Effects of Exercise Training and Feeding on Lipoprotein Lipase Gene Expression in Adipose Tissue, Heart, and Skeletal Muscle of the Rat

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Lipoprotein lipase (LPL) is found in adipose tissue and muscle, and is important for the uptake of triglyceride-rich lipoproteins from plasma. This study examined the regulation of LPL in adipose tissue and muscle by exercise training in combination with the fed or fasted state. After training male rats on a treadmill for 6 weeks, LPL activity, mass, and mRNA levels were measured in adipose tissue, heart, soleus, and extensor digitorum longus (EDL) muscles and compared with levels in sedentary rats. Tissue LPL was measured as the heparin-released (HR) and cellular-extracted (EXT) fractions 16 hours following the last bout of exercise, during which time some animals were fasted and others were allowed free access to food. Training led to an increase in HR LPL activity and LPL protein mass in soleus and EDL, but had no effect on adipose tissue and heart LPL. The increase in soleus LPL with exercise was in the HR fraction only, whereas the increase in EDL LPL with training was in both the HR and EXT fractions. All these changes in LPL activity were accompanied by similar changes in LPL immunoreactive mass. However, there were no changes in LPL mRNA levels with training. Feeding induced a large increase in adipose tissue LPL activity and mass in both the HR and EXT fractions; however, there was no change in mRNA levels. In heart, feeding yielded a decrease in HR but no consistent change in EXT activity or mass, and a consistent decrease in mRNA levels. As compared with control rats, trained rats demonstrated different responses to feeding in all tissues, especially in soleus and EDL. Whereas feeding had no effect on LPL in soleus and EDL of control rats, feeding induced a decrease in HR and EXT LPL in the soleus of trained rats. In addition, feeding yielded a significant decrease in EXT LPL of the EDL of trained rats. Thus, these data demonstrate that adipose tissue and heart LPL are highly regulated by feeding and are not responsive to long-term exercise training. On the other hand, skeletal muscle LPL is increased in trained rats, but this increase is blunted considerably by feeding following the last bout of exercise. These changes in LPL activity and mass are mostly unaccompanied by changes in LPL mRNA levels, demonstrating that much physiologic regulation occurs posttranscriptionally.

LIPOPROTEIN LIPASE (LPL) is a central enzyme in lipid metabolism, and is responsible for catabolism of the triglyceride core of circulating chylomicrons and very–low-density lipoproteins into nonesterified fatty acids (NEFA) and glycerol.[1] In addition to this important role in lipid clearance, LPL is expressed predominantly in adipose tissue and muscle and provides NEFA substrate to these tissues. In adipose tissue, little lipid synthesis occurs de novo, and most of the triglyceride found in the cell comes from NEFA derived from LPL-mediated catabolism of circulating triglycerides.[2,3] Muscle tissue stores relatively little lipid yet uses NEFA for metabolic energy. In addition to the use of circulating NEFA, muscle derives NEFA from the LPL-mediated hydrolysis of circulating triglyceride-rich lipoproteins,[4] and thus LPL is important for the provision of energy substrate for muscle.

The regulation of LPL is complex. Under many physiologic conditions, adipose tissue and muscle LPL are regulated inversely,[5] and the mechanism of regulation has been reported to be at multiple levels of gene expression.[6] For example, several studies have examined the regulation of LPL during the fed state, and found that there is an increase in adipose tissue LPL due to posttranslational translocation of LPL from the rough endoplasmic reticulum to the Golgi complex, coincident with a maturation of *N*-linked oligosaccharides.[7,8] In rats, the increase in adipose LPL with feeding is accompanied by a decrease in heart LPL, also due to posttranslational changes in the enzyme.[8] The effects of both short- and long-term exercise have been examined in animals and humans (reviewed in Nikkilä[9]). Most studies have observed increases in LPL activity in heart muscle of acutely exercised animals and increases in skeletal muscle with prolonged exercise training. However, other studies examining the response of LPL in adipose and muscle tissue have been inconsistent, with one study suggesting that the LPL response to short-term exercise is different due to the fed versus fasted state of the rat.[10] In addition, no studies have examined the mechanism of exercise-mediated changes in LPL in rats by measuring both LPL protein mass and LPL mRNA levels.

This study was designed to examine the regulation of LPL in response to long-term exercise training and to determine the response of LPL expression to feeding and fasting. In addition to measuring LPL gene expression in adipose tissue and heart, LPL expression was assessed in soleus and extensor digitorum longus (EDL), which are muscles composed of different fiber types and therefore vary in the level of LPL expression[11] and in the metabolic need for NEFA substrate.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats were purchased at a weight of 120 to 140 g, housed under standard conditions, exposed to a 12-hour

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light/dark cycle, and given unlimited access to Purina (St Louis, MO) rat chow and water. The animals were then randomized to a sedentary (control) group and an exercise (trained) group. Rats in the trained group were gradually conditioned using a conveyor-belt treadmill (Harvard Apparatus, S Natick, MA). Initially, animals were exercised for 15 minutes at a speed of 10 m/min with no incline. Duration and intensity of the exercise were gradually increased and eventually stabilized at a duration of 90 to 120 min/d, six sessions per week, at an incline of 8 degrees and a speed of 25 m/min. Control sedentary rats were handled and transported along with trained rats, and after a 6-week training regimen animals were killed. All trained rats were killed, along with control rats, after 6 weeks. For experiments involving fasting, rats were denied access to food at 4 PM and were killed between 9 and 11 AM the next morning. Fed rats were allowed free access to standard laboratory chow during this time and were killed at the same time as fasted rats. These studies were performed in six different experiments involving 10 to 12 rats per experiment.

Animals were killed by CO_2 anoxia followed by decapitation. The epididymal fat pad and heart were immediately removed along with soleus and EDL muscles. A portion of the tissue samples was immediately frozen in liquid N_2 for subsequent RNA isolation. The remaining samples were placed into iced medium 199 and processed within 1 hour for measurement of LPL activity and immunoreactive mass as described later.

Measurement of LPL Activity and Mass

LPL expression was determined as described by us previously.[11] For all tissues, LPL expression was measured in the heparin-released (HR) fraction, as well as in the fraction remaining after heparin release. For HR LPL, the tissue was minced and incubated for 30 minutes at 37°C in phosphate-buffered saline containing 10 U/mL heparin. The phosphate-buffered saline/heparin was then removed and the tissue was transferred to a tissue grinder, where it was disrupted in the presence of a solution containing deoxycholate/Nonidet P-40, as described previously.[12] Aliquots of HR and cellular-extracted (EXT) samples were then frozen for subsequent enzyme-linked immunosorbent assay, and assayed promptly for LPL activity.

LPL catalytic activity was measured using an emulsified ^{3}H-triolein substrate as described previously.[13] After incubation of 100 μL sample with 100 μL substrate for 30 minutes, liberated ^{3}H-NEFA were separated from glycerides using the method reported by Belfrage and Vaughn.[14] The EXT sample was diluted 10-fold to remove any inhibitory effects of the detergent. For LPL activity, 1 U represents 1 nEq NEFA released/min/10^6 cells for adipose tissue and 1 nEq NEFA released/min/g for muscle. LPL immunoreactive mass was measured by enzyme-linked immunosorbent assay, using a polyclonal chicken anti-LPL antiserum as described previously.[12] LPL immunoreactive mass was expressed as nanograms of LPL per 10^6 cells for adipose tissue and nanograms of LPL per gram muscle. Cell number was determined using the method reported by DiGirolamo et al.[15]

Measurement of LPL mRNA Levels

LPL mRNA levels were determined using Northern blotting and competitive reverse transcriptase–polymerase chain reaction (RT-PCR), as described previously.[11,16] For Northern blotting, total RNA was extracted using methods reported by Chomczynski and Sacchi,[17] and the quantity of RNA was verified by ethidium bromide staining of rat RNA bands on a minigel. After analysis on a 1% agarose gel, total RNA (20 μg) was transferred to nylon and

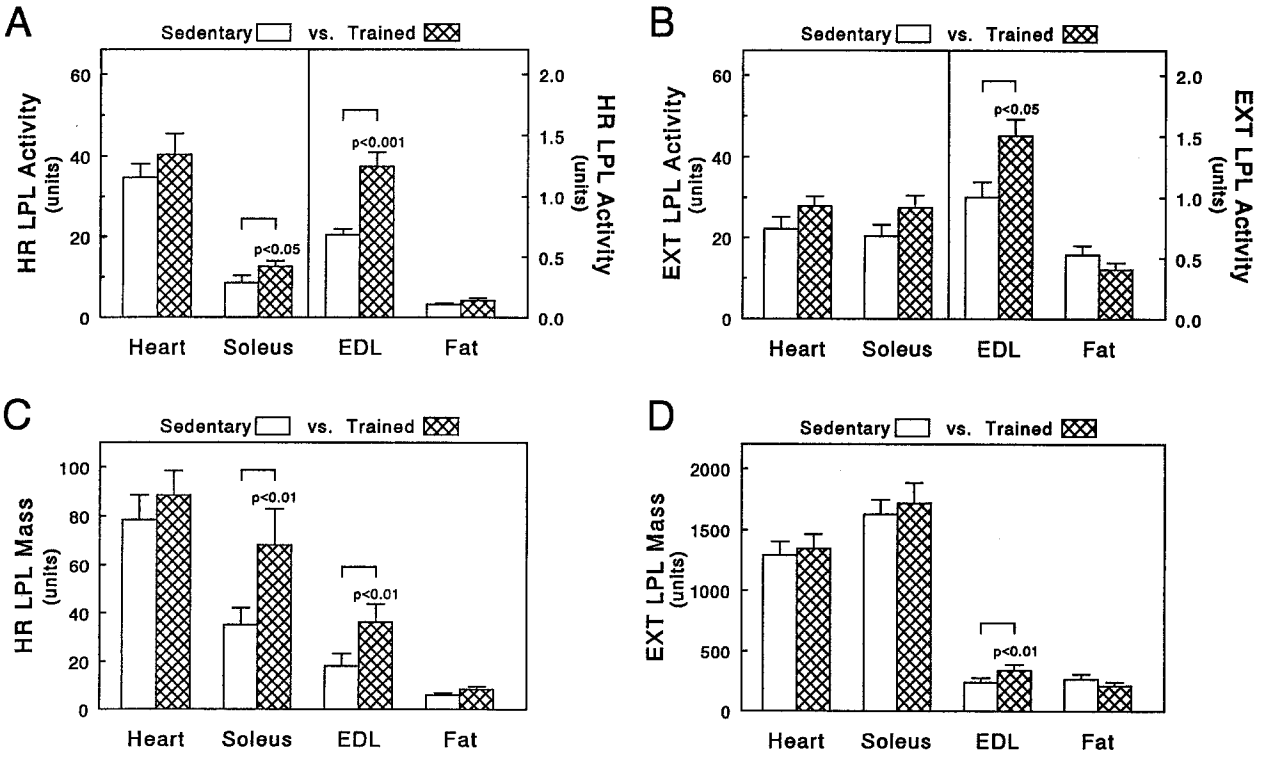

Fig 1. Effect of exercise training on adipose tissue and muscle LPL in fasted rats. (A) and (B) LPL activity in HR and EXT. (C) and (D) LPL immunoreactive mass in HR and EXT. LPL activity is expressed as nEq NEFA released/min/10^6 cells for adipose tissue and as nEq NEFA released/min/g for muscle, and LPL immunoreactive mass is expressed as ng LPL/10^6 cells for adipose tissue and as ng LPL/g for muscle. n = 14 to 22 for each group.

blotted with the random-primed,[18] ^{32}P-labeled human LPL cDNA,[19] followed by the ^{32}P-labeled probe for γ-actin.[20] The autoradiographs were exposed for 1 to 3 days, and the images were quantified using an Imagestore 5000 scanner and analyzed using the Gelbase/Gelblot software (Ultraviolet Products, San Gabriel, CA), as described previously.

Quantitative competitive RT-PCR was used to measure LPL mRNA level in soleus and EDL, and was performed as described previously.[11,21,22] In brief, equal quantities of extracted total RNA were added to increasing quantities of a rat LPL cRNA construct, which contained primer sites for rat LPL and an internal deletion of 19 nucleotides and was therefore distinguishable on an agarose gel. For soleus, 4 ng total RNA was used for the reaction; for EDL, 50 ng total RNA was used. Primer sites for the RT-PCR were located at nucleotides 1303 to 1322 and 1589 to 1608 of the LPL cDNA. These primer sites span an intron in the LPL gene, thus avoiding contamination with genomic DNA. Following the RT reaction, PCR was performed for 35 cycles at 55°C. The resulting ethidium bromide–stained gel was imaged and analyzed as described earlier. The ratio of LPL mRNA product to cRNA standard was plotted against the number of copies of cRNA added, yielding the equivalence point between cRNA and LPL mRNA. The data were expressed as the number of copies of LPL mRNA per microgram total RNA.

Statistics

All data are expressed as the mean ± SEM, and differences between groups were analyzed using one-way ANOVA. The n value for each group, which represents the number of animals, is stated in the figure legends.

RESULTS

Training resulted in significant differences in rat weight and fat pad mass as compared with those of sedentary animals. After a 6-week exercise regimen, control rats weighed 330 ± 6.8 g and fat pad mass was 2.93 ± 0.19 g, whereas trained rats weighed 302 ± 6.6 g and had a fat pad mass of 2.15 ± 0.10 g ($P < .05$ for both weight and fat pad mass). Fat cell size was also significantly different (270 ± 13.3 and 220 ± 8.2 pL, $P < .005$) for sedentary and trained animals, respectively. Weights of soleus, EDL, and heart were not significantly different between control and trained groups. Overnight fasting did not result in any significant differences in weights of the tissues in comparison to those of fed animals.

Effects of Exercise Training

As described earlier, rats were trained for 6 weeks and were killed 24 hours after the last bout of exercise. LPL activity and immunoreactive mass in chronically exercised rats in comparison to nonexercised controls are shown in Fig 1. Long-term exercise training had no significant effect on LPL activity or immunoreactive mass in heart and adipose tissue. On the other hand, training resulted in an increase in skeletal muscle LPL. In soleus, there was a 48% increase in HR activity along with a similar increase in HR mass, but no significant effect on EXT LPL. In EDL, the response to training was similar, with increase in HR activity and mass, although the increase in HR activity of 82% was proportionally greater than the increase in soleus HR activity. In addition, exercise training yielded an increase in EXT LPL in EDL. In all cases, increases in LPL activity in trained rats were accompanied by parallel changes in LPL mass.

To determine whether these changes in LPL activity and mass were accompanied by changes in LPL mRNA levels, Northern blotting and quantitative RT-PCR were performed. Northern blots from adipose tissue and heart demonstrated no difference in LPL mRNA level in trained rats as compared with control rats (Fig 2). The level of mRNA expression in EDL and soleus was less than that in the other tissues, such that quantitation was performed using RT-PCR. There was no effect of exercise training on the level of LPL mRNA in any of the tissues. Thus, training resulted in an increase in skeletal muscle LPL activity and LPL mass with no change in LPL mRNA, suggesting posttranscriptional regulation (Fig 2).

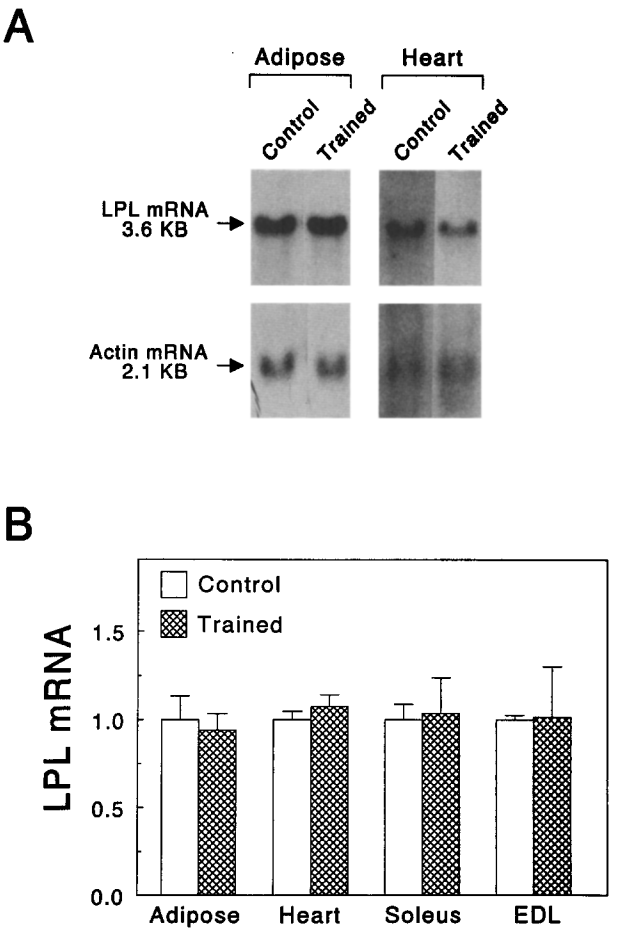

Fig 2. Effect of exercise training on LPL mRNA levels in adipose tissue, heart, and soleus. For adipose tissue and heart, Northern blotting was used to quantify LPL mRNA levels, whereas quantitative RT-PCR was used for soleus and EDL. (A) Representative Northern blots of RNA extracted from adipose tissue and heart of control and trained rats (all fasted). (B) Summary of the effect of exercise training on LPL mRNA levels in all tissues. All data are expressed as a percent of control (untrained) mRNA levels. Each bar represents data (mean ± SEM) from 4 to 10 animals.

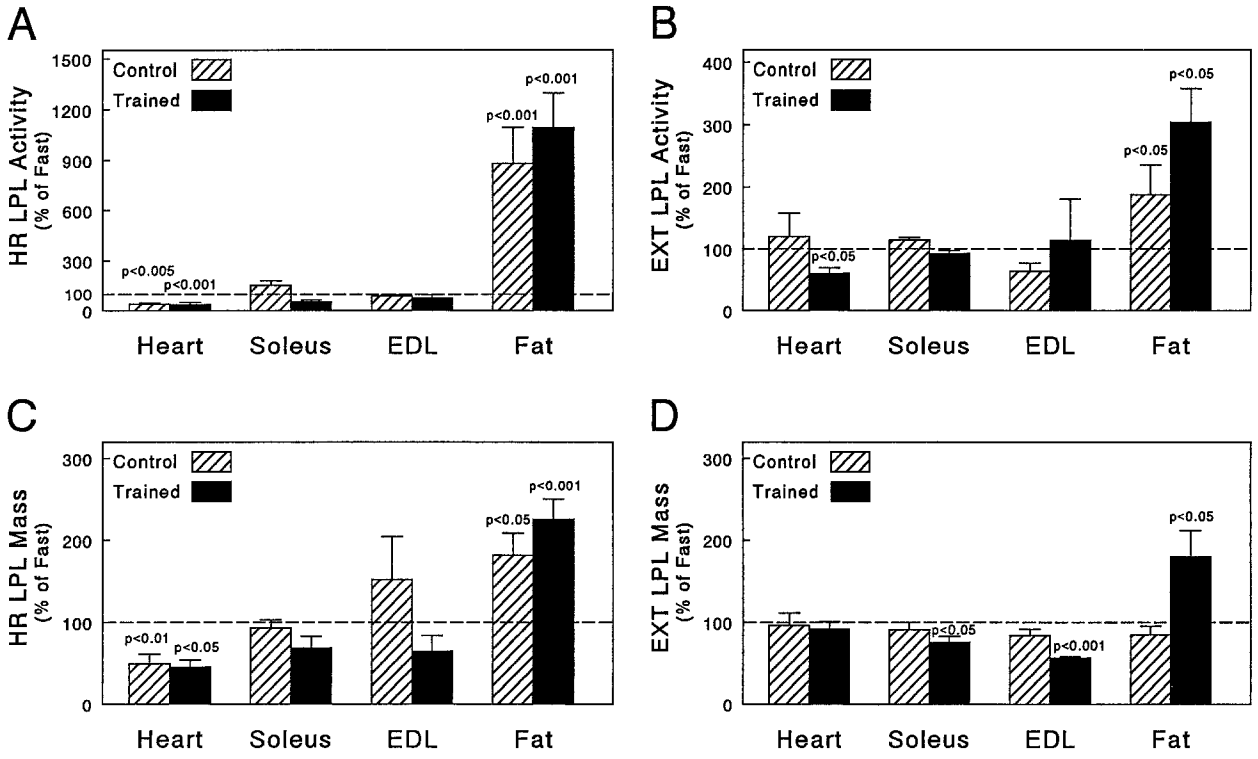

Fig 3. Effect of feeding on LPL activity and mass in control and trained rats. Data are expressed as the percent of activity in fasted rats of the same group (control or trained); n = 8 to 12 for each tissue.

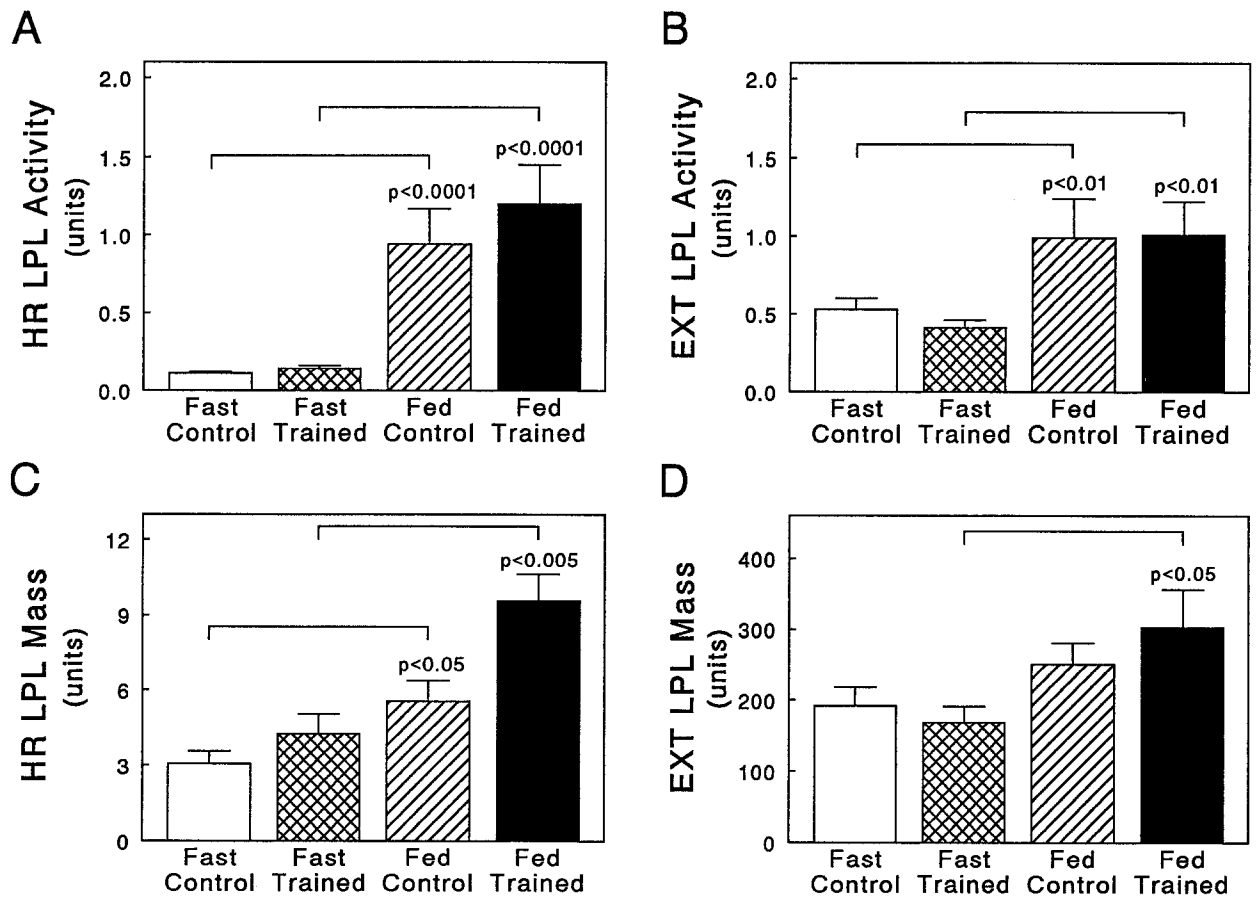

Fig 4. Effect of feeding and training on adipose tissue LPL activity and mass; n = 10 to 15 for each group.

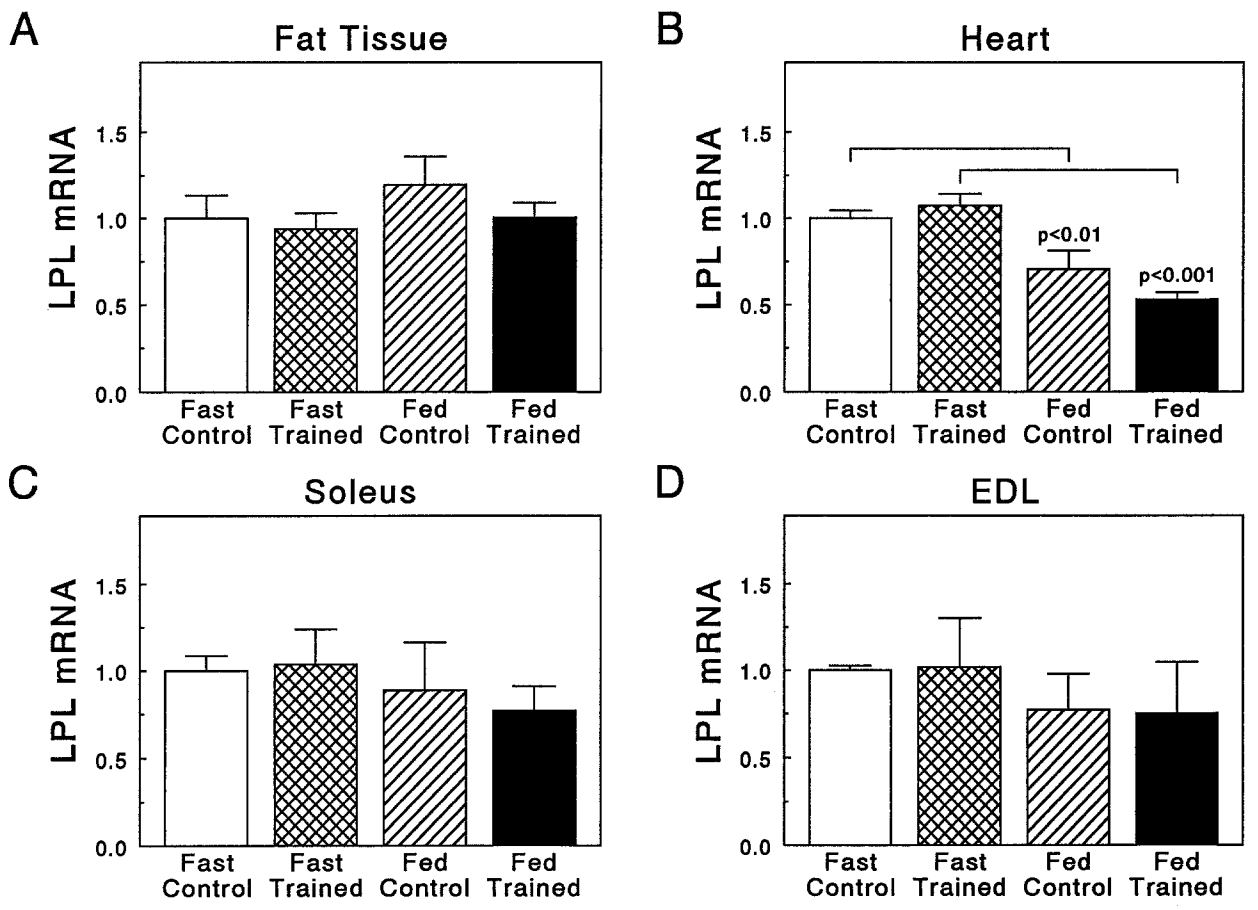

Fig 5. Effect of feeding and training on LPL mRNA levels in muscle and adipose tissue. LPL mRNA levels of different groups of rats are expressed as fractions of the mRNA level in fasted control rats. Each bar represents data (mean ± SEM) from 3 to 10 animals.

Effects of Feeding in Chronically Trained Rats

In a previous study, we examined the effects of feeding on LPL expression in heart, soleus, and EDL of sedentary rats.[11] We found that HR LPL activity and mass in heart were decreased to approximately 50% of fasting levels, and there was no change in EXT in heart. In addition, feeding had no significant effect on either HR or EXT in soleus and EDL.[11] To determine whether training modified the LPL response to feeding, LPL activity and mass were compared in control and trained rats. In control and trained rats, heart and adipose tissue LPL responded similarly to feeding. Adipose tissue LPL activity and mass increased in HR and EXT (Fig 3). However, in control rats, the increase in EXT adipose activity was not accompanied by an increase in EXT mass, whereas there was an increase in EXT mass in trained rats. Therefore, this difference in adipose EXT response to feeding may suggest different cellular mechanisms of regulation. Heart LPL decreased in HR to a similar degree in both trained and control rats. However, in trained rats, heart LPL demonstrated a 35% decrease in EXT activity without an accompanying change in LPL mass, which was not seen in control rats. In control rats, neither soleus nor EDL were affected by feeding. However, feeding resulted in a significant decrease in EXT mass in soleus and EDL in trained rats (Fig 3). Thus, in every tissue examined, trained rats demonstrated differences in response to feeding when compared with control rats. However, these differences were complex, were only in the EXT component of LPL, and suggested that feeding results in different mechanisms of regulation in trained rats.

Feeding Is the Predominant Influence on Adipose Tissue and Heart

The effects of feeding, fasting, and exercise training on adipose tissue LPL activity and mass are shown in Fig 4. In the HR fraction, feeding induces a large increase in LPL activity and a lesser increase in LPL mass in both control and trained rats. In the EXT fraction, both control and trained rats demonstrated increased EXT LPL activity with feeding, along with parallel increases in EXT LPL mass. In adipose tissue, there was no difference between control and trained rats in the response to feeding. The effects of these conditions on LPL mRNA in adipose tissue were examined by Northern blotting, and the data are expressed in relation to the level of γ-actin mRNA. Neither training nor feeding nor the combination affected LPL mRNA level in adipose tissue (Fig 5A).

The effects of feeding, fasting, and training on heart LPL are shown in Fig 6. Feeding induced similar decreases in HR activity of heart in both control and trained rats, and

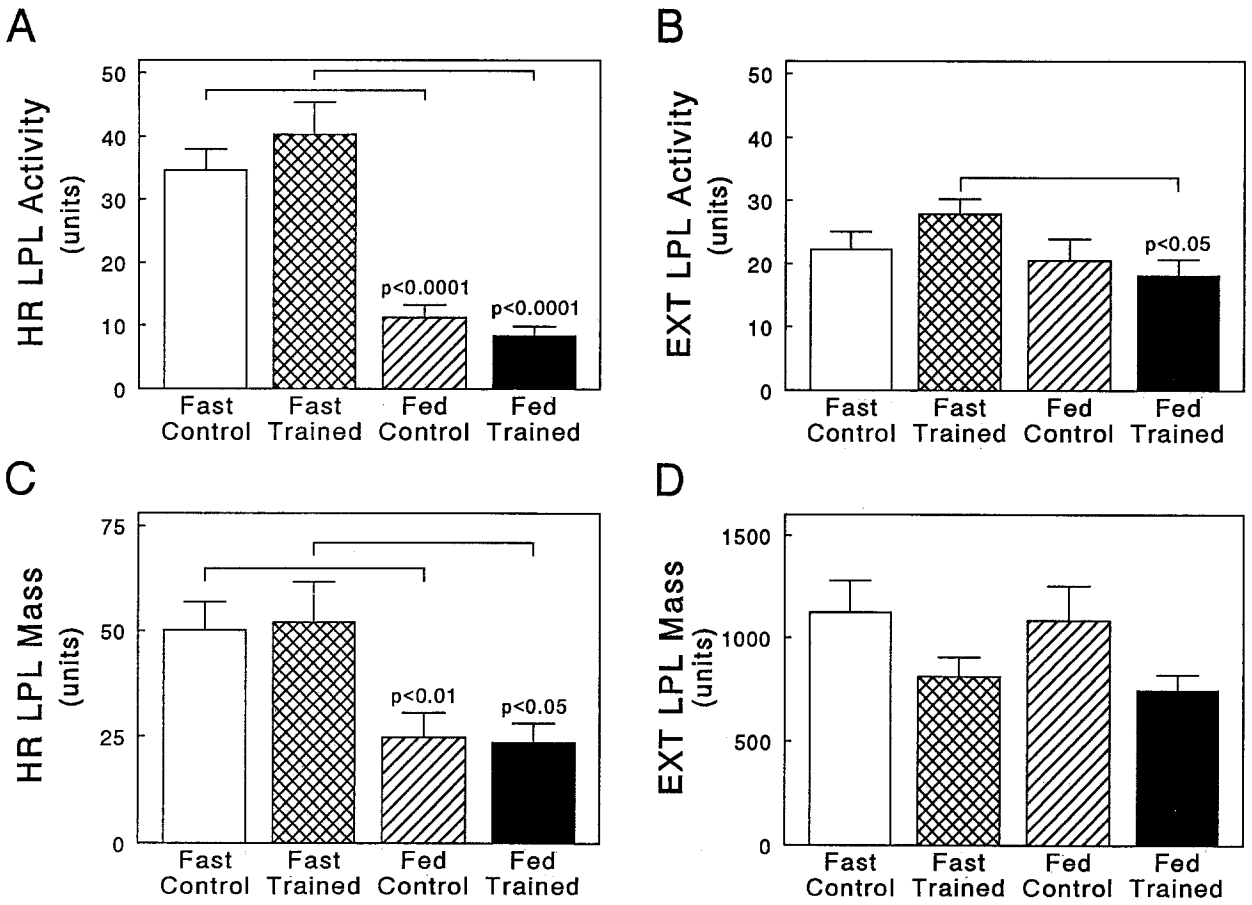

Fig 6. Effect of feeding and training on heart LPL activity and mass; n = 10 to 15 for each group.

LPL HR mass in heart changed in parallel with HR activity. In EXT, trained rats demonstrated a small but significant decrease in LPL activity in response to feeding. However, EXT mass did not change in parallel with EXT activity. Heart EXT mass was approximately 25% lower in trained rats and did not change with feeding.

As described previously by us and others,[11,23] heart LPL mRNA decreased by approximately 30% in sedentary rats in response to feeding. Similar decreases in heart LPL mRNA levels were observed in trained rats, and there were no significant differences in the response of heart LPL mRNA between control and trained rats (Fig 5B).

Training-Induced Increase in Soleus and EDL LPL Is Blunted by Feeding

The effects of feeding and training on soleus LPL activity are shown in Fig 7. In trained rats, HR LPL activity was 148% higher than in control rats. However, this was only observed in the fasted state. Whereas feeding had no effect on HR LPL activity in control rats, feeding induced a significant decrease in HR LPL in trained rats. The increase in HR activity by training was accompanied by an increase in HR LPL mass. EXT LPL activity in soleus was slightly but significantly increased in trained rats, but this increase was not accompanied by an increase in LPL mass. Soleus EXT LPL mass was unaffected by training but was decreased by feeding in trained rats. Soleus LPL mRNA levels were not affected by either feeding or training, as shown in Fig 5C.

The response of EDL to training and feeding was examined. HR LPL was increased in response to training (Fig 8). However, in contrast to soleus, the increase in HR was observed in both the fasted and fed state. In the fasted state, HR LPL activity was increased by 84%, whereas in the fed state, it was increased by 63%. EXT activity in EDL was also increased in trained rats; however, this was only observed in the fasted state. In contrast to soleus, feeding significantly decreased EDL EXT activity in both control and trained rats. Therefore, when one compares the feeding response of EDL with that of soleus, EDL HR activity is not decreased by feeding, whereas EDL EXT LPL is more sensitive than soleus EXT LPL. LPL mRNA levels were examined in EDL by RT-PCR. There was no significant change in response to feeding or training (Fig 5D).

DISCUSSION

In response to physical exercise, muscle tissue has an increased need for fuel, which must be supplied by either glucose or NEFA. At the start of exercise, the working skeletal muscle consumes glycogen stores and begins to use NEFA to satisfy its energy needs.[9] Depending on the duration and intensity of the exercise, muscles may obtain

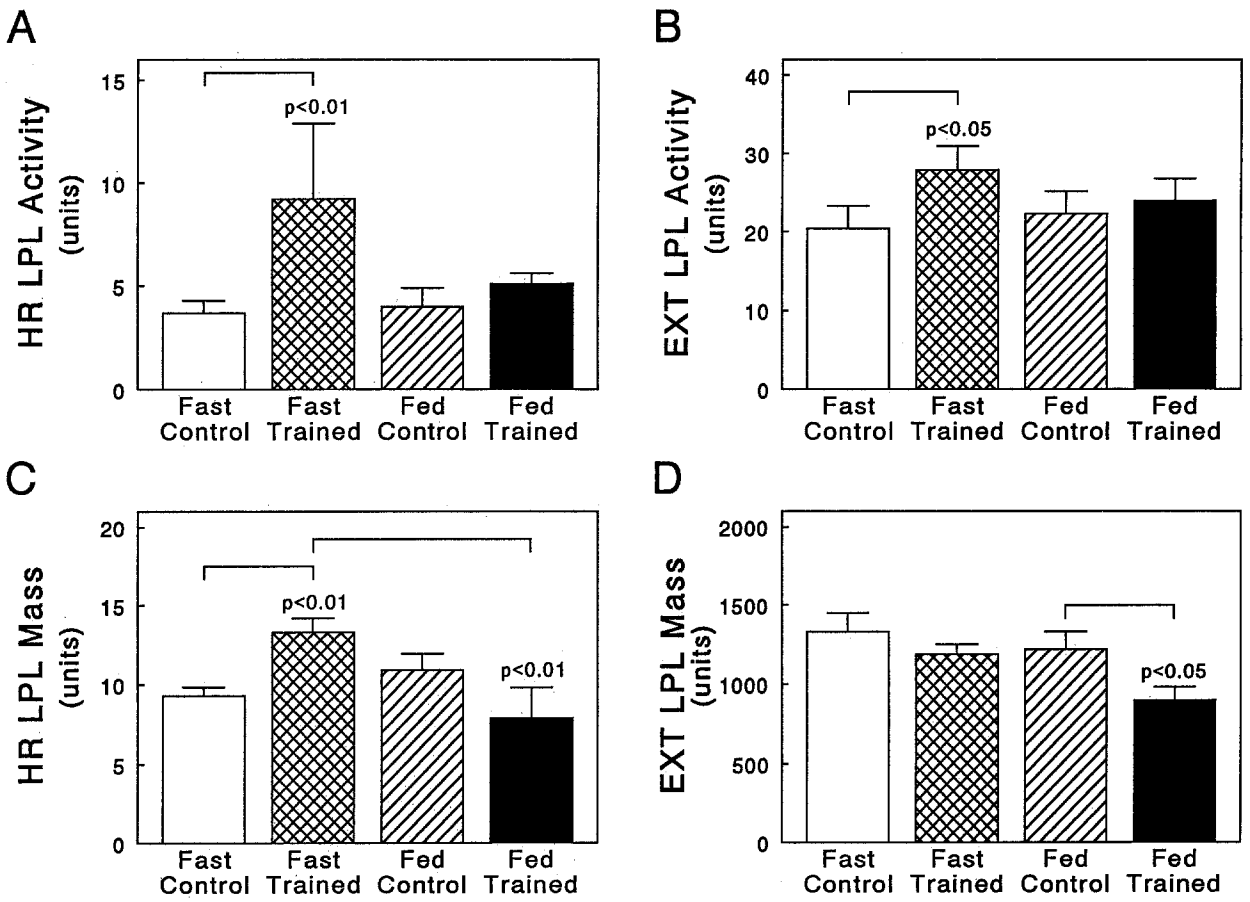

Fig 7. Effect of feeding and training on soleus LPL activity and mass; n = 7 to 10 for each group.

up to 80% of their energy from NEFA.[24] Much of the increased energy demand of muscle during exercise is met through an increase in oxidation of plasma NEFA, along with hydrolysis of endogenous muscle triglyceride.[25,26] Following completion of exercise, lipoprotein triglyceride decreases and reaches a nadir after 24 hours.[9,27,28] These data suggest that LPL plays a lesser role during exercise in providing the working muscle with NEFA, but is important in regenerating muscle triglyceride stores following completion of exercise.

This study was intended to examine the mechanism of regulation of LPL in rat adipose tissue and muscle in response to exercise training in the presence and absence of feeding. As described previously,[5,8] adipose tissue and heart LPL were regulated in an inverse fashion in response to feeding and were regulated comparatively little by training. However, in adipose tissue, the combination of feeding and training led to a synergistic increase in HR LPL, with a particular increase in LPL mass (Fig 4C). The predominant mechanism of regulation of adipose tissue LPL with feeding involved posttranslational changes in the LPL protein, resulting in transport from the rough endoplasmic reticulum to the Golgi complex and changes in glycosylation.[8] Thus, the increases in HR LPL appeared to be due to posttranslational events, perhaps resulting in an increase in LPL secretion. All of these studies were performed with epididymal fat pads, and other fat depots may respond differently.[29] In heart, this study (as did others[9]) found no change in LPL activity with training. However, heart EXT LPL mass was decreased in both fasted and fed trained rats (Fig 6D) with no change in LPL mRNA levels.

Skeletal muscle LPL in soleus and EDL was increased by training in fasted rats. No changes in LPL mRNA levels were observed, suggesting that these changes were also posttranscriptional. We have previously observed that soleus and EDL LPL did not change in response to feeding in sedentary rats.[11] However, when the effect of feeding was studied in trained rats, many of the changes in LPL due to training were blunted. Soleus HR activity increased by approximately twofold in trained-fasted rats, but decreased by the same amount in trained-fed rats. Interestingly, feeding had no effect on EDL HR activity in either control or trained rats. However, EDL EXT activity and mass were lower in response to feeding, especially in trained animals. Again, no changes in LPL mRNA were observed in EDL, demonstrating the posttranscriptional nature of these changes.

When the effects of feeding in trained rats was examined, there were important differences between soleus and EDL. Soleus, which was composed of predominantly type I fibers, was less affected by feeding than EDL. Because soleus relied heavily on triglyceride fatty acids in the postexercise

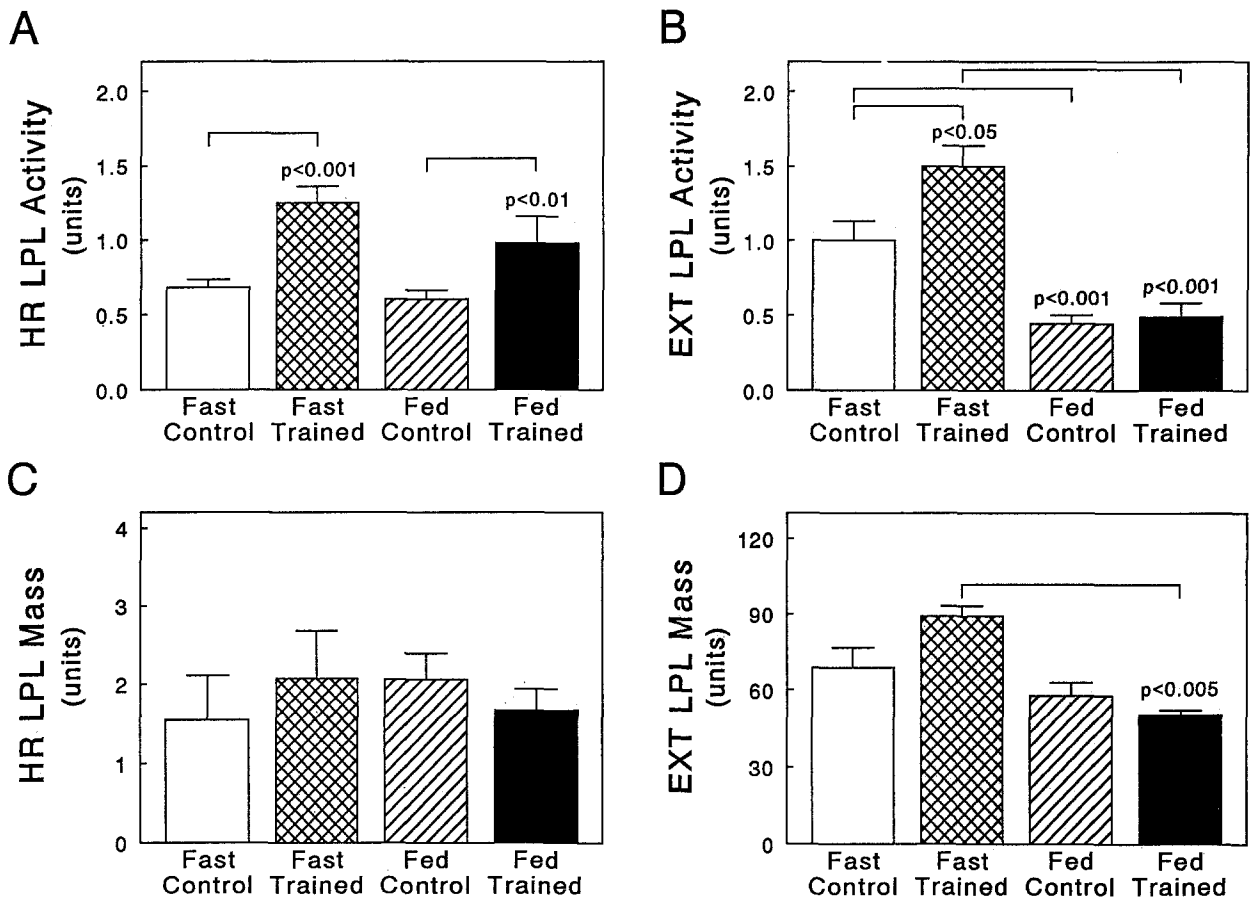

Fig 8. Effect of feeding and training on LPL activity and mass in EDL muscle; n = 5 to 8 for each group.

period for energy, fasting likely resulted in a high level of LPL, which then decreased when food was available to meet the oxidative needs in the postexercise period (Fig 7A). EDL was not as dependent on triglyceride fatty acids, since most of its fibers were glycolytic. Therefore, HR LPL in EDL was not affected by feeding in the postexercise state (Fig 8A). However, the maintenance of HR in EDL was at the expense of EXT, which decreased considerably in fed-trained rats (Fig 8B). This decrease in EXT in parallel with an increase in HR may have been due to a translocation of LPL enzyme from an intracellular pool to the more functional HR pool.

Previous studies have examined LPL activity during and after short- or long-term exercise. In response to short-term exercise in animals, LPL was increased in heart, decreased in fat, and unchanged in skeletal muscle,[9] unless the exercise was of long duration, which would then result in an increase in skeletal muscle LPL.[9,10,30,31] In dogs, this increase in skeletal muscle LPL following exercise was abolished by feeding.[10] Animal data on the response to long-term training have been inconsistent, with some studies showing increases in LPL activity with exercise[32,33] and other finding no difference in LPL between trained and sedentary rats.[30,34,35] Studies in humans have consistently shown 30% to 70% increases in skeletal muscle LPL activity with exercise.[36-38] Although many studies of LPL did not separate the HR activity from the intracellular fraction, one study[39] found a significant increase of the intracellular fraction of muscle LPL with no change of the HR fraction during strenuous exercise. On the other hand, in obese Zucker rats, training produced an increase only in HR LPL in muscle.[40]

A few studies have examined the mechanism of LPL regulation in response to exercise. Ladu et al[41] measured LPL activity and mRNA levels in rats immediately following a 2-hour swim. LPL activity was decreased in fat, increased in soleus, and unchanged in other muscles. LPL mRNA levels in fat and soleus paralleled changes in LPL activity, and there were increases in LPL mRNA levels in red and white vastus lateralis, although there were no changes in LPL activity. In human athletes who were detrained for 2 weeks, muscle LPL (both HR and EXT) decreased and adipose LPL (only HR) increased.[37] These changes in LPL occurred in the absence of any consistent change in LPL mRNA, suggesting posttranscriptional mechanisms of regulation.

These studies have several major implications for the regulation of LPL. First, regulation of muscle LPL by feeding and exercise training, as with adipose tissue LPL, is largely posttranscriptional. In both adipocytes[42-45] and myocytes,[46] inhibitors of glycoprotein processing result in an inhibition of LPL activity. Therefore, it is likely that this

process is in some way regulated during common physiologic changes. In addition, skeletal muscle LPL is regulated differently from heart LPL. Whereas heart LPL is inhibited in response to feeding, skeletal muscle LPL is less sensitive to feeding. However, feeding blunts the increase in skeletal muscle LPL due to exercise. These changes in LPL likely play a role in the reestablishment of muscle triglyceride stores following exercise.

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