Lipoprotein lipase activity in skeletal muscles of the rat: effects of denervation and tenotomy

E. SMOL,1 E. ŻERNICKA,2 D. CZARNOWSKI,3 AND J. LANGFORT2
1Department of Physiology, Academy of Physical Education, 40-065 Katowice; 2Department of Applied Physiology, Medical Research Centre, 02-106 Warsaw; and 3Department of Physiology, Medical School of Białystok, 15-230 Białystok, Poland

Received 20 April 2000; accepted in final form 13 October 2000

Smol, E., E. Żernicka, D. Czarnowski, and J. Langfort. Lipoprotein lipase activity in skeletal muscles of the rat: effects of denervation and tenotomy. J Appl Physiol 90: 954–960, 2001.—The effects of denervation, tenotomy, or tenotomy with simultaneous denervation on the activity of heparin-releasable and intracellular, residual lipoprotein lipase (LPL) and triacylglycerol (TG) content were examined in rat skeletal muscles. An influence of muscle electrostimulation on denervated and tenotomized muscles was also evaluated. Activity of both LPL fractions was decreased in denervated and/or tenotomized soleus and red portion of gastrocnemius muscles. It was accompanied by a slight elevation of the intracellular TG content. Electrostimulation increased activities of both fractions of LPL in red muscles from intact hindlimbs. In stimulated denervated muscles without or with simultaneous tenotomy, activity of two LPL fractions was also enhanced, but control values were reached only in denervated soleus muscle. Electrical stimulation had no pronounced effect on LPL activity in tenotomized muscles. In conclusion, denervation and/or tenotomy decreases LPL activity in red muscles, indicating reduction of the muscle potential to utilize circulating TG. Electrostimulation only partly restores the diminished LPL activity in denervated muscles, without any effect in tenotomized ones. Thus, to maintain LPL activity in resting muscle, intact innervation and tension are needed.

lipolysis; triacylglycerol

DENERVATION INDUCED BY PERIPHERAL nerve section and tenotomy after Achilles tendon cut cause muscle atrophy and induce numerous biochemical alterations. Muscle inactivity that follows denervation or tenotomy is accompanied by reduced mitochondrial enzyme activities (1, 35) and decreased skeletal muscle ability to oxidize glucose and fatty acids, leading to a decline in intramuscular high-energy phosphates (5). An inhibition of insulin-stimulated glucose transport and decreased rates of glycolysis and glycogen synthesis were also found in denervated or tenotomized muscles (8, 22). These metabolic changes occur within a few hours after denervation or tenotomy in skeletal muscles of different fiber types, being the most pronounced in muscles composed predominantly of slow-twitch fibers (32, 33).

There is evidence that muscle electrostimulation at least partly reverses some biochemical alterations caused by denervation or tenotomy, e.g., it increases glucose uptake and glucose utilization by myocytes (17, 33). It was also reported that elimination of neural activity in tenotomized muscles by simultaneous denervation can limit the loss of myofibrillar ATPase and other mitochondrial oxidative enzyme activities and reduce another metabolic changes induced by tenotomy (29).

Studies on the metabolic effects of muscle denervation or tenotomy concern mainly protein or carbohydrate metabolism, whereas much fewer data are available on lipid metabolism. An increase in diacylglycerol content and alterations in phospholipid composition of muscle cells were reported after denervation (13, 31, 34). However, to our knowledge, there is no information on the effects of denervation or tenotomy on the muscle potential to use the circulating plasma triacylglycerols (TG).

It is generally accepted that uptake of plasma TG, in the form of chylomicrons and very-low-density lipoproteins, requires their prior hydrolysis through the action of lipoprotein lipase (LPL). This enzyme is synthesized in several tissue cells, e.g., in the myocytes, released, and then transferred across the endothelium where it binds to the luminal surface of endothelial cells. The proteoglycan-bound fraction is the active enzyme, often defined as the heparin-releasable LPL (HLPL), whereas the fraction remaining within the myocytes is considered as the residual, most probably inactive, LPL (RLPL).

It was previously reported (6) that in skeletal muscles LPL activity depends on the muscle type, being highest in red muscles composed mostly of slow-twitch oxidative fibers, e.g., the soleus, and the lowest in white muscles, consisting of fast-twitch glycolytic fibers. These differences in LPL activity are probably due to differences in the contractile muscle activity and preferences for lipid substrate as the energy source in...
redd and white muscle fibers. Many investigators documented that enhanced physical activity increases LPL activity in skeletal muscles engaged in the effort (6, 7, 16, 21, 23, 24), suggesting that activity of this enzyme is controlled by local alterations within the contracting muscles (7). This hypothesis has been confirmed by recent findings of Hamilton et al. (12), who demonstrated that, in resting rats, local electrostimulation of a motor nerve causes a significant rise in LPL activity, accompanied by an increase in LPL mRNA and immunoreactive protein mass. On the other hand, when hindlimbs were immobilized for a few days, a decrease in LPL activity was found (12). However, the effect of a short-term reduction of contractile activity by local muscle immobilization on LPL activity is still poorly recognized.

Therefore, the purpose of this study was 1) to determine the effect of muscle inactivity induced by denervation, tenotomy, and tenotomy with simultaneous denervation on the activity of two fractions of LPL, i.e., HLPL and RLPL, as well as on TG content in the rat skeletal muscles of different fiber composition, and 2) to find out whether increased muscle contractile activity by electrostimulation could reverse changes in the muscle LPL activity and TG content.

MATERIALS AND METHODS

Animal protocols. Male Wistar rats weighing 180–200 g were used in this study. The animals were kept (10 rats in cage) in a temperature-controlled room at 24°C with a light period from 0600 to 1800 and received a standard laboratory chow and water ad libitum. Food was withheld 12–15 h before the animals were killed. Two series of experiments were performed.

In the first series, the rats were randomly divided into three main groups: 1) with muscles from one hindlimb denervated by the sciatic nerve cut (n = 8), 2) with muscles from one hindlimb tenotomized by Achilles tendon section (n = 8), and 3) with muscles from one hindlimb tenotomized and simultaneously denervated (n = 8).

Muscles from the contralateral intact hindlimb served as controls. In preliminary experiments, a group of 8 rats with both hindlimbs intact was additionally examined to check whether in the rat the contralateral hindlimb of denervated or tenotomized ones can really serve as controls.

The experimental procedure was approved by the Ethical Committee of the Medical Research Centre, Polish Academy of Sciences in Warsaw, Poland. Both denervation and tenotomy were performed under light ether anesthesia. For 12 h after surgery the animals were left free in their cages. Then they were killed by decapitation. To minimize diurnal variations in LPL activity, all experiments were performed between 9 AM and noon. Muscles from both intact and operated hindlimbs were quickly removed, washed with sterile 0.95% saline, blotted dry, frozen in liquid nitrogen, and stored at −70°C until further analysis. Three types of muscles were examined: soleus, composed mainly of slow-twitch oxidative fibers; the red portion of gastrocnemius, containing predominantly fast-twitch glycolytic-oxidative fibers; and the white portion of gastrocnemius, composed mainly of fast-twitch, glycolytic fibers.

In the second series of experiments, tenotomy, denervation, or tenotomy with simultaneous denervation was fol-lowed by muscle electrostimulation. The rats were randomly assigned to three groups, identical to those in the first series: unilaterally denervated (n = 16), unilaterally tenotomized (n = 16), and unilaterally tenotomized and simultaneously denervated (n = 16).

Electrostimulation was applied to the operated muscles 12 h after the surgery (8 rats from each group). For technical reasons, it was impossible to stimulate simultaneously both intact and operated muscles, so electrostimulation of muscles from the contralateral intact hindlimbs was performed on the remaining eight rats from each group. Additionally, muscles electrostimulation was performed on intact control rats (n = 5).

Before the electrostimulation, rats were anesthetized by an intraperitoneal injection of pentobarbital sodium (50 mg/kg body wt). The skin was removed from a hindlimb, the sciatic nerve was prepared for stimulation, and then the gastrocnemius-soleus-plantaris muscle group was exposed to isometric contractions as previously described by Gorski et al. (11). In tenotomized rats, the Achilles tendon was fixed before stimulation. The nerve was stimulated with supramaximal tetanic pulses (7 V, 0.05-ms duration, delivered in 100-ms trains at 100 Hz) with 1 pulse/s for 30 min. Immediately after electrostimulation, hindlimb muscles were removed, blotted dry, frozen, and stored at −70°C.

Biochemical assays. LPL activity in skeletal muscle samples was determined by measuring the release of [14C]oleic acid emulsion of glycerol-tri[14C]oleate in a Tris-buffer medium containing albumin and pooled human serum as an activator, according to Taskinen et al. (30). For determination of HLPL activity, muscle samples weighing 5–10 mg were incubated with gentle shaking at 28°C in 200 μl of Krebs-Ringer-0.1 M Tris-HCl buffer at pH 8.4, containing 1% bovine serum albumin and 5 IU of heparin. After 40 min, the tissue was removed from the medium, 500 μl of the triolein substrate mixture were added, and the incubation was continued for a further 120 min.

To determine the activity of RLPL, the samples of muscle tissue removed from the medium after 40 min of incubation were homogenized in 500 μl of Krebs-Ringer-Tris-HCl buffer. Samples of the homogenate (200 μl) were incubated with 500 μl of substrate mixture for 120 min.

At the end of incubation, duplicate samples of the eluate and homogenate were taken from the incubation mixture and extracted with 3.25 ml of methanol-chloroform-heptane (1.41:1.25:1.0, vol/vol/vol). Then, 1.0 ml of 0.05 M potassium carbonate buffer was added (pH 10.05). After mixing, 1.0 ml of the upper phase was used for scintillation counting (LKB Wallac 1211). Each assay included two blank tubes. LPL activity was expressed as micromoles free fatty acids (FFA) per gram protein per hour.

Muscle TG contents were determined according to the enzymatic method of Eggstein and Kreutz (9) after an overnight extraction of muscle samples in chloroform-methanol mixture (2:1). TG content was expressed as micromoles per gram.

Total protein content was measured by the method of Lowry et al. (18) in another muscle sample taken from the same muscle as for determination of LPL activity. Then, LPL activity (both fractions) was expressed relative to protein content of the tissue. The total protein concentration averaged 140–155 mg/g in the soleus and 125–135 mg/g in the gastrocnemius (red portion).

Statistical analysis. All data are presented as means ± SE. Two-way analysis of variance (ANOVA-MANOVA) followed by Tukey's test was used to verify significance of differences
between experimental and intact muscles as well as between unstimulated and stimulated muscles.

RESULTS

Marked differences in the activity of LPL were found between the soleus and the red and white portions of the gastrocnemius muscles. Activities of both fractions of LPL were the highest in the soleus, lower in the red portion of gastrocnemius, and undetectable in the white portion of gastrocnemius.

Activities of two LPL fractions in the soleus and red portion of gastrocnemius muscles taken from the intact contralateral hindlimbs of the rats undergoing denervation and tenotomy did not differ from those found in the muscles obtained from the control group of intact (unoperated) animals (the latter were 35.62 ± 1.28 and 43.75 ± 0.44 μmol FFA·g protein⁻¹·h⁻¹ for HLPL and RLPL, respectively, in the soleus and 11.60 ± 0.78 and 24.85 ± 1.19 μmol FFA·g protein⁻¹·h⁻¹ for HLPL and RLPL, respectively, in the red portion of gastrocnemius muscles).

Denervation caused a marked decrease in activities of both HLPL and RLPL in the soleus muscle (Fig. 1A) and in the red portion of gastrocnemius (Fig. 2A) compared with the respective muscles of the intact, contralateral hindlimbs. Activities of HLPL and RLPL were also significantly reduced in the soleus (Fig. 1B) and in the red portion of gastrocnemius muscles (Fig. 2B) taken from the tenotomized hindlimbs compared with the values in the contralateral intact ones. After tenotomy accompanied by simultaneous denervation, activities of both LPL fractions were diminished both in the soleus (Fig. 1C) and in the red portion of gastrocnemius (Fig. 2C) compared with the intact muscles.
Electrostimulation of muscles from the intact hindlimbs resulted in a significant elevation of the activities of two LPL fractions both in the soleus and red portion of gastrocnemius (Figs. 1 and 2). Similar results were obtained for LPL activities in the soleus (65.40 ± 1.17 and 86.95 ± 1.16 μmol FFA·g protein⁻¹·h⁻¹ for HLPL and RLPL, respectively) and the red portion of gastrocnemius (18.92 ± 1.10 and 44.01 ± 1.06 μmol FFA·g protein⁻¹·h⁻¹ for HLPL and RLPL, respectively) after stimulation in intact, control rats.

In denervated hindlimbs, electrostimulation markedly increased activity of HLPL in the soleus as well as in the red portion of gastrocnemius compared with the respective unstimulated, denervated muscles. Activity of RLPL in both muscles examined tended to increase after electrostimulation, but this effect was significant only in the soleus. After electrostimulation, activities of both LPL fractions in denervated muscles were close to those found in unstimulated, intact muscles (Fig. 1A).

Electrostimulation slightly but significantly (P < 0.05) increased activity of HLPL in tenotomized soleus, whereas activity of RLPL remained unchanged (Fig. 1B). In the red portion of the gastrocnemius, electrostimulation after tenotomy had no pronounced effect on either fraction of LPL (Fig. 2B).

When electrostimulation was applied to tenotomized and simultaneously denervated soleus, only the residual fraction of LPL increased significantly (Fig. 1C), whereas in the red portion of gastrocnemius activities of two LPL fractions were elevated compared with the unstimulated muscles (Fig. 2C). However, activity of neither LPL fraction reached the values found in the intact, stimulated muscles.

The effects of denervation and/or tenotomy per se and those followed by electrostimulation on TG content in the soleus and red and white portion of gastrocnemius are summarized in Table 1. The TG content remained unchanged or was only slightly elevated in all examined muscles from the experimental groups compared with the intact muscles. Electrostimulation caused only a slight but significant decrease (P < 0.05) in TG content in the soleus and red portion of gastrocnemius obtained from the animals that underwent tenotomy or tenotomy with denervation. None of the procedures applied had any effect on TG content in the white portion of the gastrocnemius.

**DISCUSSION**

The present study demonstrated a marked reduction in the activity of HLPL and RLPL fractions in the soleus and red portion of gastrocnemius muscles after denervation or tenotomy as well as tenotomy with simultaneous denervation. Activity of the muscle LPL in the intact hindlimbs remained unchanged, and it was similar to that found in muscles from the control unoperated rats. These findings suggest that a decrease in LPL activity of denervated and/or tenotomized muscles is directly caused by local alterations after the sciatic nerve cut or Achilles tendon section.

It is known that the effects of denervation and tenotomy depend on time. The main change occurring after these procedures is an immediate loss of muscle contractile activity, which is maintained for at least a few days (up to 7 days) after the sciatic nerve cut and/or Achilles tendon section. According to Baldwin et al. (3), muscle unloading leads to a decreased capacity for oxidation of long-chain FFA. It has been also reported that, in inactive muscles, accumulation of lipids increases (10, 20), thus suggesting a decline of FFA combustion under these conditions.

**Table 1. Triacylglycerol content in the soleus and gastrocnemius muscles (red and white portion) after denervation, tenotomy, and tenotomy with simultaneous denervation and electrostimulation**

<table>
<thead>
<tr>
<th></th>
<th>Triacylglycerol Content, μmol/g wet wt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Soleus</td>
</tr>
<tr>
<td>Intact muscles from contralateral to denervated hindlimbs</td>
<td></td>
</tr>
<tr>
<td>Unstimulated</td>
<td>10.32 ± 0.60</td>
</tr>
<tr>
<td>Stimulated</td>
<td>9.89 ± 0.32</td>
</tr>
<tr>
<td>Muscles after denervation</td>
<td></td>
</tr>
<tr>
<td>Unstimulated</td>
<td>11.11 ± 0.28</td>
</tr>
<tr>
<td>Stimulated</td>
<td>11.05 ± 0.33</td>
</tr>
<tr>
<td>Intact muscles from contralateral to tenotomized hindlimbs</td>
<td></td>
</tr>
<tr>
<td>Unstimulated</td>
<td>10.25 ± 0.20</td>
</tr>
<tr>
<td>Stimulated</td>
<td>9.49 ± 0.16†</td>
</tr>
<tr>
<td>Muscles after tenotomy</td>
<td></td>
</tr>
<tr>
<td>Unstimulated</td>
<td>10.94 ± 0.17</td>
</tr>
<tr>
<td>Stimulated</td>
<td>10.06 ± 0.24†</td>
</tr>
<tr>
<td>Intact muscles from contralateral to denervated hindlimbs</td>
<td></td>
</tr>
<tr>
<td>Unstimulated</td>
<td>10.20 ± 0.20</td>
</tr>
<tr>
<td>Stimulated</td>
<td>9.99 ± 0.16</td>
</tr>
<tr>
<td>Muscles after tenotomy and denervation</td>
<td></td>
</tr>
<tr>
<td>Unstimulated</td>
<td>10.31 ± 0.20</td>
</tr>
<tr>
<td>Stimulated</td>
<td>9.47 ± 0.20†</td>
</tr>
</tbody>
</table>

Values are means ± SE, n = 8. *Significant difference between operated and intact contralateral hindlimbs, P < 0.05; †significant differences between electrically unstimulated and stimulated muscles, P < 0.05.
In our study, only a slight elevation of TG content was found in muscles immobilized by denervation or tenotomy. This finding together with the reduced LPL activity suggests reduced ability to utilize lipids. It seems, therefore, that muscle inactivity leading to a diminished demand for FFA as an energy source can be mainly responsible for the decreased LPL activity in denervated and/or tenotomized muscles. Contrary to this assumption, Gorin et al. (10) reported increased LPL activity after a complete block of neuromuscular activity by botulinum toxin injection. However, the above-quoted authors found increased LPL activity only in the fast-twitch, glycolytic muscles 2 wk after neuromuscular block.

Although in the present study both denervation and/or tenotomy markedly affected activities of both fractions of LPL in red muscles (soleus and gastrocnemius), a decline in the activity of HLPL fraction of the enzyme directly involved in the hydrolysis of the plasma TG was more pronounced than that of the intracellular RLPL fraction. This finding remains in agreement with the data reported by Simolo et al. (27), who also noted a greater reduction in the activity of HLPL than residual RLPL in muscles taken from athletes during their detraining period.

It was demonstrated that the enzymatic changes induced by denervation depend on the muscle fiber composition and metabolic pathways. Sesodia et al. (25) reported that, in the soleus, denervation leads mainly to diminution of oxidative enzyme activities, with only slight changes in the glycolytic enzyme activities. On the other hand, Simard et al. (26) reported significant reduction of the activities of glycolytic enzymes with a concomitant elevation of hexokinase activity in a white muscle. It was suggested that in immobilized muscles their shortening may affect a magnitude of alterations in the oxidative enzyme activities (4).

In the present study, decreases in HLPL in the soleus were similar after denervation and/or tenotomy, whereas activity of the RLPL was more reduced in tenotomized than in denervated muscles ($P < 0.05$). It seems, therefore, that the decrease in LPL activity is due mainly to a loss of contractile muscle property after denervation and/or tenotomy; however, some other factors, such as, e.g., shortening or unloading that follow tenotomy, may contribute to these changes, which are expressed particularly when the intracellular fraction of the enzyme is considered.

It can be speculated that the fall in LPL activity, and especially of its active fraction, found in the present study is due partly to enhanced detachment of LPL molecules from their binding sites in the capillaries after local alterations induced by muscle inactivity. A diminished rate of LPL synthesis and increased rate of intracellular degradation or inactivation of the enzyme can be other reasons for the decreased LPL activity. As it was previously reported, muscle inactivity after denervation or tenotomy results in an inhibition of protein synthesis and activation of intracellular proteolysis (1, 5). In line with this assumption is the report of Hamilton et al. (12), who found decreased LPL activity accompanied by reduction of LPL mRNA and immunoreactive protein mass in immobilized muscles. It may be, therefore, suggested that denervation and/or tenotomy reduces production of LPL in myocytes.

Another important finding of the study is that 30 min of electrical muscle stimulation caused marked enhancement in the activity of both LPL fractions in red skeletal muscles of intact hindlimbs. Hamilton et al. (12) also reported an increased LPL activity in stimulated muscles, however, these changes were found in white muscles and after a long-term electrostimulation. Mackie et al. (19) documented that, after 25 min of electrical stimulation, the uptake of $[^{14}C]TG$ from chylomicrons markedly increases in the rat skeletal muscles of different fiber types. On the basis of a close correlation between muscle LPL activity and the uptake of circulating TG, the above-mentioned authors concluded that the rise of LPL activity after a short-term muscle electrostimulation is associated with enhanced plasma TG uptake. The results of the present study showing that electrical stimulation causes a simultaneous enhancement in the activity of both fractions of LPL in red skeletal muscles remain in agreement with the above statement, adding some new information to them. It seems likely that electrostimulation, similarly to an acute exercise, not only activates LPL synthesis but also accelerates release of this enzyme from the intracellular pool to the sites of its action in the capillaries (7, 21, 24).

Surprisingly, we have failed to find any marked alterations in TG content in the intact soleus and in the red portion of gastrocnemius muscles after electrostimulation. These results indicate that, during electrostimulation, the rate of TG synthesis may be equal to the rate of intracellular TG hydrolysis, suggesting that the supply of FFA from the plasma is sufficient to replenish the intracellular TG pool. This assumption is in agreement with the report of Hopp and Palmer (15), who found only slight changes in TG content after a short-term electrostimulation in the rat skeletal muscles.

The present investigation revealed that increased muscle contractile activity by electrostimulation markedly enhanced activities of both LPL fractions in the soleus and red portion of gastrocnemius muscles submitted to previous denervation and to tenotomy with simultaneous denervation. However, in these muscles, electrical stimulation was less effective in increasing LPL activity than in control intact muscles, so activity of this enzyme was not fully restored.

It seems likely that reduced intracellular LPL synthesis under condition of reduced muscle activity, as well as an impairment of translocation of this enzyme to capillaries, induced by denervation with/or without tenotomy, may be responsible for a less pronounced effect of electrostimulation in these muscles compared with intact ones. It is interesting, however, that similar electrostimulation applied to tenotomized muscles had no pronounced effect on LPL activity. Thus it may be suggested that after tenotomy some other, not so...
easily reversible changes occur, e.g., accelerated intracellular proteolysis or structural disturbances (2), caused by muscle shortening. They may be responsible for the lack of pronounced alterations in LPL activity after electrostimulation applied to tenotomized muscles. This suggestion may be confirmed by the finding that tenotomy with simultaneous muscle denervation, when shortening was eliminated, caused LPL activity to be partly restored by electrostimulation.

Muscle electrostimulation, applied in the present study, resulted in a slight decrease in TG content in the red muscles submitted to denervation and/or tenotomy. One might suppose that diminished delivery of FFA from the plasma, caused at least partly by a decrease of LPL activity as well as by an inhibition of glucose transport, could lead to enhanced mobilization of FFA from intramuscular TG pool, which is not fully compensated by synthesis of these lipids. This hypothesis may be supported by the data reported by other authors (14, 28) who demonstrated a marked decrease in TG content in the red portion of gastrocnemius after short-term electrostimulation when the tissue was incubated in the medium without FFA.

To summarize, muscle denervation, tenotomy, and tenotomy with simultaneous denervation result in a rapid decrease in LPL activity accompanied by a slight elevation of intramuscular TG content. These findings indicate a decline of muscle potential to take up and utilize fatty acids derived from the plasma TG after reduced contractile activity. Enhancement of this activity by muscle electrostimulation increases activities of both LPL fractions in intact, control muscles and partly restores the activity of this enzyme in red muscles denervated or tenotomized with simultaneous denervation. Electrostimulation has no influence on tenotomized muscles, in which shortening occurs. These findings suggest that muscle contractile activity plays a predominant role in determining LPL activity in skeletal muscles. However, it seems likely that, apart from the above, some other local alterations, caused by muscle immobilization in a shortened position, may also affect the enzyme activity.

We are grateful to H. Kaciuba-Uscilko and K. Nazar for valuable comments in all stages of the study.

Financial support of D. Czarnowski by the Medical School of Białystok (Grant 3-18-694) is kindly acknowledged.

REFERENCES


