IGF-I has no effect on postexercise suppression of the ubiquitin-proteasome system in rat skeletal muscle

ANTHONY J. KEE,1 ALAN J. TAYLOR,2 ANTHONY R. CARLSSON,1 ANDRE SEVETTE,1 ROSS C. SMITH,1 AND MARTIN W. THOMPSON2
1Department of Surgery, Royal North Shore Hospital, University of Sydney, St. Leonards, New South Wales 2065; and 2School of Exercise and Sport Science, University of Sydney, Lidcombe, New South Wales 1825, Australia

Received 11 October 2001; accepted in final form 21 December 2001

Kee, Anthony J., Alan J. Taylor, Anthony R. Carlsson, Andre Sevette, Ross C. Smith, and Martin W. Thompson. IGF-I has no effect on postexercise suppression of the ubiquitin-proteasome system in rat skeletal muscle. J Appl Physiol 92: 2277–2284, 2002; 10.1152/japplphysiol.01030.2001.—Both exercise and insulin-like growth factor I (IGF-I) are known to have major hypertrophic effects in skeletal muscle; however, the interactive effect of exogenous IGF-I and exercise on muscle protein turnover or the ubiquitin-proteasome pathway has not been reported. In the present study, we have examined the interaction between endurance exercise training and IGF-I treatment on muscle protein turnover and the ubiquitin-proteasome pathway in the postexercise period. Adult male rats (270–280 g) were randomized to receive 5 consecutive days of progressive treadmill exercise and/or IGF-I treatment (1 mg kg body wt−1 day−1). Twenty-four hours after the last bout of exercise, the rate of protein breakdown in incubated muscles was significantly reduced compared with that in unexercised rats. This was associated with a significant reduction in the chymotrypsin-like activity of the proteasome and the rate of ubiquitin-proteasome-dependent casein hydrolysis in muscle extracts from exercised compared with unexercised rats. In contrast, the muscle expression of the 20S proteasome subunit β-1, ubiquitin, and the 14-kDa E2 ubiquitin-conjugating enzyme was not altered by exercise or IGF-I treatment 24 h postexercise. Exercise had no effect on the rates of total mixed muscle protein synthesis in incubated muscles 24 h postexercise. IGF-I treatment had no effect on muscle weights or the rates of protein turnover 24 h after endurance exercise. These results suggest that a suppression of the ubiquitin-proteasome proteolytic pathway after endurance exercise may contribute to the acute postexercise net protein gain.

protein synthesis; protein degradation; insulin-like growth factor I; muscle adaptation

EXERCISE HAS POTENT EFFECTS on skeletal muscle protein synthesis and proteolysis (23). During resistance exercise, muscles are placed under high forces, and there is an adaptive response of increase in the mass of contractile proteins (hypertrophy) to overcome those forces. In contrast, high levels of muscle mass are not required to maintain endurance exercise, and, consequently, it leads to little change in skeletal muscle mass. There is evidence to suggest that the muscle’s acute response to endurance exercise is catabolic. Rates of protein synthesis are moderately reduced or unchange (3, 6, 10), whereas there is a concomitant increase in rates of muscle protein degradation during and in the few hours after exercise (6, 11, 16). However, despite these acute responses to endurance exercise, muscle mass over the long term is maintained or in some cases increased (23). This must be achieved through a period of protein anabolism in the “recovery” phase after exercise, but the roles of proteolysis and protein synthesis in this process are still to be fully elucidated.

Although it has long been recognized that exercise can lead to an acute increase in protein breakdown, there is still little knowledge about which proteolytic pathways are involved. Both Ca2+-activated (calpains) and lysosomal (cathepsins) proteases have been implicated in the exercise-induced increase in proteolysis, although this is usually in association with exercise-induced muscle damage (29). More recent studies suggest that the ubiquitin-proteasome pathway is also involved in the exercise-induced proteolysis (22, 27). For example, exercise has been shown to increase proteasome activity in rat muscle (22) and the expression of one component of this pathway (ubiquitin) in human muscle (27). However, in another study, the mRNA expression of components of the ubiquitin-proteasome pathway was reduced by passive leg cycling in spinal cord injury patients (31). Clearly, more extensive investigations are required to clarify the role of the ubiquitin-proteasome pathway in exercise-induced alterations in protein metabolism, particularly in the postexercise recovery period. Thus, in the present study, we have measured the rates of protein turnover and the activity of the ubiquitin-proteasome pathway in rats 24 h after the final bout of an endurance exercise training regimen.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

http://www.jap.org 8750-7587/02 $5.00 Copyright © 2002 the American Physiological Society

2277
The ubiquitin-proteasome pathway is not only involved in the general turnover of the majority of cellular proteins but also plays a key role in the control of major biological events, such as cell cycle progression, transcriptional control, signal transduction, receptor downregulation, and class I antigen presentation (21). In this pathway, polyubiquitin chains are formed covalently attached to proteins that are to be degraded in a multiple-step process that requires ATP, the ubiquitin-activating enzyme (E1), and one of the ubiquitin-conjugating enzymes (E2), which functions either alone or in the presence of a ubiquitin-protein ligase (E3) (21). After polyubiquitinylation, the targeted proteins are then recognized and degraded by the 26S proteasome. The association of a proteolytic core, the 20S proteasome, with two 19S regulatory complexes forms the 26S proteasome. The 19S regulatory complex confers ATP and ubiquitin dependency to proteolysis and stimulates the proteolytic and peptidase activities of the 20S proteasome core (30).

The dramatic muscle hypertrophy of transgenic mice overexpressing the insulin-like growth factor I (IGF-I) gene in skeletal muscle (8) has confirmed the central role of IGF in skeletal muscle proliferation and differentiation. These results, together with the reports of an exercise-induced increase in muscle IGF-I expression (12, 33), have led to the suggestion that muscle-derived IGF-I is largely responsible for exercise-induced hypertrophy. Exogenous IGF-I administration has also been shown to reduce muscle disuse atrophy when combined with resistance exercise (24). In the present study, we have examined whether the administration of exogenous IGF-I can augment the anabolic effect of endurance exercise in a nonatrophic model.

MATERIALS AND METHODS

Materials and Methods

Materials. Recombinant human (rhIGF-I) and rat IGF-I were provided by Gropep (Adelaide, Australia). [U-14C]phenylalanine and [methyl-14C]casein were supplied by APBiotech (Sydney, Australia). The fluorogenic protease substrate Suc-Leu-Leu-Val-Tyr-7-aminomethylcoumarin (Suc-LLVY-AMC) and the protease inhibitors phenylmethylsulfonyl fluoride (PMSF), trans-epoxysuccinyl-L-leucylamido-(4-guanidino)butane (E64d), and pepstatin A were from Sigma-Aldrich (Sydney, Australia). The rats were anesthetized (3.6 mg pentobarbitone/kg body wt ip), and both epitrochlearis muscles were carefully dissected and placed into incubation medium for measurement of protein turnover. The weights of a number of lower limb muscles were also recorded: tibialis anterior, soleus, whole gastrocnemius, and extensor digitorum longus (EDL). Care was taken to remove the muscles from tendon to tendon, and any excess fascia was carefully trimmed away. Finally, blood (4–5 ml) was obtained by cardiac puncture, and plasma was stored for assessment of glucose and hormone levels.

For the measurement of protease activity, fresh muscle tissue (400–500 mg) was homogenized in 10 volumes of ice-cold buffer (50 mM Tris-HCl, pH 8.0, containing 10% glycerol, 1 mM EDTA, 1 mM EGTA, 50 mM E64d, 2.5 mM pepstatin A) with the use of a Polytron homogenizer (model PT10St “OD” S, Kinematica) and was centrifuged at 100,000 g for 1 h. The resulting pellet was resuspended in reaction media and used immediately.

rhIGF-I treatment. rhIGF-I was administered to the animals as a subcutaneous injection (1.0 mg/kg body wt) immediately postexercise. The IGF-I administration began on the first exercise day and continued for the 5 days of the exercise period. Animals not receiving IGF-I were given equal volumes of sterile IGF-I vehicle as a placebo (5 mM HCl, 0.5 mg/ml BSA).

J Appl Physiol • VOL 92 • JUNE 2002 • www.jap.org
Measurement of protein turnover in incubated muscles. Epitrochlearis muscles (40–60 mg) were incubated at 37°C in a standard Krebs-Henseleit medium (120 mM NaCl, 25 mM NaHCO₃, 4.8 mM KCl, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 0.5 mM CaCl₂, pH 7.4) containing 5 mM glucose, equilibrated, and maintained under pressure with O₂/CO₂ (19:1). After 30 min of preincubation, the muscles were transferred to fresh medium and incubated for a further 2 h.

The rate of total mixed muscle protein synthesis was determined by incubating the muscles in a medium containing 0.5 mM [U-¹⁴C]phenylalanine [specific radioactivity in the medium, 500 disintegrations·min⁻¹ (dpm)·nmol⁻¹] as described previously (28). Muscle protein mass was determined by using the biocinchoninic acid method (25). Rates of phenylalanine incorporation were converted into tyrosine equivalents by multiplying by 0.77, the molar ratio of tyrosine to phenylalanine in rat muscle proteins (28). Net protein breakdown was determined by measuring the rate of tyrosine release from the epitrochlearis muscles into the medium (28). As tyrosine is not synthesized or metabolized in muscle (15), and muscle tyrosine concentration is not altered during incubation (results not shown; Ref. 15), tyrosine release is a measure of the degradation of all muscle proteins. However, as tyrosine released into the incubation medium can be taken up for protein synthesis, the rates of protein breakdown were calculated as the sum of the rate of protein synthesis (in tyrosine equivalents) and the rate of tyrosine release.

Proteasome activity. The chymotrypsin activity of the crude muscle extracts was determined by measuring the rate of hydrolysis of the fluorogenic peptide Suc-LLVY-AMC, as described by Farout et al. (14). The reaction mixture (200 μl) contained 50 mM Tris·HCl, pH 8.0, 1 mM dithiothreitol, 50–80 μg protein, and 40 μM Suc-LLVY-AMC. Incubations were performed at 37°C for 30 min. The fluorescence was continuously monitored on a RF-1501 Shimadzu spectrofluorometer (excitation 370 nm, emission 420 nm), and initial steady-state rates of hydrolysis were used. To assess the specificity of the measurements, crude muscle extracts were preincubated for 30 min at 37°C with the proteasome inhibitor lactacystin (50 μM), the serine protease inhibitor PMSF (100 μM), or the cysteine protease inhibitor E64d (50 μM). Assays were performed in duplicate, and the mean results are presented. The variation among duplicates was <12%.

Casein degradation. The activity of the ubiquitin-proteasome-dependent proteolytic pathway in crude extracts of gastrocnemius muscles was assessed by measuring the ATP-ubiquitin-dependent degradation of [methyl-¹⁴C]casein, as previously described (19). Muscle extracts (100 μg protein) were incubated at 37°C for 45 min in the presence of 40,000 dpm [methyl-¹⁴C]casein, 50 mM Tris·HCl, pH 7.8, 20 mM NaCl, 2 mM dithiothreitol, and 9 mM MgCl₂, in a total volume of 200 μl. The incubations were performed with and without ATP (5 mM) and ubiquitin (2.5 μg/ml). To assess the specificity of the hydrolysis, some muscle extracts were preincubated for 30 min at 37°C with the proteasome inhibitor lactacystin (50 μM). The reactions were stopped with the addition of 500 μl of 10% (wt/vol) TCA. The samples were left on ice for at least 1 h and centrifuged, and the release of acid-soluble peptides was measured by using a Tricarb Packard β-limited scintillation counter. Corrections were made for radioactivity appearing in the acid-soluble supernatant after incubation of [methyl-¹⁴C]casein in the absence of muscle extracts. This was generally <1% of the radioactivity in the TCA-soluble supernatants from incubations with muscle extracts. Assays were performed in duplicate, and the mean results are presented. The variation among duplicates was <7%. The appearance of acid-soluble peptides was found to be linear for at least 60 min of incubation (data not shown).

Northern blot analysis. Gastrocnemius muscles were rapidly excised, frozen in liquid nitrogen, and stored at −80°C. Total RNA was extracted from frozen tissue (100–200 mg) as described (7), and 10 μg were electrophoresed in 1% (wt/vol) agarose gels containing formaldehyde. RNA was vacuum transferred to a nylon membrane (GeneScreen, NEN Research Products, Boston, MA) and covalently bound to the membrane by ultraviolet cross-linking. The membranes were hybridized with cDNA probes encoding chicken polyubiquitin (1), the human β-1 20S proteasome subunit (2), rat 14-kDa E2 ubiquitin-conjugating enzyme (32), and mouse 18S rRNA. The hybridizations were performed at 65°C with [³²P]cDNA fragments labeled by random priming. After they were washed at the same temperature, the membranes were placed in a FujiFilm FLA-3000 phosphor imager (Sydney, Australia), and the radiographic signals were quantified by using the IProcess (FujiFilm) imaging software (version 1.96). The data were normalized with the corresponding 18S rRNA signals to correct for small variations in RNA loading.

Serum measurements. Plasma glucose was determined by using a commercially available kit (Peridochrom, Boehringer Mannheim, Sydney, Australia). Serum insulin was determined by using a rat-specific RIA (LINCO Research, St. Charles, MO). Total IGF-I (endogenous rat IGF-I plus rhIGF-I) concentrations were determined by RIA, as previously described (17). The polyclonal antibody used in the assay for total IGF-I was shown to react equipotently with recombinant rat IGF-I and rhIGF-I (data not shown).

Data treatment and statistical analysis. Results are presented as means ± SE. Levene’s test was performed to ensure homogeneity of variance together with the Kolmogorov-Smirnov (Lilliefors) test to check for normality of the data (SPSS 8.0 software, Chicago, IL). All data presented satisfied the above tests, and, therefore, parametric analysis was employed.

With the use of a 2 × 2 factorial design, with the factors being exercise and IGF-I treatment, each at two levels (with or without), ANOVA of means and interactions of factors were examined (SPSS 8.0). If statistically significant differences were detected by ANOVA, individual comparisons were made between groups by using the post hoc Fisher’s least significant difference test. Pearson’s correlation coefficients were calculated between serum insulin levels and the rate of protein turnover and ubiquitin-proteasome-dependent activity, and between rates of proteolysis in incubated muscles and proteasome activities and ATP-dependent casein hydrolysis in crude muscle extracts. The α-level was set at 0.05.

RESULTS

Effect of exercise and IGF-I on body and muscle weights. Five days of treadmill exercise significantly reduced the normal rate of body weight increase (17.1 ± 2.6 and 38.7 ± 2.5 g, means ± SE for the combined exercise and nonexercise groups; P < 0.001, 2-way ANOVA). IGF-I treatment had no effect on body weight, and there was no interaction between IGF-I and exercise (results not shown).

In general, exercise and IGF-I treatment had little effect on absolute muscle weights (data not shown) and muscle weights relative to body weight (Table 1), although there was a small (8%) but significant increase in relative soleus muscle weight with exercise (2-way ANOVA).
Validation of proteasome activity in crude muscle extracts. The assay conditions (pH 8.0, EDTA, EGTA, protease inhibitors) were chosen to allow measurement of chymotrypsin-like (ChT-L) activity of the proteasome without interference from other major proteases. To assess the specificity of the assay, a number of control experiments were performed on extracts from gastrocnemius muscles. First, to assess the efficiency of isolating the proteasome fraction, the pellet and the supernatant resulting from the 100,000 g centrifugation step were assayed for proteasome activity by using Suc-LLVY-AMC. More than 99% of the activity present in the crude homogenate was located in the pellet, and this activity was almost completely inhibited (95–100%) by the proteasome inhibitor lactacystin (data not shown). Second, the degradation of Suc-LLVY-AMC by the crude extract was not affected by inhibitors of cysteine and serine proteases (E64d or PMSF, respectively; data not shown). These experiments confirmed that, under the incubation conditions utilized, the proteasome is the major protease responsible for hydrolysis of the fluorogenic substrate by the crude muscle extracts.

Effect of exercise and IGF-I on protein synthesis and proteolysis in incubated muscles. Five days of treadmill exercise and/or IGF-I treatment had no effect on the rate of mixed muscle protein synthesis 24 h after the last exercise bout (Fig. 1). In contrast, exercise training resulted in a 23% decrease in the rate of proteolysis in incubated epitrochlearis muscles 24 h postexercise (2-way ANOVA) (Fig. 1). IGF-I treatment had no effect on protein breakdown 24 h postexercise, and there was no interactive effect between the two treatments (P = 0.345; 2-way ANOVA).

Effect of exercise and IGF-I on total proteolysis in incubated muscles. The magnitude of the suppression of proteolysis in the incubated epitrochlearis muscles (24%) (compare Figs. 1 and 2). The magnitude of the suppression of the proteasome activity (21–23%) was similar to the suppression of total proteolysis in the incubated epitrochlearis muscles (24%) (compare Figs. 1 and 2). rhIGF-I treatment had no effect on the ChT-L activity of the proteasome. There was a significant correlation between proteasome activity in muscle extracts and the rate of proteolysis in incubated muscles (gastrocnemius, r = 0.377, P = 0.033; soleus, r = 0.421, P = 0.016; EDL, r = 0.513, P = 0.003).

Effect of exercise and IGF-I on ubiquitin-proteasome-dependent proteolysis in skeletal muscle. In this study, the activity of the ubiquitin-proteasome proteolytic pathway was assessed by measuring the degradation of [methyl-14C]casein by extracts of gastrocnemius muscles from exercised and IGF-I-treated rats (Table 2). The rates of casein degradation approximately doubled with the addition of exogenous ATP and ubiquitin.
for all treatment groups, highlighting the importance of these two factors in regulating the activity of this proteolytic pathway. Muscle extracts from exercised rats had significantly less proteolytic activity, with and without added ATP/ubiquitin, compared with nonexercised rats. In contrast, there was no effect of IGF-I treatment on casein degradation. The addition of the proteasome inhibitor lactacystin decreased the rate of proteolysis by the muscle extracts to levels well below those observed in the absence of ATP/ubiquitin and lactacystin. Furthermore, when the proteasome was inhibited, the exercise-associated decrease in proteolysis was no longer present. These results indicate that the lower rate of casein degradation in the muscle extracts from exercised vs. nonexercised rats is entirely due to a decrease in the activity of the ubiquitin-proteasome proteolytic pathway.

Table 2. Effect of exercise and IGF-I treatment on the degradation of [methyl-14C]casein in gastrocnemius muscle extracts

<table>
<thead>
<tr>
<th></th>
<th>-Ub -ATP -Lac</th>
<th>+Ub +ATP -Lac</th>
<th>+Ub +ATP +Lac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ex +IGF</td>
<td>15.9 ± 1.1*</td>
<td>29.8 ± 1.7*</td>
<td>5.9 ± 0.7</td>
</tr>
<tr>
<td>Ex -IGF</td>
<td>15.9 ± 1.0*</td>
<td>33.4 ± 2.3*</td>
<td>4.5 ± 0.7</td>
</tr>
<tr>
<td>-Ex +IGF</td>
<td>20.0 ± 1.1</td>
<td>45.2 ± 3.8</td>
<td>6.9 ± 1.6</td>
</tr>
<tr>
<td>-Ex -IGF</td>
<td>19.2 ± 1.1</td>
<td>41.3 ± 3.8</td>
<td>5.7 ± 1.7</td>
</tr>
</tbody>
</table>

Values are means ± SE in ng/g protein min⁻¹ of a single muscle extract from 5 rats per treatment group. Incubations were performed with (+) and without (−) ATP (5 mM), ubiquitin (Ub; 2.5 μg/ml), and lactacystin (Lac; 50 μM) as described in MATERIALS AND METHODS. *Significant effect of exercise (P < 0.005) but not IGF-I treatment on the rate of [methyl-14C]casein degradation (2-way ANOVA).

Experiment, the ubiquitin-proteasome proteolytic pathway in muscle is responsible for at least 80% of the total rate of casein degradation. There was a significant correlation between ATP-dependent casein hydrolysis and the rate of proteolysis in incubated muscles (r = 0.485, P = 0.03).

**Effect of exercise and IGF-I on the expression of components of the ubiquitin-proteasome pathway.** Representative Northern blots for the 20S proteasome subunit β-1, ubiquitin, and the 14-kDa E2 ubiquitin-conjugating enzyme in rat gastrocnemius muscle are shown in Fig. 3. The level of expression for each transcript was quantified densitometrically by using the experiment.
18S rRNA signals to correct for small variations in RNA loading (data not shown). There was no significant difference among groups ($P > 0.05$, ANOVA) for the expression of ubiquitin, 14-kDa E2, or the β-1 20S proteasome subunit.

Effect of exercise and IGF-I on serum insulin, IGF-I, and glucose. Serum insulin, glucose, and IGF-I concentrations were measured 24 h postexercise (see Table 3). At 24-h postexercise, there was no significant effect of exercise training or IGF-I treatment on serum glucose or total IGF-I (endogenous and exogenous) levels (2-way ANOVA). However, exercise-training and IGF-I treatment significantly decreased insulin concentrations 24 h after the final exercise bout, but there was no interactive effect between the two treatments ($P = 0.704$, 2-way ANOVA). There was also no significant correlation between insulin levels and the rate of proteolysis in incubated muscles or the ubiquitin-proteasome pathway activities in the crude muscle extracts ($P > 0.1$, $r = 0.23$).

**DISCUSSION**

Exercise effects. There is a biphasic response of protein turnover to exercise. During endurance exercise of sufficient duration and intensity, there is a net loss of muscle protein, which is followed by a period of net positive protein balance, resulting in maintenance or a slight increase in muscle mass (23). It is commonly thought that the postexercise-positive protein balance is mediated largely through a postexercise increase in protein synthesis that is augmented by a rapid return of proteolysis to basal levels (23). The results of the present study suggest that a suppression of proteolysis and the ubiquitin-proteasome proteolytic pathway after endurance exercise to below basal levels may contribute to the postexercise net protein gain.

The activity of the proteasome and the ubiquitin-proteasome proteolytic pathway were assessed in crude muscle extracts by measuring the hydrolysis of an artificial proteasome substrate (Suc-LLVY-AMC) and the ATP-dependent hydrolysis of casein, respectively. The hydrolysis of Suc-LLVY-AMC does not require prior ubiquitinylation, whereas casein needs to be ubiquitinylation before it can undergo ATP-dependent proteasomal degradation (19). Therefore, the rate of ATP-dependent degradation of casein is a measure of the total functional capacity of the ubiquitin-proteasome proteolytic pathway in the crude muscle extracts. This includes both the multistep process of ubiquitinylation and the degradation of the polypeptide chain by the 26S proteasome complex. That proteasome activity and ATP-dependent degradation of casein in crude extracts were significantly correlated with the exercise-induced suppression of proteolysis in incubated muscles strongly suggests that the ubiquitin-proteasome proteolytic pathway was at least partially responsible for the suppression of proteolysis 24 h postexercise. Further work is required to determine whether this effect is mediated at the level of ubiquitin conjugation and/or the protease activity of the 26S proteasome itself.

There is little information regarding the effect of exercise on the ubiquitin-proteasome system. Some investigators have suggested that this pathway is activated in the postexercise period (22, 27). However, this conclusion was based solely on either free ubiquitin levels in muscle (27) or proteasomal activities in muscle extracts (22). Others have shown a decrease in the mRNA levels of components of the ubiquitin-proteasome pathway in muscles from spinal cord injury patients after 12 wk of exercise training (31). In the present study, there was no effect of exercise training on muscle expression of ubiquitin, the 14-kDa E2 conjugating enzyme, or the β-1 subunit of the 20S proteasome in muscle 24 h postexercise. The reasons for the discrepancies among studies are unclear but may be due to the length of exercise training or differences among species. Regardless, it would appear that changes in the level of mRNA expression were not responsible for the suppression of proteasome activity or ubiquitin-proteasome proteolytic activity observed in the present study. Lack of correlation between mRNA levels and the rate of proteolysis in muscle has been observed in a number of previous studies (9, 13). Furthermore, Lecker et al. (18) recently showed that there was a disparity between mRNA levels of 14-kDa E2 and E3a and the activities of these enzymes in muscles from diabetic rats. These findings emphasize difficulties in extrapolating changes in levels of mRNA expression with enzyme activity for the ubiquitin-proteasome pathway in skeletal muscle and highlight the need for studies examining the site(s) of regulation of this pathway in vivo.

The mediators of the hypertrophic effect of exercise are still incompletely understood. It is known that nutritional intake has a major influence on the postexercise hypertrophic response. Indeed, exercise leads to an enhanced nutrient-induced increase and decrease in muscle protein synthesis and proteolysis, respectively (23). The postprandial increase in circulating amino acids appears to be largely responsible for the enhanced rate of protein synthesis after exercise (4), whereas both insulin and amino acids have been reported to diminish the postexercise response of muscle protein breakdown (4, 5). In the present study, exercise suppressed insulin concentrations, and there was no significant correlation between insulin levels and the rate of proteolysis or the ubiquitin-proteasome pathway activities in the crude muscle extracts. Therefore,
it is unlikely that insulin was responsible for the suppression of total proteolysis and the ubiquitin-proteasome pathway 24 h postexercise. Further studies are required to explore the potential mechanisms for suppression of proteolysis during recovery from exercise.

**IGF effects.** The reasons for the lack of effect of rhIGF-I on muscle weight and muscle protein turnover in this study are unclear. The IGF-I dosing regimen used in this study was based on the studies of Roy et al. (24) in hypophysectomized hindlimb-suspended rats, where IGF-I treatment combined with exercise reduced the loss of muscle tissue. It is possible that, in animals with normal levels of IGF-I, such as the rats in the present study, supplemental IGF at the dose used is unable to increase the rate of muscle growth or effect changes in muscle protein turnover.

The present results suggest that the ubiquitin-proteasome pathway may be actively suppressed in situations in which there is a net gain of muscle protein such as in the postexercise period. The mechanism(s) for this effect is unclear, but it is unlikely to be simply due to changes in insulin concentrations. These results add to the recent studies implicating the ubiquitin-proteasome pathway in the process of myofibrillar degradation and remodeling during muscle contraction and after exercise (20, 26).

**REFERENCES**


