A reexamination of the effect of exercise on rate of muscle protein degradation

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Kasperek, George J., Guy R. Conway, Dorothy S. Krayeski, and Jack J. Lohne. A reexamination of the effect of exercise on rate of muscle protein degradation. Am. J. Physiol. 263 (Endocrinol. Metab. 26): E1144–E1150, 1992.—The purpose of this study was to examine the effect of exercise on the rate of protein degradation in rat skeletal muscle. The rates of total and myofibrillar protein degradation were determined by the measurement of the rates of release of tyrosine and 3-methylhistidine, respectively, from the perfused single rat leg. This method measures the rate of protein degradation in the entire lower leg and does not suffer from the limitations inherent in methods that rely on urinary excretion. The rate of total protein degradation was increased by exercise and involved increased flux through the lysosomal pathway, while the breakdown of myofibrillar protein was unchanged. The changes in the rates of protein degradation during the recovery period were greatly influenced by energy intake. Again the rate of myofibrillar protein degradation was unchanged or slightly increased during the recovery period, after either level or downgrade running. Exercise did prevent the increase in the rate of total protein degradation caused by food restriction, which may have important implications in weight reduction diets.

increased rates of urea and 3-methylhistidine excretion after exercise and increased amino acid oxidation during exercise led researchers to the conclusion that the rate of protein degradation was increased by exercise (reviewed in Refs. 3, 8, 17, 18). The increased rate of urinary excretion of 3-methylhistidine suggested that this increase in the rate of protein degradation during exercise is due to increased degradation of myofibrillar protein in skeletal muscle. The observed increase in the urinary excretion of 3-methylhistidine for several days after exercise consisting of downgrade running suggested that the increased rate of myofibrillar protein degradation continued into the recovery phase (11) and may be due to muscle damage, because running downgrade causes more muscle damage than running on the level (1). The use of the urinary excretion of 3-methylhistidine as an index of skeletal muscle protein degradation was criticized because 3-methylhistidine is found in both skin and gut (24, 26). This criticism prompted other studies in which the rates of total and myofibrillar protein degradation were assessed by measuring the release of tyrosine and/or 3-methylhistidine, respectively, directly from muscle. The results of these experiments suggest that the conclusions regarding the effect of exercise on skeletal muscle protein degradation may not be correct. Exercise was found to cause an increase in the rate of total protein degradation and no change in the rate of myofibrillar protein degradation when measured in the rat soleus muscle incubated in vitro (15). In another study using the perfused rat hindquarter (2), the rate of myofibrillar protein degradation was found to be increased after exercise in one experiment and not in another, while the rate of total protein degradation was unchanged. Electrical stimulation of the muscle caused a decrease in the rate of myofibrillar protein degradation (4), but no change in the rate of total protein degradation (2). The purpose of the study described here was to try to resolve the controversy concerning the effect of exercise on the rate of skeletal muscle protein degradation. To accomplish this, the rates of both total and myofibrillar protein degradation were measured immediately after treadmill running or during electrically stimulated muscle contractions to determine how the rates of protein degradation were changed during exercise. These same parameters were measured on the day after exercise to determine how protein degradation was changed during the recovery period. The single leg perfusion was chosen as the technique by which to measure the rates of protein degradation, because the rates of protein degradation measured with it measure the rate of protein degradation in all muscles of the lower leg, and the 3-methylhistidine release is only from muscle and not from other tissues such as skin and gut.

Methods

Animals. Male Sprague-Dawley rats (Charles River, Wilmington, MA) were fed Agway NIH-07 Rat Ration either ad libitum or as described in the tables legends. They had free access to water, were housed in individual wire cages, and had the lights on from 0300 to 1500 h. The temperature was 20–23°C. To keep the weights of the rats in the different experimental groups equal, the rats were weighed and assigned to groups the day before the experiment. The animal weights are given in the tables and figure legends. Rats were exercised in the morning by running at 25 m/min on a motor-driven treadmill. The rats ran down an 18° grade or as denoted in the table legends. The rats were run for 200–215 min or until they were unable to continue running. The run times are given in the tables and figure legends.

Perfusion. The rats were prepared for perfusion by a modification of the procedure described by Hood and Terjung (10). In this procedure, a single leg is perfused, which results in a preparation in which a large portion of the perfused mass is muscle. The rats were anesthetized with 90 mg/kg ketamine and 10 mg/kg xylazine injected intraperitoneally. A midline incision was made between the skin and the abdominal wall. The skin was separated from the abdominal wall as far down as the thigh. The epigastric, ipsilateral abdominal, and superior vesicle arteries were cannulated. A midline incision was made to open the peritoneal cavity. The intestines were carefully reflected to permit access to the great vessels, two ligatures ~3 cm apart were placed about the lower colon, and the segment between them was excised. Ligatures were loosely placed around the aorta and vena cava just below the insertion of the iliolumbar vessels. The iliolumbar vessels were ligated securely. Ligatures were also loosely placed around the common iliac and the external iliac
vessels to secure the catheters once perfusion began. The skin was removed from the perfused leg then replaced to prevent the release of 3-methylhistidine from the skin into the perfusate. The skin was replaced to prevent dehydration. Heparin (1,000 U) was injected into the carotid artery. The ligatures about the aorta and vena cava were quickly tightened, and the animal was transected just anterior to the last secured ligature. The upper half was decapitated to ensure mortality. The hemi-corpus was transferred to the perfusion chamber, which was maintained at 35°C, and a catheter was quickly and carefully guided into the femoral artery ~4 mm beyond the inguinal ligament. The venous catheter was then inserted into the left iliac vein and the tip guided into the femoral vein ~4 mm beyond the inguinal ligament. The catheters were secured by the ligatures already in place.

The perfusion media consisted of Krebs-Henseleit buffer, washed bovine erythrocytes (30% hematocrit), 3% bovine serum albumin, 5.5 mM glucose, 0.15 mM pyruvate, 0.2 mM cytochrome oxidase, 0.1 μM/ml insulin, and 1.0 mM chloroquine in the experiments in which chloroquine was added. The perfusate was oxygenated by passing through Silastic tubing that was in a jar that was continually flushed with 95% oxygen-5% carbon dioxide saturated with water vapor. The perfusion was the single-pass type, and the rate of perfusion was measured by timed collections of the venous effluent. The measured flow rate was 3.6-4.0 ml/min and was used to calculate the rate of release of tyrosine and 3-methylhistidine [rate of release = (concentration of venous effluent − concentration of perfusate from reservoir) × flow rate]. Pilot studies (data not shown) demonstrated that this flow rate was adequate to maintain muscle ATP levels, and the calculated rates of protein degradation were not different at flow rates of 3 ml/min. The weights of the rats in the different experimental groups within an experiment were kept the same so the rates of release of tyrosine and 3-methylhistidine could be expressed per leg. Ten-milliliter samples of perfusate were collected at the times indicated in the figures and tables. After centrifugation to remove the red blood cells, the supernatant was made 10% in trichloroacetic acid. The perfusate was centrifuged again to remove the precipitated protein, and was stored at 4°C until analyzed for tyrosine and 3-methylhistidine.

The rate of release of tyrosine when protein synthesis was inhibited by cycloheximide was used as an indicator of the rate of total protein degradation. As in previous experiments with isolated muscle (12), the rate of tyrosine release varied between experiments. The reason for this variation is unknown, but because of it, comparisons between experiments should not be made. Tyrosine was measured fluorometrically (27). The rate of release of 3-methylhistidine, which is found in actin and myosin and is not degraded by muscle, was used as an indicator of the rate of myofibrillar protein degradation. 3-Methylhistidine was measured fluorometrically (excitation 380 nm, emission 470 nm) after separation of the fluorescent derivative by high-performance liquid chromatography using a C18 column, and a mobile phase of 29% acetonitrile in water (28). In some experiments, the gastrocnemius muscle was removed at the end of perfusion and quick frozen by clamping in aluminum tongs cooled in liquid nitrogen. The frozen muscles were stored at −80°C until analyzed. The muscle was assayed for cathepsin D activity using [14C]methemoglobin as substrate, as previously described (6). ATP was assayed by an enzymatic method using a Shimadzu UV-160 spectrophotometer (16).

Electrical stimulation. In some experiments, the muscles of the lower leg were made to contract by electrically stimulating the sciatic nerve. In these experiments, the sciatic nerve was exposed, and the Achilles tendon was ligated and severed before the rat was transected. After perfusion had begun, an electrode was attached to the sciatic nerve, and the Achilles tendon was attached to a force transducer. The tension on the Achilles tendon was adjusted to produce the maximal force when the muscle was stimulated to contract. Contraction was caused by simulation with square-wave trains, as described in Table 4.

Results

Protein degradation immediately after exercise. In the first experiment, the rates of release of tyrosine and 3-methylhistidine were measured as a function of perfusion time. The purpose of this experiment was to determine the optimum perfusion time for the measurement of the rate of protein degradation and to determine how the rate of protein degradation was affected immediately after exercise. Figure 1 shows that the rate of tyrosine release from exercised rats compared with rested controls was elevated 15 min after the start of the perfusion and remained significantly elevated over control values for 90 min. The rate of tyrosine release and thus the rate of total protein degradation was increased by 24% after 30 min of perfusion. The rate of release of 3-methylhistidine (Fig. 2) was not different from that of controls at any time point during the perfusion. This lack of change in the rate of 3-methylhistidine release demonstrates that the rate of degradation of myofibrillar protein is not changed immediately after exercise. The reason for the downward drift in the rates of tyrosine and 3-methylhistidine release as a function of perfusion time is unknown but cannot be entirely due to washout, as no difference was observed in the free tyrosine content in muscle after 30 min of perfusion (1,262 ± 89 nmol/g protein) and the unperfused contralateral gastrocnemius taken before perfusion (1,362 ± 46 nmol/g protein).

In the second experiment, the involvement of lysosomes in total protein degradation was measured by perfusing with chloroquine, an inhibitor of the lysosomal pathway. Figure 3 again shows tyrosine release to be increased by exercise relative to controls. After 30 min of perfusion without chloroquine, the rate of release of tyrosine was 41% greater than controls, but the addition of chloroquine to the perfusate caused the rates of tyrosine release to coalesce after an additional 45 min of perfusion in the presence of chloroquine. This inhibition of the increased rate of total protein degradation after exercise...
The purpose of this experiment was to determine how the rates of both total and myofibrillar protein degradation were changed during the 2 days that followed an exhaustive exercise bout, and to determine whether eccentrically biasing the exercise had any effect on the rate of muscle protein degradation. The controls were fed the same amount of food as the exercised rats to compensate for the reduced food intake of the rats that were exercised. In this experiment, rats larger than in the previous experiments were used because we established that larger rats are more susceptible to muscle damage than smaller rats (13). The contribution of muscle damage to changes in rates of protein degradation after exercise were evaluated by including a group in which the exercise was eccentrically biased by running the rats downgrade. Running downgrade caused more muscle damage than running on the level (1). The data in Table 1 again show that the rates of total protein degradation were increased relative to controls immediately after exercise. In this experiment, the larger rats could not run as long as in the previous experiments, but the rates of protein degradation were increased relative to controls by 41% for those that ran downgrade, and by 44% for those that ran on the level. The rates of myofibrillar protein degradation were not statistically changed immediately after exercise in agreement with the previous experiment. There were no differences in the rates of protein degradation between rats that ran downgrade or on the level, suggesting that muscle damage is not an important contributor to the rate of total or myofibrillar protein degradation 1 and 2 days after exercise.

The rate of tyrosine release and thus the rate of total protein degradation 1 day after either level or downgrade running was decreased compared with the pair-fed controls (Table 1). This decrease was largely due to a 23% increase in the rate of tyrosine release in the pair-fed control compared with the fed controls. The rate of total protein degradation 2 days after exercise was the same for all groups, but was ~14% greater than the value for the fed control. The rate of 3-methylhistidine release from the perfused hindlimbs was not different for any of the groups (Table 1). It thus appears that the major difference in the rate of protein degradation in the recovery phase may be due to changes in food intake. To further investigate this hypothesis, an experiment was performed in which both the exercised and control rats were starved the day after exercise. In this experiment, the exercise consisted only of downgrade running because no differences between

**Table 1. Muscle protein degradation after level and downgrade running**

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>D</th>
<th>L</th>
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<th>D + 1</th>
<th>L + 1</th>
<th>C + 2</th>
<th>D + 2</th>
<th>L + 2</th>
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<td>11</td>
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<td>11</td>
<td>11</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Wt, g</td>
<td>379±2</td>
<td>384±2</td>
<td>370±4</td>
<td>331±2</td>
<td>342±5</td>
<td>333±2</td>
<td>344±3</td>
<td>352±4</td>
<td>347±3</td>
</tr>
<tr>
<td>Run time, min</td>
<td>129±9</td>
<td>89±15</td>
<td>161±10</td>
<td>144±8</td>
<td>109±6</td>
<td>103±8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-Methylhistidine Tyrosine</td>
<td>29±2</td>
<td>36±2</td>
<td>34±2</td>
<td>39±2†</td>
<td>36±2</td>
<td>39±3</td>
<td>44±2†</td>
<td>39±3</td>
<td>42±3</td>
</tr>
</tbody>
</table>

Values are means ± SE. Values for 3-methylhistidine and tyrosine are nanomoles per leg per hour. Rats were divided into 3 groups of 3 treatments. Treatments were exercise by running 18° down grade, level running, and pair-fed controls designated by a D, L, or C, respectively. Controls were fed the same amount of food as the exercised rats to compensate for the reduced food intake of the rats that were exercised. Run time, was the same as the exercised rats, which was 28.0±0.7 g the day before exercise, 12.3±1.1 g 1 day after exercise, and 22.4±0.9 g 2 days after exercise. The rats ran at 25 m/min. C, D, and L were perfused immediately after running D and L. C + 1 D + 1, and L + 1 were perfused 1 day after running D + 1 and L + 1. C + 2, D + 2 and L + 2 were perfused 2 days after running D + 2 and L + 2. After 30 min of single-pass perfusion, 10 ml of perfusate were collected for analysis. * Statistically significant (P < 0.05) difference from same day (group) control. † Statistically significant (P < 0.05) difference from same treatment in group perfused immediately after running. ‡ Statistically significant (P < 0.05) difference between same treatments in groups perfused 1 and 2 days after running.
level and downgrade running were found in the previous experiment. Smaller rats were also used in this experiment. Table 2 shows the same trends as Table 1; however, the apparent decrease of 14% in the rate of total protein degradation of the exercised rats compared with the starved controls was not statistically significant. There was no difference in the rate of 3-methylhistidine release between the starved groups; however, both values were significantly greater than that of the fed control. The increased level of cathepsin D activity found in the muscle of the exercised group suggests that the exercise was strenuous enough to cause muscle damage.

Although the results of this experiment were inconclusive, it was apparent that the amount of food consumed the day after exercise had a major influence on the rate of protein degradation. To further examine the effect of food consumption and exercise on the rate of protein degradation, another experiment was performed. In this experiment, a rested and an exercised group that were starved the day before exercise were included. Withholding food the day before exercise resulted in a 78% increase in food consumption the day after exercise compared with the rats that were fed the day before exercise. The pair-fed controls were fed the same amount as those that ran. As in previous experiments, the rate of tyrosine release from the exercised rats tended to be smaller than from the pair-fed controls in the rats that had been fed before exercise. Although the decrease was not statistically significant when all four groups were compared, a significant difference was found when only the two groups that were fed the day before exercise were compared. Table 3 also shows that when food consumption is increased the day after exercise had a major influence on the rate of protein degradation in a contracting muscle necessitated a change in experimental model because it is technically difficult to perfuse a running rat. The rate of total and myofibrillar protein degradation was determined by measuring the rate of release of tyrosine and 3-methylhistidine from the rat hindlimb that was electrically stimulated to contract. Pilot studies showed that mild stimulation, such as 2 or 4 twitches/s or one 100-ms train/s, did not produce changes in tyrosine or 3-methylhistidine release; thus we adopted a protocol consisting of stimulating with two 100-ms trains/s, which was near the maximal stimulation that could be sustained for 20 min. Table 4 shows that stimulating with two 100-ms trains/s for 20 min resulted in decreases of 41 and 20% in the rates of myofibrillar and total protein degradation, respectively. This procedure also resulted in a decrease in the muscle ATP level from 5.7 ± 0.2 to 4.2 ± 0.2 mmol/mg.

**DISCUSSION**

Many of the conflicting results on the effect of exercise on protein degradation may be due to differences in the methodologies used to measure the rate of protein degradation, different types of protein being degraded, and the time the measurements were made. The purpose of the studies described in this paper is to resolve these conflicts of the rate of protein degradation in a contracting muscle.

**Table 2. Effect of fasting on muscle protein degradation after downgrade running**

<table>
<thead>
<tr>
<th>Fed Control</th>
<th>Starved Control</th>
<th>Starved D + 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Wt, g</td>
<td>253±4</td>
<td>216±2</td>
</tr>
<tr>
<td>Run time, min</td>
<td>200±2</td>
<td>200±2</td>
</tr>
<tr>
<td>3-Methylhistidine</td>
<td>19±1*</td>
<td>37±2</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>2,230±100</td>
<td>2,430±100</td>
</tr>
<tr>
<td>Cathepsin D, cpm/mg</td>
<td>363±10</td>
<td>388±16</td>
</tr>
</tbody>
</table>

Values are means ± SE. Values for 3-methylhistidine and tyrosine are nanomoles per leg per hour. D + 1 rats were run at 25 m/min 18° downgrade and were perfused 1 day postexercise. Fed D + 1 and C ate ad libitum the day before D + 1 ran and 12.5±1.2 and 12.5±0.2 g the day after D + 1 ran, respectively. Starved D + 1 and C were starved the day before D + 1 ran, and ate 22.2±1.1 and 22.5±0.2 g the day after D + 1 ran, respectively. * Statistically significant (P < 0.05) difference from respective control. † Statistically significant (P < 0.05) difference from fed.

**Table 3. Effect of food consumption and exercise on muscle protein degradation**

<table>
<thead>
<tr>
<th></th>
<th>Fed</th>
<th>Starved</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>D + 1</td>
<td>C</td>
</tr>
<tr>
<td>n</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Wt, g</td>
<td>343±4</td>
<td>360±5*</td>
</tr>
<tr>
<td>Run time, min</td>
<td>160±11</td>
<td>149±11</td>
</tr>
<tr>
<td>3-Methylhistidine</td>
<td>37±2</td>
<td>44±3*</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>4,150±100</td>
<td>3,600±190*</td>
</tr>
<tr>
<td>Cathepsin D, cpm/mg</td>
<td>304±5</td>
<td>384±8*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Values for 3-methylhistidine and tyrosine are nanomoles per leg per hour. Rats in the D + 1 group were run at 25 m/min 18° downgrade and were perfused 1 day postexercise. Fed D + 1 and C ate ad libitum the day before D + 1 ran and 12.5±1.2 and 12.5±0.2 g the day after D + 1 ran, respectively. Starved D + 1 and C were starved the day before D + 1 ran, and ate 22.2±1.1 and 22.5±0.2 g the day after D + 1 ran, respectively. * Statistically significant (P < 0.05) difference from respective control. † Statistically significant (P < 0.05) difference from fed.
by systematically examining many of the different parameters that can be varied in the study of the effect of endurance exercise on the rate of protein degradation in muscle. In all of the experiments described in this study, the rate of protein degradation was measured by perfusing one skinned rat hindlimb. This method was chosen because it is more physiological than the incubated isolated muscle preparation. The muscle is skinned to eliminate the release of 3-methylhistidine from skin into the perfusate. The single leg was used because a greater portion of the perfused mass is muscle than in the perfused hemicorpus preparation. The rate of total protein degradation was determined from the rate of release of tyrosine in the presence of the protein synthesis inhibitor cycloheximide. 3-Methylhistidine is found in actin and is not reused or oxidized in the rat; thus its rate of release into the perfusate is a measure of myofibrillar protein degradation. With the use of these techniques, the rates of total and myofibrillar muscle protein degradation can be measured without the complications caused by changes in kidney clearance found in methods that rely on urinary excretion.

Measurements of the rate of protein degradation are made immediately after exercise in an attempt to determine what happened during the exercise bout. The assumption is that changes in the rate of muscle protein degradation caused by exercise will persist for the 20 min it takes to start the perfusion and during the perfusion. Figure 1 demonstrates that the latter part of this assumption is correct by showing that the observed increase in tyrosine release after exercise is observed at all time points after exercise. The similar findings of increased tyrosine and no or small increases in 3-methylhistidine release using the perfusion technique and the incubated muscle technique (15) in which no anesthesia is used and the time from exercise to the beginning of the measurements is only 5 min suggests that the changes in the rate of protein degradation caused by exercise are preserved and can be measured using the perfused single hindlimb technique. The data in Figs. 1 and 3, and in Table 1, show that exercise causes an increase in the rate of tyrosine release, which indicates that the rate of total muscle protein degradation is increased by exercise. This agrees with our previous finding using the isolated soleus muscle (15), but is not consistent with the findings of Balon et al. (2), who observed the increase in tyrosine release after exercise to be due to a decreased rate of protein synthesis. The reason for this difference is likely due to differences in the time after exercise that the measurements were made. The measurements made by Balon et al. (2) were made 30 min postexercise, and it was demonstrated (15) that the increase in the rate of protein degradation rapidly returns to control values if the rat is allowed to rest after exercise. The data in Fig. 2 and Table 1 show the lack of an exercise effect on the rate of 3-methylhistidine release. This is in agreement with our previous study using the isolated soleus muscle (15) and supports our conclusion that the rate of myofibrillar protein degradation is not significantly changed during an exercise bout. This must mean that the increased urinary excretion of 3-methylhistidine observed after exercise in rats (9, 11) occurs after the completion of the exercise bout or comes from nonmuscular tissue. This will be discussed subsequently.

Our finding of an increase in the rate of total protein degradation and no change in the rate of myofibrillar protein degradation during exercise makes teleological sense. One might expect that during the increased energy demand of exercise, protein in the muscle not used for contraction would be broken down to amino acids, whose rate of oxidation during exercise is known to be increased (19, 23, 29). On the other hand, it would not make sense to degrade the proteins in the motor units responsible for the continuation of the exercise. The metabolic fate of the amino acids liberated by the increased protein degradation is not known; however, as stated above, it is likely that they are oxidized in muscle, either to produce energy directly or to replenish tricarboxylic acid cycle intermediates, which are lost due to the increased activity of pyruvate dehydrogenase, and phosphoenolpyruvate carboxykinase (7). For example, the exercise-induced increase in the activity of branched-chain keto acid dehydrogenase (14), the rate-limiting enzyme in the oxidation of the branched-chain amino acids, would cause increased production of succinyl-CoA from valine and isoleucine (14).

The mechanism responsible for the increased degradation of muscle protein during exercise is unknown. In an earlier study (6) we observed an increase in "free" cathepsin D and interpreted this as an indication of the involvement of lysosomes in the increase in the rate of protein degradation caused by exercise. However, chloroquine failed to bring the rate of tyrosine release from the isolated soleus muscles of exercised rats to control levels (15), which indicates that the lysosomes are not involved. This was surprising, because it is well established that the lysosomal pathway is involved in the degradation of the nonmyofibrillar, but not the myofibrillar, proteins (22). The effect of chloroquine on the exercise-induced increase in tyrosine release was examined using the perfused single leg technique. The data in Fig. 3 show that when chloroquine was added to the perfusate, the rate of tyrosine release from the perfused legs of exercised and control rats coalesced. This is good evidence for the involvement of the lysosomal pathway in the exercise-induced increase in the rate of degradation of noncontractile muscle protein. The reason for the discrepancy between the experiments using isolated muscles and perfused single legs is not known, but it may be that the soleus, being a type 1 muscle, is not a good representative of the total leg musculature.

Another way to study the rate of protein degradation during exercise is by electrically stimulating the muscle to contract. We found electrical stimulation of the muscle resulted in a small decrease in the rates of both tyrosine and 3-methylhistidine release. Although the rate of 3-methylhistidine release was not decreased to as great an extent as previously reported (4), our findings of a larger decrease in the rate of 3-methylhistidine release compared with tyrosine release qualitatively agrees with the report (4) of a large decrease in 3-methylhistidine and no change in tyrosine (2) release after electrical stimulation.
These observations of the rates of both tyrosine and 3-methylhistidine release responding differently when measured immediately after exercise and during electrical stimulation suggest that electrical stimulation is not a good model in which to study the effect of exercise on muscle protein degradation.

The second part of this study was an investigation of the rate of protein degradation during recovery from exercise. An increase in the urinary excretion of urea and 3-methylhistidine after exercise could be interpreted as resulting in increased protein degradation during the recovery phase after exercise (see Refs. 8 and 18 for reviews). In particular, the large increase in the urinary excretion of 3-methylhistidine that persisted for several days after downgrade running suggested that there was an increase in muscle protein degradation and that it may be due to muscle damage that occurred during the exercise bout (12). However, the data in Tables 1–3 are not consistent with this hypothesis. The rate of 3-methylhistidine release was not different from controls in rats that had run either downgrade or on the level. Downgrade running eccentrically biases the exercise, which results in greater muscle damage (1). The reason no changes in 3-methylhistidine release were found in this experiment, but increases in urinary 3-methylhistidine were found previously (11), is likely due to differences in protocol. In this experiment, the controls were pair fed, and in the earlier experiment, the exercised rats served as their own controls by having their urinary 3-methylhistidine rate measured the day before they were exercised (11).

A second experiment was performed (data in Table 2) to verify that this conclusion was also valid in smaller rats. In this experiment, cathepsin D levels were elevated the day after exercise, which is an indication of muscle damage. Food consumption as a variable was eliminated by starving all rats for 1 day. Again the rate of 3-methylhistidine was not increased the day after exercise compared with starved controls. As expected, the rate of 3-methylhistidine release was increased by fasting (20, 21).

The effect of changing the food intake on the release of tyrosine and 3-methylhistidine after exercise was examined in another experiment. In this experiment, one group of rats was fasted the day before exercise to increase their food intake the day after exercise. The results, shown in Table 3 with the fed rats, were about the same as in the previous experiments, although in this experiment the small increase in 3-methylhistidine release was statistically significant. The rats that were fasted the day before exercise increased their food intake to ~80% of normal the day after exercise. This increase in food consumption compared with the rats that had eaten the day before exercise resulted in a decrease in the rate of 3-methylhistidine release, but there was no difference between those that had exercised and the pair-fed controls. It is now clear that, although there may be a small increase in the rate of 3-methylhistidine release during the recovery phase (days after exercise), the observed large increase in the urinary excretion of 3-methylhistidine during recovery from exercise is due to decreased food intake. These findings are in agreement with studies in humans in which increased rates of protein degradation were observed during exercise (25, 30), but not 2 h after exercise (5).

One of the surprising findings of this study was the observation of a decrease in the rate of tyrosine release the day after the animals were exercised. The decrease in tyrosine release the day after exercise was significant in the rats with restricted food intake (Table 1), but with a complete fast the tyrosine decrease did not reach significance with this number of rats (Table 2). Fasting and refeeding upset normal metabolism in the rat, making it difficult to interpret data in Table 3. However, one possible explanation that is consistent with our hypothesis, that exercise prevents the increase in the rate of protein degradation associated with reduced food intake, is that the increased food consumption the day after exercise in the previously fasted rats compared with the rats that had eaten the day before exercise obliterated the effect observed in the rats that ate the day before exercise. Taken together, the results of these experiments suggest that exercise does not decrease the rate of protein degradation the day after exercise, but instead prevents an increase in the rate of protein degradation caused by fasting. The mechanism responsible for this exercise-induced muscle protein sparing is unknown, but it is likely a conservation mechanism whose role is to conserve muscle protein when energy reserves are low. Thus the decrease in the rate of protein degradation may be due to a further decrease in energy reserves caused by exercise, since the energy expended by the exercise in these experiments is between 8 and 12 kcal, which is equivalent to 2–3 g of rat food. Consistent with this hypothesis is the fact that the rate of tyrosine release was not increased in the rats that were completely starved (see Table 2) compared with rats that were restricted to ~12 g of food. Further experiments are necessary to determine the relative contribution of the energy deficit caused by exercise and the exercise itself to this decreased rate of total muscle protein degradation. This exercise-induced muscle protein sparing from the increased degradation caused by fasting could have direct implications in dieting and weight loss. However, caution should be used when interpreting these results, and the conclusions should not be generalized, since they are derived from experiments involving only one day of exercise and one day of fasting.

In conclusion, the results of this study are consistent with an increase in the rate of noncontractile protein degradation and no change in the rate of myofibrillar protein degradation during exercise. This increase in the rate of nonmyofibrillar protein degradation involves the lysosomal pathway. During the recovery period after either level or downgrade running there was little or no change in the rate of myofibrillar protein degradation that was due to decreased food intake. The increased rate of total protein degradation, which was due to restricted food intake, was prevented by an exercise bout.

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