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# The significance of lipoprotein lipase in rat skeletal muscles

**Meng H. Tan,[1] Tsunako Sata, and Richard J. Havel[2]**

Cardiovascular Research Institute and Department of Medicine, University of California, San Francisco, CA 94143

**Abstract** Lipoprotein lipase was assayed in extracts of acetone–ether powders of rat skeletal muscles. Enzyme activity in soleus had typical characteristics of lipoprotein lipase in other tissues: inhibition by molar NaCl and protamine sulfate and activation by the human apolipoprotein, R-glutamic acid. Activity in muscles with predominantly red fibers (soleus, diaphragm, lateral head of gastrocnemius and anterior band of semitendinosus) was higher than in those with predominantly white fibers (body of gastrocnemius and posterior band of semitendinosus). No effect of a 24 hour fast upon enzyme activity was observed in ten skeletal muscles, but activity decreased substantially in four adipose tissue depots and increased slightly in heart muscle with fasting. Four minutes after intravenous injection of labeled lymph chylomicrons, skeletal muscles with predominantly red fibers incorporated several times more chylomicron triglyceride fatty acids than those with predominantly white fibers. Estimated lipoprotein lipase activity in total skeletal muscle was about two-thirds that in total adipose tissue of rats fed ad libitum. After a 24 hour fast, total activity in skeletal muscle was about twice that in adipose tissue. These data suggest that a substantial fraction of lipoprotein lipase is in skeletal muscle of rats and that this tissue, especially its red fibers, is an important site of removal of triglycerides from the blood.

**Supplementary key words** triglycerides · plasma · adipose tissue · heart · muscle · chylomicrons

Lipoprotein lipase (LPL) is a tissue-bound triglyceride hydrolase (glycerol-ester hydrolase EC 3.1.1.3) that catalyzes the hydrolysis of triglyceride in plasma lipoproteins and in artificial lipid emulsions when an apolipoprotein cofactor is present. The enzyme (presumably the fraction situated on the endothelial surface of blood capillaries) mediates the removal of plasma triglycerides in extrahepatic tissues, including heart (1), adipose tissue (1), lung (2), and mammary gland (3). Although plasma triglycerides are utilized by heart, adipose tissue (which forms about 7% of body weight in rat (4)) is usually considered to be the primary site of triglyceride catabolism. Skeletal muscles, which form about 45% of body weight in the rat (4), utilize considerable amounts of lipids as fuel. Plasma free fatty acids, mobilized from adipose tissue or derived from hydrolysis of plasma triglycerides in capillaries in which LPL is present, are readily oxidized by muscle (5). However, direct utilization of plasma triglyceride fatty acids by skeletal muscles evidently depends upon the content of active LPL in their capillary beds.

LPL activities of adipose tissue and heart have been extensively studied under various physiological conditions (6) but that of skeletal muscle has not. Murine skeletal muscles contain triglyceride (7, 8) as well as monoglyceride (8) hydrolases. Some studies, using emulsified triglycerides (Ediol or Intralipid) as substrate, have shown that LPL is present in the rat diaphragm (9–11). LPL also has been reported present in rat thigh muscles (12–15), but the enzyme was not fully characterized. An enzyme active against tributyrin has also been demonstrated in the pectoralis muscle of pigeon (16). This enzyme is present in greater quantities in the muscles with predominantly red fibers than those in which white fibers predominate. This distribution is in keeping with evidence that red muscle fibers readily utilize fat as fuel, whereas white fibers mainly utilize carbohydrate (5). Recently, we (17) and others (18, 19) have reported the activity of LPL to be considerably higher in leg muscles containing mainly red fibers than in those with mostly white fibers. In the present study, we have characterized and quantified LPL in rat skeletal muscles of different fiber types. To evaluate the physiological significance of LPL in skeletal muscle, the activity of the enzyme in 10 skeletal muscles, cardiac muscle and 4 adipose tissue depots was determined in the fed and fasted states.

Abbreviation: LPL, lipoprotein lipase.

[1] Centennial Fellow, Medical Research Council of Canada. Present address: Department of Medicine, Dalhousie University, 5849 University Avenue, Halifax, NS, Canada B3H 4H7.

[2] Address reprint requests to Dr. R. J. Havel, Cardiovascular Research Institute, U. C. Medical Center, San Francisco, CA 94143.

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## METHODS

### Assay for tissue lipoprotein lipase activity

Male Sprague-Dawley rats, 200–250 g, were either fed ad libitum with standard Purina chow (Ralston Purina Co., St. Louis, MO) or fasted for 24 or 48 hr. Tissues were removed from rats lightly anesthetized with ether. Adipose tissues were obtained from the epididymal fat pad and the perirenal, omental, and abdominal subcutaneous regions. Left ventricular muscle of the heart and skeletal muscles were obtained as indicated. Visible fat on the muscles was always removed before the tissues were rinsed with cold 0.15 M NaCl and blotted dry. Muscle tissues were minced before acetone–ether powders were prepared. In experiments where LPL activity of different skeletal muscles and adipose tissues was measured, some of the tissues were frozen immediately and stored at −20°C overnight before acetone–ether powders were prepared.

The assay was a modification of that originally described by Nilsson-Ehle, Tornqvist, and Belfrage (20). Tissue grinders, Duall size 22 (Kontes, Vineland, NJ), powered by a motor, were used to homogenize the tissues. Minced tissue was weighed after it was added to the homogenizer. For adipose tissues, 50 μl of a medium containing cold, fasting human serum–Tris HCl (0.078 M, pH 7.4), 1:3 (v/v) was added to each homogenizer tube. For both skeletal and heart muscle, 0.5 ml of $NH_4OH$–$NH_4Cl$ buffer (0.05 M, pH 8.1) was added to each homogenizer tube and the specimens were homogenized for 1 min. Four ml of ice-cold acetone were then added and the contents rehomogenized for another min. The samples were then centrifuged at 2000 rpm for 10 min at 4°C. The acetone was decanted and the sediment was homogenized and centrifuged twice more with the same volume of acetone and once with cold diethyl ether. The acetone–ether powder was dried at room temperature under nitrogen. It was then homogenized in $NH_4OH$–$NH_4Cl$ buffer (0.05 M, pH 8.1) containing heparin (20 U/ml) in a volume of 1 ml/20 mg tissue. The suspension was left on ice for 20 min after which it was centrifuged for 20 min at 2000 rpm at 4°C. The clear supernatant fluid was used as the enzyme source in routine assays.

The substrate was prepared within 30 min of use by sonication of the mixture (described below) with a Branson sonifier-cell disruptor (Branson Instruments Co., Danbury, CT). Labeled tri-[9,10-$^3$H]oleoylglycerol (Amersham/Searle Corporation, Arlington Heights, IL) and unlabeled triolein (Sigma grade, Sigma, St. Louis, MO) were mixed to give 30 μmol of trioleoylglycerol with a specific activity of $1.5 \times 10^6$ cpm/μmol. The triolein mixture, together with egg lecithin (Sigma) (10% w/w), was evaporated under nitrogen. After removal of the solvents under nitrogen, the lipids were reconstituted in Tris buffer (0.2 M, pH 8.3) in a total volume of 6 ml containing pooled 5% human serum, 10 mM calcium chloride, and 0.7 mM bovine serum albumin (Fraction V, Sigma).

To maximize reproducibility, the sonication procedure was performed in a standardized manner. The flat macrotip of the sonifier was centered and immersed about ¼ in below the surface of the mixture, contained in a standard 25-ml liquid scintillation vial suspended in a beaker of ice. Sonication was carried out at setting 3 (60 W) for a total of 4 min. This was performed for 0.5 min periods for a total of eight times over 8 min. A translucent emulsion was obtained (particle size 500–1000 Å by negative staining electron microscopy (21)).

Incubations were started by addition of 100 μl of enzyme solution to 100 μl of the substrate mixture in 13 × 100 mm stoppered culture tubes. Incubations were routinely for 20 min at 37°C. Reactions were terminated and free fatty acids (FFA) were extracted as described by Belfrage and Vaughan (22) by addition of 3.25 ml of a heptane–chloroform–methanol mixture and 1.05 ml of potassium carbonate–borate–hydroxide buffer (0.05 M, pH 10.0). Two ml of the upper phase were used for assay of radioactivity in Aquasol (New England Nuclear, Boston, MA) by liquid scintillation spectrometry. Total release of FFA was calculated after correction for efficiency of extraction and quenching in each assay. Enzyme activity was expressed in units of μmol/g wet weight of tissue × $hr^{-1}$ (units). When the same substrate emulsion was used, duplicate values differed by less than 5%. When acetone–ether powders of soleus muscle were extracted three times with ammonia buffer containing heparin as described, 73.9 ± 1.3% (mean ± SEM, n = 4) of the total activity recovered was in the first extract.

To monitor interassay variation owing to differences in substrate emulsion, a postheparin plasma sample from a normal human control subject was used as a standard in each assay. 0.2 ml portions of the postheparin plasma were stored at −20°C until the time of assay. Each sample was diluted with nine volumes of $NH_4OH$–$NH_4Cl$ buffer (0.05 M, pH 8.1) before assay. One hundred μl of the diluted plasma was used as enzyme solution and the assay was performed as described above. The interassay coefficient of variation for 21 consecutive assays was 10.3%.



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The role of specific human apolipoproteins (R-glutamic acid, $R_2$-alanine, R-serine and the arginine-rich protein) in the activation of lipoprotein lipase in skeletal muscle was studied by methods described previously (23, 24) but using the present assay. Control samples containing no apoprotein were included in each assay. The apolipoproteins were added in increasing amounts directly to the substrate and the final concentration was expressed as $\mu$g protein/ml of medium. For these assays no human serum was used in the preparation of the substrate. Apolipoproteins were prepared as described previously (24).

**Metabolism of chylomicron triglycerides**

Chylomicrons were obtained from intestinal lymph of rats fed a fat-rich meal and [9,10-$^3$H]palmitate as described by Mjøs et al. (25). The chylomicrons, containing about 0.03 mmol of triglycerides and 4 $\mu$Ci of esterified [$^3$H]palmitate together with about 1 $\mu$Ci of $^{131}$I-labeled human serum albumin in 0.5 ml of 0.15 M NaCl, were injected into the tail vein of fed rats. After 2 min the rats were anesthetized with diethyl ether and then bled 4 min after injection from the abdominal aorta (about 12 ml of blood was obtained). Muscles were rapidly removed, weighed, and homogenized in at least 20 vol of ethanol–acetone 1:1 (v/v). After extraction of lipids overnight, the extracts were filtered through filter paper and made to known volume. The precipitated protein, collected on the filter paper, was assayed for $^{131}$I by scintillation spectrometry. Portions of the extracts were evaporated to dryness, taken up in chloroform, and assayed for $^3$H by liquid scintillation spectrometry, as such and after removal of phospholipids with silicic acid. Samples of blood serum were analyzed similarly. Content of $^3$H in tissue lipids was calculated, after correction for $^3$H in total lipids and neutral lipids contained in trapped plasma. Trapped plasma was estimated from content of $^{131}$I (26). Content of $^3$H remaining in lipids of blood plasma was estimated on the assumption that plasma volume was 4.5% of body weight (25).

## RESULTS

**Characteristics of the assay and enzyme**

The soleus muscle from fed rats was used as the enzyme source in these experiments. Reaction velocity was linear for 40 min at saturating levels of substrate, and for at least 10 min at low levels (**Fig. 1**). Velocity was maximal at 2.5 $\mu$mol/ml and at this concentration, used in all subsequent studies, a linear response to increasing amounts of tissue up to 3 mg (wet weight) was obtained. Optimal pH was between 8.0 and 8.5.

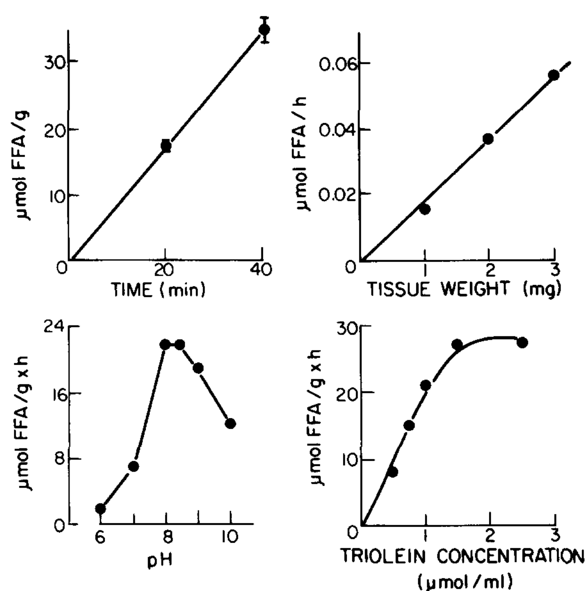


**Fig. 1.** Characteristics of the assay of LPL in rat soleus muscle. Enzyme was extracted from acetone–ether powder of soleus muscle from fed rats with ammonium buffer containing 20 units of heparin per ml. Unless indicated in the individual experiments where time of incubation, wet weight of tissue per assay, pH, and substrate concentration were studied, the following standard conditions apply: incubation time, 20 min; wet weight of tissue per assay, 2 mg; pH 8.1; substrate concentration, 2.5 $\mu$mol/ml.

When no heparin was present in the extracting or incubating medium, only a fraction of the enzyme activity was measured (**Fig. 2**). As little as 5 U heparin/ml incubation medium increased enzyme activity fivefold. Optimal heparin concentration was

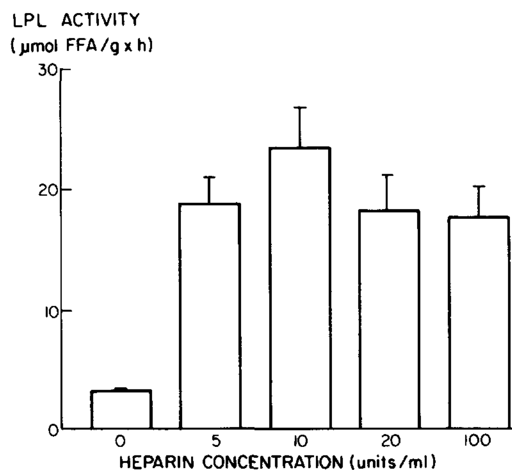


**Fig. 2.** Effect of heparin on the activity of LPL extracted from acetone–ether powder of soleus muscle from fed rats. Ammonium buffer (0.05 M, pH 8.1) containing various amounts of heparin was used to extract the enzyme from the powder. Extraction was for 20 min at 4°C. Assay was under the standard conditions described for Fig. 1.



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TABLE 1. Characteristics of the lipase in soleus muscle

| Condition of Assay[a] | Enzyme Activity (relative to complete system) | |
|---|---|---|
| | Fed Rat (N = 3)[c] | Fasted Rat (N = 4)[c] |
| Complete system | 100% | 100% |
| No serum | 11.2 ± 1.9[d] | 12.4 ± 0.8 |
| NaCl (1M)[b] | 3.7 ± 1.2 | 12.1 ± 1.2 |
| Protamine sulfate (1 mg/ml)[b] | 16.2 ± 5.8 | 7.4 ± 2.2 |

[a] Extract of acetone–ether powder from soleus muscles of fed rats or rats fasted 24 hr were incubated with substrate for 20 min at 37°C under various conditions. In each assay 2 mg wet weight of tissue was used.
[b] The enzyme extract was preincubated with either 1M NaCl or protamine sulfate for 20 min at 4°C.
[c] Number of experiments is indicated in parentheses.
[d] Mean ± SEM.

10 U/ml. In the absence of serum, enzyme activity was reduced by almost 90%. Activity was largely inhibited by 1 M NaCl and protamine, 1 mg/ml (**Table 1**). In these experiments the enzymes were preincubated with the inhibitors for 20 min at 4°C. The controls were treated similarly but without the inhibitors. In the fasted rat the LPL from the soleus muscle of fasted rats had similar characteristics (Table 1).

The human apolipoprotein, R-glutamic acid, was by far the most potent activator of hydrolysis of triglycerides by the unpurified enzyme from rat soleus muscle. Stimulation was detectable at 0.5 $\mu$g/ml of incubation medium (**Fig. 3**) and was fourfold at 7 $\mu$g/ml. The $R_2$-alanine apolipoprotein produced a slight increase in activity. Neither the R-serine nor the arginine-rich apolipoprotein had a detectable effect.

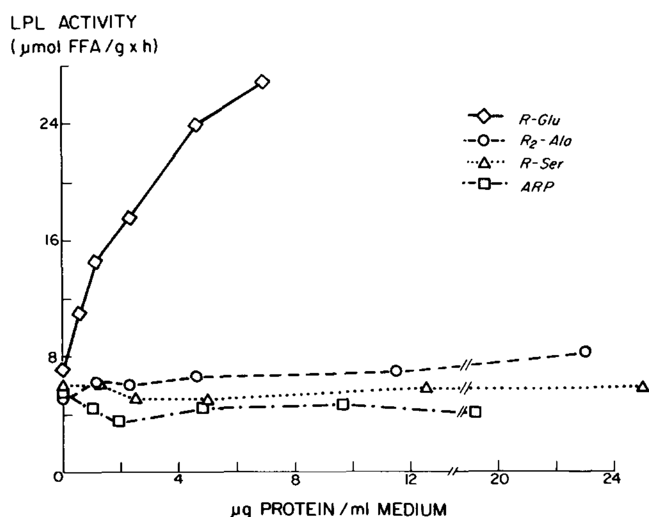


**Fig. 3.** Effect of human apolipoproteins on the activity of LPL from rat soleus muscle. Acetone–ether powder of muscle from fed rats was used as enzyme source. Apolipoproteins were added directly to the substrate and final concentration is expressed as $\mu$g protein per ml medium. For these assays no human serum was used in preparation of the substrate. Control samples contained no apolipoproteins.

## LPL activity in skeletal muscles of different fiber types

The soleus, which contains only red and intermediate fibers, had the highest LPL activity (**Fig. 4**). The activity in the diaphragm, which contains mainly red fibers, was intermediate and that of the posterior band of the semitendinosus, which contains very few red fibers, was only about 3% of that of the soleus.

LPL activity in the lateral head of the gastrocnemius (which contains predominantly red fibers) was higher than that in the body (which contains predominantly white fibers) (**Table 2**). Similarly, activity in the anterior band of the semitendinosus (which contains predominantly red fibers) was higher than that in the posterior band of the muscle (which contains predominantly white fibers).

## LPL activity in various tissues

All skeletal muscles in the rat are heterogeneous, each containing different proportions of red, intermediate, and white fibers. LPL activities in 10 different skeletal muscles, together with those of adipose tissue from 4 different sites and that of the heart, are shown in **Table 3.** In order to minimize biological variations, rats were killed at 10 AM and all 15 tissues were obtained from the same rat. Eight of these tissues were frozen immediately and were then stored at −20°C overnight before their LPL activities were assayed. They were only thawed once. The remaining 7 tissues were assayed for their LPL activities the day of the experiment. In each group of four animals, the same tissue was assayed fresh on two occasions and after being stored at −20°C overnight on two occasions.

In the fed state, the soleus and diaphragm were

TABLE 2. Lipoprotein lipase activity in different parts of the same muscle

| Muscle | | Enzyme Activity | Significance[b] |
|---|---|---|---|
| | | *µmol FFA/g × hr* | |
| Gastrocnemius: (N = 10) | lateral head | 6.06 ± 1.41[a] | $P < 0.001$ |
| | body | 0.46 ± 0.04 | |
| Semitendinosus: (N = 8) | anterior band | 2.88 ± 0.19 | $P < 0.003$ |
| | posterior band | 1.18 ± 0.09 | |

The number of experiments is shown in parentheses. In each experiment two different parts of the same muscle were used. Extract of acetone–ether powder was incubated with substrate for 20 min at 37°C.
[a] Mean ± SEM.
[b] Statistical analysis was by the unpaired $t$ test.



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TABLE 3. Lipoprotein lipase activity in various tissues of the rat

| Tissue | Fed | Fasted 24 hr |
|---|---|---|
| Adipose tissue | | |
| Epididymal fat pad | 92.6 ± 18.2[a] | 32.8 ± 3.9[b] |
| Subcutaneous fat | 42.3 ± 6.8 | 17.1 ± 1.7[b] |
| Omental fat | 55.0 ± 4.4 | 12.4 ± 1.2[b] |
| Perirenal fat | 51.5 ± 3.7 | 6.7 ± 1.4[b] |
| Heart | 63.3 ± 4.5 | 78.7 ± 5.8 |
| Skeletal muscle | | |
| Pectoralis major | 2.6 ± 0.2 | 2.6 ± 0.3 |
| Triceps longus | 2.6 ± 0.5 | 1.1 ± 0.1[c] |
| Latissimus dorsi | 3.2 ± 0.4 | 2.1 ± 0.2 |
| External oblique | 2.4 ± 0.3 | 1.1 ± 0.2[c] |
| Gluteus maximus | 2.3 ± 0.5 | 1.4 ± 0.3 |
| Psoas major | 2.0 ± 0.2 | 1.1 ± 0.4 |
| Vastus medialis | 2.4 ± 0.4 | 2.1 ± 0.2 |
| Semimembranosus | 1.9 ± 0.5 | 1.8 ± 0.3 |
| Soleus | 31.4 ± 5.6 | 31.7 ± 7.8 |
| Diaphragm | 14.1 ± 2.4 | 11.7 ± 2.0 |

For each rat, seven tissues were assayed on the day the animal was killed. The remaining eight tissues were quick frozen and thawed once the following day when the LPL activity was assayed. In each group of animals, the same tissue was assayed fresh on two occasions and after being stored at −20°C overnight on two other occasions. Extracts of acetone–ether powder of the tissue were incubated with substrate at 37°C for 20 min.
Statistical analysis was by unpaired $t$ test.
[a] μmol FFA/g × hr (mean ± SEM, n= 4).
[b] $P < 0.01$.
[c] $P < 0.05$.

conspicuous by their high LPL activity. The other skeletal muscles studied had activities ranging from 1.9 to 3.2 units. The heart had the highest activity of all the muscles studied. Adipose tissues from each of four different sites also had higher activities than those of skeletal muscles.

Fasting for 24 hr decreased LPL activity in adipose tissue from all sites (Table 3) whereas it increased activity in the heart. Mean activity in most skeletal muscles was reduced, but this was significant in only two (Table 3). A more extensive evaluation of the effect of fasting for 24 and 48 hr showed no effect upon LPL activity in soleus muscle (**Fig. 5**).

### Total LPL activity in adipose tissue and skeletal muscles of rat (Table 4)

To estimate the total LPL activity in adipose tissues and skeletal muscles in the rat, the following formula was used:

$$\text{Total LPL activity} = \frac{\text{LPL activity}}{\text{of tissue}} \times \frac{\text{\% body weight}}{\text{of tissue}} \times \frac{\text{body weight}}{\text{of animal}}$$

For these estimations, the LPL activity of adipose tissue was the mean value from four different sites and that for skeletal muscles was the mean value for 10 different muscles. It was assumed that the fraction of total LPL activity assayed was the same in all sites and that adipose tissue and skeletal muscle systems comprise 7% and 45% of body weight, respectively (4). Table 4 summarizes the findings. In the fed state more LPL activity was in adipose tissue than in skeletal muscle. After the 24 hr fast, activity in skeletal muscle was twice that of adipose tissue.

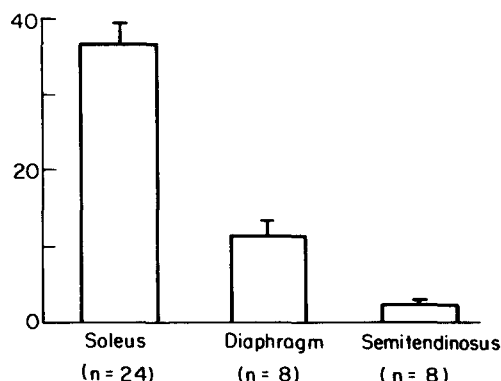


**Fig. 4.** LPL activity in skeletal muscles of different fiber types. The soleus contains only red and intermediate fibers, the diaphragm contains mainly red fibers but also intermediate and white fibers, and the posterior band of the semitendinosus contains mainly white fibers but also a few intermediate and red fibers. The standard assay conditions described for Fig. 1 were used. Values represent mean ± SEM. Number of muscles studied is shown in parentheses.

### Fat content of muscle

The amount of adipose tissue in the soleus and semimembranosus muscles was estimated microscopically and chemically. In the former estimation, after visible fat was dissected from the surface of

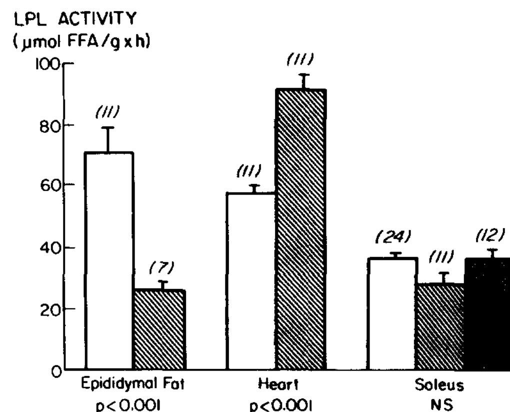


**Fig. 5.** Effect of fasting on LPL activities in different rat tissues. Open bars, rats fed ad libitum; single hatched bars, rats fasted for 24 hr; double hatched bars, rats fasted for 48 hr. Number of rats is indicated in parentheses. Assay conditions were as described for Fig. 1. Values represent mean ± SEM. Statistical analysis was by unpaired $t$ test. NS, not significant.



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TABLE 4. Lipoprotein lipase activity (units) in total adipose tissues and skeletal muscles of eight rats

| Fed | | | 24-hr Fast | | |
|---|---|---|---|---|---|
| Rat | Adipose Tissue | Muscle | Rat | Adipose Tissue | Muscle |
| 1 | 966 | 609 | 5 | 276 | 552 |
| 2 | 1173 | 585 | 6 | 340 | 874 |
| 3 | 856 | 727 | 7 | 263 | 512 |
| 4 | 1255 | 824 | 8 | 406 | 602 |
| Mean | 1063 | 691 | Mean | 322 | 635 |

For each of the four rats in the two groups, the LPL activity of adipose tissues from four different depots and of 10 different skeletal muscles (see Table 3) was assayed. The total LPL activity in adipose tissue and skeletal muscles was estimated as described in the text.

the muscle, the tissue was fixed with formalin. Thin sections of the muscle were then prepared and stained with hematoxylin–eosin. The amount of adipose tissue in each section was then estimated by examination under light microscopy. In three preparations of each muscle the amount of adipose tissue was estimated to be about 1%. Content of triglycerides was determined as follows. After initial homogenization with 0.5 ml of $NH_4OH-NH_4Cl$ buffer (0.05 M, pH 8.1), the muscle was extracted with chloroform–methanol 2:1 and the triglycerides in the chloroform phase were determined (27). The mean triglyceride concentrations in soleus (N = 3) and semimembranosus (N = 3) were 2.5 and 1.8 $\mu$mol/g wet weight, respectively.

### Incorporation of chylomicron–lipids into muscles

About 95% of the [$^3$H]palmitate in lipids of the chylomicrons injected was in neutral lipids. During the 4 min after injection, 55–63% of the $^3$H was removed from the blood. Soleus and predominantly red portions of the gastrocnemius and semitendinosus muscles contained more [$^3$H]palmitate than did white portions of the latter two muscles (**Table 5**). Somewhat more than one-half was in neutral lipids (presumably triglycerides) and the remainder in phospholipids. Per unit weight, much more $^3$H was recovered in lipids of the heart than in any of the skeletal muscles.

TABLE 5. Recovery of $^3$H in muscle lipids 4 min after injection of labeled chylomicrons into fed rats

| | Total Lipids | Neutral Lipids |
|---|---|---|
| | *% of injected $^3$H/g of tissue* | |
| Soleus | 0.167 ± 0.039[a] | 0.103 ± 0.047 |
| Gastrocnemius (lateral head) | 0.0731 ± 0.024 | 0.0508 ± 0.017 |
| Gastrocnemius (body) | 0.0247 ± 0.013[b] | 0.0128 ± 0.0067[b] |
| Semitendinosus (anterior band) | 0.0821 ± 0.097 | 0.0457 ± 0.041 |
| Semitendinosus (posterior band) | 0.0270 ± 0.014 | 0.0149 ± 0.0023[c] |
| Heart | 2.13 ± 0.33 | 1.47 ± 0.34 |

[a] Mean values ± SD, n = 5.
[b] Significantly lower than lateral head of gastrocnemius ($P < 0.01$).
[c] Significantly lower than anterior band of semitendinosus ($P < 0.05$).

## DISCUSSION

The lipase assayed as LPL in skeletal muscle has not been characterized fully heretofore. Borensztajn et al. (19) stated that the activity that they assayed required the presence of serum. The triglyceride hydrolase that we have assayed in the rat soleus muscle has properties typical of LPL. It is activated by serum and inhibited by 1 M sodium chloride and by protamine sulfate (1 mg/ml). Triglyceride hydrolase in the semimembranosus and body of the gastrocnemius was similarly inhibited by salt (52% inhibition) and protamine (56% inhibition). However, activation by serum was slight (11% activation). This may be related to the low activity of the enzyme in these muscles and to the presence of plasma in blood capillaries of the specimens. The conditions of the assay are such that it is likely that our values reflect mainly LPL in all cases.

Apolipoprotein R-glutamic acid appears to be the major cofactor in human serum for LPL of the soleus. Preparations of lipoprotein lipase from other tissues—cow's milk, rat adipose tissue, and purified lipoprotein lipase from human and rat plasma—are also stimulated by this protein (24). As in other tissues (24), the $R_2$-alanine apolipoprotein slightly stimulated the enzyme from the soleus.

Small amounts of adipose tissue are present on the epimysium and endomysium of skeletal muscles. We removed all visible fat from the epimysium before the muscles were used for assay for LPL. The remaining adipose tissues constituted about 1% of the specimen, much too little to influence our results appreciably.

The present results confirm that more LPL is present in muscles with predominantly red fibers than in those with mainly white fibers.[3] Thus, the soleus, which contains only red and intermediate fibers in the rat (28), and the diaphragm, which contains about 60% red fibers (29), have much higher activity of LPL

[3] These observations were made in various strains of albino rats. Histochemical staining for succinic dehydrogenase activity in our Sprague-Dawley rats (Tan, M. H. and V. E. Sangalang: unpublished observations) gives similar distributions to those reported in references 28–30.



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than the posterior band of the semitendinosus, which contains almost entirely white fibers (30). This distinction also applies to differentiation of fiber types within individual muscles. The lateral head of the gastrocnemius contains more red fibers (28) and more LPL activity than the body of the muscle. A similar situation obtains in the semitendinosus. These observations extend those of Borensztajn et al. (19) on the rat quadriceps. In some tissues with high activity of LPL, an appreciable fraction appears to be functional on the capillary surface (31). If this is so in skeletal muscle, capillaries of red muscles and those surrounding red fibers in mixed muscles should have substantial capacity to hydrolyze plasma triglycerides. In part, the higher activity may simply reflect the greater capillary density of red than of white muscles (32), but known variations in capillary density cannot account for the large observed differences in enzyme activity. Rather, it appears that these differences constitute one of many functional adaptations related to the oxidative and glycolytic capacities of red and white fibers (5).

Our data indicate that skeletal muscles may contain a substantial fraction of the total enzyme in the rat. Unlike Borensztajn, Otway, and Robinson (11), Cryer et al. (15) and Chernick, Linder, and Scow (18), we did not find that a 24 hr fast affected LPL activity in skeletal muscles. However, because of the fall in enzyme activity in adipose tissue with fasting, the total activity in skeletal muscles became predominant in the fasting state. These estimates suffer from the limitation that the fraction of total activity that reflects functional enzyme in the capillary bed is unknown, but our observations on the rapid incorporation of chylomicron triglyceride fatty acids into lipids of leg muscle suggest that an appreciable fraction of the enzyme is functional. Predominantly red-fibered parts of the gastrocnemius and semitendinosus muscles incorporated about three times as much of the labeled lipid as did those parts with predominantly white fibers. However, comparison of the LPL activities of these muscles and of the soleus with those of the heart in relation to their incorporation of chylomicron triglycerides suggests an equal or greater importance of other factors in triglyceride utilization. The heart incorporated more than ten times as much lipid as did the soleus, whereas LPL activity was only twice as great. The difference between the uptake (as contrasted with incorporation) of lipid may be even greater, because more of the triglyceride fatty acid taken up is likely to have been oxidized rapidly in the contracting heart muscle than in the relatively quiescent soleus (26). The much greater blood flow in heart muscle (about tenfold greater (33)) may underlie the apparent discrepancy. Differing blood flow to red and white fibers of resting skeletal muscles (34) may also have contributed to the differences that we observed within portions of the same muscle.

Jones and Havel (26) found that, per unit weight, incorporation of chylomicron triglyceride fatty acids by adipose tissue of fasted rats was about sixfold greater than that of skeletal muscle. Our observations on LPL activity of these tissues in fasted rats (about eightfold greater in adipose tissue than in skeletal muscle (Table 3)) suggest that, in these tissues with similar blood flow (33), the activity of this enzyme determines the relative rates of triglyceride utilization.

The contribution of LPL in skeletal muscle to clearance of chylomicron triglycerides cannot be estimated precisely from the limited data obtained in this research, but it is likely to be appreciable. If the incorporation of injected triglyceride fatty acid into gastrocnemius and semitendinosus muscles (~0.05%/g) is taken to represent the average for all skeletal muscles, about 13% (0.09%/g) of the triglyceride fatty acid removed from the blood during the 4 min following injection was in this tissue. These data are consistent with the observations of Kaijser and Rössner (35) on the uptake of emulsified triglycerides into human forearm muscle. Uptake of plasma triglycerides by skeletal muscle is likely to be of particular importance in the fasting state, when activity in adipose tissue is low, and in other states of reduced utilization of carbohydrates.

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