Aortic Lipoprotein Lipase Activity in Relation to Species, Age, Sex, and Blood Pressure

By Samuel Mallov, Ph.D.

It has been suggested that the function of the enzyme lipoprotein lipase (LPL), which occurs in a number of tissues and organs, is to hydrolyze the triglyceride moieties of circulating chylomicrons and/or low density beta-lipoproteins at the surfaces of tissues or within cells. The free fatty acids (FFA) liberated may be used either catabolically for the production of energy or anabolically for the synthesis of new triglycerides and other lipids. Since circulating lipoproteins may be involved in the etiology of atherosclerosis, it has been proposed that LPL activity may also play a role in atherogenesis. Thus arteries possessing low LPL activities may be more susceptible to the deposition of circulating lipoprotein lipids and consequent atherosclerotic plaque formation than arteries with relatively high LPL activities; in the latter case, the greater enzyme activity may promote a more rapid hydrolysis of triglyceride, with a consequent rapid utilization of the liberated fatty acids, and thus prevent direct deposition of unhydrolyzed lipid. Therefore, it would be of interest to learn whether an inverse relationship actually exists between arterial LPL activity and the tendency for arteries to become atherosclerotic in a number of different circumstances. Hence, determinations were made of the activities of the enzyme in the aortas of animals under conditions associated with a lesser or greater tendency for atherosclerosis to develop. Specifically, aortic LPL activity was measured in two animal species showing markedly different tendencies to develop experimental atherosclerosis. Both males and females of these species were used since, in some species at least, males are more apt to exhibit the disease than females. Young and old animals of the same species were used since the incidence of atherosclerosis generally increases with age. Finally, hypertensive as well as normotensive animals were used inasmuch as hypertension is associated with an increased incidence and severity of atherosclerosis in humans and in some animals.

Methods

Holtzman albino rats and New Zealand white rabbits of both sexes and of three age groupings were employed. At the time of sacrifice, the young rats and rabbits were 4 to 5 weeks, the adult rats 10 to 12 weeks and the adult rabbits 18 to 20 weeks old, while the older animals were all over one year of age. The rats were maintained on Purina Laboratory Chow, while the rabbits were given Purina Rabbit Chow without antibiotics, ad libitum.

Hypertension was produced in a number of rats and rabbits by tying a figure-of-eight ligature around the left kidney and removing the right kidney at the same time, following the induction of anesthesia with ether. Sham operations were performed on the controls. Other rats were made hypertensive by weekly intramuscular injections of 5 mg of desoxycorticosterone trimethylacetate (DCA) and replacement of drinking water with 1% NaCl solution. The DCA-salt hypertensive rats were sacrificed six to eight weeks after the initial DCA injections, and the renal hypertensive rats and rabbits, three to four months after operation. Blood pressures of the rats were determined every two weeks on nonsedated animals by means of the microphonic manometer tail-cuff method, while blood pressures of the rabbits were taken directly by cannulating the femoral arteries, following the induction of light pentobarbital anesthesia. Blood pressures of the rabbits were recorded on a calibrated Grass polygraph with the aid of a pressure transducer.
The animals were sacrificed by exsanguination following the induction of deep anesthesia with pentobarbital. The entire aorta, extending from the aortic arch to the junctions of the external iliac artery, was removed from the rats, and sections of both thoracic and abdominal aorta were removed from the rabbits. These tissues were placed in physiological saline solution in petri dishes standing on ice, and all extraneous fat and connective tissue then dissected away. The cylindrical pieces were slit open lengthwise, washed twice in fresh portions of ice-cold saline, and blotted with filter paper. One hundred and twenty to 140 mg sections were then removed, weighed rapidly on a Roller-Smith torsion balance, and carried in beakers over ice to a cold room (2 to 3°C) where they were homogenized in cold saline with precooled mortars and pestles and a little clean white sand. In the young rats, it was necessary to combine the aortas from two rats to obtain enough tissue for homogenization as a single sample. Each homogenate was diluted to a final volume of 12 ml. All of the 0.9% NaCl solution used was preadjusted to a pH of 8.5.

The homogenates were then removed from the cold room, and 1 ml aliquots of these were added to 25 ml Erlenmeyer flasks containing media that had been preincubated at 37°C for 30 minutes. Each Erlenmeyer flask now contained, in addition to the tissue homogenate, 1 ml of 10% coconut oil emulsion (Ediol) in physiological saline at pH 8.5, 1 ml of 10% bovine albumin (pH 8.5), 1 ml of serum obtained from dogs fasted overnight, and 1 ml of 0.9% saline at pH 8.5. Oil emulsions preincubated with serum, form chylomicron-like particles, suitable as a substrate for determination of LPL activity. A pH of 8.5 has been reported to be optimum for LPL activity. The actual pH of the contents of the Erlenmeyer flasks, as measured, was 8.2. After the addition of the aliquots of homogenates to the media, and mixing, 1 ml aliquots were removed from the Erlenmeyer flasks for initial FFA determinations by the method of Dole. The flasks were then incubated at 37°C under air, in a Dubnoff metabolic incubator, with shaking, for 1 hr, at the end of which time additional 1 ml aliquots were removed for final FFA determinations. Controls containing 1 ml of 0.9% NaCl in place of 1 ml of homogenate were simultaneously incubated in order to correct for any FFA formed by factors other than those present in the aortic tissue homogenates. The differences between the final FFA and initial FFA values, expressed as μEq of FFA formed per gram of wet tissue per hour of incubation, were recorded as indices of enzyme activities.

In those experiments in which inhibition of lipoprotein lipase activity was determined, the incubation flasks contained, in addition to the usual components, either 5 mg/ml of protamine sulfate, or 1 M NaCl, the total volume of medium remaining the same.

To determine the effects of hypertension and age on dry weight and nitrogen content of aortic tissue, sections of aorta from hypertensive animals, old animals, young animals, and controls for hypertensives, were dessicated in an oven at 95°C overnight, and reweighed. Sections of wet and dried tissue were digested with sulfuric acid and hydrogen peroxide and, following dilution, Nesslerized for determination of total tissue nitrogen.

Results

LIPOPROTEIN LIPASE ASSAY

In order to determine whether there was a direct relationship between tissue lipase activity and FFA formed during incubation, in our assay system, different quantities of aortic tissue homogenate from the same animal were incubated with the usual substrate. As indicated in figure 1, a direct proportionality was observed between the quantity of tissue (and therefore of lipase) present in the incubation flask and the quantity of FFA liberated.

EFFECT OF AGE

Although the differences were not large, consistently smaller (about 14% less) LPL activities were shown by aortas of old male rats.
TABLE 1

Lipoprotein Lipase Activities of Homogenates of Rat and Rabbit Aortas

<table>
<thead>
<tr>
<th>Species</th>
<th>No.</th>
<th>Sex</th>
<th>Age class</th>
<th>Body weight</th>
<th>Type of animal</th>
<th>Lipase activities of aorta homogenates μ Eq FFA/g wet tissue/hr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total aorta</td>
</tr>
<tr>
<td>Rats</td>
<td>40</td>
<td>M</td>
<td>young</td>
<td>74 ± 2.2</td>
<td>normal</td>
<td>24.8 ± 1.76 *</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>M</td>
<td>young</td>
<td>116 ± 1.3</td>
<td>normal</td>
<td>25.2 ± 1.66</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>M</td>
<td>adult</td>
<td>323 ± 8.2</td>
<td>normal</td>
<td>24.9 ± 1.66</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>F</td>
<td>adult</td>
<td>261 ± 4.9</td>
<td>normal</td>
<td>24.5 ± 2.02</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>M</td>
<td>old</td>
<td>504 ± 6.8</td>
<td>normal</td>
<td>21.8 ± 0.86</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>M</td>
<td>old</td>
<td>454 ± 6.0</td>
<td>controls for renal hypertensives</td>
<td>21.3 ± 0.71</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>M</td>
<td>old</td>
<td>454 ± 7.3</td>
<td>renal hypertensives</td>
<td>27.3 ± 1.96</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>M</td>
<td>adult</td>
<td>299 ± 5.1</td>
<td>controls for DCA hypertensives</td>
<td>23.8 ± 1.83</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>M</td>
<td>adult</td>
<td>204 ± 6.0</td>
<td>DCA hypertensives</td>
<td>31.6 ± 1.42</td>
</tr>
<tr>
<td>Rabbits</td>
<td>12</td>
<td>M</td>
<td>young</td>
<td>701 ± 34.6</td>
<td>normal</td>
<td>14.9 ± 1.08</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>M</td>
<td>adult</td>
<td>3049 ± 64.6</td>
<td>normal</td>
<td>13.3 ± 1.22</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>F</td>
<td>adult</td>
<td>2911 ± 79.5</td>
<td>normal</td>
<td>11.3 ± 0.76</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>M</td>
<td>old</td>
<td>3593 ± 97.4</td>
<td>normal</td>
<td>8.9 ± 0.48</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>F</td>
<td>adult</td>
<td>2397 ± 117.4</td>
<td>renal hypertensives</td>
<td>17.3 ± 2.15</td>
</tr>
</tbody>
</table>

* Values are expressed as means ± standard errors of the means. The following groups are statistically significantly different: all normal young vs. all normal old rats (P<0.03); young vs. old male rabbits (P<0.01); DCA-salt hypertensive vs. control rats (P<0.01); renal hypertensive vs. control rats (P<0.01); renal hypertensive vs. control rabbits, abdominal aortas (P<0.01). Renal hypertensive vs. control rabbits, thoracic aortas (P<0.02); rats vs. rabbits (male, young, adult, or old) (P<0.01).

than by those of young male rats (table 1). Since there was no difference in activity between the two groups of young male rats studied, as well as between the two groups of old male rats (normals, and normal controls for the renal hypertensive rats), the data were combined in each of these cases. A comparison of the two groups indicated that there was a statistically significant difference between the lipase activities of the aortas of the young and old rats (P < 0.03). The aortas of the older rabbits showed distinctly lower lipase activities (41%) than those of the young rabbits (P < 0.01) (table 1). No significant differences were found in any of the experiments between the lipase activity of the thoracic aorta and abdominal aorta of the rabbits. The results were expressed in terms of LPL activity per unit of wet tissue weight. Determination of some dry tissue weights and total tissue nitrogens, as per cent of wet tissue weights, showed that the aortas of old rats and rabbits had significantly greater dry tissue and nitrogen contents than did the aortas of young rats and rabbits, respectively (table 2). Hence, if the LPL activities are expressed on a dry tissue weight or tissue nitrogen basis, the differences between the old and young animals are even greater than when the activities are expressed on a wet tissue weight basis.

EFFECT OF SEX

No significant differences in LPL activities were observed between either adult male and female rats or adult male and female rabbits (table 1).

SPECIES DIFFERENCE

A species difference was clearly manifested. Rabbit aortas exhibited markedly less LPL activities than did rat aortas. The activities were approximately twice as great in rats as in rabbits, when young males, adult males, adult females or old males of the two species were compared (table 1). These differences are somewhat reduced, but still significant, when comparison is made of LPL activities on a dry tissue weight or tissue nitrogen basis, since rabbit aortas have somewhat higher water contents than rat aortas (table 2). For example, aortas of old rats had a 21% greater dry weight content, but a 14% greater LPL activity, than aortas of old rabbits.
EFFECT OF HYPERTENSION

Aortas from both renal hypertensive and DCA-salt hypertensive rats showed significantly greater LPL activities than did aortas from their respective controls \((P < 0.01)\). The same was true for the aortas of renal hypertensive rabbits and their controls (table 1). No differences were observed in dry weight or nitrogen contents between aortas of DCA-salt hypertensive rats (which had the severest hypertension) and the aortas of their controls. This is assumed also to be true for the other hypertensive animals. Hence, the differences in LPL activities between hypertensive animals and controls remain the same when expressed on a dry tissue weight or tissue nitrogen basis as when expressed on a wet tissue weight basis. The blood pressures and heart weight per body weight ratios for the hypertensive and normotensive rats are given in table 3. In the DCA-salt hypertensive rats and renal hypertensive rabbits, the blood pressures were not only significantly elevated, but the hearts were hypertrophied, when compared with the controls. In the renal hypertensive rats, no significant hypertrophy of the hearts was observed.

EFFECT OF LIPOPROTEIN LIPASE INHIBITORS

It has been reported that both protamine sulfate and \(1 \times \) NaCl inhibit LPL specifically and significantly, although perhaps not completely. In order to test whether the lipase activity we were measuring was largely LPL activity, these inhibitors were added to eight incubation flasks containing homogenates of aorta from different animals, while eight other vessels with the same homogenates contained no inhibitors. It was found that protamine sulfate produced a mean inhibition of 50% (range, 36 to 67%), and \(1 \times \) NaCl a mean inhibition of 42% (range, 33 to 67%). These results indicate that at least a large part of the activity measured was due to LPL.

Discussion

A number of reports have appeared in the literature suggesting the involvement of LPL (the clearing factor) in the etiology of atherosclerosis. Thus, higher postabsorptive levels of triglyceride, higher levels of triglyceride and chylomicrons following a fatty meal, and lower postheparin LPL activities in plasma have been reported to occur in atherosclerotics than in normal subjects. These reports have been based on studies with blood or plasma. It has been demonstrated however, that LPL is present predominantly if not exclusively in tissues other than blood, and is only released into the circulation following the iv injection of heparin (and other substances), or sometimes after a fatty meal or the infusion of fat emulsions in vivo. Hence, the deficiencies of clearing activity or of LPL in the circulation in atherosclerotics may simply reflect a deficiency of the enzyme in the tissues. It is natural to be concerned with the tissue most immediately involved in atherosclerosis, arterial tissue.

Although some of our results are in accordance with the hypothesis that arterial LPL activity may play a part in atherogenesis, others are not. A species difference clearly exists with respect to aortic LPL activity. It is well-known that rats are quite resistant to the development of atherosclerosis, while the

### Table 2

**Effects of Age and Hypertension on Water and Nitrogen Content of Aortic Tissue**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Type</th>
<th>No. of animals</th>
<th>Aortas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dry weight, as % of wet tissue wt</td>
</tr>
<tr>
<td>Rat</td>
<td>normal old males</td>
<td>10</td>
<td>30.7 ± 0.29 *</td>
</tr>
<tr>
<td>Rat</td>
<td>normal young males</td>
<td>26</td>
<td>23.4 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>DCA hypertensive males</td>
<td>12</td>
<td>28.5 ± 0.47</td>
</tr>
<tr>
<td></td>
<td>controls for DCA hypertensives</td>
<td>12</td>
<td>28.8 ± 0.33</td>
</tr>
<tr>
<td>Rabbits</td>
<td>normal old males</td>
<td>6</td>
<td>23.4 ± 0.38</td>
</tr>
<tr>
<td>Rabbits</td>
<td>normal young males</td>
<td>6</td>
<td>17.7 ± 0.43</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Total nitrogen, as % of wet tissue wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>4.26 ± 0.074 *</td>
</tr>
<tr>
<td>Rat</td>
<td>3.67 ± 0.066</td>
</tr>
<tr>
<td></td>
<td>4.21 ± 0.075</td>
</tr>
<tr>
<td></td>
<td>3.56 ± 0.084</td>
</tr>
<tr>
<td>Rabbits</td>
<td>2.57 ± 0.065</td>
</tr>
</tbody>
</table>

* Values are expressed as means ± standard errors of the means.

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disease is readily inducible in rabbits. In our experiments rat aortas did indeed manifest much higher LPL activities than did rabbit aortas. Perhaps such high activity is protective. Zemplenyi and Grafnetter also found that when they incubated minced aortas with lipemic human serum, significantly higher activities were shown by minces of rat aorta than by those of rabbit. On the other hand, when these authors increased the tendency of rabbits to develop atherosclerosis by dietary procedures, the rabbit aortas actually showed significantly increased rather than decreased LPL activities.10

Our experiments also showed that in the case of both rats and rabbits, aortas of old animals possessed significantly less LPL activity than did the aortas of young animals of the same species. This too is suggestive of the possibility of a protective effect of high activity of the enzyme. Zemplenyi and Grafnetter have reported, however, that while minces of aortas from 24-month-old rats showed lower lipase activities than did minced aortas from 7-month-old rats, they showed no such reduced activities in comparison with the vessels of young (2-month-old) rats.17 We did not find aortas of mature adult, but not old, rats to have lower activities than aortas of very young rats; and in the case of rabbits, adult animals showed a decrease in aortic LPL activity when compared with young ones, this decrease becoming much more marked with advancing age. Drury, in contrast to our findings, reported that intact sections of aorta from 15- to 18-month-old rats manifested greater lipase activities (with coconut oil emulsion as substrate) than did sections of aorta from 3- to 4-month-old rats.18 However, when rats were first injected with heparin in vivo, the lipase activities of the aortas of young rats, as measured in vitro, increased, so that they were now greater than those of the older animals. We cannot explain the differences between our results and those of Zemplenyi and Grafnetter. Although the experimental techniques and substrates were somewhat different, one might expect the results to have been qualitatively the same. Our experiments with homogenates were somewhat different than those of Drury, who used sections of aorta and therefore determined activity due to enzyme released from the tissue slices into the medium or present at the surface of the tissue sections, whereas we measured total tissue lipase activity.

Our data, indicating similar LPL activities in aortas of males and females of both species of animals, argue against the idea that premenopausal human females show a smaller incidence of atherosclerosis than do males as a consequence of possessing higher arterial LPL activities.

Finally, contrary to our expectations, we...
found hypertension to be associated with a significant rise, rather than fall, in aortic LPL activity in both rats and rabbits. These results also are not in accord with the hypothesis that hypertension is accompanied by an increased incidence and severity of atherosclerosis as a consequence of decreased arterial LPL activity. The change in aortic LPL activity due to hypertension is of interest; it has been found in our laboratory and by others that a number of other functional alterations occur in the walls of aortas as a consequence of hypertension. The increased LPL activity in these walls is consonant with our findings that hypertrophied and metabolically overactive hearts also exhibit increased LPL activities, and that aortas of hypertensive rats become hypertrophied. Perhaps increase in size and/or metabolic activity of a number of tissues is accompanied by increased concentrations of an enzyme which may permit the tissues to extract larger supplies of FFA for metabolic purposes.

In our study (and in studies by others of other tissues) there was no certainty that LPL was the only lipolytic enzyme whose activity was being measured, although the substrate used is relatively specific for LPL and the pH was near the optimum for this enzyme. Studies with specific LPL inhibitors indicated that the latter caused 42 to 50% inhibitions. Since the inhibitors have never been reported to cause complete inhibition of the enzyme in tissues, it is difficult to know, from the above results, how much of the LPL present was being inhibited. We have found that heart homogenates, which are relatively rich in LPL, show 60 to 72% inhibition of LPL activity in the presence of protamine sulfate, and 87 to 89% inhibition in the presence of 1 M NaCl. Korn and Zemplenyi et al. have obtained evidence for the presence of lipases and/or esterases other than LPL in arterial walls. It is probably safe to say that at least 42 to 50%, and probably more, of the aortic lipase activity we measured was due specifically to the enzyme, which, although not purified, has been characterized as "lipoprotein lipase" on the basis of its properties.

Summary

It has been suggested that lipoprotein lipase (LPL) activity may play a role in the etiology of atherosclerosis. To determine whether there is an inverse relationship between arterial LPL activity and the tendency for arteries to become atherosclerotic, determinations were made of the activities of the enzyme in homogenates of the aortas of animals under conditions associated with a greater or lesser tendency for atherosclerosis to develop. It was found that the LPL activities of the aortas of old rats and rabbits were significantly lower than those of young animals of the same species, respectively. Rats, which are resistant to the development of atherosclerosis, manifested about twice the aortic LPL activities that goats did, the latter animals being notorious for the ease with which they develop the disease. This was true regardless of the age and sex of the animals compared. These results support the hypothesis that low arterial LPL activity may be associated with increased atherogenesis. Aortas of female rats or rabbits, however, showed no greater LPL activities than did aortas of males of the same species. Renal and desoxycorticosterone-salt hypertensions were accompanied by significant increases rather than decreases in aortic LPL activity. The latter results are not consonant with the aforementioned hypothesis.

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References

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