabbits and N-LDL = LDL from nondiabetic cholesterol-fed rabbits.
0.15% Na$_2$EDTA and 0.04% Na$_2$PO$_4$, were kept on ice until plasma was separated, and were stored at 4°C.

**Alloxan Infusion**

To generate the six diabetic rabbits for influx studies, 14 rabbits were infused with freshly prepared 5% alloxan monohydrate (Sigma, St. Louis, MO) in physiological saline over a period of 15 minutes, through a catheter inserted via the marginal ear vein. If the rabbits weighed less than 3 kg, the dose of alloxan was 150 mg/kg; and if they weighed more, 200 mg/kg. To counteract hypoglycemia caused by insulin released from necrotic beta cells in the pancreas, the rabbits were provided with 5% sucrose instead of water for the first 24 hours. One rabbit died within 24 hours. Twelve rabbits became diabetic, that is, fasting blood glucose of 250 mg/dl or more, as determined with Dextrostix (Ames Division, Miles Laboratories, Incorporated, Elkhart, IN). To avoid high death rates within the first weeks after alloxan infusion, diabetic rabbits were injected daily subcutaneously with 2 to 4 units of NPH insulin (U-100N, Lilly, Indianapolis, IN). In spite of this, an additional three rabbits died within the first week. The remaining nine rabbits were severely diabetic with severe lipid abnormalities; that is, at some time they all had plasma triglycerides above 3000 mg/dl as determined enzymatically (Cat. No. 701912, Boehringer-Mannheim Diagnostics, Indianapolis, IN). Such rabbits have postabsorptive plasma glucose of 320 to 550 mg/dl compared to 130 to 150 mg/dl in non-diabetic cholesterol-fed rabbits as determined enzymatically (Cat. No. 124001, Boehringer-Mannheim Diagnostics). To achieve differences in plasma triglycerides and thereby possible differences in sizes of LDL, different daily doses of insulin (0 to 13 units) were given to the different rabbits the last 2 weeks before the influx study. In spite of the relatively high doses of insulin given to some of the rabbits, such rabbits still were severely hyperglycemic during most of the day. The six rabbits used for influx studies were diabetic at 5 to 14 weeks; the other three died.

**Iodination of Low Density Lipoprotein**

LDL were prepared from freshly drawn rabbit blood containing 2 mg/ml of the anticoagulant Na$_2$EDTA, 40 µg/ml of the antibiotic gentamicin sulfate (Sigma), 0.1 mg/ml of the antibiotic gentamicin sulfate (Sigma), and the protease inhibitors e-amino-n-caproic acid (2.6 mg/ml), benzamidine (10 µg/ml), and aprobin (10 kallikrein units/ml), all from Sigma. Sequential ultracentrifugation at 4°C was performed in solven densities of 1.019 and 1.063 g/ml with a Beckman 50 Ti rotor (Beckman Instruments, Incorporated, Palo Alto, CA) for at least 4.5-40 g-hour (average); fractions were collected by tube slicing. Before iodination, LDL were equilibrated with phosphate-buffered saline (PBS) (11.4 mg/ml Na$_2$HPO$_4$, 7H$_2$O; 1.0 mg/ml NaH$_2$PO$_4$, 1.0 mg/ml Na$_2$EDTA, and 5.3 mg/ml NaCl, pH 7.4) containing 0.1 mg/ml Na$_2$EDTA by prepacked PD-10 gel filtration columns (Sephadex G-25M, Pharmacia, Piscataway, NJ). LDL were iodinated according to the modification by Bilheimer et al. of McFarlane's iodine monochloride method as follows. In 1.6 ml PBS with EDTA containing 2 to 13 mg LDL protein, pH was adjusted to 10 with 0.4 ml of 1 M glycine buffer (pH=10). Then, 1.4 to 5.0 mCi $^{125}$Iodine (Amersham, Arlington Heights, IL) or 1.0 to 1.8 mCi $^{131}$Iodine (Amersham) was added before iodine monochloride (15 mmol/mg protein), prepared as described by Goldstein et al. Unbound iodine was removed with PD-10 columns, followed by extensive dialysis overnight at 4°C against PBS with EDTA and Na$_2$PO$_4$ (0.2 mg/ml). To minimize possible self-irradiation damage, primarily from $^{131}$I, in the course of the six pairs of rabbits, rabbit albumin (10× the LDL protein) was added after the PD-10 column procedure and before dialysis. Iodination efficiency averaged 25% for $^{125}$I and 12% for $^{131}$I, which converts to 1.8 and 0.9 mol, respectively, of iodine per mole of LDL. Labeled LDL were used for influx experiments immediately after preparation, that is, about 72 hours after donor blood was drawn and less than 24 hours after iodination.

**Equivalency of $^{125}$Iodine and $^{131}$Iodine**

The conclusions of the present study are based on the assumption that LDL, whether iodinated with $^{125}$I or $^{131}$I, are transferred into the arterial intima-media at the same rate. To test this assumption, aliquots of LDL from a nondiabetic cholesterol-fed rabbit were iodinated with $^{125}$I or $^{131}$I, were mixed, and were injected intravenously into three nondiabetic cholesterol-fed rabbits for arterial uptake measurements. Injected doses were 3.6-1.0 mCi with 218±94 µCl $^{125}$I and with 35±15 µCl $^{131}$I.

**Protocol for Influx Experiment**

In six pairs of rabbits, LDL were isolated simultaneously from a diabetic (D-LDL) and a nondiabetic (N-LDL), cholesterol-fed rabbit. D-LDL were iodinated with $^{125}$I and N-LDL with $^{131}$I, or vice versa. After dialysis, the two labeled LDL preparations were mixed and filtered through 0.22-µm filters (Millipore Corporation, Bedford, MA) before aliquots (2.6±0.3 ml, n=12) of this mixed dose (171±21 µCl $^{125}$I and 35±15 µCl $^{131}$I) were injected intravenously into the same two rabbits. Blood samples were drawn at regular intervals until the rabbits were killed 4 to 6 hours later by intravenous injection of a 6% pentobarbital solution (50 to 100 mg/kg). The thorax was opened, and the rabbit was perfused with 1 liter of saline introduced into the left ventricle of the heart after the inferior vena cava was severed. Subsequently, the aorta was excised, the adventitia was removed, and the vessel was opened longitudinally and rinsed with saline. The aorta was fixed with pins on a corkboard, the area was outlined on graph paper, and the vessel was divided into the aortic arch (from the heart to the first intercostal arteries), the thoracic aorta (to the diaphragm), and the abdominal aorta (to the bifurcation). The intima-media was stripped from the remaining media in each of these parts.

**Analyses**

An aliquot of the dose (10 µl), plasma aliquots (200 µl), and aortic intima-media layers were counted in a Beckman Gamma 8000 counter immediately after the experiment. To stop enzyme reactions in these samples, 2 ml of methanol was added within 1 hour after the samples were obtained. After counting, the same volume of chloroform was added to the samples, the aortic samples were
minced, and the lipids were extracted during a 24-hour period. After centrifugation, the precipitate was washed twice with chloroform/methanol (1:1, vol/vol). To the combined washes were added half a volume of chloroform and 20% of the final volume as water. The upper phase, (“aqueous phase”) was removed, and the lower phase (“lipid phase”) was washed twice with upper phase. The combined aqueous phase was washed twice with chloroform. The radioactivities in the protein precipitate, the lipid phase, and the aqueous phase were determined. The distribution of label in the LDL doses in the presence of added carrier plasma between those three phases was 92 ± 1%, 5 ± 0.8%, and 4 ± 0.4% (n = 6), respectively. The equivalent distributions for plasma samples and aortic samples were 95 ± 0.3%, 3 ± 0.5%, 2 ± 0.4% (n = 6), and 88 ± 1%, 5 ± 1%, 7 ± 1% (n = 21), respectively. Aliquots of the LDL doses were electrophoresed on 1% agarose (universal electrophoresis film, Corning Medical, Palo Alto, CA); 96 ± 1% (n = 11) of radioliodine comigrated with LDL. Other aliquots were delipidated, and apolipoproteins were separated by 5% sodium dodecyl sulphate-polyacrylamide gel electrophoresis; 90 ± 1% (n = 4) of radioliodine comigrated with LDL apolipoprotein B. All the above radioliodine distribution determinations gave similar results whether a 125I or a 131I was used or whether the LDL was from a diabetic or a non diabetic rabbit.

LDL were isolated from plasma by sequential ultracentrifugation at 10°C and densities of 1.019 and 1.063 g/ml with a Beckman 50.3 Ti rotor for at least 2.4 - 10² g · h (average). Lipids in LDL fractions and in plasma samples were extracted into hexane from 43% ethanol in water. Total cholesterol in these extracts, as well as in the lipid phase from aortic samples (see above), was determined according to the method of Zak et al. after saponification.

Calculations
Aortic intimal clearance (nl · cm⁻² · h⁻¹) was calculated by the "sink" method: dividing the radioactivity in the intima-media layer (cpm/cm²) by the average radioactivity in plasma during the 4- to 6-hour influx period (cpm/ml) and by the length of the influx period (hours). In three cholesterol-fed rabbits, the plasma contaminations (measured with ¹²⁵I-LDL) were 11.0 ± 1.8, 5.0 ± 0.8, and 5.6 ± 0.5 nI/cm² for the arch, thoracic, and abdominal aorta, respectively (details reported elsewhere). The corresponding corrections were made before calculations of intimal clearance. In two other cholesterol-fed rabbits, the sink assumption for iodinated LDL uptake into aortas was found to be appropriate for up to 6 hours (reported elsewhere).

The calculations of intimal clearances assume that the process of lipoprotein uptake by the artery is a linear, non-saturable process. Earlier studies in our laboratory have provided evidence for this assumption. In the present study, when intimal clearances of D-LDL and N-LDL were compared in diabetic and non diabetic rabbits, the plasma concentration of nonautologous LDL was much lower than that of autologous LDL. Furthermore, the plasma concentration of nonautologous LDL decreased during the influx period, whereas that of autologous LDL was constant, since the animal was in a steady state. From the crossover experiments shown in Table 4, it appears that the relative intimal clearance (D-LDL/N-LDL) for the same pair of LDL was similar whether measured in a diabetic or a non diabetic rabbit. In the diabetic rabbit, N-LDL had a low plasma concentration, whereas D-LDL had a low plasma concentration in the nondiabetic rabbit. The differences in plasma concentrations between the two types of LDL, therefore, did not appear to affect the magnitude of arterial intimal clearance.

Glycosylation of Low Density Lipoprotein
The extent of glycosylation of LDL from diabetic and non diabetic cholesterol-fed rabbits was determined by Curtiss and Witztum with two radiolimune assays with the monoclonal antibodies GSC11 and GSC9 and synthetic pure glucolipidine as a standard. Protein in the LDL fractions was determined by the method of Lowry et al. with dry bovine serum albumin (Miles Laboratories, Inc., Naperville, IL) as a standard.

Fractional Catabolic Rate for Low Density Lipoprotein
FCR in eleven normal rabbits were determined simultaneously for D-LDL and N-LDL. Aliquots (0.1 ml) of the mixed doses (7.8 ± 2.3 μCi ¹²⁵I and 1.4 ± 0.4 μCi ¹³¹I, n = 6), also used for influx studies, were injected intravascularly into six normal rabbits. Blood samples were drawn regularly over the ensuing 3 to 4 days until less than 6% (average 3.4%) of the initial radioactivity was left in plasma. Biologically screened LDL were also used to determine FCR. One hour after injection of the mixed doses into non diabetic cholesterol-fed rabbits, 4 ml of plasma were obtained (4.8 ± 1.0 μCi ¹²⁵I and 1.0 ± 0.1 μCi ¹³¹I) and injected into the remaining five normal rabbits. An aliquot of the dose (10 μl), as well as plasma aliquots (200 μl) for the 3 to 4 days, were counted simultaneously as described above.

Fractional irreversible removal or FCR was calculated accordingly to the method of Matthews (K₂, p. 45). Curves for plasma radioactivities for the 3 to 4 days were fitted to two exponentials by an iterative least-squares technique with a computer program as described by Delf et al. From the two exponentials, b₁ and b₂, and the intercepts with the Y-axis, C₁ and C₂, the FCR (h⁻¹) was calculated as:

\[
\text{FCR} = \frac{1}{C₁/b₁ + C₂/b₂}
\]

Size of Low Density Lipoprotein
The size of all LDL used for influx studies was estimated by gel filtration on a Superose 6B (Pharmacia) column. The column was 1.6 cm × 50 cm, and the elution buffer was PBS with Na₂EDTA and NaCl. The flow rate was 1 ml/minute. After dialysis against PBS with Na₂EDTA but before iodination, an aliquot of the isolated LDL fraction (200 μl) was applied to the column at room temperature, and LDL in the eluate were determined by spectrophotometric measurement of the absorption at 280 nm. The elution volume (Vₑ) of all LDL was determined twice, and the average value was used. In three of the six experiments, the elution volume of the LDL was also
determined after iodination and intravenous injection. To do so, plasma samples (200 \( \mu \)l) from the six rabbits obtained 10 minutes after injection of the dose and just before death of the animal were applied to the Superose 6B column. Fractions of 2 ml were collected and counted. Recoveries on the column were 95.5\( \pm \)1.1\% (n=24).

The void volume (\( V_v \)) and total volume (\( V_t \)) of the column were determined with very low density lipoproteins (VLDL) (d<1.019 g/ml) from cholesterol-fed rabbits and radiiodine, respectively. The column was calibrated with tryptophan (Sigma), rabbit albumin (Sigma), and high density lipoproteins (HDL) and LDL from nondiabetic cholesterol-fed rabbits. The apparent molecular weights of 3 \( \cdot \) 10\(^4\) g/mol for LDL and 2.3 \( \cdot \) 10\(^4\) g/mol for HDL as reported by Rudel et al.\(^{(26)}\) were used. The molecular weights of the four calibrators were plotted on semilogarithmic paper against:

\[
K_w = \frac{V_v - V_s}{V_t - V_s}
\]

This gave a linear relationship. From the \( V_s \) of the various LDL, \( K_w \) was calculated, and the molecular weight was subsequently obtained from the graph. Since the actual molecular weights of the LDL were not measured, only the relative molecular weights are given in the Results section.

**Statistics**

The values are given as means\( \pm \)standard error. Paired and unpaired t tests\(^{(27)}\) (pp. 83–106) and linear regression analysis\(^{(27)}\) (pp. 149–193) were used. Analysis of covariance was performed with the SAS program.\(^{(28)}\)

**Results**

**Fractional Catabolic Rate for Low Density Lipoproteins**

For five of the six pairs of LDL used for influx studies, the LDL from the diabetic cholesterol-fed rabbit were removed more slowly from plasma than LDL from the nondiabetic cholesterol-fed rabbit when injected into a normal rabbit (Figure 1, Table 1). FCR determined before and after biological screening of the mixed LDL doses were similar. The ratio between FCR for D-LDL and N-LDL was approximately the same whether the LDL dose had been screened or not. Sasaki and Cottam\(^{(29)}\) reported an FCR between 0.055 and 0.083 h\(^{-1}\), and Steinbrecher et al.\(^{(30)}\) reported an FCR of 0.086 h\(^{-1}\) when labeled normal LDL was injected into normal rabbits, values which are in close agreement with our present results.

Since glycosylation of LDL is known to reduce the FCR of such LDL in normal rabbits,\(^{(29,30)}\) the relative degree of glycosylation of D-LDL as compared to N-LDL was determined. Glycosylation of D-LDL from seven cholesterol-fed rabbits that had been diabetic for 2 to 8 weeks was, on the average, 3.5 and 2.4 times greater than that of seven N-LDL, as determined with two different antibodies against glycolylsine.

**Size of Low Density Lipoprotein**

LDL from nondiabetic cholesterol-fed rabbits were larger than LDL from diabetic cholesterol-fed rabbits with hypertriglyceridemia (Figure 2). Elution volumes on the Superose 6B column were 56.1\( \pm \)0.4 ml (n=13) for LDL from nondiabetic cholesterol-fed rabbits; 55.8 and 56.3 ml for LDL from two diabetic cholesterol-fed rabbits with almost normal plasma triglyceride (approximately 500 mg/dl); but were 56.1\( \pm \)0.4 ml (n=10) for LDL from diabetic cholesterol-fed rabbits with plasma triglyceride from 2000 to 18 000 mg/dl. These elution volumes show that the molecular weights of LDL from diabetic cholesterol-fed rabbits with hypertriglyceridemia were, on the average, 70% of that for LDL from nondiabetic cholesterol-fed rabbits. The difference in size between LDL from diabetic and nondiabetic rabbits was similar whether determined before or after iodination. No changes in size of iodinated LDL were detected during the 4- to 6-hour influx periods.

**Equivalency of \(^{125}\)Iodine and \(^{131}\)Iodine**

In the first two pairs of rabbits used for influx studies, the volume of distribution for \(^{131}\)I-LDL was 5% to 20% larger than that for the simultaneously injected \(^{125}\)I-LDL. When such LDL were reinjected into a normal rabbit, after
1 hour of biological screening in a nondiabetic cholesterol-fed rabbit, the two LDL had similar volumes of distribution. This indicates that some of the LDL iodinated with 131I may have been damaged and rapidly removed from the circulation. Therefore, for the remaining four pairs of rabbits used for influx studies, a 10-fold excess of protein as rabbit albumin was added immediately after the iodination. Subsequently, volumes of distribution for the two LDL were similar and never differed by more than 6%.

If 5% to 20% of 131I-LDL were removed from the circulation within the first 10 minutes, this would not affect the conclusions of the present study, unless the damaged LDL were taken up by arterial tissue. When no albumin was added to the iodinated LDL, arterial intimal clearance of 131I-LDL appeared to be a little smaller than that for 125I-LDL (rabbit A, Table 2). When 131I-LDL without added albumin was kept in dialysis for a week before the influx study, the result was similar (rabbit B). Arterial intimal clearances of the two differently iodinated LDL aliquots were similar when an excess of rabbit albumin was added after iodination and before the overnight dialysis (rabbit C). Taken together, the data exclude the possibility that 125I-LDL is taken up preferentially by the aorta and indicate that, in the present experimental conditions, 125I-LDL and 131I-LDL do not differ more than 10% in arterial uptake.

Table 2. Ratios of Arterial Intimal Clearance of LDL Iodinated with 125I and 131I in Cholesterol-fed Rabbits

| Rabbit | Intimal clearance 125I-LDL | Intimal clearance 131I-LDL | Mean |
|--------|--------------------------|--------------------------|------|
| A      | Arch 0.89, Thoracic 0.85, Abdominal 1.01 | | 0.92 |
| B*     | Arch 0.89, Thoracic 0.90, Abdominal 0.99 | | 0.93 |
| C†     | Arch 0.96, Thoracic 1.01, Abdominal 0.96 | | 0.98 |

The arterial cholesterol content and the magnitude of intimal clearances in rabbits A, B, and C were similar to those in the rabbits of Table 4.

131I-LDL was dialyzed for 1 week after iodination before influx study.

†Rabbit albumin (10-fold) was added after iodination and before dialysis.

Aortic intimal clearance of LDL was studied in six diabetic cholesterol-fed and six nondiabetic cholesterol-fed rabbits. Table 3 shows plasma triglyceride, total cholesterol, and LDL cholesterol concentrations.

After intravenous injection of labeled LDL, radioactivity in plasma decreased at a similar rate in diabetic cholesterol-fed and in nondiabetic cholesterol-fed rabbits; after 4 hours, 80±2% (D-LDL), 81±2% (N-LDL), and 79±2% (D-LDL), 80±2% (N-LDL) of the initial radioactivity remained in plasma in the diabetic and the nondiabetic, recipient rabbits, respectively. When arterial intimal clearances of LDL were calculated based on protein precipitable radioactivity, the values were 93±1% (n=8) of the values calculated based on total radioactivity in plasma and intima-media. The reported values are based on total radioactivity.

Five of the six LDL from diabetic cholesterol-fed rabbits had a larger arterial intimal clearance than did LDL from nondiabetic cholesterol-fed rabbits (D-LDL/N-LDL, Table 3).

| Recipients | Diabetics | Nondiabetics |
|------------|-----------|--------------|
| Experiment | Plasma Triglyceride | Plasma Cholesterol | LDL cholesterol | Plasma Triglyceride | Plasma Cholesterol | LDL cholesterol |
| 1          | 8520      | 4000         | 148           | 93           | 1610          | 190          |
| 2          | 8800      | 3870         | 66            | 79           | 1680          | 202          |
| 3          | 2760      | 4280         | 380           | 102          | 1770          | 331          |
| 4          | 14 400    | 5640         | 91            | 107          | 1830          | 288          |
| 5          | 573       | 3510         | 495           | 79           | 972           | 182          |
| 6          | 515       | 2200         | 471           | 112          | 1770          | 164          |

The values are mg/dl.
Table 4. Arterial Intimal Clearance of LDL from Diabetic and Nondiabetic, Cholesterol-fed Rabbits

| Experiment | Tissue | Cholesterol (µg/cm²) | N-LDL (nL/cm²·h⁻¹) | D-LDL/N-LDL |
|------------|--------|---------------------|-------------------|-------------|
| 1          | Arch   | 360                 | 30.8              | 1.26        |
|            | Thor   | 53                  | 5.6               | 1.27        |
|            | Abd    | 131                 | 7.9               | 1.34        |
|            | Arch   | 718                 | 40.7              | 1.45        |
| 2          | Thor   | 148                 | 6.0               | 1.54        |
|            | Abd    | 250                 | 11.7              | 1.39        |
|            | Arch   | *                   | 51.4              | 1.12        |
| 3          | Thor   | 55                  | 7.6               | 1.33        |
|            | Abd    | 58                  | 5.4               | 1.07        |
|            | Arch   | 66                  | 17.0              | 1.46        |
| 4          | Thor   | 31                  | 3.2               | 1.26        |
|            | Abd    | 35                  | 3.2               | 1.50        |
|            | Arch   | 653                 | 65.5              | 0.89        |
| 5          | Thor   | 156                 | 15.9              | 0.99        |
|            | Abd    | 109                 | 8.0               | 1.11        |
|            | Arch   | 501                 | 49.9              | 1.11        |
| 6          | Thor   | 83                  | 4.4               | 1.14        |
|            | Abd    | 109                 | 5.2               | 1.15        |

In each experiment, low density lipoproteins (LDL) were isolated simultaneously from the diabetic and the nondiabetic rabbit, were iodinated with ¹³¹I and ¹³²I, respectively, and were mixed and reinjected into the same two rabbits. N-LDL = LDL from nondiabetic, cholesterol-fed rabbits; D-LDL = LDL from diabetic, cholesterol-fed rabbits; Thor = thoracic aorta; Abd = abdominal aorta.

To compare the intimal clearance of N-LDL in diabetic and nondiabetic rabbits, a "normalized" intimal clearance (specific intimal clearance) was calculated by dividing the intimal clearance (nL·cm⁻²·h⁻¹) by the aortic cholesterol content (µg/cm²). This calculation was validated in a large sample of N-LDL from diabetic and nondiabetic rabbits studied for a different purpose. In these animals, the regression line of the intimal clearance of N-LDL in arch, thoracic, and abdominal aortas increased linearly with increasing aortic cholesterol content and passed through the origins. Specific intimal clearances for N-LDL in diabetic cholesterol-fed and nondiabetic cholesterol-fed rabbits were not statistically significantly different (Table 5). In spite of the large variation in specific intimal clearances between animals and between aortic sites within animals, the data give no indication that arteries of diabetic rabbits are more permeable to LDL than arteries in nondiabetic rabbits.

Since LDL from diabetic cholesterol-fed rabbits with hyperlipidemia were smaller than LDL from nondiabetic cholesterol-fed rabbits (Figure 2), the larger intimal clearance of D-LDL as compared to N-LDL could possibly be explained by differences in size. Figure 3 shows the relationship between relative aortic clearances and relative molecular weights of LDL in diabetic and nondiabetic rabbits. The same labeled LDL doses were used to measure clearance ratios in a diabetic cholesterol-fed and a nondiabetic cholesterol-fed rabbit and in arch, thoracic, and abdominal aortas in each of these rabbits. To assess whether the correlation between LDL molecular weight ratios and LDL intimal clearance ratios was the same for the two types of rabbits and the three different aortic sites, an analysis of covariance was performed. The analysis showed no significant differences for these variables. Therefore, we calculated the mean values of the LDL clearance ratios for the three aortic sites for each animal as a function of the molecular weight ratios.
Figure 3 shows that when the molecular weight of D-LDL was as low as 60% of the molecular weight of N-LDL, the D-LDL was cleared 40% faster by arterial tissue than N-LDL. When, on the other hand, the two LDL had similar molecular weights, the clearances by arterial tissue were also similar. Thus, the larger arterial intimal clearance of D-LDL as compared to N-LDL appears to be largely explained by a difference in size.

**Discussion**

The aim of the present study was to compare the arterial intimal clearance of LDL from diabetic cholesterol-fed rabbits with the simultaneously measured arterial intimal clearance of LDL from normal cholesterol-fed rabbits. The D-LDL were removed from total plasma at a slower rate than N-LDL, which suggests that D-LDL might have been altered chemically (e.g., glycosylation) and therefore might show altered clearance rates into arterial tissue. However, such alterations did not appear to affect arterial intimal clearance. The difference in arterial intimal clearance for the two LDL could be explained by a difference in their size. Differences in size among the six pairs of rabbits were not correlated with differences in FCR (data not shown).

Stender and Zilverstreit demonstrated an inverse relationship between the macromolecular diameters of albumin, HDL, LDL, and VLDL and the intimal clearances of these groups of particles in cholesterol-fed rabbits. A similar relationship has recently been demonstrated for HDL and LDL in humans. However, differences in arterial intimal clearance caused by a small difference in size of lipoproteins within a given lipoprotein class have not been previously observed.

In the arterial influx experiments, the molecular weight for D-LDL was as low as 60% of that of N-LDL, which is equivalent to a diameter for D-LDL of 84% (r = 0.6) of that of N-LDL if one assumes a spherical complex. Thus, the present data indicate that as little as a 16% reduction in LDL diameter on the average causes a 40% increase in LDL clearance by arterial tissue.

Since LDL from diabetic rabbits with almost normal plasma triglyceride were similar in size to LDL from nonobese rabbits, the small size of LDL in diabetic rabbits with hypertriglyceridemia appears to depend on the elevated plasma triglyceride and not on the diabetic state, per se. This is in accordance with the observation that hypertriglyceridemic humans have smaller LDL than normotriglyceridemic humans.

In vitro glycosylation of LDL causes reduced receptor-mediated uptake and degradation of LDL by isolated fibroblasts, endothelial cells, and hepatocytes and reduced FCR of LDL as compared to normal LDL in rabbits, humans, and guinea pigs. Since D-LDL in the present study was shown to be three times more glycosylated than N-LDL, it is possible that the reduced FCR for D-LDL, as compared to N-LDL, may have been caused by glycosylation, and that the reduced FCR for D-LDL may reflect a reduced interaction of such LDL with the LDL-receptor.

 Wiklund et al. suggested that LDL uptake into arterial tissue was not dependent on endothelial LDL receptors, but was mediated by other mechanisms. The observations in the present report support this suggestion. The demonstrated dependence of arterial intimal clearance on LDL size suggests a mechanism of LDL uptake by arteries as a nonspecific, molecular sieving. This mechanism is furthermore supported by the fact that the measurement of arterial intimal clearance did not seem to depend on the large difference in plasma concentrations of D-LDL and N-LDL in diabetic and nonobese rabbits. Also, in the present study, D-LDL was shown to be glycosylated, which is known to block receptor-mediated uptake into isolated endothelial cells, and since the present experiments showed no indication of reduced arterial uptake of D-LDL as compared to N-LDL, it is unlikely that LDL receptors are important for uptake into the arterial wall across endothelial cells.

In conclusion, the present results demonstrate an inverse relationship between size of LDL and arterial intimal clearance of LDL and suggest that arterial intimal clearance of LDL from diabetic cholesterol-fed rabbits is comparable, unless the two types of LDL have a different size.

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Index Terms: aortic lipoprotein uptake • arterial wall • atherosclerosis • cholesterol • diabetes • glycosylation • lipoproteins • lipoprotein size