Hydrolase Activities in the Rat Aorta

IV. Relation between Clearance Rates of Circulating 125I-Labeled Low-Density Lipoproteins and Levels of Tissue Hydrolase Activity

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SUMMARY We studied the relationships among levels of acid cholesteryl esterase (ACE) activity in kidney, liver, and aorta and clearance of 125I-labeled human low density lipoprotein (LDL) from the circulation. The premise was that levels of ACE (which is involved in the hydrolysis of the cholesteryl esters of LDL) might correspond to the rate at which LDL was removed from the circulation, or fractional catabolic rate (FCR). The models used were rats with streptozotocin diabetes and exercised (swimming) rats. In the diabetic rat, ACE specific activity was increased by 46% in kidney and 30% in liver and decreased by 25% in aorta when compared to controls. The FCR of LDL increased by 25% in the diabetic animal over control values. Insulin treatment restored all these alterations to normal. Swimming rats showed no change in ACE-specific activity of kidney or liver, but aortic ACE increased by approximately 25% over levels in sedentary rats. The FCR of radioiodinated LDL in swimmers was unchanged from normal. Return of swimmers to a sedentary existence resulted in persistence of normal ACE activity in kidney and liver and in FCR of LDL; however, previously elevated aortic ACE activity returned to normal. Thus, dynamic, irreversible removal of LDL from the circulation (FCR) is changed in the same direction as, and may be linked to, ACE activity in non-vascular tissues. Aortic ACE activity, on the other hand, is not so related to either ACE in those tissues or to FCR of LDL. One implication of this finding is that the general pattern of LDL catabolism by the rat is distinct from the local problem of LDL catabolism by the blood vessel wall.

MAJOR advances in our understanding of lipoprotein metabolism have been made in recent years (Schaefer et al., 1978). These include new information about the interactions among the various lipoprotein classes as they circulate in the blood. In particular, it has been noted that, in humans (and in the rat, to a lesser extent), progressive loss of triglyceride from the very low density lipoproteins (VLDL) by the action of lipoprotein lipase gives rise to low density lipoproteins (LDL) that are composed largely of free and esterified cholesterol (Schaefer et al., 1978). This latter lipoprotein class is considered to be an endpoint in intravascular conversions; the subsequent fate of LDL is dependent largely on tissue uptake and catabolic processes.

Coincident with these new insights has been progress in identifying cellular mechanisms responsible for uptake (Brown and Goldstein, 1976) and degradation of the LDL molecule (Takano et al., 1974; Goldstein et al., 1975). Furthermore, an acid cholesteryl esterase (ACE) localized to the lysosome (Takano et al., 1974) is involved integrally in hydrolysis of esterified cholesterol in LDL to result in free cholesterol and fatty acid, which then diffuse freely from the cell. We have identified factors that influence the level of this acid lipase and other lysosomal enzymes in smooth muscle cells of vascular tissue. Insulin-deficient diabetes mellitus in rats results in reduction of the specific activity of ACE and many other hydrolases; insulin administration restores the enzymes to normal (Wolinsky et al., 1978a). Regular swimming activity results in increased specific activity of ACE and other hydrolases in aortic tissue, but no change of those in kidney or liver (Wolinsky et al., 1979). Experimental hypertension also produces increases in hydrolase activities, including that of ACE, in vascular tissue which return to normal when the blood pressure is restored to normal (Wolinsky et al., 1978b). To date, however, it has not been clear in what way, if any, the tissue effects of these various hormonal and mechanical stimuli are related to metabolism of the LDL molecule. The present studies were undertaken to obtain data about rates of decay of LDL from the circulation in two animal models, rats with experimental diabetes mellitus and exercised rats, that could be related to previously obtained information about tissue acid lipase activities in these...
models. Additional measurements of enzyme activity in liver and kidney tissues also were obtained when needed to complement previous results. These attempts were made in the hope that a pattern would emerge that suggested a relationship between clearance of LDL and tissue enzyme levels.

Methods

We used male Sprague-Dawley rats (Hilltop Lab Animals, Inc.) 6-7 weeks old at the outset (weight, 150–175 g). Protocols used for induction of diabetes mellitus and insulin treatment and for swimming activity and return to sedentary life were identical in most respects to those described earlier (Wolinsky et al., 1978a, 1979). In brief, streptozotocin-induced diabetes was the model and the diabetes was maintained for 4–8 weeks. Of the 8-week group, a number of animals were treated with 6 units of long-acting insulin (Lente insulin; Iletin U-40, Eli Lilly) daily in late afternoon for 4–7 weeks; age-matched controls and untreated diabetics were kept for comparison. The presence of diabetes and the effectiveness of insulin were monitored with determination of blood glucose levels and body weight changes.

The exercise program consisted of a swimming period of 1 hour per day for 6–8 weeks. As previously described (Wolinsky et al., 1979), a group of rats that had swum daily for 8 weeks was returned to a nonswimming sedentary life for an additional 7 weeks. All animals were fed standard laboratory chow (#5001, Ralston Purina Co.) and were given water to drink ad libitum.

Preparation of Labeled Lipoprotein

Human LDL was isolated from normal fasting plasma in 0.1% EDTA with a Beckman 40.3 rotor and the Beckman model L5-50 ultracentrifuge as described by Havel et al. (1955). To be certain of the appropriateness of the preparation for injection and recovery from rats, the density cut used was 1.030–1.050. The protein:cholesterol ratio in six different preparations averaged 0.797 ± 0.056. Protein determinations were made using the method of Lowry et al. (1951); cholesterol measurements were done by the method of Abell et al. (1952). Human LDL was shown to be uncontaminated with other lipoproteins by use of acrylamide-gradient slab gel electrophoresis (Swaney and Kuehl, 1976). Human lipoprotein was chosen for injection into rats because of (1) greater relative purity of isolated apoprotein B in this density cut compared to the rat (2) the relative abundance of human LDL compared to rat LDL in serum, and (3) the evidence that human LDL is catabolized with the same kinetics as native LDL in the rat (Sigurdsson et al., 1978).

Following ultracentrifugation, the 1.030–1.050 density material was dialyzed overnight against a 0.9% NaCl, 0.1% EDTA solution at pH 7.4. Based on a protein determination of this fraction, concentration of the lipoprotein by filtration through a collodion bag, (UH 100/25, Schleicher and Schuell, Inc.) with a pore size of <0.005 μm and a 25,000 molecular weight cutoff was carried out to give a final protein concentration of approximately 10 mg/ml. This concentrated human LDL was then labeled by a modification of the iodine monochloride method of McFarlane (1958) as described by Langer et al. (1972). Unbound iodine was removed by dialysis against 0.9% NaCl, 0.1% EDTA, 1 mM KI solution at pH 7.4 with frequent changes of solution. Later solutions were free of KI. After dialysis overnight, the 125I-LDL preparations all contained less than 3% free 125I, as determined by precipitation with 20% trichloroacetic acid (TCA) in the presence of carrier albumin. Labeled lipoprotein lipids were determined by the method of Bligh and Dyer (1959). Lipid labeling amounted to no more than 8.0% of total counts in the LDL.

The efficiency of iodination averaged 25.0 ± 3.2% (n = 5) for human LDL. 125I-Labeled LDL was indistinguishable from nonlabeled LDL with respect to migration on acrylamide-gel electrophoresis.* For every LDL preparation, more than 91% of all radioactivity was associated with the apoprotein B; this was determined by comparing the position of the apoprotein B on stained gels with the distribution of label in slices of an adjacent unstained channel in the gel (Fig. 1). Specific activities of iodinated human LDL preparations ranged from 20 to 35 μCi/mg of LDL protein.

Each iodinated LDL preparation was passed through a 0.45-mm Millipore filter (Millipore Corp.) prior to determination of the protein concentration and injection into rats of three experimental groups. The final injection volume per rat ranged from 0.075 to 0.15 ml, depending on protein content of a given preparation, and contained a total of 5.2–9.7 μCi (0.16–0.29 mg LDL protein). Six preparations were injected from 96 to 120 hours after the initial blood sample had been obtained; one preparation was injected as long as 144 hours later.

Experimental Protocol

Rats of the various experimental groups described above were injected via the jugular vein while in a nonfasting state and under light ether anesthesia. They had been given KI in their drinking water for 48 hours previously. Blood samples (0.5 ml) were obtained from the tail 5 minutes after injection, every 20 minutes for the first hour, then at 2, 4, 6, 8, 18, 20, 22, 24, and occasionally at 26 hours, for a usual total of 12 bleedings or 6 ml. Based on calculated blood volumes of approximately 35 ml in these rats (hematocrit assumed to

* To localize the label, acrylamide-gels were frozen on dry ice and sliced with a model 190 Bio-Rad gel slicer. Each slice was placed in a scintillation vial, moistened with 0.1 ml of water, and the label extracted by incubation in 1.0 ml of an 0.5 M Protosol solution (New England Nuclear) for 3–4 hours at 50–55°C. Samples were counted in 0.7% Omnifluor (New England Nuclear) in toluene.
be 50%) and pilot studies that showed the decay curve not to be influenced by reducing bleedings to as few as five, we believe that this amount of blood loss is without effect on the results. For each bleeding, the rats were briefly and lightly anesthetized; tourniquets were placed on the tails afterward to prevent oozing. Food and water consumption and urine output were monitored while animals were maintained individually in metabolic balance cages.

Each blood sample was centrifuged and plasma counts determined on duplicate 0.1-ml samples. In one experiment, two animals of each group were injected with iodinated LDL and were exsanguinated 6 hours later. In all groups, reisolation of lipoproteins of density range 1.030–1.063 by ultracentrifugation and evaluation by acrylamide gel electrophoresis showed more than 95% of all label in the circulation at this time to be in this density cut. More than 85% of all counts were associated with apoprotein B when assessed by counting on gel slices (Fig. 2). In these preparations, more than 90% of counts were TCA precipitable. These data indicate that conversion of LDL to other lipoproteins or the presence of circulating degradation products were not major sources of error. These findings are in agreement with previous detailed analyses showing that LDL is an “endpoint” of the metabolism of circulating lipoproteins (Schaefer et al., 1978).

Determination of Plasma Volumes

For three rats each from the control, diabetic, and exercise groups, blood volume determinations were made by the Evans Blue dilution procedure (Chinard, 1951). Mixing of dye in the blood was complete at 5 minutes, as was confirmed by obtaining very similar spectrophotometric values for Evans blue concentration at 5-, 10-, and 15-minute intervals after injection. A preinjection plasma sample was used as the blank for these determinations because of the contribution of plasma to the absorbance. In each instance, Evans blue of known amount was added to the preinjection plasma and shown by subtraction of plasma blank to fall on the standard curve. The average values for plasma volume as a percentage of body weight are 4.10 for controls, 4.65 for diabetics, and 4.18 for swimmers. Final body weights were as follows (mean ± sd): controls, 433 ± 28 g; diabetics, 287 ± 30 g; insulin-treated diabetics, 481 ± 46 g; swimmers, 418 ± 34 g; and swimmer-sedentary rats, 542 ± 49 g. For insulin-treated diabetics and swimmers returned to sed-
enteric life, the assumption was made that control values of plasma volume as a percentage of body weight were most likely to be accurate.

**Calculation of Total LDL Pool**

Although not critical to the aims of this study relating to fractional catabolic rate and tissue enzymes, an attempt was made to estimate total LDL-apoprotein pools in these animals. This value permits estimation of the contribution of steady state synthesis and catabolism to circulating LDL levels (Mathews, 1957). In three separate experiments, plasma from three rats each of the control, diabetic, and swimmer groups was subjected to sequential ultracentrifugation and the density fraction of 1.030-1.050 isolated. This fraction was studied by acrylamide gel electrophoresis (Fig. 3) and protein-cholesterol ratios were calculated. As seen in Figure 3, apoprotein B (concentrated 10-fold in the 1.030-1.050 range) is the major protein constituent, with some contribution from apoprotein E. Little apoprotein B was seen in the density cut of either <1.030 or >1.050. These results agree well with those shown by Swaney and Kuehl (1976). Protein: cholesterol ratios in the 1.030-1.050 fraction averaged 1.10 for control, 1.06 for diabetic, and 1.02 for swimming animals (n = 3 for each). The average plasma LDL protein concentration, based on the assumption that this fraction contained all circulating LDL, was 5.9 mg/dl for control, 6.2 for diabetic, and 4.3 for swimming animals. The values in controls are about double those reported by Faergeman et al. (1975) in rats fed another diet. However, when our values are used in conjunction with our fractional catabolic rates, calculated steady state LDL synthesis in our rats is found to be very comparable to that obtained by that group (see below). Furthermore, our calculated protein:cholesterol ratios on this 1.030-1.050 fraction are comparable to those reported for normal rat LDL by Bar-On et al. (1976) and Havel et al. (1955), although the LDL density range was defined slightly differently in each of those studies.

When we attempted to recover more circulating LDL by expanding the density cut to 1.030-1.063, we encountered problems described by others. Whereas the apparent amount of electrophoretically identifiable apoprotein B was slightly greater as seen on acrylamide gels (Fig. 3) with none of this material now seen in the 1.063-1.21 cut, apoprotein E and other lower molecular weight proteins were increased markedly and protein values on this preparation became markedly variable from sample to sample. This reflects the previously described contamination with proteins characteristic of HDL as shown by Lasser et al. (1973) on detailed ultracentrifugal studies and by Swaney and Kuehl (1976) on detailed electrophoretic analysis. In any case, we recognize the errors inherent in attributing the protein of the 1.030-1.050 fraction solely to LDL in our animals, but show the values after duly cautioning the reader. It should be remembered, however, that uncertainties surrounding this calculation do not influence the calculation of fractional catabolic rates, the major aim of this paper. The total intravascular LDL-apoprotein pool in each animal was computed from the product of the average LDL-protein concentration of that group and the calculated plasma volume of each individual rat.

The FCR, or fraction of intravascular pool catabolized per hour, reflects irreversible loss from the circulation of the protein under study, assuming equilibration of intravascular and extravascular pools (Mathews, 1957). Curve-peeling was done after the least squares method had been used to optimize curve fitting. As others had found for the rat (Sigurdsson et al., 1978), pig (Sniderman et al., 1974), and human (Langer et al., 1972), the plasma decay could be described by a biexponential function in which $\text{FCR} = 1/(C_1/b_1 + C_2/b_2)$ (Mathews, 1957). The half-life of LDL was calculated from the terminal linear portion of the decay curve (exponential 1).

Steady state synthetic rate (or absolute catabolic rate) was calculated as follows: synthetic rate = FCR × intravascular LDL-apoprotein. This rate is expressed both in absolute terms (µg apoprotein per hour), and normalized for body weight (per 100 g), the latter in view of the large difference in body weight between control and diabetic groups.

**Figure 3** Comparison of acrylamide-gel apoprotein patterns of rat lipoproteins isolated at density 1.030-1.050 and density 1.030-1.063, shown on the left and right, respectively, of each pair of gels. C = control, D = diabetic, E = exercised rat plasmas. High molecular weight standards are on the far right. Note how much more of the smaller molecular weight proteins relative to the typical heavier LDL apoproteins are present in the broader cut (right side of each pair). All LDL preparations were concentrated 10-fold before placement on gel.
Hydrolytic Enzyme Activities in Tissues

Measurements of aortic enzyme activities were repeated in these experiments and, although not shown here, the results were very similar to those reported previously for these models (Wolinsky et al., 1978a, 1979). In summary, specific activities of all aortic acid hydrolases studied, including ACE, were decreased in animals with streptozotocin-induced diabetes mellitus and were restored to normal by insulin treatment. In swimming rats, most activities were increased above control levels, ACE being one of the most affected. After the rats had returned to a sedentary life, the level of the acid lipase, but not that of other hydrolases, returned to control levels.

Methods of preparation of kidney and liver homogenates and assay conditions for four enzymes (neutral α-glucosidase and lysosomal β-galactosidase, N-acetyl-β-glucosaminidase, and acid cholesteryl esterase) in each of these tissues in controls and swimmers are reported elsewhere (Wolinsky et al., 1979). In the present experiments, optimum conditions for enzyme assays in these tissues were similar to those of aortic tissue in all respects, not only for control, but also for diabetic and swimmer groups; insulin treatment also did not influence optimal incubation conditions. Optimal conditions reported previously for demonstration of acid cholesteryl esterase activities in aorta, liver, and kidney were identical to those found here, with the exception that the pH optimum in all three tissues of these rats had shifted from 4.5 to 4.2. [The Hilltop Lab strain of rats is identical to that previously obtained from Marland Farms (Wolinsky et al., 1978a).] For the assay, a liposome preparation incorporating radioactive cholesteryl oleate was used as substrate (Brecher et al., 1976) as described previously (Wolinsky et al., 1978a). DNA measurements were made on aliquots of each homogenate by the method of Burton (1956), using d-ribose and calf thymus DNA as standards. In several instances, aorta, liver, and kidney tissues from the same animals were removed and assayed for specific activity of acid cholesteryl esterase.

Fractionation into cortical, medullary, and glomerular fractions was carried out on kidneys obtained from control and diabetic groups. Cortex and medulla were separated by sharp dissection along the corticomedullary junction that was easily discerned after the kidneys had been divided coronally. A glomerular fraction of cortex was isolated by the method of Heald and Hollis (1976), which employs sequential filtration of cortex homogenates through metal screens. Modification of this method for our purposes included use of saline for the initial isolation of glomeruli with later substitution of a sucrose (0.25 M)-EDTA (1 mM) solution for collection and homogenization. The purity of glomerular fractions was confirmed by phase microscopy before homogenization. Specific activities of the four enzymes measured on total kidney were also determined for each kidney fraction, based on previously described assay procedures for DNA and for glycosidase and acid lipase activities (Wolinsky et al., 1978a).

Under the incubation conditions used, all enzyme activities of aorta, kidney (and kidney fractions), and liver were linear as a function of time, protein, or DNA concentration. Enzyme activities are expressed as milliunits of activity, with one milliunit defined as 1 nmol of substrate hydrolyzed per minute at 37°C. All results are presented as mean ± SD. Statistical analyses of results were determined by Student’s non-paired t-test when two groups were compared, and by Duncan’s multiple range test (Duncan, 1955) when three groups were compared. In all cases, a P value of less than 0.05 was considered significant.

Results

Diabetes Mellitus

In previous work we had found that specific activities of aortic ACE and other hydrolases were decreased significantly in animals with experimental diabetes mellitus (Wolinsky et al., 1978a). When insulin treatment was given and the glucose intolerance was controlled, full restoration of aortic enzyme levels to normal was achieved. We have confirmed these results in the present study (not shown). However, we wished to examine hydrolases in other tissues for the effects of diabetes mellitus. It should be emphasized that the aortic tissue previously studied consisted of only one cell type, the smooth muscle cell, whereas most other tissues contain more than one cell type, which will complicate interpretation (see below). In Table 1, it is seen that, in liver homogenates, specific activities of lysosomal β-galactosidase and ACE are increased significantly in diabetics compared to controls, and that these increases are restored to normal or nearly so with insulin treatment. In the case of kidney, the results are somewhat more complicated (Table 1) in that ACE activity of whole kidney homogenates was increased in diabetics compared to controls, whereas the two glycosidases studied, including lysosomal β-galactosidase, were decreased significantly. We attributed this discrepancy to a summation effect of very different activities in different components of the kidney structure (Maunsbach, 1969), and we therefore proceeded to kidney fractionation. As can be seen in Table 2, when cortex and medulla were separated, all lysosomal enzymes in the medulla then were increased, with the direction of change in glycosidases then in agreement with that of ACE. Note, however, that the degree of increase in ACE differs from that of whole kidney. In the isolated cortex, the discrepancy in direction of change in the acid lipase and the three
glycosidases studied remained. Glomeruli next were isolated from the cortex in an attempt to resolve this discrepancy further (Table 2), but no further progress was made. Enzymes in the glomeruli of diabetic animals were no different from those of control animals, with the exception of ACE, which was increased. We believe that the results in studies of whole kidney and cortex probably are due to the presence of cortical components with very different levels of hydrolase activities, particularly of the acid lipase in relation to the glycosidases. Although full resolution of these individual contributions has not been accomplished, it seems inescapable that the increase in ACE activity of kidney is a characteristic of the diabetic state, whether one examines whole kidney or any of the three components studied. Mixing experiments done on homogenates of kidneys and cortices from control and diabetic animals (Table 3) seemed to rule out the possibility of nonspecific activation of ACE in diabetes. Insulin treatment restored kidney ACE activity to normal (Table 1).

The FCR of radioiodinated human LDL clearly was increased over control values in rats with streptozotocin-induced diabetes mellitus (Fig. 4; Tables 4 and 5). The calculated half-life of the LDL molecule was correspondingly and significantly shortened. Given the estimated slight increase in circulating plasma LDL levels in diabetics, steady state synthesis also was calculated to be significantly increased over the control level, when expressed on a normalized basis (per 100 g body weight). The correlation coefficient between plasma glucose level and calculated FCR in eight diabetic rats was + 0.72 \( (P < 0.05) \).

Insulin treatment of diabetic rats for several
weeks resulted in total return of the FCR of LDL to control values (Table 5). Correspondingly, the mean half-life of circulating LDL was also at the control level (Table 4). Based on an assumed return of the pool of LDL in the plasma to normal, the expected return to normal of steady state synthesis of LDL (normalized) in insulin-treated animals was seen.

To summarize this experimental model, note that aortic ACE activity decreased at the same time that liver and kidney ACE activity increased in diabetic rats, and that directions of change were reversed with insulin treatment. Furthermore, the dynamic studies of FCR showed an increase in the diabetic and a return to normal in the insulin-treated rats, corresponding to the measured enzyme levels of the nonvascular tissues examined.

Swimming Rats

We had reported previously that specific activities of neutral α-glucosidase and lysosomal β-galactosidase and ACE were increased in aortas from rats engaged in a chronic 1-hour/day swimming regimen compared to sedentary controls (Wolinsky et al., 1979). When livers and kidneys from these same animals were studied, enzyme levels were no different from those of controls (Wolinsky et al., 1979).

When FCR of radioiodinated human LDL was measured in swimmers, no change from the rate found in age-matched sedentary controls was seen (Tables 4 and 6). The decay curves were essentially superimposable and the calculated half-lives also were similar in swimmers and nonswimmers. The estimated circulating plasma LDL level is lower in swimmers (Table 6), as is total plasma cholesterol (Wolinsky et al., 1979). In combination with the unchanged FCR, calculation of steady state LDL synthesis gives a normalized value significantly less than that of control rats.

When these same studies were carried out on swimmers returned to a sedentary life, the FCR remained the same; plasma volume and LDL pool sizes were larger due to the greater body weights of the sedentary animals (Table 6). It should be noted again that (1) aortic ACE specific activity changed in association with swimming, whereas ACE activity in two other organs did not and (2) the measured FCR of LDL did not change, again corresponding to the enzyme results obtained on nonvascular tissues.

Finally, these studies afforded us the opportunity to compare relative specific activities of ACE and other hydrolases in aorta, kidney, and liver, keeping in mind the possibility that results with our substrate may not totally reflect in vivo conditions. As can be seen in Table 1, for control rats obtained from Hilltop Labs, values for specific activities of ACE (mU/mg DNA) are much higher in liver (33.06) and kidney (4.20) than was found in aortas of these rats (0.16; Wolinsky et al., unpublished observations). These results are similar to those obtained previously for ACE and other hydrolases in these tissues (Wolinsky et al., 1979). Thus, for ACE, the level is at least 1 order of magnitude higher in kidney and at least 2 orders of magnitude greater in liver than it is in aorta. Furthermore, our findings that profiles of pH curves of ACE in these tissues obtained from the same control animal were similar (Wolinsky, 1980), and that conditions for optimization also were identical, both strongly sug-

**TABLE 3 Enzyme Activity of Mixed Control and Diabetic Kidney Homogenates**

<table>
<thead>
<tr>
<th>Proportions*</th>
<th>Acid cholesteryl esterase activity (mUf/mg DNA)</th>
<th>Predicted</th>
<th>Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Diabetic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>0.75</td>
<td>3.53</td>
<td>3.62</td>
</tr>
<tr>
<td>0.75</td>
<td>0.25</td>
<td>3.92</td>
<td>3.96</td>
</tr>
<tr>
<td>0.5</td>
<td>0.5</td>
<td>4.31</td>
<td>4.44</td>
</tr>
<tr>
<td>0.25</td>
<td>0.75</td>
<td>4.70</td>
<td></td>
</tr>
</tbody>
</table>

* Stated proportions of kidney homogenates from control and streptozotocin-diabetic rats were mixed to a final volume of 0.1 ml and incubated with 0.1 ml of substrate. The activities of different mixtures were compared with those calculated from assays performed on pure diabetic and pure control homogenates. All observed activities were within the linear range of the assay.

† nmol substrate hydrolyzed/min.

**FIGURE 4 Plasma decay curves of human 125I-labeled LDL in control and diabetic rats. Circles reflect the mean of eight animals in each group and hatched areas indicate mean ± 1 SD. C1 and C2 refer to Y intercepts of first and second exponentials, respectively (see text and Table 4 for details).**
suggest that the same enzyme(s) is being studied in all three tissues.

**Discussion**

Several assumptions underlie the present experiments, and they should be clarified before proceeding. First, human LDL was injected into rats to determine catabolic rates. Although some have reported human LDL to be catabolized at the same rate as rat LDL by the perfused rat liver (Sigurdsson et al., 1978) and when injected into rats (Chao et al., 1979), and although others have found the same half-life of approximately 15 hours for human LDL injected into the rat (Eisenberg et al., 1973), as was found here, human LDL has been shown to be catabolized at a faster rate than homologous LDL when injected into the dog (Langer et al., 1972). From our re-isolation studies, we believe that injected LDL comiled with rat LDL and was removed from the circulation in like fashion to the native lipoprotein. Second, we considered changes in cellular lysosomal ACE levels in various tissues to reflect tissue catabolic capacity. It is also possible, of course, that these enzyme levels reflect catabolism of cholesteryl-ester containing lipoprotein classes other than LDL. For example, liver and kidney lysosomes may be important catabolic sites of certain subtractions of canine high-density lipoproteins (Nakai and Whayne, 1976). In both the diabetic and swimmer rat groups studied here, changes in measured LDL levels were mirrored in direction by both total serum cholesterol and high-density lipoprotein-cholesterol levels. In diabetes mellitus, they all were increased whereas, in exercise, all decreased (Gidez, L., and Wolinsky, H., unpublished observations). It is also true that the

**Table 4 Metabolic Parameters: Control Rats**

<table>
<thead>
<tr>
<th>Plasma volume (ml)</th>
<th>Fractional catabolic rate (%/hr)</th>
<th>Biological half life (t½) (hours)</th>
<th>Total intravascular LDL-apoprotein pool (µg)</th>
<th>Synthetic rate µg/hr</th>
<th>µg/hr per 100 g body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.6</td>
<td>7.76</td>
<td>10.5</td>
<td>917.5</td>
<td>71.2</td>
<td>18.7</td>
</tr>
<tr>
<td>19.0</td>
<td>6.58</td>
<td>13.6</td>
<td>1120.1</td>
<td>73.7</td>
<td>15.9</td>
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<tr>
<td>18.8</td>
<td>6.05</td>
<td>17.4</td>
<td>1108.3</td>
<td>67.1</td>
<td>14.6</td>
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<tr>
<td>18.0</td>
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<td>1095.9</td>
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<td>17.8</td>
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<td>16.9</td>
<td>5.12</td>
<td>14.0</td>
<td>992.3</td>
<td>50.8</td>
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<tr>
<td>17.3</td>
<td>5.18</td>
<td>14.0</td>
<td>1018.8</td>
<td>52.8</td>
<td>12.5</td>
</tr>
<tr>
<td>Mean ± sd</td>
<td>17.7 ± 1.2</td>
<td>14.4 ± 2.1</td>
<td>1044.5 ± 67.7</td>
<td>62.8 ± 8.7</td>
<td>14.5 ± 2.2</td>
</tr>
</tbody>
</table>

* Based on plasma LDL-apoprotein concentration of 58.9 µg/ml.

**Table 5 Metabolic Parameters: Diabetic and Insulin-Treated Diabetic Rats**

<table>
<thead>
<tr>
<th>Plasma volume (ml)</th>
<th>Fractional catabolic rate (%/hr)</th>
<th>Biological half life (t½) (hours)</th>
<th>Total intravascular LDL-apoprotein pool (µg)</th>
<th>Synthetic rate µg/hr</th>
<th>µg/hr per 100 g body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetics</td>
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<td></td>
</tr>
<tr>
<td>14.0</td>
<td>8.98</td>
<td>10.6</td>
<td>870.0*</td>
<td>77.3</td>
<td>25.8</td>
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<td>14.1</td>
<td>7.34</td>
<td>12.2</td>
<td>878.7</td>
<td>64.5</td>
<td>21.3</td>
</tr>
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<td>12.1</td>
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<td>757.1</td>
<td>71.5</td>
<td>27.4</td>
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<td>7.78</td>
<td>10.3</td>
<td>899.3</td>
<td>70.0</td>
<td>22.6</td>
</tr>
<tr>
<td>11.4</td>
<td>7.44</td>
<td>11.4</td>
<td>713.5</td>
<td>53.1</td>
<td>21.6</td>
</tr>
<tr>
<td>11.8</td>
<td>5.53</td>
<td>13.9</td>
<td>736.5</td>
<td>40.7</td>
<td>16.0</td>
</tr>
<tr>
<td>15.2</td>
<td>6.48</td>
<td>12.1</td>
<td>948.6</td>
<td>61.5</td>
<td>18.8</td>
</tr>
<tr>
<td>13.8</td>
<td>7.37</td>
<td>11.4</td>
<td>861.3</td>
<td>63.5</td>
<td>21.4</td>
</tr>
<tr>
<td>Mean ± sd</td>
<td>13.4 ± 1.4†</td>
<td>11.4 ± 1.4†</td>
<td>833.1 ± 85.7†</td>
<td>62.8 ± 11.5</td>
<td>21.9 ± 3.6†</td>
</tr>
</tbody>
</table>

* Based on plasma LDL-apoprotein concentrations of 62.4 µg/ml for diabetics and 58.9 µg/ml for insulin-treated diabetics.

† P < 0.05 for comparison vs. control group.

‡ P < 0.05 for comparison vs. diabetic group.
lack of change in enzyme levels seen in nonvascular tissues of swimming rats does not necessarily signify that LDL catabolism remained constant; more lipoprotein could be catabolized by the same amount of enzyme. However, this study was aimed at producing large changes in tissue catabolic capacity in the hope of discerning patterns of response that could be correlated with FCR of injected LDL. That has been possible, notwithstanding possible coexistent increases in catabolic rates that were not detectible as increased enzyme levels. Finally, it could be argued that we have perturbed the rather small LDL pool in the rat by injecting $^{125}$I-labeled small LDL pool in the rat by injecting $^{125}$I-labeled material into the circulation and have thereby influenced catabolic rates. However, it will be seen that, compared to the total estimated intravascular LDL pool of approximately 1.0 mg in our mature control rats, the injected LDL-protein amounted to no more than approximately 25%.

We believe that these studies represent an initial step in attempting to link decay of circulating LDL to the catabolic capacities of various tissues, including the vascular tree. Whatever the contribution made by errors in the above assumptions, it is striking from our results that, in a variety of experimental situations, levels of ACE and other hydrolyses in the blood vessel wall do not correspond to the change or lack of change of the same enzymes present in other actively metabolizing organs. In addition, the specific activity of ACE in vascular smooth muscle cells is 1 to 2 orders of magnitude lower than that of cells in liver or kidney from the very same animals. Given the good agreement between FCR and acid lipase levels in the latter tissues, we would cautiously submit the following working postulate (Wolinsky, 1980). Nonvascular tissues may be responsible for catabolism of circulating LDL, with the rate of clearance being reflected in measured tissue lipolytic and proteolytic activities. Vascular tissue, on the other hand, is a minor factor in overall lipoprotein catabolism and, more importantly, does not follow the pattern of response of other organs. However, the catabolic capacity of arterial tissue does play a critical role in maintaining the local influx-efflux balance in the arterial wall with respect to incoming materials, particularly those containing lipid. Circulating LDL levels are only one component, albeit an important one, in this equation for the blood vessel wall. Factors that influence endothelial permeability and thereby greatly increase the rate of influx, and factors that influence metabolic activity of the arterial tissue, and perhaps limit efflux, also are potential sources of perturbation of the balance. Hypertension and diabetes mellitus, respectively, are two likely examples of these factors (Wolinsky and Fowler, 1978). Arterial tissue might be particularly vulnerable to lipid accumulation because of the small likelihood that determinants of cellular metabolic activity will be coordinated fully with occurrences of hemodynamic events that markedly affect rates of influx of circulating materials. Another pattern that is likely to be deleterious to this balance in vascular tissue might be simply the coexistence of elevated circulating LDL levels, a decreased FCR for LDL (Cnoop-Koopmans and Angel, 1978) and slightly reduced catabolic capacity of arterial tissue, as occurs in experimental hypothyroidism (Wolinsky et al., unpublished observation). The findings of increased synthesis and decreased FCR of LDL in homoygous familial hypercholesterolemia (Bilheimer et al., 1979) also deserve closer scrutiny in terms of vascular and non-vascular tissue lipase levels.
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