Resistance exercise induced mTORC1 signaling is not impaired by subsequent endurance exercise in human skeletal muscle

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Apró W, Wang L, Pontén M, Blomstrand E, Sahlin K. Resistance exercise induced mTORC1 signaling is not impaired by subsequent endurance exercise in human skeletal muscle. Am J Physiol Endocrinol Metab 305: E22–E32, 2013. First published April 30, 2013; doi:10.1152/ajpendo.00091.2013.—The current dogma is that the muscle adaptation to resistance exercise is blunted when combined with endurance exercise. The suggested mechanism (based on rodent experiments) is that activation of adenosine monophosphate-activated protein kinase (AMPK) during endurance exercise impairs muscle growth through inhibition of the mechanistic target of rapamycin complex 1 (mTORC1). The purpose of this study was to investigate potential interference of endurance training on the signaling pathway of resistance training [mTORC1 phosphorylation of ribosomal protein S6 kinase 1 (S6K1)] in human muscle. Ten healthy and moderately trained male subjects performed on two separate occasions either acute high-intensity and high-volume resistance exercise (leg press, R) or R followed by 30 min of cycling (RE). Muscle biopsies were collected before and 1 and 3 h post resistance exercise. Phosphorylation of mTOR (Ser2448) increased 2-fold (P < 0.05) and that of S6K1 (Thr389) 14-fold (P < 0.05), with no difference between R and RE. Phosphorylation of eukaryotic elongation factor 2 (eEF2, Thr56) was reduced ~70% during recovery in both trials (P < 0.05). An interesting finding was that phosphorylation of AMPK (Thr172) and acetyl-CoA carboxylase (ACC, Ser79) decreased ~30% and ~50%, respectively, 3 h postexercise (P < 0.05). Proliferator-activated receptor-γ coactivator-1 (PGC-1α) mRNA increased more after RE (6.5-fold) than after R (4-fold) (RE vs. R: P < 0.01) and was the only gene expressed differently between trials. These data show that the signaling of muscle growth through the mTORC1-S6K1 axis after heavy resistance exercise is not inhibited by subsequent endurance exercise. It is also suggested that prior activation of mTORC1 signaling may repress subsequent phosphorylation of AMPK.

mechanistic target of rapamycin complex 1; adenosine monophosphate-activated protein kinase; interference

SKELETAL MUSCLE POSSESSES remarkable plasticity and as such has the unique ability to adapt to various types of contractile activity, which ultimately results in divergent phenotypes in accordance with the specificity of training principle (5). The divergent adaptations following resistance and endurance exercise place these exercise modalities in contrasting ends of the training adaptation continuum. As such, the opposing phenotypes are likely dependent on highly specific adaptations that may be incompatible when different exercise modes are performed simultaneously (5).

This notion is supported by several studies showing negative effects on the development of strength and power (23, 31) as well as muscle fiber hypertrophy (34, 40) when both modes of exercise have been performed concurrently over longer periods of time. Collectively, these findings have given rise to the current paradigm of the training interference effect (30), yet not all studies support the existence of such a phenomenon (8, 37, 42, 43). The reasons for the discrepancies within the literature are not readily obvious but are likely related to experimental variables such as exercise intensity and volume, exercise sequence, or nutritional status.

Assessment of physical performance with hard outcome measures offers little insight into the molecular mechanisms regulating long-term training adaptations. In recent years, our understanding of the molecular events underlying the various training adaptations has increased considerably, and several distinct signaling pathways have been identified. For instance, it is well established that skeletal muscle growth to a large extent is mediated by activation of the mechanistic target of rapamycin complex 1 (mTORC1) pathway (12, 28). Similarly, peripheral adaptations responsible for mitochondrial biogenesis include induction of the peroxisome proliferator-activated receptor-γ coactivator-1 (PGC-1α) (7, 50) pathway, which in turn mediates the characteristic increase in muscle oxidative capacity seen after endurance training (38). Mechanistically, cross talk between these two pathways has been linked to the adenosine monophosphate-activated protein kinase (AMPK), an enzyme activated during energetic stress (53). Activation of AMPK by pharmacological agents has been shown to repress mTORC1 signaling (13, 39, 47) but elevate mRNA expression of PGC-1α (32) in rodent muscle.

To date, only a small number of studies have been performed in humans to examine the impact of acute concurrent exercise on the signaling pathways leading to different phenotypes. Some of the investigations found support for an interference effect (15, 16), whereas others did not (20, 35). More specifically, when concurrent exercise was performed in the fed state (20, 35) and with several hours of recovery time between exercise modes (35), mTORC1 signaling was similar compared with single-mode resistance exercise. However, from these studies and from a mechanistic perspective, it is difficult to evaluate the impact of concurrent exercise per se, since feeding in itself may influence signaling though the mTORC1 pathway (33). In contrast, when endurance (16) and sprint (15) exercise preceded resistance exercise in the fasted state, with only 15 min of rest in between, mTORC1 signaling was attenuated compared with when the exercise order was reversed. However, none of these studies included a single mode exercise for comparison. Thus, to date, no study has investigated the signaling response following concurrent exercise compared with single-mode resistance exercise in the fasted state.
METHODS

Subjects. Ten healthy moderately trained male subjects were recruited for this study. After being informed of the purpose of the study and of all associated risks, all subjects gave written consent. To be eligible for enrollment in the study, subjects were required to have performed resistance exercise two to three times per week and endurance exercise once to two times a week during the last 6 months and to have a maximal leg strength equaling four times their body weight or more. Subject characteristics are presented in Table 1. The study was approved by the Regional Ethical Review Board in Stockholm and performed in accordance with the principles outlined in the Declaration of Helsinki.

Study design. The study employed a randomized cross-over design in which each subject performed one session of resistance exercise (R) and another session of resistance exercise followed by endurance exercise (RE). The two sessions were separated by approximately 2 weeks. A schematic overview of the experimental protocols is provided in Fig. 1. All subjects were instructed to maintain their habitual diet intake and physical activity pattern throughout the entire experimental period. Subjects were instructed to refrain from physical exercise for 2 days before each trial and to record and duplicate their food intake before the first and second trials, respectively.

Pretests. Before initiation of the actual experiments, each subject’s two-legged one-repetition maximum (1RM) was determined on a leg press machine (243 leg press 45°; Gymleco, Stockholm, Sweden) after warming up on a cycle ergometer for 10 min. The 1RM was assessed by gradually increasing the load until the subject was unable to perform no more than one single repetition (90–180° knee angle). Maximal and submaximal oxygen uptake was determined on a mechanically braked cycle ergometer (model 839E; Monark, Vansbro, Sweden) with the work rate gradually increased until volitional exhaustion as described by Åstrand and Rodahl (3a). Oxygen uptake was measured continuously using an on-line system (Oxycon Pro; Erich Jaeger GmbH, Hoechberg, Germany), and heart rate (HR) was recorded continuously (Polar Electro Oy, Kempele, Finland). Following initial testing, subjects performed two familiarization sessions to minimize any training effects during the live experiments (see below).

On the day of each trial, subjects reported to the laboratory at ~7:30 A.M. following an overnight fast from 9:00 P.M. the evening before. Upon arrival, subjects were placed in a supine position and rested for 10 min before the collection of a resting blood sample from an arm vein. After subsequent administration of local anesthesia, a resting biopsy was collected from the middle portion of the vastus lateralis muscle of one leg using a Bergström needle (11) with manually applied suction.

Following blood and tissue sampling, subjects were seated in the leg press machine and performed three warm-up sets of 10 repetitions at 0, 30, and 60% of 1RM with 3 min of rest between each set. Thereafter, the subjects performed 10 sets of heavy resistance exercise (R) or resistance exercise followed by endurance exercise (RE). The resistance exercise protocol consisted of four sets of ~8–10 repetitions at 85% of 1RM, four sets of 10–12 repetitions at 75% of 1RM, and lastly two sets to volitional fatigue at 65% of 1RM with 3 min of recovery allowed between each set. The load and number of repetitions was recorded during the first trial and duplicated during the second trial. Time under tension was recorded for each set during the first trial and was used to match, as closely as possible, the repetition speed in each set during the second trial. Following resistance exercise in the RE trial, subjects rested for 15 min after which 30 min of cycling was initiated at an intensity equal to 70% of the subjects’ maximal oxygen consumption (Table 2).

In both trials, two additional muscle biopsies were collected in the contralateral leg at 1 and 3 h following resistance exercise together with blood samples. For each biopsy sampling, a new incision was made ~3–4 cm proximal to the previous one, and, after biopsy collection, samples were immediately blotted free of blood, frozen in liquid nitrogen, and stored at ~80°C for later analysis. Because of technical difficulties, tissue sampling at 3 h post resistance exercise

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ± SE</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>26 ± 2</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>85 ± 3</td>
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<tr>
<td>Height, cm</td>
<td>179 ± 2</td>
</tr>
<tr>
<td>1RM, kg</td>
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</tr>
<tr>
<td>VO2max, l/min</td>
<td>4.27 ± 0.12</td>
</tr>
<tr>
<td>Relative VO2max, ml·min⁻¹·kg⁻¹</td>
<td>50.8 ± 1.6</td>
</tr>
<tr>
<td>HRmax, beats/min</td>
<td>192 ± 2</td>
</tr>
<tr>
<td>Wattmax, W</td>
<td>308 ± 15</td>
</tr>
</tbody>
</table>

Values are presented as means ± SE for 10 subjects. 1RM, one repetition maximum; VO2max, maximum oxygen uptake; HRmax, maximum heart rate; Wattmax, maximum cycling intensity expressed in watts.

Therefore, the aim of the present investigation was to examine whether endurance exercise following a heavy resistance exercise protocol would repress molecular signaling through the mTORC1 pathway, compared with single-mode resistance exercise. To this end, muscle biopsies from moderately trained men were analyzed with regard to protein signaling and mRNA expression involved in skeletal muscle hypertrophy and mitochondrial biogenesis. It was hypothesized that concurrent exercise would impair growth-related signaling and gene expression.

Table 1. Subject characteristics

<table>
<thead>
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Values are presented as means ± SE for 10 subjects. 1RM, one repetition maximum; VO2max, maximum oxygen uptake; HRmax, maximum heart rate; Wattmax, maximum cycling intensity expressed in watts.
was unsuccessful for one subject in the R trial and for another subject in the RE trial.

Plasma analysis. Blood samples (4 ml) were centrifuged at 1,500 g at 4°C for 10 min, and the plasma obtained was stored at −20°C. Plasma samples were later analyzed for glucose and lactate concentrations as described by Bergmeyer (10).

Immunoblot analysis. Muscle samples were lyophilized, cleaned from blood and connective tissue under a dissection microscope (Carl Zeiss), and then homogenized in ice-cold buffer (80 μl/mg dry wt) containing 2 mM HEPES, pH 7.4, 1 mM EDTA, 5 mM EGTA, 10 mM MgCl₂, 50 mM β-glycerophosphate, 1% Triton X-100, 1 mM Na₃VO₄, 2 mM dithiothreitol, 20 μg/ml leupeptin, 50 μg/ml apro tinin, 1% phosphatase inhibitor cocktail (P-2850; Sigma), and 40 μg/μl phenylmethylsulfonyl fluoride. Homogenates were then cleared by centrifugation at 10,000 g for 10 min at 4°C, and the resulting supernatant was stored at −80°C.

Protein concentrations were determined in aliquots of supernatant diluted 1:10 in distilled water using a bicinchoninic acid assay (Pierce Biotechnology, Rockford, IL). Samples were diluted in Laemmli sample buffer (Bio-Rad Laboratories, Richmond, CA) and homogenized buffer to obtain a final protein concentration of 1.5 μg/μl. Following dilution, all samples were heated at 95°C for 5 min to denature proteins present in the supernatant. Samples were then kept in freezing buffer to obtain a final protein concentration of 1.5 μg/μl. Samples were then kept at −20°C until further analysis.

Details of the Western blotting procedures have been described elsewhere (3). Briefly, and with minor modifications, samples containing 30 μg total protein were separated by SDS-PAGE on Criterion cell gradient gels (4–20% acrylamide; Bio-Rad Laboratories). Electrophoresis was performed on ice at 200 volts for 120 min, after which the gels were equilibrated in transfer buffer (25 mM Tris base, 192 mM glycine, and 10% methanol) for 30 min. The proteins were then transferred to polyvinylidine fluoride membranes (Bio-Rad Laboratories) at a constant current of 300 mA for 3 h at 4°C. Following transfer, membranes were stained with the MemCode Reversible Protein Stain Kit (Pierce Biotechnology) (2) to confirm equal loading of the samples. All samples from each subject were run on the same gel.

Membranes were blocked for 1 h at room temperature in Tris-buffered saline (TBS; 20 mM Tris base, 137 mM NaCl, pH 7.6) containing 5% nonfat dry milk. After blocking, membranes were incubated overnight with commercially available primary antibodies diluted in TBS supplemented with 0.1% Tween 20 containing 2.5% nonfat dry milk (TBS-TM). After incubation with these primary antibodies, the membranes were washed with TBS-TM and incubated for 1 h at room temperature with secondary antibodies conjugated with horseradish peroxidase. Next, the membranes were washed serially (2 × 1 min, 3 × 15 min) with TBS-TM, followed by four additional washes with TBS for 5 min each. Finally, membranes with the antibodies bound to the target proteins were visualized by chemiluminescent detection on a Molecular Imager ChemiDoc XRS system, and the bands were analyzed using the contour tool in the Quantity One version 4.6.3 software (Bio-Rad Laboratories). All values are expressed relative to total levels of α-tubulin.

Antibodies. Primary antibodies raised in rabbit against phospho-protein kinase B (Akt, Ser473; no. 9271), phospho-mTOR (Ser2448; no. 2971), phospho-S6 kinase 1 (4E-BP1, Thr37/46; no. 2855), phospho-eukaryotic elongation factor 2 (eEF2, Thr56; no. 2331), phospho-AMPK (Thr172; no. 4188), phospho-acetyl-CoA carboxylase (ACC, Ser79; no. 3661), phospho-p38 (Thr180/Tyr192; no. 9211), phospho-ERK1/2 (Thr202/Tyr204; no. 1183), and phospho-calcium/calmodulin-dependent protein kinase (CaMKII, Thr386; no. 3361) were purchased from Cell Signaling Technology (Beverly, MA). Primary total antibodies for mouse α-tubulin (no. T6074) and rabbit regulated in development and DNA damage response 1 (RED1, no. ab63059) were purchased from Sigma-Aldrich (St. Louis, MO) and Abcam (Cambridge, UK), respectively. All primary antibodies were diluted 1:1,000 except for eEF2 and α-tubulin, which were diluted 1:5,000. Secondary anti-rabbit (no. 7074) and anti-mouse (no. 7076) antibodies (1:10,000) were purchased from Cell Signaling Technology.

RNA extraction and quantitative real-time PCR. Total RNA was extracted from ~2 mg lyophilized and cleaned tissue that was homogenized in PureZOL RNA isolation reagent (Bio-Rad Laboratories) according to the manufacturer’s instructions. The concentration and purity of the RNA was determined by spectrophotometry, and 1 μg RNA was used for reverse transcription of 20 μl cDNA with the iScript cDNA Synthesis Kit (Bio-Rad Laboratories). The primers for the specific genes analyzed here have been presented in a previous publication from this laboratory (50). The concentration of cDNA, annealing temperature, and PCR cycle protocol were determined for each primer pair to ensure optimal conditions for amplification. Samples were run in triplicate, and all samples from each subject were run on the same plate to allow direct relative comparisons. Quantitative real-time PCR (qRT-PCR) amplification mixtures (25 μl) contained 12.5 μl iQ SYBR Green Supermix (Bio-Rad Laboratories), 0.5 μl 10 μM forward and reverse primers, and 11.5 μl template cDNA in RNase-free water. qRT-PCR was performed with the Bio-Rad iCycler (Bio-Rad Laboratories), and relative changes in mRNA levels were analyzed by the ΔΔCt method with GAPDH used as the reference gene.

Statistical analyses. All data are expressed as means ± SE. For protein signaling, gene expression, and plasma data, a two-way
repeated-measures ANOVA (trial and time) was used for statistical analysis. For missing data points, weighted means were used in the analysis. When a significant main effect or an interaction effect was observed, Fisher’s least-significant difference post hoc analysis was performed to locate differences. For some positively skewed distributed variables, log transformation was performed before analyses. For analysis of time under tension and number of repetitions, a paired t-test was used. Statistical significance was set at \( P < 0.05 \).

**RESULTS**

**Task performance.** Eight subjects performed the same number of repetitions in both trials while two subjects performed one and two repetitions less in the RE trial. Time under tension was similar between trials (Table 2). Average heart rate during cycling corresponded to 88 ± 1% of maximal heart rate.

**Blood parameters.** Plasma concentrations of glucose were significantly decreased at both time points during recovery in the R trial (Table 3). In the RE trial, plasma levels of glucose remained unchanged until the 3-h time point at which these levels decreased but only compared with 1 h post resistance exercise (Table 3). Plasma levels of lactate were increased in both trials 1 h after resistance exercise, but more so in the RE trial.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Rest</th>
<th>1 h Post</th>
<th>3 h Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mmol/l</td>
<td>5.65 ± 0.11</td>
<td>5.03 ± 0.11*</td>
<td>5.29 ± 0.06*</td>
</tr>
<tr>
<td>Lactate, mmol/l</td>
<td>1.43 ± 0.23</td>
<td>3.51 ± 0.28*</td>
<td>1.35 ± 0.09#</td>
</tr>
</tbody>
</table>

Values are presented as means ± SE for 10 subjects. Blood was sampled at rest and at 1 and 3 h post resistance exercise. *\( P < 0.05 \) vs. rest; \( \# P < 0.05 \) vs. 1 h post; ‡\( P < 0.05 \) vs. R.

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**Table 3. Plasma concentrations of glucose and lactate**

**Fig. 2.** Phosphorylation levels of protein kinase B (Akt, 60 kDa) at Ser473 (A), mechanistic target of rapamycin (mTOR, 289 kDa) at Ser2448 (B), S6 kinase 1 (S6K1, 70 kDa) at Thr389 (C), and eukaryotic elongation factor 2 (eEF2, 95 kDa) at Thr56 (D) before and 1 and 3 h post resistance exercise in both trials. Representative immunoblots from one subject are shown above each graph. Values are normalized to α-tubulin and presented as means ± SE for 10 subjects (\( n = 9 \) for 3 h post). Symbols above lines denote differences revealed by a post hoc test when a main effect was observed. Symbols without lines denote differences revealed by a post hoc test when an interaction effect was observed. *\( P < 0.05 \) vs. rest; \( \# P < 0.05 \) vs. 1 h post; ‡\( P < 0.05 \) vs. R.
**Protein signaling.** Phosphorylation of Akt at Ser\(^{473}\) was decreased 1 h after resistance exercise in the R trial compared with resting values as well as being lower compared with the RE trial at the same time point (Fig. 2A). For phosphorylation of mTOR at Ser\(^{2448}\), statistical analysis revealed main effects of time as well as trials (\(P < 0.05\)) but no interaction effect. Further analysis of the data with paired \(t\)-tests showed no difference between R and RE trials in the fold change of mTOR phosphorylation. The significant main effect of trial was therefore likely the result of the difference in preexercise values. Post hoc analysis showed statistically significant elevations in mTOR phosphorylation at both postexercise time points (\(P < 0.01\)), with a trend (\(P = 0.057\)) for a further increase at 3 h after resistance exercise (Fig. 2B).

Phosphorylation of S6K1 at residue Thr\(^{389}\) increased over time in both trials, exhibiting 5- and 14-fold (\(P < 0.01\) for both) increases at 1 and 3 h after resistance exercise, respectively (Fig. 2C). Phosphorylation of eEF2 at Thr\(^{56}\) decreased at the 1-h time point to about 30% of preexercise values (\(P < 0.01\)) with no difference between trials (Fig. 2D). At 3 h after resistance exercise, phosphorylation of this residue remained significantly depressed in both trials (\(P < 0.01\)) and tended to decrease further compared with the 1-h time point (\(P = 0.084\)) (Fig. 2D).

Phosphorylation of AMPK at Thr\(^{172}\) was unchanged 1 h after resistance exercise in both trials. At the 3-h time point, phosphorylation of this protein was decreased 33% (\(P < 0.01\)) in both trials compared with preexercise and 1-h values (Fig. 3A). Similarly, phosphorylation of ACC at residue Ser\(^{79}\) was unaffected 1 h after resistance exercise but decreased 47% (\(P < 0.01\)) at the 3-h time point in both trials (Fig. 3B). The degree of p38 phosphorylation at Thr\(^{180}/\text{Tyr}^{182}\) increased approximately twofold in both trials at the 1-h time point (\(P < 0.01\)) and remained elevated at 3 h (\(P < 0.01\)) after resistance exercise (Fig. 3C).

Phosphorylation of 4E-BP1 at residues Thr\(^{37/46}\) showed a small but significant decrease (\(P < 0.05\)) 1 h after resistance exercise, but at the 3-h time point phosphorylation of 4E-BP1 had returned to baseline values (Fig. 4A). Phosphorylation of ERK1/2 at residues Thr\(^{202}/\text{Tyr}^{204}\) remained unchanged in both trials at all time points (Fig. 4B). Similarly, phosphorylation of CaMKII at Thr\(^{286}\) was unaltered by exercise in both trials (Fig. 4C). Totals levels of REDD1 protein were unchanged 1 h post resistance exercise but increased modestly by the 3-h time point in both trials (Fig. 5A).

**Gene expression.** There were minor but significant increases in mRNA expression of Rheb at both the 1 (\(P < 0.05\))- and 3 (\(P < 0.01\))-h time points in both trials (Table 4). Expression of mTOR, hVps34, and TSC1 was unaffected by either protocol at the 1-h time point but decreased at the 3-h time point.
The main finding of the present investigation is that endurance exercise performed subsequent to resistance exercise does not blunt growth-related signaling through the mTORC1 pathway in human skeletal muscle. This conclusion is supported by similar and positive alterations of mTOR as well as several downstream targets of mTORC1 (i.e., S6K1 and eEF2) following both modes of exercise. In addition, both modes of exercise induced similar responses at the transcriptional level with the exception of PGC-1α, showing superior expression of this gene with concurrent exercise.

Mechanistically, activation of mTORC1 results in a coordinated signaling cascade to various components within the translational machinery, which when repeated over time has been shown to correlate with muscle hypertrophy in both rodent (6) and human (46) muscle. The regulatory role of mTORC1 in muscle growth was recently confirmed by Goodman and colleagues (28, 29), who in a series of elegantly designed experiments using various genetic mouse models showed that not only is load-induced muscle growth dependent on mTORC1 signaling (28) but activation of this complex is compared with before exercise (P < 0.05) as well as with 1-h post resistance exercise values (P < 0.01; Table 4). On the contrary, both protocols resulted in elevated mRNA levels of TSC2 1 h (P < 0.01) after resistance exercise, but these levels had returned to baseline values by the 3-h time point (Table 4). Both exercise protocols resulted in minor but significant elevations in S6K1 mRNA expression at the 1-h time point (P < 0.05) compared with preexercise levels, although these levels had returned to baseline values in both trials at the 3-h time point (Table 4).

Both exercise protocols induced significant and similar elevations in mRNA expression of REDD1 at the 1-h time point (P < 0.01), although, at 3 h after exercise, mRNA levels of REDD1 had dropped below baseline values (P < 0.01; Fig. 5B). Expression of REDD2 was unchanged after both protocols at the 1-h time point but was significantly reduced in both trials 3 h post resistance exercise (P < 0.01; Fig. 5C). mRNA abundance of c-Myc increased continuously and similarly during recovery in both trials (7-fold at 1 h post vs. 26-fold at 3 h post; P < 0.01) (Fig. 5D).

Expression of PGC-1α increased in both trials at 3 h post resistance exercise but significantly more so in the RE trial (6.5 vs. 4.1-fold in R; P < 0.01) (Fig. 6A). mRNA expression of PGC-1-related coactivator (PRC) was similar in both trials but more pronounced 3 h after resistance exercise compared with the 1-h time point (P < 0.01; Fig. 6B). Both trials resulted in pronounced and similar increases in pyruvate dehydrogenase kinase 4 (PDK4) mRNA expression at the 1-h time point (~7-fold; P < 0.01) as well as the 3-h time point (~8-fold; P < 0.01) after resistance exercise (Fig. 6C).

**DISCUSSION**

The main finding of the present investigation is that endurance exercise performed subsequent to resistance exercise does not blunt growth-related signaling through the mTORC1 pathway in human skeletal muscle. This conclusion is supported by similar and positive alterations of mTOR as well as several downstream targets of mTORC1 (i.e., S6K1 and eEF2) following both modes of exercise. In addition, both modes of exercise induced similar responses at the transcriptional level with the exception of PGC-1α, showing superior expression of this gene with concurrent exercise.

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Table 4. Gene expression related to muscle growth

<table>
<thead>
<tr>
<th>Genes</th>
<th>Exercise</th>
<th>Rest</th>
<th>1 h Post</th>
<th>3 h Post</th>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Time</td>
<td>Exr</td>
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<tr>
<td>Rheb</td>
<td>R</td>
<td>0.64 ± 0.11</td>
<td>0.69 ± 0.09*</td>
<td>0.95 ± 0.15*</td>
<td>*P &lt; 0.05</td>
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<tr>
<td></td>
<td>RE</td>
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<td>0.82 ± 0.14*</td>
<td>0.76 ± 0.10*</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>mTOR</td>
<td>R</td>
<td>2.28 ± 0.50</td>
<td>2.39 ± 0.53</td>
<td>2.10 ± 0.34**</td>
<td>*P &lt; 0.05</td>
<td>NS</td>
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<tr>
<td></td>
<td>RE</td>
<td>1.90 ± 0.34</td>
<td>2.26 ± 0.40</td>
<td>1.40 ± 0.31**</td>
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<td>NS</td>
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<tr>
<td>S6K1</td>
<td>R</td>
<td>1.03 ± 0.17</td>
<td>1.10 ± 0.13*</td>
<td>1.07 ± 0.14*</td>
<td>*P &lt; 0.05</td>
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<td>0.83 ± 0.11*</td>
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<td>R</td>
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<td>0.97 ± 0.09</td>
<td>0.94 ± 0.10**</td>
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<tr>
<td></td>
<td>RE</td>
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<td>1.12 ± 0.14</td>
<td>0.82 ± 0.10**</td>
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<td>TSC1</td>
<td>R</td>
<td>1.55 ± 0.29</td>
<td>1.49 ± 0.19</td>
<td>1.46 ± 0.17**</td>
<td>*P &lt; 0.05</td>
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<tr>
<td></td>
<td>RE</td>
<td>1.43 ± 0.15</td>
<td>1.55 ± 0.15</td>
<td>0.99 ± 0.18**</td>
<td>*P &lt; 0.05</td>
<td>NS</td>
</tr>
<tr>
<td>TSC2</td>
<td>R</td>
<td>1.72 ± 0.34</td>
<td>2.97 ± 1.14*</td>
<td>2.37 ± 0.68*</td>
<td>*P &lt; 0.05</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>RE</td>
<td>1.36 ± 0.24</td>
<td>2.25 ± 0.33*</td>
<td>1.54 ± 0.37*</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are presented as means ± SE for 10 subjects (n = 9 for 3 h post). Muscle was sampled at rest and at 1 and 3 h post resistance exercise. Int, interaction; Exr, exercise; Rheb, ras homolog enriched in brain; mTOR, mechanistic target of rapamycin; S6K1, 70-kDa ribosomal protein S6 kinase 1; hVps34, human vacuolar protein sorting 34; TSC1/2, tuberous sclerosis complex 1 and 2, respectively. *P < 0.05 vs. rest; *P < 0.05 vs. 1 h post. NS, not significant.
sufficient to induce muscle hypertrophy (29). We had initially hypothesized that combining resistance and endurance exercise would impair mTORC1 signaling. However, in contrast to this hypothesis, both modes of exercise induced substantial elevations in mTOR phosphorylation at Ser2448 as well as of its immediate downstream target S6K1 at the Thr389 residue. While mTOR phosphorylation increased 2-fold, the phosphorylation level of S6K1 increased 5-fold at the 1-h time point but continued to increase until 3 h postexercise, reaching 14-fold higher values compared with baseline.

In addition to regulating translation initiation, mTORC1-mediated signaling also stimulates translation elongation by repressing phosphorylation of the eEF2 at Thr359 (51). In the present study, both exercise protocols induced dramatic reductions in eEF2 phosphorylation during recovery, and, in line with the mTOR and S6K1 phosphorylation data, we could not detect any difference in eEF2 phosphorylation between trials. Collectively, these findings indicate that the added endurance exercise does not impose an inhibitory effect on growth-related signaling, neither at the level of translation initiation nor translation elongation.

The reason for the lack of AMPK phosphorylation in this trial is not readily apparent, since we (49) and others (14, 26, 48) have shown that endurance exercise with similar intensity and duration as that used here induces robust elevations in phosphorylation and activity of this kinase. Some studies show that endurance-trained subjects with high VO_{2max} values (i.e., ≥65 ml·min^{-1}·kg^{-1}) may have a diminished AMPK response to endurance exercise (17), especially compared with untrained subjects (55). However, other studies (14, 55) have shown elevated activity and phosphorylation of AMPK in subjects with similar training status (~50 ml·min^{-1}·kg^{-1}) as those involved in this study. In addition, sampling was performed 15 min post endurance exercise (1 h after resistance exercise) in the RE trial. This time point is well within the time frame of expected elevations in AMPK phosphorylation and/or activity as shown previously (21, 49). Thus, the lack of AMPK phosphorylation in the present study is likely related to other factors than those discussed above.

Although activation of AMPK following endurance exercise is well recognized (14, 26, 36, 48, 54), the molecular interplay with mTORC1 in human muscle is much less established. The complexity of these interactions is demonstrated by several studies showing simultaneous increases in AMPK and mTORC1 signaling following endurance (9, 36, 52) and resistance exercise (21, 52) as well as after concurrent exercise (49). Furthermore, even though AMPK phosphorylation was increased in these trials, protein synthesis was elevated (21, 36, 52), a finding that is contradictory to the suggested mechanism of the interference effect (13, 39, 47). Thus, it is unclear whether or not activation of AMPK impairs mTORC1 signaling in human muscle under physiological conditions.

Unexpectedly, at the 3-h time point, phosphorylation of AMPK was actually decreased ~30% compared with baseline values. This reduction was also seen on the phosphorylation levels of ACC, although to a larger extent (~50%). This finding was present in both trials, suggesting it may be related to activation of mTORC1 induced by the resistance exercise protocol. In support of this idea, Atherton and colleagues (4) demonstrated that high-frequency electrical stimulation of rat muscle resulted in pronounced increases in phosphorylation of S6K1 at Thr389 3 h poststimulation, and, at this time point, AMPK phosphorylation at Thr172 was depressed below resting values. Similarly, in a study by Fujita et al. (27), resistance exercise combined with nutrient provision dramatically induced mTORC1 signaling while concomitantly reducing AMPK phosphorylation at Thr172. In contrast, some studies have failed to detect a decrease in AMPK phosphorylation despite elevated phosphorylation of S6K1 (17, 22). Our find-
ings may be related, at least in part, to the intensity and volume of the exercise protocol used here, which was much higher than in the previously mentioned studies.

In the present study, decreased AMPK phosphorylation coincided with maximal phosphorylation of S6K1 at Thr389 in both trials, an association also observed by others (4, 27). Dagon et al. (19) recently demonstrated a direct inhibitory phosphorylation of AMPK at Ser491 by S6K1 in mouse hypothalamic cells, and, although not yet confirmed in skeletal muscle, these findings identify a negative feedback loop in which AMPK is influenced by the mTORC1 pathway. Additional support for a regulatory role of S6K1 on AMPK activity is provided by Aguilar and colleagues (1) who showed increased phosphorylation of the Thr172 residue of AMPK in cultured myotubes of S6K1-deficient mice. Therefore, based on these observations, we propose that prior activation of mTORC1 inhibits the activity of AMPK, which would suggest bidirectional cross talk between these pathways. Furthermore, in the studies showing an inhibitory effect of AMPK on mTORC1 (13, 39, 47), pharmacological activation of AMPK was initiated before determination of mTORC1 signaling, indicating the existence of an order effect.

We also measured gene expression of several positive (hVps34, Rheb, and c-Myc) and negative (TSC1/2, REDD1/2) effectors of mTORC1 signaling, as well as expression of key components (mTOR and S6K1) of this pathway (Table 4). There were only modest fluctuations in mRNA expression of most of these genes, but, most importantly, we could not detect any differences between trials at any time point, in line with the signaling data presented above. Interestingly, the pattern of mRNA expression of the growth-related transcription factor c-Myc mirrored that of S6K1 phosphorylation (Fig. 5D), suggesting that expression of this gene may, at least in part, be regulated by mTORC1 as has been shown in Drosophila (45). These findings show that the lack of inhibition induced by endurance exercise is not only evident at the level of translation initiation and elongation but also at the level of transcription.

In addition to measuring mRNA abundance, we also analyzed total levels of REDD1 protein. Both REDD1 and REDD2 have been identified as negative regulators of mTORC1 signaling under various conditions of cellular stress (24), including energetic stress (44). Although the precise mechanism by which REDD1/2 inhibits mTORC1 signaling remains elusive, in vitro studies have shown that induction of REDD1 occurs within hours following hormonal treatment (25, 41). As such, 3 h post resistance exercise could have been an appropriate time point to detect a potential increase in expression of this protein. However, even though the RE trial arguably induced a greater energetic stress, we could not detect any differences in REDD1 protein levels between trials (Fig. 5A).

In contrast to the other genes examined, concurrent exercise resulted in superior mRNA expression of PGC-1α (Fig. 6A), a molecule shown to have a major regulatory role in mitochondrial biogenesis (38). Although an expected finding, the reason for the differential expression of this gene is not readily obvious, at least from a mechanistic perspective. Induction of PGC-1α has been linked to increased signaling by several upstream effectors such as AMPK, CaMK, and the mitogen-activated protein kinase p38 (38), none of which differed between trials in the present study. In addition to these kinases, mTOR has also been implicated in the induction of PGC-1α (18), yet, phosphorylation of mTOR and of all its immediate downstream targets was similar between protocols. Consequently, these data do not allow for any clear conclusions regarding the involvement of these kinases in the PGC-1α response seen during recovery in the RE trial.

In summary, both single-mode resistance exercise and concurrent exercise resulted in robust and positive alterations of regulatory proteins involved in translation initiation (i.e., mTOR and S6K1) and elongation (eEF2), thus promoting skeletal muscle hypertrophy (6, 28, 29, 46). These data demonstrate that moderate-intensity endurance exercise performed subsequent to a high-intensity high-volume resistance exercise protocol does not inhibit mTORC1 signaling during acute recovery in moderately trained men. This conclusion is further supported by the reduction in AMPK phosphorylation following both modes of exercise, which we propose is due to prior activation of mTORC1 induced by the resistance exercise protocol. This finding suggests that the interference effect between these pathways is bidirectional. We therefore propose that endurance exercise may be included in training regimes aiming to promote muscle growth. However, further studies are required to assess if the acute changes in the signaling pathways examined here fully reflect long-term training adaptations.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: W.A., L.W., E.B., and K.S. conception and design of research; W.A., L.W., M.P., and K.S. performed experiments; W.A., L.W., and M.P. analyzed data; W.A., L.W., M.P., E.B., and K.S. interpreted results of experiments; W.A. prepared figures; W.A. drafted manuscript; W.A., L.W., E.B., and K.S. edited and revised manuscript; W.A., L.W., M.P., E.B., and K.S. approved final version of manuscript.

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