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mTOR signaling response to resistance exercise is altered by chronic resistance training and detraining in skeletal muscle

Riki Ogasawara,1,2,3 Koji Kobayashi,4 Arata Tsutaki,4 Kihyuk Lee,4 Takashi Abe,5 Satoshi Fujita,2 Koichi Nakazato,4 and Naokata Ishii3

1The Research Organization of Science and Technology, Ritsumeikan University, Kusatsu, Shiga, Japan; 2Faculty of Sport and Health Science, Ritsumeikan University, Kusatsu, Shiga, Japan; 3Department of Human and Engineered Environmental Studies, University of Tokyo, Tokyo, Japan; 4Graduate School of Health and Sport Science, Nippon Sport Science University, Tokyo, Japan; and 5Department of Health, Exercise Science and Recreation Management, University of Mississippi, Oxford, Mississippi

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Resistance training is known to be a strong stimulus for inducing muscle hypertrophy, but only a limited literature exists that investigates the changes in phosphorylation status of signaling proteins in response to resistance exercise in animal models. In humans, resistance training-induced muscle anabolism and subsequent hypertrophy occur most rapidly during the early phases of training, becoming progressively slower with time (33, 40). On the other hand, following a detraining period, muscle adaptation responses may return to their initial levels, and the effects of retraining on muscle growth are similar to those observed during the initial phase of resistance training (33, 37). However, the mechanisms underlying such changes in the sensitivity of muscles to training stimuli are unclear.

The mammalian target of rapamycin (mTOR) signaling pathway is recognized as a key regulator of translation initiation and has been shown to be important in muscle protein synthesis and muscle hypertrophy (2, 3, 13, 31, 36). Resistance exercise is known to be one of the stimuli able to activate mTOR signaling activity (6, 25). A recent study has clearly demonstrated that mechanical load-induced muscle hypertrophy is fully dependent on mTOR signaling within the skeletal muscle (17). However, resistance exercise also activates extracellular signal-regulated kinase (ERK) 1/2 and its downstream substrate p90 ribosomal S6 kinase (p90RSK), which are both involved in a signaling pathway independent of mTOR activity (6, 22, 29, 42). The ERK signaling pathway is also considered to be a regulator of translation initiation and protein synthesis (12, 13, 39). Therefore, studies investigating mTOR and ERK signaling pathway activities should give insight into the mechanisms underlying resistance exercise-induced anabolic responses.

The purpose of the present study was to examine whether the activities (based on phosphorylation of signaling molecules) of mTOR and ERK signaling pathways are altered with training and detraining. To elucidate this adaptive response, a training protocol was designed to induce maximal muscle contraction. We hypothesized that the activation of the mTOR signaling pathway in response to muscle contraction would be attenuated after chronic training, even if muscle is contracted maximally, but would recover after short-term detraining.

METHODS

Animals. Twenty male Sprague-Dawley rats, 10 wk of age (356.1 ± 4.4 g), were obtained from CLEA Japan (Tokyo, Japan). All animals were housed individually in an environment maintained at 22–24°C with a 12-h light-dark cycle and were allowed food and water ad

THE DYNAMIC AND PLASTIC NATURE of skeletal muscle allows it to respond adaptively to changes in activity. Resistance exercise accelerates muscle anabolism, and muscle proteins gradually accumulate with repetition of this type of stimulus. Skeletal muscle also has the ability to maintain cell homeostasis and thus to respond adaptively to muscle contraction stimuli to minimize cellular disturbances during subsequent stimulation. This can be associated with the reduced anabolic response and rate of growth of skeletal muscle observed with repetition of the muscle contraction stimuli.
Rats were randomly assigned to one of four groups: 1 exercise bout (1B), 12 exercise bouts (12B), 18 exercise bouts (18B), and detraining (DT). In the DT group, rats were detrained 12 days after completion of 12 exercise sessions and then completed 1 exercise session. This study was approved by the Ethics Committee for Animal Experiments at Nippon Sport Science University.

Muscle activation. Under isoflurane anesthesia, the hair was shaved off the right lower leg of each rat, and the shaved legs were cleaned with alcohol wipes. Rats were then positioned with their right foot on the footplate (the ankle joint angle was positioned at 90°) in the prone posture. The triceps surae muscle was stimulated percutaneously with electrodes (Vitrode V, Ag/AgCl; Nihon Kohden, Tokyo, Japan), which were cut to 10 mm × 5 mm and connected to an electric stimulator and an isolator (SS-104; Nihon Kohden, Tokyo, Japan).

Resistance training protocol. Rats were acclimatized for 1 wk, and the right gastrocnemius muscle was then isometrically trained every other day (i.e., Monday, Wednesday, Friday, Tuesday, Thursday, etc.). The left gastrocnemius muscle served as an internal control. For all exercise sessions, the gastrocnemius muscle was trained by stimulating five contractions, with a 5-s interval between contractions, per set for five sets, with 5-min rest intervals. The voltage (~30 V) and stimulation frequency (60 Hz) were adjusted to produce maximal isometric tension. Before every exercise session, peak twitch torque was measured. Torque signals were collected continuously at a sampling rate of 1,024 Hz using a 16-bit analog-to-digital converter (PowerLab/16SP; AD Instruments) and analyzed using Power Lab Chart 5 software (AD Instruments). Twenty-four hours after the last exercise session, rats were anesthetized and exsanguinated. Target tissues were removed immediately after death. After the mass of each tissue was measured, tissues were rapidly frozen in liquid N2 and stored at −80°C until use.

Western blotting analysis. Muscle samples were homogenized with a polytron homogenizer in a homogenization buffer containing 100 mM Tris·HCl, pH 7.8, 1% NP40, 0.1% SDS, 0.1% sodium deoxycholate, 1 mM EDTA, 150 mM NaCl, and protease and phosphatase inhibitor cocktail (Thermo Fisher Scientific). Homogenates were centrifuged at 15,000 g for 15 min at 4°C. The supernatant was removed, and the protein concentration for each sample was determined using a protein concentration determination kit (Protein Assay II; Bio-Rad, Hercules, CA). The samples were diluted in ×3 sample buffer (1.0% vol/vol β-mercaptoethanol [β-ME], 4.0% wt/vol SDS, 0.16 M Tris·HCl, pH 6.8, 43% vol/vol glycerol, and 0.2% wt/vol bromphenol blue) and boiled at 85°C for 5 min. Using 10–15% SDS-polyacrylamide gels, 30 μg of protein were separated by electrophoresis and subsequently transferred to polyvinylidene difluoride (PVDF) membranes. After transfer, the membranes were washed in Tris-buffered saline containing 0.1% Tween-20 (TBST), and membranes were then blocked with 3% BSA in TBST for 1 h at room temperature. After blocking, membranes were washed and incubated overnight at 4°C with primary antibodies, including phospho-p90RSK (Thr573, catalog no. 9345), total p90RSK (catalog no. 9355), phospho-p70S6 kinase (Thr389, catalog no. 9205), total p70S6 kinase (catalog no. 9202), phospho-S6 ribosomal protein (Ser235/236, catal- no. 2211), phospho-S6 ribosomal protein (Ser240/244, catalog no. 2215), total S6 ribosomal protein (catalog no. 2217), phospho-4E-BP1 (Thr37/46, catalog no. 9459), and total 4E-BP1 (catalog no. 9452) (Cell Signaling Technology, Danvers, MA). Membranes were then washed again in TBST and incubated overnight with appropriate secondary antibodies at 4°C. Chemiluminescent reagents (SuperSignal West Dura; Pierce, Rockford, IL) were used to facilitate the detection of protein bands. Images were scanned using a chemiluminescence detector (AE6961; ATTO, Tokyo, Japan). After the scan, the membranes were stained with Coomassie Blue to verify equal loading in all lanes. Band intensities were quantified using a PC application (CS Analyzer; ATTO). Samples from all eight experimental conditions were run on the same gel, which allowed for the direct comparison between conditions.

Statistical analysis. Changes in protein expression and muscle wet weight were compared by two-way ANOVA [training status (group) × stimulation]. Changes in mechanical parameters were examined by one-way ANOVA. Post hoc analyses were performed using t-tests with the Benjamini and Hochberg false discovery rate correction for multiple comparisons. All values were expressed as means ± SE. Significance was accepted at P < 0.05.

RESULTS

Animal characteristics. Rat characteristics are presented in Table 1. Relative to the control muscle, neither muscle wet weight nor its value relative to body weight in the exercised muscle changed after a single bout of training. Muscle wet weight and its value relative to body weight were increased above control muscle after 12 and 18 training sessions by 8.6% (P < 0.01) and 10.7% (P < 0.01), respectively. After 12 training sessions followed by 12 days of detraining, both muscle wet weight and its relative value to body weight remained equivalent to the posttraining level (P = 0.03 vs. control muscle).

Mechanical parameters. Table 2 shows changes in twitch and tetanic parameters. The peak twitch torque (Pt) and peak tetanic rate of force development (RFD) were increased with training and were maintained at a similar level during the subsequent detraining period. However, the Pt relative to the gastrocnemius muscle wet weight and the tetanic RFD relative to Pt were not changed during training and detraining periods. Similarly, the peak twitch torque (Pt) was increased with training, but the Pt relative to the gastrocnemius muscle wet weight was not changed with training. On the other hand,
compared with the initial session, the twitch RFD was greater at both the 18th training session and the 1st training session after the 12-day detraining period, and the twitch RFD relative to the Pt was greater at the 1st training session after the 12-day detraining period than at the 18th training session.

*p70S6 kinase. Phosphorylation at Thr389 of p70S6K was elevated above control muscle (P < 0.05) 24 h after the first training session in the 1B group (Figs. 1A and 5). However, repeated bouts of exercise blunted the level of phosphorylation of p70S6K in the 12B or 18B groups, although this level was still higher than in the control muscle (P < 0.05). The p70 phosphorylation in response to a bout of exercise was restored after 12 days of detraining in the DT group. Chronic training increased total protein of p70S6K in the 12B or 18B groups, whereas a short detraining period tended to decrease the total protein of p70S6K (Figs. 1B and 5).

4E-BP1. No significant change in phosphorylation of 4E-BP1 at Thr37/46 was observed throughout the training and detraining periods (P > 0.10; Figs. 2A and 5). On the other hand, training increased total 4E-BP1 protein in the 18B group (Figs. 2B and 5).

*p90RSK. Acute muscle contraction increased phosphorylation of p90RSK at Thr573 in the 1B group, and a similar Table 2. Mechanical parameters

<table>
<thead>
<tr>
<th></th>
<th>1 Bout</th>
<th>12 Bouts</th>
<th>18 Bouts</th>
<th>DT</th>
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<tbody>
<tr>
<td>Po, mN·m</td>
<td>209.2 ± 9.5</td>
<td>265.2 ± 6.6†</td>
<td>279.0 ± 6.5†</td>
<td>293.0 ± 4.0†</td>
</tr>
<tr>
<td>Po/GST WW, mN·m/g</td>
<td>106.3 ± 4.2</td>
<td>103.8 ± 1.0</td>
<td>108.4 ± 2.5</td>
<td>106.3 ± 2.7</td>
</tr>
<tr>
<td>Pt, mN</td>
<td>90.0 ± 6.3</td>
<td>100.9 ± 4.2</td>
<td>108.0 ± 3.5†</td>
<td>107.0 ± 3.5</td>
</tr>
<tr>
<td>Pt/GST WW, mN·m/g</td>
<td>45.8 ± 3.2</td>
<td>39.5 ± 1.3</td>
<td>42.0 ± 1.8</td>
<td>38.8 ± 1.3</td>
</tr>
<tr>
<td>Tetanic RFD, N·m/s</td>
<td>3.79 ± 0.32</td>
<td>5.01 ± 0.20†</td>
<td>4.96 ± 0.20†</td>
<td>4.99 ± 0.23†</td>
</tr>
<tr>
<td>Tetanic RFD/Po, %Po/s</td>
<td>1,879.9 ± 169.8</td>
<td>1,894.80 ± 84.0</td>
<td>1,778.8 ± 54.5</td>
<td>1,700.1 ± 67.3</td>
</tr>
<tr>
<td>Twitch RFD, N·m/s</td>
<td>3.62 ± 0.28</td>
<td>4.03 ± 0.17</td>
<td>4.36 ± 0.15†</td>
<td>4.40 ± 0.17†</td>
</tr>
<tr>
<td>Twitch RFD/Pt, %Pt/s</td>
<td>4,096.6 ± 18.7</td>
<td>4,062.0 ± 30.0</td>
<td>4,040.6 ± 17.9</td>
<td>4,139.4 ± 19.6†</td>
</tr>
</tbody>
</table>

Values are means ± SE. Po, peak tetanic torque; Pt, peak twitch torque; RFD, peak rate of force development. *Significant difference vs. 18 bouts (P < 0.05). †Significant difference vs. 1 bouts (P < 0.05).
increase was observed throughout the training and detraining period in the 12B, 18B, and DT groups (all $P \leq 0.05$; Figs. 3A and 5). Chronic training increased total protein of p90RSK in the 12B or 18B groups ($P \leq 0.05$; Figs. 3B and 5). Total protein of p90RSK was still elevated above control muscle but tended to decrease after 12 days of detraining period (Figs. 3B and 5).

**S6 ribosomal protein.** Acute exercise increased ($P \leq 0.05$) rpS6 phosphorylation status at both Ser235/236 (Figs. 4A and 5) and Ser240/244 (Figs. 4B and 5) 24 h after the initial training session in the 1B group. However, repeated bouts of exercise blunted the level of phosphorylation of rpS6 at Ser235/236 in the 12B or 18B groups, although this level was still higher than the control muscle ($P \leq 0.05$), whereas no significant elevation in phosphorylation of rpS6 at Ser240/244 was observed after chronic training in both the 12B and 18B groups. As in p70S6K, the phosphorylation response of rpS6 was restored following detraining in the DT group ($P = 0.02$ vs. control muscle). Chronic training increased total protein of p90RSK in the 12B or 18B groups, whereas a short detraining period tended to decrease the total protein of p90RSK (Figs. 4C and 5).

**DISCUSSION**

Chronic muscle contraction induces a variety of metabolic and morphological adaptations in skeletal muscle to maintain homeostasis and minimize cellular disturbances during subsequent training sessions. Chronic adaptations are the result of the cumulative effects of repeated bouts of exercise, and certain molecular and cellular responses lead to specific adaptations. Therefore, acute exercise-induced molecular responses are likely to be effective predictors of training outcomes. However, it has been relatively unknown whether these responses were altered with chronic muscle contraction. In the present study, we investigated the effects of chronic muscle contraction (resistance training) and subsequent cessation of training (i.e., detraining) on muscle anabolic signaling activities. Our main finding was that, although repeated bouts of
exercise blunted the phosphorylation of the mTOR downstream target p70S6K and rpS6, short-term detraining could lead to a recovery of these. However, in our experiments, the phosphorylation level of the signaling protein 4E-BP1, downstream of mTOR, was not significantly altered with training and detraining.

Our rat isometric training model increased muscle mass (wet weight) by 8.6% after 12 training sessions and by 10.7% after 18 training sessions. The extent of muscle hypertrophy in this study was comparable to those in previous studies using direct nerve stimulation (1, 20). Some signaling proteins (i.e., p70S6K and p90RSK) are known to be phosphorylated immediately, and their peak activation state is often observed ~3 h after resistance exercise. For example, previous studies have reported that the peak activation or phosphorylation state of p70S6K is observed ~3 h after exercise (27, 32, 34). We investigated the phosphorylation state of multiple signaling proteins at 24 h after the initial training session. Therefore, we may have missed the peak activation of some or all proteins. However, endurance-type muscle contraction, which does not result in muscle hypertrophy, is also known to increase phosphorylation of p70S6K during the early phase of the recovery period but returns to basal level by 6 h after contraction (30).

Similarly, the rat squat training model has also been used as an animal resistance exercise model, which results in increased anabolic signaling activity (4, 11, 25). However, a previous study using this model reported that p70S6K phosphorylation was elevated for up to 12 h after exercise but returned to preexercise levels by 24 h after exercise (7, 11, 21), and it has not been reported to alter muscle weight as a result of repeated bouts of exercise, probably because insufficient exercise load or volume was added to muscle (14, 24). In contrast, certain signaling proteins show relatively prolonged changes in their activated or elevated phosphorylation state during a recovery period (27, 32, 34), and in agreement with these data, we observed elevated phosphorylation of p70S6K, p90RSK, and rpS6 24 h after the initial training session. Similarly, studies using direct electrical nerve stimulation have reported elevated phosphorylation of these proteins for more than 24 h after exercise (2, 18, 19). Therefore, acute resistance exercise-induced activation of anabolic signaling pathways, especially later events (i.e., 24 h after exercise) in the phosphorylation cascade, may be important for determining the net anabolic response and may be responsible for muscle hypertrophy caused by repeated bouts of resistance exercise.

Acute contraction-induced kinase phosphorylation in skeletal muscle has been shown to be attenuated after chronic, low-frequency, electrical stimulation-induced contractile activity, which leads to endurance training-like skeletal muscle adaptation (26). However, relatively little is known about changes in the activation of the mTOR signaling pathway with chronic, high-frequency, electrical stimulation-induced maximal contractile activity. In the present study, we showed attenuated phosphorylation of p70S6K and rpS6 with chronic resistance exercise-like electrical stimulation inducing a maximal isometric contraction. Although few studies examined change in phosphorylation status with training in animal study, a previous study in humans examined the anabolic signaling response to a bout of resistance exercise before and after 10 wk of resistance training (41). This study demonstrated that the duration of elevated Akt, p70S6K, and GSK3-β phosphorylation was reduced in trained participants compared with untrained participants, and rpS6 phosphorylation, which was elevated in the untrained state, was not increased after the 10-wk training period (41). Furthermore, another human study reported that the phosphorylation of p70S6K and rpS6 did not increase after a session of resistance exercise in highly resistance-trained subjects (power lifters), whereas increases in these phosphoproteins were observed in untrained subjects (8). These results suggest that the responses of mTOR signaling molecules to resistance exercise may be altered with chronic resistance training, which agree with our current results in animal model. On the other hand, in the present study, attenuated phosphorylation responses of p70S6K and rpS6 were restored to the initial postexercise levels after 12 days of detraining, and this recovery occurred without significant muscle atrophy, suggesting that attenuated specific anabolic responses can be recovered after a short-term detraining period without morphological changes to the muscle.

4E-BP1 as well as p70S6K are known to be key downstream effectors of mTOR. Multisite phosphorylation of the translational repressor 4E-BP1 results in its dissociation from eIF4E, thereby allowing eIF4E to assemble with eIF4G, facilitating the recruitment of other translation initiation factors to form the eIF4F complex and initiate cap-dependent translation (16). Previous studies showed elevated 4E-BP1 phosphorylation after resistance exercise (4, 38). However, changes in phosphorylation of 4E-BP1 and p70S6K in response to a bout of exercise do not necessarily correspond, and some previous studies have failed to find elevated phosphorylation of 4E-BP1 (9, 10, 23, 29). We also did not detect an elevated rate of 4E-BP1 phosphorylation 24 h after exercise throughout the training and detraining periods, suggesting differential regulation of 4E-BP1 and p70S6K. Future work is needed to clarify the difference in regulation of the phosphorylation status of 4E-BP1 and p70S6K.

The ERK1/2 signaling pathway has the ability to regulate proteins involved in the initiation and elongation stages of mRNA translation in an mTOR-dependent and -independent manner (28, 35, 43, 44). Recent studies indicated that ERK signaling is involved in later phase anabolic responses after resistance exercise (5, 6, 15). In the present study, we also observed that phosphorylation of p90RSK, a downstream target of ERK1/2, was increased during the late-phase recovery period after initial resistance exercise, as with p70S6K. However, different from p70S6K, increase in p90RSK phosphorylation in response to muscle contraction was not altered throughout the training and detraining period, suggesting that factors other than ERK signaling are mainly responsible for changes in mTOR activation by chronic muscle contraction and detraining.

The present study showed that the specific attenuation of protein kinase phosphorylation in the skeletal muscle with chronic resistance training was recovered after a short detraining period without muscle atrophy. These results suggest that specific signaling may become less sensitive to exercise stimulus even in muscle that is contracted maximally and that some signaling proteins in the muscle can become resensitized after a short detraining or nontraining period without a loss of muscle mass. Therefore, short-term detraining may be an effective and alternative intervention to maintain muscle re-
sponsiveness or adaptation during the late phases of resistance training.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: R.O., K.K., A.T., K.L., T.A., K.N., and N.I. conceived and designed the experiments; R.O., T.A., S.F., K.N., and N.I. performed the experiments; R.O., T.A., S.F., K.N., and N.I. analyzed the data; R.O., T.A., S.F., K.N., and N.I. interpreted the results of the experiments; R.O. drafted the manuscript; R.O., T.A., S.F., K.N., and N.I. edited and revised the manuscript; R.O., K.K., A.T., T.A., S.F., K.N., and N.I. approved final version of manuscript.

REFERENCES


