Role of local contractile activity and muscle fiber type on LPL regulation during exercise

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Role of local contractile activity and muscle fiber type on LPL regulation during exercise. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E1016–E1022, 1998.—The purpose of this study was to determine the influence of local contractile activity on lipoprotein lipase (LPL) regulation in skeletal muscle. Short-term voluntary run training increased LPL mRNA concentration and LPL immunoreactive mass about threefold in white skeletal muscles of the rat hindlimb (all P < 0.01). Training also increased total and heparin-releaseable LPL enzyme activity in white hindlimb muscles and in postheparin plasma (P < 0.05). Training did not enhance LPL regulation in a white muscle that was not recruited during running (masseter). LPL levels were already high in red skeletal muscles of control rats, and training did not result in a further rise. In resting rats, local electrical stimulation of a motor nerve to a predominantly white muscle caused a significant rise in LPL mRNA, immunoreactive mass, and enzyme activity relative to the contralateral control muscle of the same animals (all P < 0.01). Finally, LPL expression was several times greater in a red muscle (soleus) of rats with normal postural activity than rats with immobilized hindlimbs (P < 0.01). In summary, these studies support the hypothesis that local contractile activity is required for increasing LPL expression during exercise training and for maintaining a high level of LPL expression in postural muscles.

physical activity; triglyceride; gene regulation

Lipoprotein lipase (LPL) is a plasma enzyme that is synthesized primarily by muscle cells and adipocytes. Tremendous progress has been made in establishing the multifunctional consequences of high and low LPL levels. Overexpression of LPL in transgenic mice reduced plasma triglycerides and increased high-density lipoprotein cholesterol (HDL-C) concentration (20, 30), prevented hypertriglyceridemia after a high-fat or high-sucrose diet (30), and blunted the lipoprotein disorders associated with diabetes (29). In addition, overexpression of LPL, specifically in skeletal muscle, was sufficient to impart resistance to diet-induced obesity (15). A rapid catabolism of HDL apolipoprotein and decreased HDL-C was observed in primates after acute injection of an antibody against LPL (12). LPL also has a role in receptor-mediated clearance of lipoprotein remnants (4). The above effects may apply to human disease, because several studies have documented premature atherosclerosis, elevated triglyceride-rich lipoproteins, and reduced HDL-C in people with moderately reduced LPL activity caused by LPL mutations (e.g., Ref. 25). It is unique for a single enzyme to potentially impact polygenic diseases so profoundly. Thus it is surprising that the study of LPL regulation in muscle has been “neglected” (28).

In the current study, we have focused on physical exercise, because exercise may be a practical means to significantly increase skeletal muscle LPL activity and plasma triglyceride metabolism (3, 16, 26). Several research teams have initiated the difficult endeavor of explaining how LPL is regulated during exercise. A valuable contribution to understanding compartmentalization of the LPL enzyme has been documented (24). Additionally, Sép et al. (27) made the important finding that the time course of LPL mRNA and protein after acute exercise in humans was temporally related, consistent with the suggestion that pretranslational events may be important (17). However, other studies (23, 31) concluded that skeletal muscle LPL mRNA concentration did not change after running in rats or 2 wk of inactivity in endurance athletes (32). We have extended the understanding of LPL regulation during exercise in a series of systematic experimental designs that have taken into consideration the large amount of information available about recruitment patterns in different muscles (1, 2, 13, 18, 33). The overall aim was to determine the role of local contractile activity on LPL regulation in different muscle fiber types. First, LPL mRNA, LPL protein mass, LPL activity, and compartmentalization of LPL activity were determined in a wide range of muscle types in sedentary and voluntary run-trained rats. We focused on the following questions. Does voluntary running in rats mimic human exercise by increasing LPL mRNA and LPL protein content in limb muscles? Is there a difference in LPL regulation between red and white muscle fiber types during voluntary running? Results led us to additional experiments to answer fundamental questions never studied before. Does intense and prolonged exercise (voluntary running) change LPL regulation in skeletal muscles that are not recruited during exercise (masseter muscle)? A positive answer to this last question would provide strong evidence for the first time that systemic factor(s) regulate LPL during exercise. Other researchers have often suggested this possibility (17, 19, 27), because studies in resting animals (8) have shown a high degree of LPL regulation by systemic factors (hormones, cytokines, growth factors). Then, we determined the effect of stimulating a peripheral motor nerve to determine whether local contractile activity was a sufficient stimulus to increase local LPL expression. Finally, we studied the effect of short-term hindlimb immobilization to determine
whether the high level of LPL expression in the soleus was because of muscle contractions during postural activities. Taken together, these studies support the hypothesis that the effects of local contractile activity are necessary and sufficient to increase LPL expression during exercise.

**MATERIALS AND METHODS**

**Animals**

Female Sprague-Dawley rats (Harlan) at a weight of ~175 g were maintained in a controlled environment in accordance with National Institutes of Health guidelines. All rats were housed with a 12:12-h light-dark cycle (light from 0700) and fed standard low-fat rat chow ad libitum (Harlan Teklad F6 Rodent Diet). Animals were anesthetized by injection of a mixture of ketamine (54 mg/ml), xylazine (2.2 mg/ml), and acepromazine (3.5 mg/ml) into the gluteal muscles (1.4 ml/kg) before invasive procedures.

**Description of Voluntary Run Training Model**

Rats were allowed access to voluntary running wheels for 14–20 consecutive days. This mode of exercise training was used because rats voluntarily run at the high wheel speeds (3) required to recruit all fiber types (2, 33), while avoiding the potentially confounding effects associated with stress from forced exercise (10). Calibrated bicycle computers recorded the average speed, distance, and duration of running activity. More than 90% of the voluntary running activity occurred during the dark cycle when rats are normally active (data not shown; Ref. 3). Running distance increased gradually each day during the 1st wk and reached a plateau during the 2nd wk. This plateau was maintained for ~2–3 mo in a subset of nine rats (data not shown). For the 4 days before tissue collection, the average running distance was steady at 11.4 ± 0.7 km/day, and the running speed was 56 ± 1 m/min. Therefore, these rats ran at a high intensity for a cumulative duration of 3.4 h/day.

Tissue was collected from rats 2–4 h after voluntary running at 0900–1100. A subset of runners rested for 25–27 h before being killed. Red muscles were obtained, including the rectus femoris (RF), plantaris, pure white muscles (e.g., the vastus intermedius with a small red portion of adjacent vastus medialis). Predominantly white muscles included the rectus femoris (RF), plantaris, pure white vastus lateralis, mixed vastus lateralis, and the superficial masseter (a jaw muscle). The myosin isof orm for these five white muscles has been shown (7) to range between 60 and 100% type IIB, with the remainder mainly type IID/X (30–0%). The two red skeletal muscles ranged between 60 and 90% type I, and the remainder was mostly type IIA myosin. Throughout this study, we have used the more simple nomenclature of “red” and “white” because there was no need for a more complicated subdivision of the muscles to describe the observed changes in LPL.

**Electrical Stimulation**

Low-frequency motor nerve stimulation was used as a model of intense unilateral contractile activity and metabolic demand. Miniature battery-operated stimulators (14) were implanted in the abdominal cavity of anesthetized rats. Electrodes were carefully tied next to the common peroneal nerve (on the upper surface of the gastrocnemius) for stimulation of the tibialis anterior (TA) muscle. Stimulation (10 Hz, 3 V, 0.25-ms pulses) commenced ≥4 days after surgery. Stimulators were activated 4 h each day, and rats remained asleep or performed normal cage activities during the involuntary contractions. Muscle was harvested after a 24-h rest period after 28 consecutive days of stimulation. The resting control TA muscle (no stimulation) was obtained from the contralateral leg.

**Hindlimb Immobilization**

Hindlimbs of rats were placed in plaster casts (5) for 7 days, and rats were housed individually. The soleus muscles from casted and control rats were obtained to determine the effects of complete loss of postural support by this muscle.

**LPL mRNA**

Northern blot analysis was performed with total RNA extracted from powdered muscle by the procedure of Chomczynski and Sacchi (6). Isolated RNA was denatured and electrophoresed, then transferred to nylon membrane (Hybond-N+, Amersham) by capillary action, and then ultraviolet cross-linked. Membranes were prehybridized for 2–3 h at 68°C in 12 ml hybridization buffer (QuickHyb, Stratagene). Radioactively labeled probe with a specific activity of 1–4 × 106 counts·min⁻¹·cpm·mg⁻¹ DNA was prepared by random priming rat LPL cDNA with α-32P-labeled deoxyctydine 5'-triphosphate (3,000 Ci/mmol). After hybridization (1–3 × 10⁶ cpm/ml) for 2 h at 68°C, the membrane was washed two times for 15 min with 2× standard sodium citrate (SSC)-0.1% SDS at 20°C and then washed once for 30 min with 0.1 × SSC-0.1% SDS at 55°C. The radiolabeled membrane was subjected to autoradiography with intensifying screens, and the integrated optical density (IOD) was quantified. The IOD from 18S rRNA was subsequently determined by a probe with one-third the specific activity of LPL. In no case was there a treatment effect on 18S IOD. Dose-response analysis with increasing amounts of RNA was performed for each muscle group to verify that the IOD quantitatively reflected changes in LPL mRNA concentration. Between 5 and 20 µg of total RNA were used when comparing different muscles so that the IOD would be in the linear range of the assay. Values were expressed as LPL IOD/18S IOD.

**LPL Immunoreactive Protein Mass**

A sandwich enzyme-linked immunosorbent assay (ELISA) was used to measure LPL protein mass in selected muscles to determine whether the alterations in LPL mRNA resulted in LPL protein expression. The primary or “capture” antibody was polyclonal chicken anti-bovine LPL (“egg A”) generously provided by Dr. John Goers. Antigen injection and affinity-purification procedures were similar to previous descriptions (11). Antibody was adsorbed onto 96-well microtiter plates (Nunc MaxiSorp) at a concentration of 2 µg/ml at 37°C for 4 h. Excess antibody was removed, and wells were washed with Tris-buffered saline containing 0.05% Tween-20 and 0.25% BSA. Plates were incubated with blocking buffer (0.1 M H3BO4, 0.12 M NaCl, 0.05% Tween-20, 1 mM EDTA, 0.25% BSA, 0.05% NaN3) for 1 h at 22°C. Muscle homogenates (described below in LPL enzyme activity) were first diluted in PBS with 0.05% Tween-20 detergent, 1 mg/ml heparin, and 0.4% BSA and then added to wells in duplicate at two concentrations for 1 h at 4°C. The SD2 monoclonal antibody (gift from Dr. John Brunzell) was diluted 1:10,000 in blocking buffer (0.105 µg/ml) and incubated in wells for 3 h at 22°C. The SD2 was raised in mouse and detects both monomeric and dimeric LPL (21). Goat anti-mouse IgG conjugated to hors eradish peroxidase (Sigma, 1:8,000 in blocking buffer) was added for 2 h after the wells were washed thoroughly. Color was developed by the addition of 100 µl of 3,3′,5,5′-
tetramethylbenzidine peroxidase enzyme immunoassay substrate (Bio-Rad). The reaction was stopped after 10 min with 100 µl of 1 M H₂SO₄, and the optical absorbance at 450 nm was measured. The optical absorbance from two dilutions of each sample and from serial dilutions of a reference standard obtained from rat postheparin plasma was used to calculate the LPL immunoreactive mass (units/mg tissue), where a unit is defined as immunoreactive mass of 1 µl standard.

LPL Enzyme Activity

Three general sources of LPL enzyme were assayed: muscle homogenates, postheparin eluates from fresh tissue, and plasma after intravenous heparin injection. For preparation of tissue homogenates, whole muscles were flash frozen and finely powdered under liquid nitrogen and homogenized intermittently with a polytron (Brinkman Instruments, Westbury, NY) at 0°C for 3 × 15-s bursts. The homogenization buffer consisted of 0.05 M Tris-HCl (pH 8.1), α-proteinase (2 µg/ml), leupeptin (10 µg/ml), benzamidine (1 mM), pepstatin (1 µg/ml), EDTA (5 mM), BSA (1 mg/ml), phenylmethylsulfonyl fluoride (0.4 mM), and heparin (5 U/ml, 0.025 mg/ml). Muscles were homogenized at a concentration of 100 mg/ml buffer. For heparin-releasable experiments, fresh tissue was obtained from selected muscles (RF, soleus, and masseter) and adipose tissue surrounding a kidney and uterus. A tissue mass of ~50 mg was weighed, minced into ~10-mg pieces, and soaked immediately in PBS containing 100 U/ml heparin (Sigma) for 30 min at 37°C. Studies of a wide range of heparin concentrations (0–200 U/ml) demonstrated that maximal extracellular LPL activity was released from soleus and RF at this heparin dosage (data not shown).

Postheparin plasma was obtained from a separate group of rats 15 min after injection of 300 U/kg heparin into a catheterized jugular vein. LPL activity in plasma was calculated as total lipase activity minus the activity remaining with 1 M NaCl. We confirmed that high salt content inhibited >95% of the lipase activity specific to LPL by preincubation with monodonal anti-rat LPL antibody (9). Muscle tissues were not obtained from rats injected with heparin.

LPL enzyme activity was measured with a [¹³C]triolein-glycerol stable substrate (22). LPL activity was measured by the rate of hydrolysis of [¹³C]triolein containing substrate emulsified with lecithin, in the presence of heat-inactivated rat serum as the source of apolipoprotein C-II and fatty acid-free albumin (22). Assays were performed at 37°C for 60 min. Serial dilutions of representative samples in each assay verified that LPL activity was linear with time and amount of substrate in the range that assays were performed. The assay was specific to LPL because lipase activity was not detectable when incubated with monoconal anti-rat LPL antibody (9) before addition of substrate.

Statistics

One-way analysis of variance (ANOVA) was used to compare the effects of voluntary running and immobilization.

Table 1. LPL mRNA levels in sedentary control and voluntary run trained muscles

<table>
<thead>
<tr>
<th>Treatment</th>
<th>White Muscle</th>
<th>Red Muscle</th>
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<tbody>
<tr>
<td></td>
<td>Masseter</td>
<td>WV</td>
</tr>
<tr>
<td>Sedentary</td>
<td>2.0 ± 0.2</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>Run trained</td>
<td>1.7 ± 0.2</td>
<td>2.5 ± 0.5</td>
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Values are means ± SE reported as relative values (LPL IOD/18S IOD) determined by Northern blots; n = 5–8. LPL, lipoprotein lipase; WV, white vastus lateralis; RF, rectus femoris; VL, mixed vastus lateralis; VIm, vastus intermedius with portion of red vastus medialis. RF-2 muscle removed 25–27 h after exercise; all other muscles removed 2–4 h after exercise. *P < 0.01 by one-way ANOVA.

Fig. 1. Northern blot analysis of lipoprotein lipase (LPL) mRNA and 18S rRNA content in rectus femoris (RF) muscle. Total RNA (20 µg) was separated, transferred to nylon membrane, and hybridized with rat LPL cDNA probe as described in MATERIALS AND METHODS. C, caged sedentary control (n = 7); R, voluntary run trained (n = 6).

Student’s paired t-test was used for comparisons of the stimulated TA muscle and paired contralateral TA muscle in the resting leg. Results were considered significant at P < 0.05 and are presented as means ± SE.

RESULTS

Voluntary Run Training

LPL mRNA. Quantitative Northern blot analysis showed that LPL mRNA levels were consistently upregulated in the RF muscle of trained rats 2–4 h after running compared with sedentary controls (Fig. 1). LPL mRNA in controls and runners for rats killed 2–4 h after exercise and 25–27 h after exercise are shown in Table 1. LPL mRNA percent changes 2–4 h after running are summarized in Fig. 2A. Compared with controls, LPL mRNA was ~150–200% greater in white hindlimb muscles of runners (P < 0.01), not different in the white masseter muscle, and was not different in the three red muscles. Finally, LPL mRNA levels in the RF muscle of sedentary controls was not different from those of runners that rested 25–27 h before muscles were harvested (Table 1).

LPL protein mass. The LPL mass was measured in three muscles in controls and runners. The LPL mass was 179% greater (P < 0.01) in the RF of runners than in controls (Fig. 2B) and was not significantly different between the run-trained and sedentary control animals in the masseter and soleus muscles (Fig. 2B; Table 2).

LPL enzyme activity in muscle homogenates. Total LPL activity was ~150% greater (P < 0.01) in the RF and pure white vastus lateralis of runners compared with controls (Fig. 2C; Table 3). LPL activity was not different between runners and controls for any of the three red muscles or the masseter.
Heparin-releasable LPL enzyme activity. Postheparin LPL activity (Fig. 3) in plasma and the RF muscle was significantly higher in runners than in controls ($P < 0.05$). There was no difference in heparin-releasable enzyme activity in the masseter, soleus, or adipose tissue.

Electrical Stimulation

Unilateral electrical stimulation was performed to test the hypothesis that intense local muscle contractions in a nonexercising animal would be sufficient to increase local LPL expression. The expression of LPL mRNA, protein, and activity was significantly greater in the TA muscle, which was stimulated 4 h/day compared with the inactive TA muscle in the contralateral leg (Table 4; $P < 0.01$). This average may have underestimated the peak rise in LPL, because muscle was sampled after a 24-h rest period.

Immobilization

Hindlimb immobilization was used in this study to determine whether the normal contractile activity of a postural muscle is sufficient to induce high LPL expression. Relative to controls with normal cage activity, LPL mRNA concentration, mass, and activity were significantly lower in the soleus muscles of immobilized rats without postural activity (Table 4; $P < 0.01$ for mRNA and mass; $P < 0.05$ for activity).

**DISCUSSION**

This study includes the most complete information available on the role of local contractile activity on LPL regulation. Findings from multiple muscles covering a wide range of functions and fiber types are summarized below. Short-term voluntary running greatly increased LPL mRNA, LPL mass, total LPL activity, and heparin-releasable LPL activity only in predominantly white skeletal muscles recruited during running (Figs. 2 and 3). The rise in LPL mRNA was attenuated significantly within 25–27 h after exercise (Table 1), although LPL activity remained relatively high during this time (Table 3). There was no discernible effect of prolonged (3.5 h/day) and high-intensity run training on LPL regulation at any level in red muscles or in white muscle not recruited during running (Figs. 2 and 3).

**Fig. 2.** LPL mRNA (A), protein mass (B), and total enzyme activity (C) percent differences due to voluntary running. WV, white vastus; RF, rectus femoris; VIm, vastus intermedius with a portion of red vastus medialis. Muscles are grouped according to functional utilization: superficial masseter (a white muscle from the jaw that is not recruited during running); WV and RF (2 white hindlimb muscles recruited during intense running); and VIm, soleus, and heart (3 red muscles with high contractile activity in control animals and runners). Bars are mean percent differences ± SE between sedentary control and voluntary run trained animals. LPL levels in WV and RF are significantly higher in runners than in sedentary controls; $*P < 0.01$ by one-way ANOVA.

**Table 2.** LPL protein mass in sedentary control and voluntary run trained muscles

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Masseter</th>
<th>RF</th>
<th>Red Muscle</th>
</tr>
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<tbody>
<tr>
<td>Sedentary</td>
<td>0.156 ± 0.014</td>
<td>0.162 ± 0.014*</td>
<td>0.900 ± 0.060</td>
</tr>
<tr>
<td>Run trained</td>
<td>0.152 ± 0.014</td>
<td>0.452 ± 0.042*</td>
<td>0.860 ± 0.060</td>
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Values are means ± SE measured in units/mg tissue; $n = 12$. *$P < 0.01$ by one-way ANOVA.
Thus increased postheparin plasma LPL activity (Fig. 3) reflected changes in white skeletal muscle with a high level of recruitment during running. Indirect electrical stimulation of a predominantly white muscle in resting animals also produced a local increase in LPL mRNA, LPL mass, and LPL activity (Table 4). The fall in LPL mRNA, LPL mass, and LPL activity in the soleus during short-term hindlimb immobilization showed that this muscle type was responsive to contractions associated with postural activity (Table 4). Therefore, both red and white muscles were responsive to physiological forms of contractile activity.

Identifying the effect of training on LPL mRNA abundance has been a recent goal of several studies to determine whether future investigation should focus on posttranslational processes interacting with “exercise signals.” The very large increases in LPL mRNA (Fig. 2) suggested that posttranslational processes played a large part in the increased LPL protein and activity. Although human studies found that skeletal muscle LPL mRNA concentration increased significantly (26, 27), two reports (23, 31) concluded that rat skeletal muscle LPL mRNA concentration did not change after treadmill training. Like an earlier study (23), we found no increase in white hindlimb muscles using treadmill speeds of 15–25 m/min (23, 31). This may partly be explained by observations that relatively slow trotting (<40 m/min) does not recruit white muscle significantly (2, 18, 33). Furthermore, rats had rested 16–24 h before muscles were harvested (23, 31), which could have reduced the ability to detect transient LPL mRNA changes as found for RF in the current study (Table 1). It is not known whether LPL mRNA differences between the current investigation (with female rats) and prior studies (with male rats) were also because of gender differences or the pattern of running activity. Nevertheless, the current findings are consistent with human studies (26, 27). In summary, this study provides strong evidence that daily voluntary exercise induced a large rise in LPL levels in rat hindlimb muscles as a result of posttranslational processes.

The observation that training increased LPL regulation in white hindlimb muscles, but not in red muscles, may be explained by the normal daily duration of contractile activity in control rats. Measurements of motor-unit activity (13) demonstrated that white muscle fibers were rarely recruited (~2 min/day) in rats housed in standard laboratory cages. White muscles had relatively low LPL mRNA, protein, and activity in control rats. Rats running at ~56 m/min recruit all fiber types in the hindlimb (1, 2, 18, 33). LPL levels in muscles that were heavily recruited in control rats (24 h/day for heart and ~7 h/day for soleus; Ref. 13) were not increased further by training. Thus voluntary running for 3.5 h/day would have greatly increased the relative number of muscle contractions in white hindlimb muscles and probably changed the metabolic homeostasis of white muscle more than red muscle.

The above discussion implies that high LPL expression in the soleus required posturally related muscle contractions. The immobilization experiment tested

<table>
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<tr>
<th>Treatment</th>
<th>White Muscle</th>
<th>Red Muscle</th>
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<tr>
<td></td>
<td>Masseter</td>
<td>WV</td>
</tr>
<tr>
<td></td>
<td>128 ± 22</td>
<td>61 ± 6*</td>
</tr>
<tr>
<td></td>
<td>105 ± 11</td>
<td>153 ± 20*</td>
</tr>
<tr>
<td>Sedentary</td>
<td>492 ± 112</td>
<td>524 ± 46</td>
</tr>
<tr>
<td>Run trained</td>
<td>550 ± 80</td>
<td>495 ± 39</td>
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Values are means ± SE in units of nmol·min⁻¹·g⁻¹ for total enzyme activity in muscle homogenates; n = 5–8 for all muscles except RF, and soleus n = 12. RF-2 muscle removed 25–27 h after exercise; all other muscles removed 2–4 h after exercise. *P < 0.01 by one-way ANOVA.

Table 4. Effect of immobilization and stimulation on LPL regulation

<table>
<thead>
<tr>
<th>LPL mRNA, LPL IOD/18S IOD</th>
<th>LPL Mass, units/mg</th>
<th>LPL Activity, nmol·min⁻¹·g⁻¹</th>
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</thead>
<tbody>
<tr>
<td>Soleus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10.6 ± 1.2†</td>
<td>1.16 ± 0.08†</td>
</tr>
<tr>
<td>Immobilized</td>
<td>4.1 ± 0.3†</td>
<td>0.24 ± 0.02†</td>
</tr>
<tr>
<td>Contralateral</td>
<td>2.6 ± 0.5†</td>
<td>0.30 ± 0.02†</td>
</tr>
<tr>
<td>Stimulated</td>
<td>8.3 ± 0.2†</td>
<td>0.70 ± 0.04†</td>
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Values are means ± SE; n = 5 for immobilization; paired t-test of stimulated leg and control leg in 12 rats, n = 12. IOD, integrated optical density; TA, tibialis anterior. *P < 0.05; †P < 0.01, one-way ANOVA.
whether inherently high recruitment of the soleus during postural activity, as indicated by motor-unit activity (13) and blood flow (1, 18), was the primary reason for high LPL expression in the soleus. The high LPL mRNA, mass, and activity (Table 4) in the soleus of control rats were not maintained at a high level in rats after hindlimbs had been immobilized for 7 days. This suggests that the relatively high LPL levels in the soleus of control rats were primarily because of the recruitment during normal cage activities (1, 13, 18). Interestingly, there was no difference in the amount of LPL in the immobilized soleus and TA muscle of control rats (Table 4), and thus no effect of fiber type remaining.

It has been shown many times before that LPL levels are low in white muscles and high in red skeletal muscles. LPL mRNA abundance in the soleus was not much different from some white muscles after voluntary run training, and the changes in white muscle were too transient to be ascribed to a fiber-type conversion or changes in capillary density (Table 1). Because running was entirely voluntary, one could also logically conclude that imposing an inactive lifestyle (cage restriction) on otherwise active rats amplified differences in LPL regulation between fiber types. Results in Table 4 showed a reversal in the normal fiber-type expression of LPL in the TA and soleus muscles, such that the stimulated TA muscle had much greater LPL mRNA, mass, and activity than the immobilized soleus muscle. Therefore, the present study raises the interesting possibility that the primary cause of well-known differences in LPL levels between fiber types was local contractile activity.

At present, we do not know specifically how muscle senses “exercise signals” to induce LPL expression. The two general categories of stimuli that have been logically suggested (3, 17, 19, 27) are “local signals” related to contractile activity and “systemic signals” related to plasma hormones and other humoral factors. Many researchers have speculated that LPL increases during intense “whole body exercise” because of one of the many humoral factors shown to regulate LPL activity in resting animals (8). Thus it is surprising that previous exercise studies have not measured the changes in LPL regulation in muscles not recruited during exercise. Induction of LPL activity in a noncontracting muscle would provide a strong rationale for the humoral hypothesis. This study shows no increase in LPL mRNA, mass, total activity, or heparin-releasable activity in the masseter muscle. We interpret this as evidence that local contractile activity is a necessary component for the exercise-induced increase in LPL expression under the current experimental conditions. LPL mRNA, protein mass, and activity in the stimulated TA muscle were two- to threefold greater compared with the resting contralateral TA muscle taken from the same animals (Table 4). The most probable interpretation of this finding was that influences from the motor nerve or muscle contractions were sufficient to induce LPL expression. Further studies will be needed to determine whether there is a synergistic interaction between systemic factors and local contractile activity.

LPL activity changes during voluntary run training were tissue specific (an increase in white hindlimb muscles but no change in adipose, heart, or red skeletal muscles). A prior voluntary running study (3) reported that LPL activity did not change in the soleus and increased in a fast-twitch red muscle (red vastus lateralis) that had relatively low LPL activity in control rats. There are many investigations of LPL activity changes during exercise, but the results regarding tissue specificity have been equivocal. For example, some studies of acute swimming and forced running have reported a significantly greater LPL activity in the heart and soleus, but other studies using the same modes of exercise have reported no change in heart and soleus. It remains to be determined whether forcing a rat to swim or run sometimes increases LPL activity in the heart and soleus because of contractile activity or because of systemic factors.

Skeletal muscle LPL has been shown to play a crucial role in the optimization of lipids in arterial blood. Kiens and Lithell (16) found that changes in the plasma lipoprotein profile associated with endurance training were largely caused by local adaptations in trained skeletal muscle. The present study found that exercise training had a significant effect on LPL mRNA, protein concentration, total enzyme activity, and heparin-releasable activity in skeletal muscles with a large increase in contractile activity. Findings from the stimulation experiment were consistent with the hypothesis that influences from the motor nerve or muscle contractions were sufficient to induce LPL expression. Failure for voluntary run training to change LPL expression in the masseter muscle was consistent with the hypothesis that local contractile activity was necessary for the exercise-induced increase in LPL expression. Large differences in LPL levels between fiber types in control rats are a result of contractile activity. Overall, these studies have shown that local contractile activity is a major determinant of LPL regulation in skeletal muscle.

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