Elevation of myostatin and FOXOs in prolonged muscular impairment induced by eccentric contractions in rat medial gastrocnemius muscle

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The purpose of this study was to investigate the effects of ECs on protein synthesis and/or protein degradation signaling pathways after contraction-induced muscle injury. We employed our originally developed device with two EC modes—one with an angular velocity of 30°/s (defined as slow) and the other with an angular velocity of 180°/s (defined as fast)—to modulate the intensity of ECs in rat gastrocnemius muscle. Since we previously showed that repeated bouts of slow (30°/s) eccentric exercise cause an increase in muscular size and strength (34), we hypothesized that the protein synthesis signaling pathway is activated by slow ECs. Since high EC velocity causes severe muscle damage, we speculated that the protein degradation signaling pathway is activated by high EC intensity. The immediate increase in the levels of catalytic events was significantly higher in SLOW than in the control group (control; n = 12), slow ECs group (SLOW; 30°/s; n = 12), and control group (control; n = 12). ECs comprised four sets of five forced dorsiflexions combined with electrical stimulation of the plantar flexors. Isometric tetanic torque was measured before and after ECs. Tissue contents of Akt(P) (P, phosphorylated), mammalian target of rapamycin (mTOR)(P), 70-kDa ribosomal protein S6 kinase (P70S6k), P70S6k(P), forkhead transcription factor 1 of the O class (FOXO1), FOXO1(P), FOXO3, FOXO3(P), myostatin, and activin receptor type IIB (ActRIIB) were measured.

Elevation of myostatin and FOXOs in prolonged muscular impairment induced by eccentric contractions in rat medial gastrocnemius muscle. J Appl Physiol 108: 306–313, 2010. First published December 3, 2009; doi:10.1152/japplphysiol.00278.2009.—This study aimed to investigate torque deficit and activation of protein synthesis and/or protein degradation signaling pathways during the early and recovery phase after high- and low-velocity eccentric contractions (ECs). Male Wistar rats (n = 36) were randomly divided into fast angular velocity ECs group (FAST; 180°/s; n = 12), slow ECs group (SLOW; 30°/s; n = 12), and control group (control; n = 12). ECs comprised four sets of five forced dorsiflexions combined with electrical stimulation of the plantar flexors. Isometric tetanic torque was measured before and after ECs. Tissue contents of Akt(P) (P, phosphorylated), mammalian target of rapamycin (mTOR)(P), 70-kDa ribosomal protein S6 kinase (P70S6k), P70S6k(P), forkhead transcription factor 1 of the O class (FOXO1), FOXO1(P), FOXO3, FOXO3(P), myostatin, and activin receptor type IIB (ActRIIB) were measured.

The immediate increase in the levels of catalytic events was significantly higher in SLOW than in the control. The ratio of FOXO1 against total FOXO1, the ratio of FOXO3a against total FOXO3a, and the myostatin protein content on days 2 and 7 were significantly higher in SLOW than in the control. The ratio of FOXO1 against total FOXO1, the ratio of FOXO3a against total FOXO3a, and the myostatin protein content on days 2 and 7 were significantly higher in SLOW than in the control, while that of ActRIIB on day 7 was significantly lower in SLOW than in the other two groups. These results suggest that EC intensity plays a key role in impairment of muscular function and activation of protein synthesis and/or protein degradation signaling pathways.

hypertrophy; recovery; protein metabolism; speed; atrophy-related protein

APPROPRIATE intensity and magnitude of eccentric contractions (ECs) of skeletal muscle are known to cause skeletal muscle hypertrophy (12, 20, 21, 40). On the other hand, it has been shown that excessive ECs damage muscle fibers (4, 10, 13, 14, 27, 33, 41). The velocities and number of muscle contractions, range of motion or muscle length, and amount of work or peak active tension of muscles have been found to contribute to hypertrophy and/or muscle strain injury (7, 9, 19, 29, 45).

Skeletal muscle contractions, including ECs, promote the activation of the insulin-like growth factor 1 (IGF1)-induced Akt (hypertrophy and atrophy) (43, 44). Activated Akt can also phosphorylate FOXOs and inhibit their transcriptional functions (42). In previous studies, the FOXO1 protein and MURF-1 mRNA levels transiently increased within 24 h after exercise (26, 32); the increased mRNA levels soon returned to their initial level. The immediate increase in the levels of catalytic molecules suggests an association between protein synthesis and muscle regeneration. Since torque deficit was found to decrease within 2 days after exercise in the above-mentioned studies, the exercises selected in these studies might have been less effective in inducing catalytic events.

The purpose of this study was to investigate the effects of ECs on protein synthesis and/or protein degradation signaling pathways after contraction-induced muscle injury. We employed our originally developed device with two EC modes—one with an angular velocity of 30°/s (defined as slow) and the other with an angular velocity of 180°/s (defined as fast)—to modulate the intensity of ECs in rat gastrocnemius muscle. Since we previously showed that repeated bouts of slow (30°/s) eccentric exercise cause an increase in muscular size and strength (34), we hypothesized that the protein synthesis signaling pathway is activated by slow ECs. Since high EC velocity causes severe muscle damage, we speculated that the protein degradation signaling pathway is activated by fast ECs. In addition, we measured the tissue content of myostatin and activin receptor type IIB (ActRIIB) for further confirmation of protein synthesis or occurrence of catalytic events. Myostatin, which belongs to the transforming growth factor-β (TGF-β) superfamily, functions as a negative regulator of muscle growth and plays critical roles in controlling muscle mass (hypertrophy and atrophy) (43, 44). Activated Akt is identified as the primary myostatin receptor, binds to myostatin and is activated by the myostatin signaling pathway, thereby blocking muscle growth (44).

MATERIALS AND METHODS

Animals. Male Wistar rats (age, 11 wk; body mass, 344–398 g; n = 36) were randomly assigned to the following three groups: a fast ECs
group (FAST; angular velocity, 180°/s; n = 12), a slow ECs group (SLOW; angular velocity, 30°/s; n = 12), and a control group (control; n = 12). The angular velocities in the FAST and SLOW groups were chosen in accordance with conditions set in previous human studies (8, 9). The rats were each housed in individually ventilated cage (IVC) systems (Tecniplast, Milan, Italy) maintained at 22–24°C with a 12:12-h light-dark cycle. Rats were provided water and food ad libitum. No significant differences were found in weight between the groups (Table 1). The right hindlimbs of all the animals were shaved, and each rat was then anesthetized with isoflurane (aspiration rate, 450 ml/min; concentration, 2.0%). The anesthetized animals were used for inducing ECs and measuring torque. For the control rats, only isometric tetanic torque was measured. At either 2 or 7 days after ECs, six rats in each group were dissected and weighed. This study was approved by the Ethical Committee for Animal Experiments at the Nippon Sport Science University (ID: 008-A01).

**Measurement of isometric tetanic torque.** Isometric tetanic torque was measured as previously reported (34, 41). Maximal isometric plantar flexion torque was measured with a dynamometer at the ankle joint angle of 0° (defined as the angle at which the sole of the foot and the tibial bone are orthogonally positioned). Before the measurement, the right hindlimb was shaved for electrical stimulation. The rats were anesthetized and placed prone on a platform with their knee extended. The triceps surae muscle was stimulated supramaximally (pulse duration, 0.4 ms; frequency, 100 Hz; intensity, ~35 V) with self-adhesive surface electrodes (7.5 mm × 7.5 mm) connected to an electric stimulator and an isolator (SS-104J; Nihon Koden). Measurements were made before ECs and on days 1, 2, 3, 5, and 7 after ECs. 

**ECs of the medial gastrocnemius muscle.** The EC system of the medial gastrocnemius muscle was similar to that observed in our previous study (34). After measurement of the isometric tetanic torque, the FAST and SLOW rats were anesthetized and placed prone on the isokinetic dynamometer. The triceps surae muscle of the right hindlimb was then electrically stimulated. ECs were induced at 30°/s (SLOW) or 180°/s (FAST). The range of forced lengthening contractions was from 0° to 45°. The ECs comprised four sets of five contractions, and the interval between each set was 5 min. The control group was anesthetized only and received no other treatment. After the last measurement of isometric tetanic torque, the muscle specimens were dissected, weighed, immediately frozen in liquid N2, and stored at −80°C until analysis.

**Western blot analysis.** The medial gastrocnemius muscle was macerated in liquid N2 and homogenized in a buffer containing 50 mM Tris·Cl (pH 7.4), 150 mM NaCl, 5 mM EDTA, 0.5% SDS, 1% deoxycholate, 0.1% Triton X-100, 1% Nonidet P-40 (NP-40), 0.05% mercaptoethanol, 10 mg/ml phenylmethylsulfonyl fluoride (PMSF), 0.5 mg/ml leupeptin, 0.2 mg/ml aprotinin, and 1 mM Na3VO4. The homogenate was centrifuged at 15,000 g for 25 min at 4°C. Protein concentrations were determined using a protein concentration determination kit (Protein Assay II; Bio-Rad, Richmond, VA). A 25-μg total protein extract from each sample was mixed with sample buffer, boiled, loaded on the same SDS-polyacrylamide gel (12.5%), and electrophoresed at 20 mA. The samples were electrophoretically separated at 180 mA for 90 min, and separated proteins were then transferred onto polyvinylidene difluoride (PVDF) membranes (ATTO, Tokyo, Japan). The membranes were blocked for 1 h with PBS containing 5% skimmed milk and then incubated overnight at 4°C with the following primary antibodies (dilution, 1:1,000): monoclonal anti-Akt (P) (no. 4051; Cell Signaling Technology, Danvers, MA), polyclonal anti-mTOR (P) (no. 2971; Cell Signaling Technology), polyclonal anti-PI3K (P) (no. 9202; Cell Signaling Technology), monoclonal anti-FOXO1 (P) (no. 9461; Cell Signaling Technology), monoclonal anti-FOXO3 (no. 2497; Cell Signaling Technology), polyclonal anti-FOXO3 (P) (no. 9466; Cell Signaling Technology), polyclonal anti-myostatin (AB3239; Millipore, Billerica, MA), polyclonal anti-ActRIIB (no. sc25453; Santa Cruz Biotechnology, Santa Cruz, CA), and α-tubulin loading control (no. ab7291, Abcam, Cambridge, MA). The membranes were then washed two times and incubated with the secondary antibody at room temperature. Horseradish peroxidase (HRP)-conjugated goat anti-rat immunoglobulin G (IgG) or anti-rabbit IgG (dilution, 1:1,000) was used as the secondary antibody. Chemiluminescent reagents were used for detecting the secondary antibody (SuperSignal West Dura; Pierce Protein Research Products, Rockford, IL). Chemiluminescent signals were detected using a chemiluminescence detector (AE6961; ATTO) and quantified using a personal computer with image analysis software (CS Analyzer; ATTO). The band densities were expressed relative to those obtained for the control.

**Statistics.** All values are expressed as means ± SD. One-way ANOVA followed by Bonferroni test was used to compare the body mass, muscle wet weight, and mechanical parameters except time course data. Two-way ANOVA followed by Bonferroni test was used to test the time course changes in isometric tetanic torque (time × groups) and protein analysis. Significance level was set at P < 0.05.

**RESULTS**

**Body mass of rats and wet weight of triceps surae muscle.** No significant changes were observed in both the body mass of rats and the wet weights of the medial gastrocnemius, lateral gastrocnemius, plantaris, and soleus muscles between the groups (Table 1).

**Changes in produced ankle joint torque.** Table 2 shows isometric tetanic torque and isometric tetanic torque per body mass in all groups before treatment; no significant difference was observed among any of the groups. We calculated torque deficit from before ECs to immediately after the ECs (Table 2). Statistical analysis revealed no significant difference in the torque deficit between the FAST and SLOW groups. We further calculated the maximum torque produced during 20 ECs (peak ankle joint torque). The peak ankle joint torque was significantly higher in FAST than in SLOW (P < 0.05; Table 2).

**Table 1. Body mass and muscle wet mass of triceps surae muscle**

<table>
<thead>
<tr>
<th></th>
<th>Body mass, g</th>
<th>Medial gastrocnemius, mg</th>
<th>Lateral gastrocnemius, mg</th>
<th>Plantaris, mg</th>
<th>Soleus, mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 2</td>
<td>Fast</td>
<td>Slow</td>
<td>Fast</td>
<td>Slow</td>
</tr>
<tr>
<td></td>
<td></td>
<td>364.5 ± 20.70</td>
<td>370.3 ± 16.23</td>
<td>815.0 ± 45.60</td>
<td>825.8 ± 59.92</td>
</tr>
<tr>
<td></td>
<td></td>
<td>362.4 ± 20.70</td>
<td>370.3 ± 16.23</td>
<td>815.2 ± 47.82</td>
<td>815.2 ± 47.82</td>
</tr>
<tr>
<td></td>
<td>Day 7</td>
<td>Fast</td>
<td>Slow</td>
<td>Fast</td>
<td>Slow</td>
</tr>
<tr>
<td></td>
<td></td>
<td>367.2 ± 10.43</td>
<td>368.5 ± 10.84</td>
<td>818.4 ± 57.09</td>
<td>849.3 ± 52.37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>367.2 ± 10.43</td>
<td>368.5 ± 10.84</td>
<td>810.7 ± 35.70</td>
<td>810.7 ± 35.70</td>
</tr>
</tbody>
</table>

Values are means ± SDs. Fast, fast eccentric contractions group (angular velocity 180°/s); Slow, slow eccentric contractions group (angular velocity 30°/s). No significant differences.
Table 2. Mechanical parameters in the rat ankle joint

<table>
<thead>
<tr>
<th></th>
<th>Fast</th>
<th>Slow</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isometric tetanic torque, mN·m</td>
<td>113 ± 10.5</td>
<td>116 ± 13.3</td>
<td>107 ± 11.5</td>
</tr>
<tr>
<td>Isometric tetanic torque per body mass, mN·m/g</td>
<td>0.309 ± 0.0319</td>
<td>0.319 ± 0.0348</td>
<td>0.313 ± 0.0321</td>
</tr>
<tr>
<td>Torque deficit, %</td>
<td>82.6 ± 13.3</td>
<td>81.1 ± 15.6</td>
<td></td>
</tr>
<tr>
<td>Peak ankle joint torque, mN·m</td>
<td>256 ± 30.1</td>
<td>207 ± 38.8*</td>
<td></td>
</tr>
<tr>
<td>Peak ankle joint torque per body mass, mN·m/g</td>
<td>0.698 ± 0.0860</td>
<td>0.571 ± 0.102*</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SDs. *P < 0.05, Slow vs. Fast.

2). On the other hand, the time course of isometric tetanic torque in FAST significantly decreased compared with that in SLOW (days 1, 3, and 5, P < 0.05; day 2, P < 0.01; Fig. 1). In addition, a significant torque deficit in FAST was observed from 1 to 5 days after the ECs compared with the pretreatment value (day 3, P < 0.05; days 1, 2, and 5, P < 0.01; Fig. 1); however, such a deficit was not observed in SLOW.

Protein content. Two or seven days after the ECs, the medial gastrocnemius muscle was removed for Western blot analysis. The tissue content of Akt(P) in SLOW, but not in FAST, was significantly higher than that in the control (P < 0.05; Fig. 2A), while that of Akt was no significant difference among the groups (Fig. 2B). In addition, we further calculated the ratio of Akt(P) to total protein (Fig. 2C). The ratio of Akt(P) showed a significant increase in SLOW compared with the control group. There was no significant difference in the tissue content of mTOR(P), mTOR, and the ratio of mTOR (P) to total protein between the groups (Fig. 3). P70S6k(P) on days 2 and 7 in SLOW were significantly higher than that in the control (day 2, P < 0.05; day 7, P < 0.001; Fig. 4A), and P70S6k(P) on day 7 in SLOW was also significantly higher than that in FAST on day 7 and SLOW on day 2 (P < 0.05). The tissue content of P70S6k on day 2 was not significantly different among all groups, while that on day 7 in SLOW showed significant increase compared with that in FAST, control, and SLOW on day 2 (compared with FAST, P < 0.05; compared with SLOW and control, P < 0.01; Fig. 4B). The ratio of P70S6k(P) to total protein of SLOW on day 2 was also significantly higher than that of FAST and the control (compared with FAST, P < 0.01; compared with control, P < 0.05), and the ratio on day 7 was significantly higher than that of the control (P < 0.05; Fig. 4C).

FOXO1 on both days 2 and 7 showed significantly enhanced expression in FAST than in the other two period-matched groups (compared with SLOW on day 7, P < 0.01; compared with SLOW on day 2 and period-matched controls, P < 0.001; Fig. 5A). Similarly, the tissue content of FOXO3 on both days 2 and 7 showed significant increase in FAST compared with the other two groups (day 7, P < 0.01; day 2, P < 0.001; Fig. 6A).
On the other hand, no significant difference was observed in the muscle content of FOXO1(P) among all the groups (Fig. 5B). The tissue content of FOXO3(P) significantly decreased in FAST and SLOW compared with the control (compared with SLOW on day 2, \( P < 0.05 \); day 7, \( P < 0.01 \); compared with FAST on day 2, \( P < 0.001 \); Fig. 6B). The results of the ratio of FOXO1 and FOXO3 to total protein on days 2 and 7 were similar to the content of FOXO1 and FOXO3, respectively (Figs. 5C and 6C).

The muscle content of myostatin on days 2 and 7 was significantly higher in FAST than in the other two period-matched groups (\( P < 0.001 \); Fig. 7A), while that of ActRIIB was significantly lower in SLOW than in the other two groups (compared with FAST, \( P < 0.01 \); compared with control, \( P < 0.05 \); Fig. 7B).
Fig. 5. Muscle contents of forkhead transcription factor 1 of the O class (FOXO1) (A), phosphorylated FOXO1 [FOXO1(P)] (B), and the ratio of [FOXO1 – FOXO1(P)] to FOXO1 (C) as determined by Western blotting. Black bars, FAST; gray bars, SLOW; open bars, control. The muscle content of FOXO1 (A) on days 2 and 7 was significantly higher in FAST than in the other two groups, while that of FOXO1(P) did not differ significantly between the groups (B). The ratios of FOXO1 to total protein in FAST on days 2 and 7 were significantly higher than that in the control (C). Values are expressed as means ± SDs. *P < 0.05, **P < 0.01, ***P < 0.001.

Fig. 6. Muscle content of FOXO3 (A), phosphorylated FOXO3 [FOXO3(P)] (B), and the ratio of [FOXO3 – FOXO3(P)] to FOXO3 (C) as determined by Western blotting. Black bars, FAST; gray bars, SLOW; open bars, control. The muscle content of FOXO3 (A) on days 2 and 7 was significantly higher in FAST than in the other two groups, while that of FOXO3(P) in FAST and SLOW significantly decreased compared with the control (B). The ratios of FOXO3 to total protein in FAST on days 2 and 7 were significantly higher than that of the other two groups (C). In addition, the ratio of FOXO3 to total protein of SLOW on day 7 was significantly lower than that of the control. Values are expressed as means ± SDs. *P < 0.05, **P < 0.01, ***P < 0.001.
myostatin

\[ \text{myostatin} \]

\[ \alpha\text{-tubulin} \]

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**DISCUSSION**

In the present study, we attempted to evaluate the effects of ECs on the protein synthesis and/or protein degradation signaling pathways after muscle injury. We confirmed that isometric tetanic torque showed a prolonged decrease in FAST but not in SLOW. This result was supported by the fact that peak EC torque in FAST was significantly higher than that in SLOW, suggesting that the ECs had a greater impact on the medial gastrocnemius muscle of FAST rats. Activation of the protein synthesis signaling pathways [Akt(P) and P70S6k(P)] was seen only in SLOW. In contrast, the protein degradation signaling pathways (FOXO1 and FOXO3) were significantly activated only in FAST. We also found that the myostatin level in the muscle was significantly elevated in FAST. Since the levels of these hypertrophy/atrophy-related proteins were already elevated by day 2 after either slow or fast ECs and continued until day 7, we suggest that the response of muscle adaptations for each contraction velocity starts relatively early.

In this study, we observed a slower recovery of isometric tetanic torque in FAST than in SLOW rats (Fig. 1). This finding is consistent with that of Chapman et al. in which fast-velocity ECs were shown to induce greater muscle damage in human elbow flexors than slow-velocity ECs (8, 9). Chapman et al. confirmed that the work performed during fast exercise was greater than that performed during slow exercise (8, 9). In addition, we also confirmed that the peak ankle joint torque in FAST was significantly larger than that in SLOW (Table 2). Since previous animal studies have indicated that the extent of EC-induced injury is associated with peak active tension or amount of work (7, 19, 29, 45), we believe that these parameters are major factors responsible for the severity of contraction-induced muscle damage.

We should pay careful attention for 1) the velocity-torque relationship in isokinetic eccentric contractions and 2) muscle fiber recruitment. With regard to the velocity-torque relationship, it has been suggested that cross-bridge detachment and/or a muscle’s elastic elements provide greater force in ECs with higher velocity, but these speculations are controversial (25). Although the tension development during EC increases from slow to fast angular velocity in vitro, several reports have shown that the tension development remained similar or slightly decreased under in vivo voluntary contractions (25). In this study, we used anesthetized animals, which may have resulted in our observation of higher torque with faster angular velocity contractions. Next, we would like to consider the muscle fiber recruitment. There is a possibility that the slow protocol might activate a lower percentage of the medial gastrocnemius than did the fast protocol because of the different motor unit populations. Since the torques pre-EC and immediately after ECs were almost the same before and after ECs (Table 2). We also confirmed that the obtained torque in this study is almost maximal since we could not obtain higher force with higher voltage and frequency. Even if we have these suggestive results, we also think that it is hard to exclude the possibility of differences in the activated motor unit populations in these two protocols.

One of the important signal transduction pathways involved in muscle fiber hypertrophy and protein synthesis after exercise is the IGF-1-induced Akt/mTOR pathway (5, 37). This pathway has also been reported to play a role in muscle regeneration (18, 30, 31). In this study, we also analyzed the muscle contents of Akt(P) and mTOR(P) and observed a significant increase in the tissue content of Akt(P) only in SLOW (Figs. 3A and 4A), while the increase in the muscle content of mTOR(P) in SLOW was not significant. The same tendency of [Akt(P) upregulation without mTOR(P) activation] has also been reported by other researchers (6, 11). We previously reported that 10 sessions (20 days) of slow ECs caused an increase in muscular hypertrophy (34), and we believe that a similar effect of ECs was also observed in one bout of muscular exercise in this study. Furthermore, we found evidence in this study that Akt(P) may enhance protein synthesis independent of mTOR, as discussed by Creer et al. (11). In addition,
we observed that P70S6k(P) in SLOW was increased on days 2 and 7, which is consistent with a previous study that used a similar rat electrical stimulation model (3). These results suggest that the Akt/mTOR/P70S6k pathway was activated only in SLOW.

On the other hand, the muscle content of FOXO1 and FOXO3 significantly increased in FAST compared with that in the other two groups on days 2 and 7 (Figs. 5A and 6A). It has been shown that myotube atrophy is induced by Akt-directed dephosphorylation of FOXO1 and FOXO3 (39); dephosphorylated FOXO1 and FOXO3 relocate in the nucleus and activate the genes associated with cell metabolism (24). Our data showing reduction in the tissue content of FOXO3(P) and elevation of the ratios of dephosphorylated FOXO1 and FOXO3 against the total in FAST also support the dephosphorylation of FOXO proteins (Figs. 5C and 6C). In the SLOW rats, the same atrophic tendency of FOXO1 and FOXO3 was not observed. Pardo et al. (36) have shown that mechanical stress on the diaphragm muscle causes decreases in FOXO1 and FOXO3 DNA binding. We believe that the protein degradation pathway includes selectively activated FOXOs in the FAST contraction group in accordance with the mechanism described by Pardo et al. Further, we believe that this is the first report showing that ECs cause an increase in the muscle content of atrophy-related molecules. However, the muscle mass observations must be treated carefully. There was no significant change in muscle mass among the three groups after either day 2 or day 7 (Table 1). Since we previously reported that repeated bouts of SLOW ECs caused an increase in muscle mass (34), we think that the one bout of FAST ECs might not have been enough to elicit muscle mass change. We also believe that repeated FAST ECs would result in reduction of muscle mass.

The present study showed changes in the muscle contents of myostatin and ActRIIB, which were found to be associated with the extent of muscular damage. The muscle content of myostatin significantly increased in FAST while that of ActRIIB significantly decreased in SLOW (Fig. 7, A and B). Myostatin is known to be produced by the muscle fibers and to inhibit muscle hypertrophy, and ActRIIB is the primary ligand-binding receptor for myostatin (43, 44). Thus, our results suggest that slow ECs induce muscular hypertrophy and that fast ECs induce an atrophic response. With regard to the high-angular velocity ECs group (FAST), it has been reported that FOXOs regulate the expression of myostatin in C2C12 myotubes (1) and that the Akt-mTOR signaling pathway is inhibited in myostatin-overexpressing rats (2). Considering the results of these previous studies, we propose that FOXOs, Akt, P70S6k, and myostatin are closely associated with high-velocity ECs.

In conclusion, fast ECs cause a decrease in muscular strength and activation of the protein degradation signaling pathways. In contrast, the protein synthesis signaling pathways are activated after slow ECs. We conclude that the velocities of ECs are related to the severity of muscle damage and that prolonged torque deficit is associated with activation of the protein degradation process. Furthermore, we think that there should be a pivotal “threshold” EC strength for resultant protein metabolism.

GRANTS
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DISCLOSURES
No conflicts of interest are declared by the authors.

REFERENCES


