Lipoprotein Lipase and Hepatic Triacylglycerol Lipase Activities in Peripheral and Skeletal Muscle Lymph

Juren Huang, Charles H. Sloop, Paul S. Roheim, and Laurence Wong

We studied the interstitial fluid concentration of two lipid-metabolizing enzymes (lipoprotein lipase and hepatic triacylglycerol lipase) to determine their importance in interstitial modification of filtered lipoproteins. Despite the use of a very sensitive lipase assay (1 nmol of fatty acid release/ml/hr), lipase activities in plasma and in peripheral and skeletal muscle lymph from control dogs were below the sensitivity of our assay. After heparin injection, hepatic triacylglycerol lipase and lipoprotein lipase activities in plasma were similar. However, the postheparin hepatic triacylglycerol lipase activities in peripheral and skeletal muscle lymph were only 1.4% and 1.1%, respectively, those of plasma. This concentration is considerably less than the lymph concentration of albumin, which has a similar size to the lipases but has a lymph concentration of 30% to 40% of plasma. Lipoprotein lipase activity in peripheral lymph and skeletal muscle lymph was 2.7% and 4.8%, respectively, of plasma activity. Since lipoprotein lipase has a similar size as hepatic triacylglycerol lipase, the disproportionate amount of lipoprotein lipase in lymph as compared to hepatic triacylglycerol lipase could be due to heparin crossing the capillary endothelium and displacing lipoprotein lipase from peripheral cells. Injection of radioactive heparin confirmed that it does cross into the interstitial space in sufficient concentrations to displace lipase from peripheral cells. We conclude that most of the lipase found in lymph after heparin injection is derived from peripheral cells and not from plasma. Furthermore, hepatic triacylglycerol lipase does not play a role in high density lipoprotein remodeling in interstitial fluid. Therefore, it seems likely that the considerable remodeling of high density lipoprotein that we found previously results from its interaction with peripheral cells.

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heparin crossing the interstitial space and displacing de novo synthesized LPL into the lymph. Finally, we will provide evidence to suggest that heparin is capable of crossing the interstitial space, although in much smaller amounts than would be predicted from its molecular size.

Methods

Materials

Radioactive nickel and sodium borohydride were purchased from New England Nuclear (Boston, MA). Unless otherwise stated, all other chemicals were purchased from Sigma Biochemical (St. Louis, MO). Solvents were purchased from Baker (Philipsburg, NJ). Liquid scintillation fluid was obtained from Amersham (Arlington Heights, IL). Sodium heparin injectable (from bovine lung, 1000 IU/ml) was obtained from LyphoMed Inc. (Melrose Park, IL). The heparin used had a measured molecular mass of approximately 10 000 Da. Purified milk lipoprotein lipase was a gift from George Melchior, The Upjohn Company, Kalamazoo, MI.

Analytical Methods

Our lipase assay has been described previously. The assay could detect lipase activity down to the release of 1 nmol of fatty acid per unit of time. This sensitivity was sufficient to detect 0.001% of the peak plasma LPL and HTL activity. For this study, the incubations were for 1 hour. To assay for HTL activity, all the reagents contained 1 M of NaCl. In addition, the pH was shifted to 8.6 from 8.2. Under normal conditions, 100 μl of a 1:200 dilution of postheparin plasma was used for the assay. Lymph was used either undiluted or at a 1:10 dilution. For the assay of preheparin plasma, 100 μl of plasma was used undiluted. The activator used in LPL assays consisted of 25 μl of a heat-inactivated plasma (in a total assay volume of 400 μl). Postheparin lipase was prepared by injecting 100 IU/kg body weight heparin intravenously into a dog and collecting the plasma 30 minutes after the heparin injection. LPL activity was calculated by subtracting postheparin lipolytic activity from HTL activity that was determined in the presence of 1 M of sodium chloride.

The titration of heparin was based on the method described by Hatton et al. Briefly, 10 ml of heparin (1000 IU/ml) was incubated with 1 ml of a 1 M Tris HCl (pH 8.0), 10 mCi of tritiated sodium borohydride, and 2.5 ml of sodium cyanoborohydride for 4 hours at 25°C. The sample was then diluted with 1 ml of saline, and 2.5 ml each was filtered through PD-10 (Sephadex G-25) columns. The eluate was then dialyzed twice with 1 l each of saline. The tritiated heparin contained 600 IU/ml of heparin and had a radioactivity of 1.1×10^7 dpm/ml. After titration, the heparin was tested for its ability to displace lipases in the rat. Radioactive and nonradioactive heparin released essentially identical amounts of the lipases. The heparin concentration was determined by the method of Smith et al.

Collection of Lymph and Heparin Infusion

Peripheral lymph was collected by methods which have been described previously. Control, heart worm-free dogs were anesthetized with sodium pentobarbital (30 mg/kg) and were placed on their sternums with their hind legs pronated. A 2 cm incision was made directly over the lateral saphenous vein. The subcutaneous tissue on either side of the vein was ligated to distend the prenodal lymphatics, which were then dissected for cannulation. Flared polyethylene tubing (0.58 mm i.d.×0.97 mm o.d., Clay Adams) was used for the cannulas. This preparation collects prenodal lymph derived mainly from subcutaneous connective and adipose tissue on the dorsum of the paw (peripheral lymph).

Skeletal muscle lymph was collected by the method of Bach and Lewis adapted to the dog. Collection of peripheral lymph prevents it from contaminating femoral lymph and allows collection of lymph derived primarily from skeletal muscle. Small blebs of sodium fluorescein solution were placed directly under the surface of skeletal muscles exposed by an incision over the femoral triangle. This allowed direct visualization of individual skeletal muscle lymphatics and their union with the femoral lymphatic. In several experiments, injection of 50 μl of concentrated Evans blue dye solution into the dorsum of the paw almost immediately turned the peripheral lymph dark blue, while there was no detectable color change in simultaneously collected skeletal muscle lymph. In agreement with previous investigators, the total plasma protein concentration of skeletal muscle lymph was higher than that of peripheral lymph. Lymph flows were approximately 1 ml/hr for skeletal muscle and 2 ml/hr for peripheral lymph.

For constant heparin infusion experiments, a priming dose of 100 IU/kg was administered to the dogs, followed by a constant infusion of 1.65 IU/kg/min for the duration of the experiment. All the experiments involving animals or animal surgery were reviewed and approved by the Animal Care and Use Committee of Louisiana State Medical Center.

Results

The LPL or HTL activities in the plasma and lymph of control dogs were below the detectable range of our assay. Lipase activity was detected both in plasma and lymph only after intravenous injection of heparin (100 IU/kg body weight). The plasma distributions of HTL and LPL activities were approximately equal.

To determine if there were any activators or inhibitors of lipase activity in peripheral or skeletal muscle lymph, we added them to lipases obtained from two sources: purified milk LPL and postheparin plasma from normal dogs. The data are summarized in Table 1. When 100 μl of a 1:100 dilution of dog postheparin lipolytic activity (PHLA) plasma was used (Table 1, column 1) in the presence of 50 μl or 80 μl of undiluted lymph, no detectable difference in lipolytic activity was noted. When purified milk LPL was used, the presence of an activator of LPL (heat-inactivated plasma, 25 μl in a total assay volume of 400 μl) caused the milk lipase to hydrolyze 110 μmol of fatty acid per milliliter per hour (Table 1, column 2) which was unchanged by the addition of lymph. Thus, it would appear that lymph contained no
The concentration of activators of LPL in lymph is approximately 10 times higher than the lymph ordinate on the right. In vertebrates, the peak plasma lipolytic activity was 12.47 μmol/ml/hr fatty acid was released. This is similar to the peak peripheral lymph lipase activity, 0.226 μmol/ml/hr and that of skeletal muscle lymph, 0.791 μmol/ml/hr. LPL activity accounted for most of the lymph lipolytic activities.

Valid measurements of lipase lymph/plasma concentration ratios require steady-state conditions. One method of achieving this is to maintain constant plasma heparin concentration. This approach was first used by Porte and Bierman to study triacylglycerol turnover in humans. Preliminary experiments with 35S-heparin demonstrated that the plasma half-life of heparin was 42 minutes. The decrease of plasma heparin concentration followed a single exponential decay. Therefore, a suitable infusion rate could be calculated to maintain plasma concentration relatively constant. The result of one such experiment is shown in Figure 2.

Figure 2. Lipoprotein lipase and hepatic triacylglycerol lipase activities in the plasma of an animal undergoing a constant infusion of heparin. • = the plasma heparin level during the infusion period, ○ = the lipoprotein lipase activity in the plasma, and △ = the plasma hepatic triacylglycerol lipase activity in plasma. Note that there was a peak of lipase activity at 30 minutes after a plateau level of 9 μmol fatty acid release per milliliter per hour up to 150 minutes. In contrast, the hepatic triacylglycerol lipase activity rose to a plateau level at 15 minutes and stayed constant during the remainder of the time.

When plasma heparin concentrations were stable, lipase activities in the lymph were monitored. In the peripheral lymph, the LPL activity increased steadily to 90 minutes before reaching a plateau. On the other hand, the HTL activity plateaued at 60 minutes. This observation was reproduced in three other dogs. The data are presented in Figure 3. In contrast to the plasma lipase activities where LPL and HTL activities were similar, the LPL activity was approximately twice that of the HTL.

The other major interstitial fluid (skeletal muscle lymph) was next studied. Skeletal muscle lymph HTL activity was similar to that found in peripheral lymph during the constant infusion of heparin both in concentration and in the shape of the plateau (Figures 3 and 4). A plateau was reached at 60 minutes into the constant infusion. The LPL activity, however, increased steadily and did not reach a plateau until 120 minutes after the start of the heparin injection.
Figure 3. Lipoprotein lipase (LPL) and hepatic triacylglycerol lipase (H-TGL) activities in the peripheral lymph of an animal undergoing a constant infusion of heparin. O=the LPL activity, and △=the H-TGL activity. Note that in contrast to the plasma LPL, the peripheral lymph LPL did not show a peak followed by a plateau.

Figure 4. Lipoprotein lipase (LPL) and hepatic triacylglycerol lipase (H-TGL) activities in the skeletal muscle lymph of an animal undergoing a constant infusion of heparin. O=the LPL activity and △=the H-TGL activity in the skeletal muscle lymph. Note that in contrast to Figure 2, the LPL activity was nearly four times as high as that of the H-TGL.

Figure 5. Heparin levels in the peripheral and skeletal muscle lymph of an animal undergoing a constant infusion of heparin in the plasma. In contrast to lipoproteins or albumin, the level of heparin in the skeletal muscle lymph was the same as that of the peripheral lymph. The lymph to plasma ratio is 0.2. The molecular weight of the heparin used was approximately 10 000 Da. -heparin levels in plasma, =heparin levels in the skeletal muscle lymph, and △=heparin levels in peripheral lymph. Note that the scale for the plasma heparin levels is plotted on the right axis.

The concentration of skeletal muscle LPL was twice as high as that of the peripheral lymph LPL and four times higher than the skeletal muscle lymph H-TGL lipase activity.

We were able to achieve plateau levels of LPL and H-TGL activities in both peripheral and skeletal muscle lymph and plasma during the constant heparin infusion. Therefore, we could calculate the lymph/plasma concentration ratios of the two lipases. The results are summarized in Table 2. The lymph concentration for LPL was 2.7% of plasma for peripheral lymph and 4.8% of plasma for skeletal muscle lymph. The lymph H-TGL concentration was 1.4% of plasma for peripheral lymph and 1.1% of plasma for skeletal muscle lymph.

Since both lipases have approximately the same size, one would expect similar lymph/plasma concentration ratios for the lipases in both lymph samples. One possible reason for greater LPL lymph concentration is that heparin may filter through to the interstitium and displace nascent, de novo synthesized lipoprotein from the peripheral or skeletal muscle cells. To test this hypothesis, radioactive heparin was injected intravenously as a bolus and as an infusion to maintain a constant level of plasma heparin. These experiments were exactly the same as the constant infusion of heparin experiments described above except that radioactive heparin was substituted for nonradioactive heparin. Peripheral and skeletal muscle lymph were collected and monitored. The result shown in Figure 5 indicates that heparin crossed from the plasma into the interstitium. The concentration of heparin in lymph was 20% that of plasma.

At these plasma concentrations, the heparin concentration in peripheral and skeletal muscle lymph is insufficient to displace all LPL from the cell surface. Our preliminary experiments have shown that when heparin was injected into animals at 10 IU/kg (10 times less than the dose used in these experiments), not all the LPL was released (data not shown). Therefore, we suspected that there was more releasable LPL in the skeletal muscle lymph. To test this hypothesis, the plasma concentration

<table>
<thead>
<tr>
<th>Lipase activities</th>
<th>L/P ratio</th>
<th>L/P ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>9.05±0.43</td>
<td>7.28±0.63</td>
</tr>
<tr>
<td>Peripheral lymph</td>
<td>0.24±0.03</td>
<td>0.10±0.02</td>
</tr>
<tr>
<td>Skeletal muscle lymph</td>
<td>0.44±0.05</td>
<td>0.08±0.01</td>
</tr>
</tbody>
</table>

The values are given as μmoles of fatty acid released/ml/hr and are the means±1 SD of four different animals.

The protocol for the constant infusion of heparin is described in the Methods section. The levels of LPL and H-TGL activities were measured after the lipase levels reached a steady-state level (see Figures 2 to 4). LPL=lipoprotein lipase, H-TGL=hepatic triacylglycerol lipase, and L/P ratio=lymph to plasma ratio.
Thus, very low density lipoprotein (VLDL) will be remodeled through the action of lipoprotein lipase (LPL), and high-density lipoprotein (HDL) will be remodeled through their interaction with LPL or hepatic triacylglycerol lipase (HTL). During this remodeling, part of the VLDL phospholipid transfers to form HDL. Little is known about the presence or absence of lipases in the interstitial space. Reichl and his coworkers were not able to detect any LPL activity when heparin was not introduced in human peripheral lymph. These data agree with our findings. However, postheparin LPL activity in dog cardiac lymph has been reported to reach 10% to 30% that of plasma. Since our previous articles suggest considerable remodeling of HDL in the interstitial space, we thought it important to determine if some of this remodeling could be due to interactions of lipoproteins with lipases. As we could not find any lipase activities in lymphs of dogs not injected with heparin, we wondered whether this could be due to the presence of inhibitors of lipase activities in the lymph. Our experiments, summarized by Table 1, suggest that there were no inhibitors to either LPL or HTL. However, there were activators of LPL, and their concentration was 10% of plasma.

After a bolus injection of heparin, lipase activities could be detected in both the peripheral and skeletal muscle lymph. However, the activities were lower than expected for molecules of the size of both lipases. The data, summarized by Figure 1 and Table 1, suggest that there were no inhibitors to cause the low level of lipase activity. The data also illustrate the difficulties of trying to calculate lymph concentrations of lipase as a fraction of plasma, since the lipases peak and decay at different rates.

During the constant infusion of heparin, there was an initial surge of plasma LPL activity. The lipase activity peaked at 30 minutes and then fell to a steady-state level (see Figure 2). These data indicate that the injection of heparin caused an initial release of LPL from all sources linked by the vasculature. This initial release was followed by a rapid uptake of the LPL. After this event, the level of LPL remained constant. This would imply, although it does not prove, that the rate of removal and the release of LPL are in steady state. HTL activity also remained constant at a steady level during the period of constant infusion (Figure 2). Therefore, the rate of removal must also be in rough equilibrium with the rate of release. Porte and Bierman had similar findings in PHA-L in human subjects that were being given a constant infusion of heparin.

Since we were able to maintain relatively constant levels of lipase activity in both the peripheral and skeletal muscle lymph (Figures 3 and 4), we were able to calculate lymph lipase concentrations as a fraction of plasma.

### Table 3. Plasma and Lymph Postheparin Lipolytic Activities during Constant Heparin Infusion and at 1 Hour After Bolus Injection of 500 IU/kg of Heparin

<table>
<thead>
<tr>
<th>Lipase activities</th>
<th>Continuous infusion</th>
<th>After bolus injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LPL</td>
<td>HTL</td>
</tr>
<tr>
<td>Plasma</td>
<td>11.2±3.15</td>
<td>7.28±0.63</td>
</tr>
<tr>
<td>Peripheral lymph</td>
<td>0.19±0.05</td>
<td>0.09±0.01</td>
</tr>
<tr>
<td>Skeletal muscle lymph</td>
<td>0.28±0.05</td>
<td>0.06±0.01</td>
</tr>
</tbody>
</table>

The values are given as μmoles of fatty acid released/μl/hr and are the means±1 SD of three determinations.

LPL = lipoprotein lipase, HTL = hepatic triacylglycerol lipase.

Discussion

We have previously described the concentration of lipoproteins and apolipoproteins in the interstitial fluid of both control and cholesterol-fed dogs. The concentrations of apolipoprotein (apo) B containing particles are 2% to 7% of their plasma counterparts. In general, these values agreed with those observed by Reichl and coworkers in their studies of human interstitial fluid. Interstitial HDL is present in 10% to 15% of its plasma concentration. It is enriched in free cholesterol, and at least some of the HDL is discoidal in nature.

Although the peripheral lymph model is considered a good model for interstitial fluids, it represents approximately 30% to 40% of the total interstitial space (skin and connective tissues). In this study, we extended the model to include lymph derived from skeletal muscle, which represents another 30% to 40% of total interstitial space. Thus, taken together, data derived from peripheral and skeletal muscle lymph should be representative of most of the interstitial space.

Lipoproteins in the vasculature are constantly being remodeled through their interaction with LPL or HTL. Thus, very low density lipoprotein (VLDL) will be remodeled through the action of LPL, and HDL will be remodeled through the action of HTL. During this remodeling, part of the VLDL phospholipid transfers to form HDL. Little is known about the presence or absence of lipases in the interstitial space. Reichl and his coworkers were not able to detect any LPL activity when heparin was not introduced in human peripheral lymph. These data agree with our findings. However, postheparin LPL activity in dog cardiac lymph has been reported to reach 10% to 30% that of plasma. Since our previous articles suggest considerable remodeling of HDL in the interstitial space, we thought it important to determine if some of this remodeling could be due to interactions of lipoproteins with lipases. As we could not find any lipase activities in lymphs of dogs not injected with heparin, we wondered whether this could be due to the presence of inhibitors of lipase activities in the lymph. Our experiments, summarized by Table 1, suggest that there were no inhibitors to either LPL or HTL. However, there were activators of LPL, and their concentration was 10% of plasma.

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Since we were able to maintain relatively constant levels of lipase activity in both the peripheral and skeletal muscle lymph (Figures 3 and 4), we were able to calculate lymph lipase concentrations as a fraction of plasma.
concentrations. Based on their molecular size and the known albumin concentration in lymph (30% to 40% of plasma), we expected much higher concentrations of lipase activities in the peripheral lymph and skeletal muscle lymph. In actuality, the lymph to plasma (l/p) ratio for HTL was relatively constant for both skeletal and peripheral lymph. However, the actual concentrations were only 1.1% and 1.4% of plasma, 40-fold less than had been expected (Table 2). There is evidence that LPL may exist as a dimer in solution. In postheparin plasma, it probably exists as a complex with heparin, thereby making the actual size of LPL closer to that of HDL (200 000 Da). It is likely that HTL may behave the same way. The concentration of apo A-I in peripheral lymph is 15% to 20% that of plasma. Therefore, either LPL or HTL is larger than low density lipoprotein (apo B in lymph is 5% to 8% of plasma), or factors other than molecular sieving are responsible for the transport of extremely small amounts of HTL from the vascular space into the interstitial space. Our finding that there are undetectable levels of either LPL or HTL in the control dog makes it likely that neither enzyme is responsible for the extensive remodeling of interstitial HDL.

The concentration of LPL is somewhat higher than that of HTL in the lymph. They are 2.7% and 4.8%, respectively, of plasma in the peripheral and skeletal muscle lymph (Table 2). This level is still not the 30% to 40% concentration of plasma that was expected if albumin is used as a guide, nor the 15% to 20% if apo A-I is used. Again, factors other than molecular sieving must be at work to account for plasma’s low interstitial concentrations. The most likely explanation for the inability of the lipases to permeate the interstitial space is that the lipases are trapped by the glycoalyx at the surface of the vasculature. If LPL, like HTL, is relatively impermeable, then the somewhat higher levels of LPL l/p ratio needs explanation. This is especially true in the skeletal muscle lymph. A number of investigators have found that LPL can be synthesized by skeletal muscle and adipose tissue. Therefore, it was our hypothesis that the somewhat higher concentrations of LPL found in the skeletal muscle lymph and peripheral lymph were due to heparin crossing the interstitium and displacing the de novo synthesized LPL. To test this hypothesis, radioactive heparin was infused into dogs in constant infusion experiments. The results shown in Figure 5 demonstrate that heparin does indeed penetrate the capillary wall and enter the interstitial space.

The concentration of heparin in lymph was 20% that of plasma. This value is nearly five times lower than expected since the molecular mass of heparin is 10 000 Da. Even assuming that heparin does exist as a rigid rod and not a folded-up globular protein, the “effective” size could not be 20 times (the size of HDL as determined by apo A-I) more than the actual size. Again, factors other than sieving, such as trapping by the glycoalyx, must be at work. This concentration of heparin was insufficient to displace all LPL in the interstitial space. Therefore, a fivefold concentration of heparin was introduced into the plasma in some later constant infusion experiments. As predicted, the level of LPL rose in the skeletal muscle lymph. LPL concentration did not increase in the plasma or peripheral lymph (Table 3).

Taken together, our results demonstrate that LPL exists in bound form in the interstitium. HDL is probably not remodeled to any great extent by lipases within the interstitial space as a result of interactions with HTL. Therefore, it seems likely that most of the modifications of lymph HDL result from its interaction from peripheral cells.

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