Moderate-Intensity Strength Exercise to Exhaustion Results in More Pronounced Signaling Changes in Skeletal Muscles of Strength-Trained Compared With Untrained Individuals

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Abstract

Lysenko, EA, Popov, DV, Vepkhvadze, TF, Sharova, AP, and Vinogradova, OL. Moderate-intensity strength exercise to exhaustion results in more pronounced signaling changes in skeletal muscles of strength-trained compared with untrained individuals. J Strength Cond Res XX(X): 000–000, 2018—The aim of our investigation was to compare the response pattern of signaling proteins and genes regulating protein synthesis and degradation in skeletal muscle after strength exercise sessions performed to volitional fatigue in strength-trained and untrained males. Eight healthy recreationally active males and 8 power-lifting athletes performed 4 sets of unilateral leg presses to exhaustion (65% 1 repetition maximum). Biopsy samples of m. vastus lateralis were obtained before, 1 and 5 hours after cessation of exercise. Phosphorylation of p70S6kThr389, 4EBP1 Thr37/46, and ACCSer79 increased, whereas phosphorylation of eEF2Thr56 and FOXO1Ser256 decreased only in the trained group after exercise. Expression of DDIT4, MURF1, and FOXO1 mRNAs increased and expression of MSTN mRNA decreased also only in the trained group after exercise. In conclusion, moderate-intensity strength exercise performed to volitional fatigue in trained and untrained males. Eight healthy recreationally active males and 8 power-lifting athletes performed 4 sets of unilateral leg presses to exhaustion (65% 1 repetition maximum). Biopsy samples of m. vastus lateralis were obtained before, 1 and 5 hours after cessation of exercise. Phosphorylation of p70S6kThr389, 4EBP1 Thr37/46, and ACCSer79 increased, whereas phosphorylation of eEF2Thr56 and FOXO1Ser256 decreased only in the trained group after exercise. Expression of DDIT4, MURF1, and FOXO1 mRNAs increased and expression of MSTN mRNA decreased also only in the trained group after exercise. In conclusion, moderate-intensity strength exercise performed to volitional fatigue changed the phosphorylation status of mTORC1 downstream signaling molecules and markers of ubiquitin-proteasome system activation in trained individuals, suggesting activation of protein synthesis and degradation. In contrast to the trained group, signaling responses in the untrained group were considerably less pronounced. It can be assumed that the slowdown in muscle mass gain as the athletes increase in qualification cannot be associated with a decrease in the sensitivity of systems regulating protein metabolism, but possibly with inadequate intake or assimilation of nutrients necessary for anabolism. Perhaps, the intake of highly digestible protein or protein-carbohydrate dietary supplements could contribute to the increase in muscle mass in strength athletes.

Key Words muscle biopsy, mTOR, mRNA translation, proteolysis

Introduction

Skeletal muscle adapts to strength training by increasing both the cross-sectional area of muscle fibers and muscle volume, thereby increasing muscle strength (1). One of the most important signaling events that trigger a hypertrophic response in skeletal muscles is activation of mammalian target of rapamycin complex 1 (mTORC1). mTORC1 regulates mRNA translation by phosphorylating p70 ribosome protein S6 kinase (p70S6k) and 4E-binding protein (4E-BP), which leads to activation of several translation initiation and elongation factors (14). The importance of mTORC1 in adaptation of skeletal muscle to strength training is confirmed by several studies. In particular, increased phosphorylation of the mTORC1 downstream target p70S6kThr389 after a strength exercise session correlates highly with muscle hypertrophy after 14 weeks of strength training (36). Conversely, administration of the mTORC1 inhibitor rapamycin completely blocks exercise-induced increases in protein synthesis in human skeletal muscle (6).

The increase in muscle mass and strength in response to strength training is reduced in trained athletes. This could be explained by data indicating that in trained athletes, the increase in the protein synthesis and degradation rate after a strength exercise session is less pronounced in comparison...
with untrained people (28). On the other hand, it was pre-
viously shown that after 8 weeks of strength training, both
the protein synthesis response and activation of mTORC1-
dependent signaling induced by a strength exercise session is
more pronounced and less prolonged compared with those
in untrained individuals (34,42). It is not clear what changes
in skeletal muscle of trained individuals can provoke more
pronounced and less prolonged responses after strength
exercise. Importantly, adaptive changes caused by several
weeks and several years of strength training may differ. Com-
parison of the signaling responses to a strength exercise ses-
sion in the muscles of strength-trained athletes and
untrained people has not previously been conducted.

The primary aim of our investigation was to compare the
response pattern of signaling proteins and genes regulating
protein synthesis and degradation in skeletal muscle after
strength exercise sessions performed to volitional fatigue in
strength-trained and untrained males. The secondary aim was
to compare the expression of the signaling proteins and genes
in muscle from trained and untrained individuals at rest and after
strength exercise sessions performed to volitional fatigue.

METHODS

Experimental Approach to the Problem
We compared expression of signaling proteins and mRNAs in
muscle from trained and untrained individuals. Untrained and
strength-trained participants performed the strength
exercises session with the same relative intensity. It is known
that the time to reach fatigue may depend on training status
and specialization (30). Therefore, to equate the physiologi-
cal cost of exercise, each group performed an equal number
of sets to volitional fatigue. Importantly, the correct evalua-
tion of 1 repetition maximum (1RM) is crucial for calculation
of relative load. The volunteers in the trained group initially
had experience in performing various exercises with the
maximum load, which could markedly improve the accuracy
of determining the IRM. However, this feature of trained
volunteers can be considered as part of a complex of adap-
tive changes that arise in response to strength training.
Biopsies from m. vastus lateralis were obtained before and
at 2 time points after the strength exercise session. This
approach allowed us to compare the content of signaling
proteins and mRNAs in untrained and trained muscle as well
as the response to the strength exercise session.

Subjects
Eight healthy males who were recreationally active (mean \(\pm\) SD:
median age 27 [interquartile range: 21–33] years; height 181
[175–182] cm; body mass 74 [69–75] kg; and body mass index
(BMI) 22.1 [21.0–23.3]; untrained) and 8 trained men involved
in power-lifting competitions (age 29 [24–35] years; height 176
[172–182] cm; body mass 92 [83–102] kg; and BMI 29.2
[270–328]; trained) were recruited for the experiment. All
subjects were older than 18 years. Subjects were informed of
the purpose of the study, the experimental procedures to be used,
and potential risks, and gave written consent to participate before
commencing the study. The study was approved by the Human
Ethics Committee of SSC RF Institute of Biomedical Problems
of Russian Academy of Sciences, Moscow, Russia and complied
with the guidelines of the Declaration of Helsinki.

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</table>
Procedures

Preliminary Experiments. During the first visit to the laboratory, participants performed familiarization exercises using a leg press machine (5 sets with a gradually increasing load). Before the second visit, all participants refrained from any physical activity for 3 days. During the second visit, the 1RM for the leg press was determined. For this purpose, participants performed a warm-up with a light weight and then performed one repetition with a gradually increasing weight (2-minute rest between attempts) until they were unable to move the weight; the last proper repetition was designated the 1RM. After 15-minute rest, the exercise session (3 sets to exhaustion; 65% 1RM) was performed to ensure further familiarization with the load to be used during the experiment.

Main Study. All participants refrained from any physical activity for 5 days before the day of the experiment. Participants arrived at the laboratory at 07:00 and consumed a standardized light breakfast (1,135 kJ; 8 g protein, 50 g carbohydrate, and 4 g fats). From 09:00, they laid on a couch for 30 minutes. Next, venous blood was obtained from the v. intermedia cubiti via a catheter, and a microbiopsy from m. vastus lateralis was obtained under local anesthesia (2 ml 2% lidocaine) (16). After the biopsy, participants performed a warm-up with a light weight (33% 1RM), followed by 4 sets of unilateral leg presses to exhaustion (65% 1RM, 4-minute rest between sets). Venous blood samples were obtained immediately after cessation of exercise and again 15 minutes later. Biopsy of the m. vastus lateralis was performed 1 and 5 hours after cessation of exercise. Muscle samples were quickly blotted with gauze to remove superficial blood, frozen in liquid nitrogen, and stored at −280°C until further analysis. A standardized lunch (4,849 kJ; 37 g protein, 126 g carbohydrate, and 67 g fats) was provided 75 minutes after exercise. Because the lunch volume was overabundant for the untrained group and their muscle mass was lower, we did not insist that untrained volunteers consume the provided volume of food. However, the difference in food consumption between the groups was not controlled.

M. Quadriceps Volume. Determination of the volume of the m. quadriceps was performed on the basis of the regression model obtained in a previous study by comparing the anthropometric parameters (thigh length, pelvis circumference, proximal, middle and distal thigh circumferences) with the volume of muscles determined by MR tomography (39). All anthropometric parameters were measured before the first biopsy.

Measurement of Blood Hormone Levels. Blood cortisol and testosterone levels were evaluated using enzyme-linked immunosorbent assays (ELISA) using commercial ELISA-Cortisol and ELISA-Testosterone kits (Immuno-Tek, Moscow, Russia).

Table 2. Physiological characteristics of the volunteers in the untrained and trained groups.1

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Age, y (min–max)</th>
<th>Quadriceps volume, cm³</th>
<th>Leg press 1RM, kg</th>
<th>Specific force, kg cm⁻²</th>
<th>Total work volume (reps x sets x load), kg</th>
<th>Relative work volume, reps x sets x %1RM</th>
<th>MHCI content, %</th>
<th>MHCIIa content, %</th>
<th>MHCIIx content, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untrained (U)</td>
<td>8</td>
<td>27 (21–35)</td>
<td>1,567 (1,413–1,684)</td>
<td>135 (117–152)</td>
<td>0.086 (0.075–0.093)</td>
<td>6,075 (5,063–7,776)</td>
<td>4,682 (4,334–5,006)</td>
<td>29 (21–31)</td>
<td>35 (33–47)</td>
<td>34 (31–38)</td>
</tr>
<tr>
<td>Trained (T)</td>
<td>8</td>
<td>29 (24–35)</td>
<td>2,132 (1,695–2,250)</td>
<td>209.3 (175–225)</td>
<td>0.102 (0.097–0.106)</td>
<td>9,869 (8,528–11,583)</td>
<td>4,963 (4,599–5,081)</td>
<td>34 (21–32)</td>
<td>55 (48–63)</td>
<td>34 (14–29)</td>
</tr>
</tbody>
</table>

*1RM = 1 repetition maximum; MHCI = myosin heavy chain I; MHCIIa = myosin heavy chain IIa; MHCIIx = myosin heavy chain IIx. Each value represents the median and interquartile range. **Significant differences between the groups (p < 0.05).
cDNA was synthesized from 1 μg of total RNA using the MMLV Reverse Transcriptase kit (Evrogen, Moscow, Russia).

**RNA Extraction.** Frozen samples (~20 mg) were sectioned (20-μm slices) using an ultratome (Leica Microsystems, Wetzlar, Germany), and RNA was extracted using an RNeasy Mini Kit (Qiagen, Hilden, Germany). RNA concentration and purity were measured using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, USA). After DNase treatment (Fermentas, Vilnius, Lithuania), RNA was extracted using an RNeasy Mini Kit (Qiagen, Hilden, Germany), and RNA was extracted using an RNeasy Mini Kit (Qiagen, Hilden, Germany). After DNase treatment (Fermentas, Vilnius, Lithuania), cDNA was synthesized from 1 μg of total RNA using the MMLV Reverse Transcriptase kit (Evrogen, Moscow, Russia).

**Real-Time Polymerase Chain Reaction.** Real-time polymerase chain reaction (PCR) was performed using the Rotor-Gene Q cycler (Qiagen). The annealing temperature was optimized for each primer pair. The thermal profile included an initial heat denaturing step at 95°C for 5 minutes, followed by 45 cycles of denaturation at 95°C for 15 seconds, annealing (56–60°C) for 30 seconds, and extension at 72°C for 30 seconds. Amplified genes were quantified via real-time fluorescence using the EvaGreen Master Mix (Syntol, Moscow, Russia). The specificity of amplification was monitored via melting curve analysis and agarose gel (1%) electrophoresis. Each sample was run in triplicate, and a nontemplate control was included in each run. Target gene mRNA expression levels were calculated using the efficiency-corrected ΔCt method with the formula: 1/ΔE × (Cttarget/Ctcontrol). Polymerase chain reaction efficiency (E) was calculated using standard curves corresponding to target and reference genes (RPLP0 and GAPDH). Each standard curve included 6 points (diluted PCR product), with triplicate data obtained for each point. Primer sequences are shown in Table 1.

**Myosin Heavy Chain Contents.** Myosin heavy chain contents were determined as previously described (19). Frozen tissue was homogenized in 10 volumes of ice-cold phosphate buffer, mixed with sample buffer (5% β-mercaptoethanol, 2.5% SDS, 10% glycerol, 62.5 mM Tris [pH 6.8], and 0.1% bromophenol blue), boiled for 2 minutes, and separated in acrylamide gels (separating gel: 30% glycerol, 8% acrylamide, 200 mM Tris-HCl [pH 8.80], 100 mM glycine, and 0.4% SDS; stacking gel: 30% glycerol, 4% acrylamide, 70 mM Tris-HCl [pH 6.80], 4 mM EDTA, and 0.4% SDS). Running buffer contained 0.12% β-mercaptoethanol. Separation using the Mini-PROTEAN Tetra Cell system (Bio-Rad, Hercules, CA, USA) was performed for 5 hours with a constant current (10 mA per gel). Gels were stained with Coomassie blue and visualized with the ChemiDoc Imaging System (Bio-Rad). Image processing was performed using Image Lab 5.0 (Bio-Rad).

**Western Blot Analysis.** Frozen samples (~10 mg) were homogenized in ice-cold RIPA buffer containing protease and phosphatase inhibitors (50 mmol·L⁻¹ β-glycerophosphate, 50 mmol·L⁻¹ NaF, 1 mmol·L⁻¹ Na3VO4, 20 μg·mL⁻¹ aprotinin, 50 μg·mL⁻¹ leupeptin, 20 μg·mL⁻¹ pepstatin, and 1 mmol·L⁻¹ PMSF). Samples were centrifuged for 10 minutes at 10,000g and 4°C. The supernatant was collected and stored at −80°C until analysis. Protein content was analyzed using the bicinchoninic acid assay. Samples were mixed with Laemmli buffer, loaded onto a 10% polyacrylamide gel (20 μg protein per lane), and electrophoresis was performed in the Mini-PROTEAN Tetra Cell system (Bio-Rad) at 20 mA per gel. Proteins were transferred to nitrocellulose membranes for 30 minutes at 25 V using the Trans-Blot Turbo system (Bio-Rad). Membranes were stained with Ponceau S to verify consistent protein loading, followed by washing and incubation in 5% nonfat dry milk for 1 hour. Next, membranes were incubated overnight at 4°C with anti-phospho-p70S6K1Thr389 (#9205; 1:200), anti-p70S6K1Thr421/Ser424 (#9204; 1:200), anti-phospho-FOXO1Ser256 (sc-101681; 1:200), anti-phospho-4E-BP1Thr37/46 (#2855; 1:1000), anti-phospho-Erk1/2Thr202/Tyr204 (#4377; 1:500), or anti-Erk1/2 (9107; 1:1000) (all from Cell Signaling Technology, Leiden, The Netherlands); anti-phospho-FOXO1Ser256 (sc-101681; 1:1000) (Santa Cruz Biotechnology, Dallas, TX, USA).

### Table 3. Venous blood cortisol and testosterone levels before, immediately after, and 15 minutes after termination of the exercise in trained (T) and untrained (U) groups.*

<table>
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<th>Before</th>
<th>Immediately after</th>
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<tbody>
<tr>
<td>Cortisol, nM·L⁻¹</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>U</td>
<td>429.5 (337.0–548.5)</td>
<td>404.0 (360.0–695.5)</td>
<td>574.0 (411.0–766.0)</td>
</tr>
<tr>
<td>T</td>
<td>344.5 (200.5–532.3)</td>
<td>393.5 (257.5–598.5)</td>
<td>514.5 (358.0–693.8)</td>
</tr>
<tr>
<td>Testosterone, ng·mL⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U</td>
<td>15.7 (12.7–19.7)</td>
<td>16.8 (14.6–23.0)</td>
<td>14.9 (12.9–19.1)</td>
</tr>
<tr>
<td>T</td>
<td>10.9 (6.2–15.8)</td>
<td>10.30 (6.9–19.4)</td>
<td>7.90 (5.7–16.7)</td>
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</tbody>
</table>

*Each value represents the median and interquartile range. †Significant differences from initial levels.
anti-FOXO1 (ab52857; 1:2500), anti-phospho-ACC<sup>Ser79/222</sup> (ab68191; 1:1000), anti-phospho-eEF2<sup>Thr56</sup> (ab115165; 1:1000), anti-eEF2 (ab33523; 1:500), anti-eIF2B epsilon (ab32713; 1:500), anti-Fbx32 (ab168372; 1:1000), or anti-MURF1 (ab98657; 1:2500) (all from Abcam, Cambridge, United Kingdom). The next day, membranes were incubated for 1 hour with an HRP-linked secondary antibody (Cell Signaling Technology) and washed with PBS-Tween 20 after each step (3 times for 5 minutes each). After incubation of membranes with an enhancing chemiluminescence substrate (Bio-Rad), luminescent signals were captured using the ChemiDoc Imaging System (Bio-Rad). Densitometry was performed using Image Lab 5.0 (Bio-Rad). All values were expressed as the ratio of staining intensity for target protein to the intensity of all proteins in the corresponding electrophoresis lane in gels stained with Coomassie blue as was previously recommended (10).

**Statistical Analyses**

Data were expressed as the median and interquartile range because the number of samples was small ($n = 8$). For the same reason, the analysis of normality of distribution was not performed. To compare post-exercise Western blot values with initial values, Friedman’s with Dunn’s multiple comparison tests were used. To compare initial mRNA levels and levels 5 hours post-exercise (assessed by real-time PCR), the Wilcoxon matched-pairs signed-rank test was used. Comparison of physiological indices and the amounts of different proteins and mRNAs between the untrained and trained groups was performed using the Mann-Whitney test. Cohen’s $d$ was calculated to measure the magnitude of practical effect (37). Analysis of correlations between the parameters was performed using the Spearman rank test. The level of significance was set at $p \leq 0.05$. Statistical analysis was performed using Graph Pad Prism 7 software.

**Results**

The 1RM for the unilateral leg press ($p = 0.001$), the volume of the $m$. quadriceps femoris ($p = 0.02$), and the specific force (ratio of 1RM to muscle volume) ($p = 0.001$) were significantly higher in the trained group than in the untrained group (Table 2). The trained group performed a higher total
work (38% more; \( p < 0.001 \)) during the strength exercise session, but the relative work (repetitions \( \times \%1RM \)) was similar for both groups (Table 2). There was a strong correlation between total work performed and \( m. \) quadriceps volume (\( r = 0.86, \ p < 0.0001 \)).

Venous blood testosterone level was unchanged in both groups after exercise, but the cortisol level in the trained group increased by 33% 15 minutes after exercise (\( p < 0.001; \) Table 3).

Phosphorylation of p70S6k\( ^{\text{Thr389}} \) after exercise was unchanged in the untrained group, but a significant time-dependent effect was observed in the trained group (\( p < 0.05; \) Figure 1A, G). Phosphorylation at another site within the protein (\( \text{Thr421/Ser424} \)) increased in both groups after exercise: by 6.2- and 1.9-fold for untrained and trained, respectively, at 1 hour; and by 3.4- and 2.8-fold for untrained and trained, respectively, at 5 hours (\( p < 0.01; \) Figure 1C, G). Phosphorylation of \( 4\text{EBP1}^{\text{Thr37/46}} \) in the trained group increased 1.5-fold at 5 hours post-exercise (\( p < 0.05; \) Figure 1B, G). There was weak but significant correlation between post-exercise increases of p70S6k\( ^{\text{Thr389}} \) and \( 4\text{EBP1}^{\text{Thr37/46}} \) in all participants (\( r = 0.33; \ p = 0.046 \)). Phosphorylation of eukaryotic translation elongation factor 2 (\( \text{eEF2}^{\text{Thr56}} \)) at 5 hours post-exercise decreased 0.71-fold in the trained group (\( p < 0.05; \) Figure 1D, G), but there was no change in the untrained group. The activation of AMP-activated protein kinase (AMPK) was evaluated by measuring phosphorylation of its substrate, acetyl coenzyme A carboxylase (ACC). ACC\(^{\text{Ser79}} \) increased 3.4-fold only in the trained group at 1 hour after cessation of the strength exercise session (\( p < 0.01; \) Figure 1F, G).

Expression of DNA damage inducible transcript 4 (\( \text{DDIT4} \), also known as \( \text{REDD1} \)) mRNA decreased 0.24-fold at 5 hours post-exercise in the trained group only (\( p < 0.01; \) Figure 2D). Phosphorylation of forkhead box protein O1 (\( \text{FOXO1}^{\text{Ser256}} \)) decreased 0.28-fold at 5 hours after exercise in the trained group (\( p < 0.01; \) Figure 1E, G). Strength exercise resulted in a significant increase in mRNA expression of muscle RING-finger protein-1 (\( \text{MURF1} \), also known as \( \text{TRIM63} \)) (2.4-fold) and \( \text{FOXO1} \) (1.6-fold), and a significant decrease (3.6-fold) in Myostatin (\( \text{MSTN} \)) mRNA expression, at 5 hours post-exercise in the trained group only (\( p < 0.05 \) for all; Figure 2B, F, C); no changes in mRNA expression of \( \text{eEF2} \) and F-box protein 32 (\( \text{FBXO32} \)) were observed (Figure 2A, E).

Comparison of trained and untrained muscle tissue at rest revealed no differences between the groups in terms of expression of the proteins AMPKa1/2, p70S6k, \( \text{MURF1} \), \( \text{FBXO32} \), and eukaryotic translation initiation factor 2B epsilon (\( \text{eIF2B}\epsilon \)). By contrast, 51% less \( \text{eEF2} \) and 45% more FOXO1 were observed in the trained group compared with the untrained group (\( p < 0.05; \) Figure 3). Moreover, expression of \( \text{MSTN} \) mRNA at rest in the trained group compared with the untrained group was higher (\( p < 0.05; \) Figure 2C). Electrophoretic separation of skeletal MHC isoforms revealed 20% more MHCIIa (\( p = 0.005 \)) and 13.5% less MHCIIx (\( p =
0.003) in the trained group than in the untrained group (Table 2).

**DISCUSSION**

To investigate muscle adaptation to prolonged strength training, we compared well-trained powerlifters with untrained subjects. It is known that time to fatigue may depend on training status and specialization (30). Therefore, to equate physiological cost of the exercise, each group performed an equal number of sets to volitional fatigue. The total work performed by the trained group during the test session was 38% higher than that in the untrained group, whereas relative work was similar. Furthermore, the number of sets and number of repetitions until exhaustion was similar for both trained and untrained subjects.

As expected, a greater *m. quadriceps* volume and 1RM were observed in trained individuals than in untrained individuals. Moreover, significantly greater specific force in the trained group could be associated with a greater ability to recruit muscular fibers compared with untrained subjects. Taking into consideration the strong correlation between muscle volume and total work performed during the strength exercise session, the higher work performed by the athletes was due to the larger muscle mass involved. We assume that involving more muscle mass in the work caused more pronounced metabolic stress in the athletes. Indeed, blood cortisol, a well-known marker of exercise-induced stress (8,20), was increased only in the trained group. Previously, it was shown that high-intensity strength exercise increased phosphorylation of p70S6k<sup>Thr389</sup> in recreationally active subjects (5,7,17), in moderately trained subjects (after 10 weeks of strength training) (42), and in strength-trained athletes (13). However, we found no increase in p70S6k<sup>Thr389</sup> phosphorylation in the untrained group after the strength exercise session. One of the possible reasons for this was the feeding state of the participants. Indeed, in a number of cited studies, an increase in the phosphorylation of p70S6k<sup>Thr389</sup> was observed after exercise in a fasted state (5,7,13,17), whereas food intake per se may increase the level of phosphorylation of p70S6k<sup>Thr389</sup> (17). In our study, volunteers received a standardized breakfast 2 hours before the first biopsy, which could slightly increase the phosphorylation levels of p70S6k<sup>Thr389</sup> and eEF2<sup>Bc</sup> (17). Thereby, exercise-induced changes of these parameters in both groups might be suppressed. On the other hand, the untrained individuals in our study generated a lower specific force, which may be associated with poorer neural control and reduced ability to recruit muscle fibers during a strength exercise session, and as a result lower mTORC1 activation.

![Figure 3. Pre-exercise abundance of signaling proteins p70S6k, eEF2, AMPKα1/2, MURF1, FBXO32, FOXO1, and eEF2Bc in skeletal muscles of trained and untrained individuals. Each value was normalized to the staining intensity of all proteins in the electrophoresis lane and to pre-exercise levels. Data represent the median and interquartile range. The median in the untrained group was set as 100%. Cohen’s d represents the magnitude of the changes between the groups. *Significant differences between groups (p < 0.05).](image-url)
It was shown that despite higher levels of p70S6k in predominantly fast-twitch *extensor digitorum longus* muscle fibers, the basal phosphorylation of the kinase was higher in slow-twitch *soleus* muscle, suggesting that fast-twitch muscle fibers have higher potential for increased p70S6k phosphorylation in rats (18). Moreover, it was shown that human fast-twitch muscle fibers demonstrated more pronounced increases in p70S6k^{Thr389} in response to strength exercise than slow-twitch muscle fibers (35). In our study, the trained group revealed a significantly higher MHCIla content. Thus, the absence of an increase in p70S6k^{Thr389} phosphorylation in the untrained group could be partially explained by differences in MHC contents between the groups.

eEF2 is a key regulator of translation elongation and is dephosphorylated and upregulated in a p70S6k-eEF2k-dependent and p90RSK-eEF2k-dependent manner (41). In our investigation, eEF2^{Thr56} phosphorylation decreased after the strength exercise session (a marker of activation) only in the trained group, but there was no correlation with p70S6k^{Thr389}. It is likely that the activation of eEF2 was mediated not only by the mTORC1-dependent mechanism but also by p90RSK.

Earlier, it was suggested that AMPK activation can potentially reduce activation of mTORC1 and its targets (3). In our investigation, phosphorylation of ACC^{Ser79} (a specific marker of AMPK activity (15)) increased after the exercise only in the trained group. However, there was no correlation between ACC^{Ser79} and the phosphorylation levels of any mTORC1 target proteins. Parallel activation of AMPK and mTORC1 in our study confirms the previously suggested assumption that activation of AMPK does not prevent the activation of mTORC1 after strength exercise in the postprandial state (42).

Previously, a link between AMPK activation and mRNA abundance of genes regulating ubiquitinin-proteasome system activity (*FOXO1, MURF1*, and *FBXO32*) has been shown (21,23,24). We found that increased abundance of *MURF1* and *FBXO32* mRNA was observed after the strength exercise session only in the trained group. A few studies showed activation of the ubiquitin-proteasome system after strength exercise (9,26), but other studies did not (2,33). Apparently, such differences could be explained by differences in exercise protocols. In particular, it was shown that eccentric and concentric contractions resulted in different expression levels of *MURF1* and *FOXO1* mRNA (25). In our investigation, in contrast to the untrained group, upregulation of the markers of the ubiquitin-proteasome system in the trained group was recorded. This observation could be explained by more pronounced metabolic changes in trained group (AMP accumulation and glycogen depletion), which were related to AMPK activation.

Differences in signaling responses to a strength exercise session between the trained and untrained groups might also be explained by differences in basal expression of signaling proteins and their genes between trained and untrained skeletal muscles. Indeed, some differences in proteome and transcriptome previously were shown between the trained and untrained skeletal muscles (27,29) that may be partly due to different fiber composition (4,29). Unexpectedly, in our experiment, the amount of eEF2 protein was significantly lower in the trained group than in the untrained group, whereas no changes in eEF2 mRNA abundance at rest or in eEF2 mRNA expression after exercise were found. We hypothesized that different rates of proteolysis might affect eEF2 content. This is in accordance with higher levels of FOXO1 at rest in the muscles of trained individuals obtained in our investigation. A previous study showed that 10 weeks of strength training increased the abundance of *FOXO1* and *FBXO32* mRNA in the basal state (33). We did not observe any differences in mRNA abundance of *FOXO1* and *FBXO32* at rest between the groups, but the higher abundance of FOXO1 in the trained group might be explained by exercise-induced increases in *FOXO1* mRNA expression. In addition, we found significantly higher content of *MSTN* mRNA at rest in trained muscle, despite similar exercise-induced decreases in its expression in trained and untrained groups. This may be due to an increase in the content of the FOXO1 protein at rest, an important regulator of the expression of the *MSTN* gene (40). In addition, it was previously shown that the use of a high-protein diet led to an increase in *MSTN* in the blood plasma after 8 weeks of strength training in young subjects (26). Assuming that an increase in the *MSTN* content in this study was associated with an increase in the expression of its gene, a higher level of *MSTN* expression in our study could also be associated with a high-protein diet used by power-lifters.

The choice of time points for biopsy could significantly affect the changes in the parameters studied. In our study, biopsies were taken 1 and 5 hours after the exercise session. Previously, it was shown that the activation of mTORC1 can be observed immediately after, 1, 2, 3, and 5 hours after the end of strength exercise session (2,12,22,38,42). But the most evident activation of mTORC1 in these studies was observed at different time points. This may depend on the fitness level of volunteers and the experimental conditions. The differences between the groups in the activation of the signaling in response to the exercise, observed in our study, could also be due to differences in the dynamics of changes in these parameters between trained and untrained volunteers.

In conclusion, the moderate-intensity strength exercise to volitional fatigue changed the phosphorylation of mTORC1 target proteins and the phosphorylation and expression of markers of the ubiquitin-proteasome system activation in the muscles of trained volunteers. The absence of changes in the untrained group could be due to poorer neural control and reduced ability to recruit muscle fibers as well as the lower content of MHCIla than in the trained group. As a result, metabolic changes caused by strength exercise in the untrained group were reduced. Another possible reason for the absence of changes in the untrained group was the
nonoptimal choice of biopsy time points. The most striking differences between the trained and untrained skeletal muscle were revealed at rest. Differences in the content of eEF2 and FOXO1 proteins at rest in muscle tissue potentially can affect the rates of protein synthesis and breakdown. The reduced content of eEF2 in trained skeletal muscle can potentially increase the in the rate of protein synthesis after a strength exercise session.

**PRACTICAL APPLICATIONS**

The smaller specific forces and less pronounced signaling responses in the untrained group revealed that training of untrained individuals should focus on improving exercise technique and optimizing muscle fiber recruitment during exercise. It seems that improved motor control and recruitment of muscle fibers in the trained individuals might lead to an increase in regulation of anabolic response after strength exercise. The approach used in our study (each set performed to volitional fatigue) may not have the expected effect in untrained individuals, at least at the initial stages of the training process. It was shown in our study that the signaling response in the muscles of trained volunteers was activated more than that in untrained volunteers. Based on this, it can be assumed that the slowdown in muscle mass gain as the athletes increase in qualification cannot be associated with a decrease in the sensitivity of systems regulating protein metabolism, but possibly with inadequate intake or assimilation of nutrients necessary for anabolism. Perhaps, the intake of highly digestible protein or protein-carbohydrate dietary supplements could contribute to the increase in muscle mass in strength athletes.

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**REFERENCES**


