Aerobic Exercise Alters Skeletal Muscle Molecular Responses to Resistance Exercise

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ABSTRACT

LUNDBERG, T. R., R. FERNANDEZ-GONZALO, T. GUSTAFSSON, and P. A. TESCH. Aerobic Exercise Alters Skeletal Muscle Molecular Responses to Resistance Exercise. Med. Sci. Sports Exerc., Vol. 44, No. 9, pp. 1680–1688, 2012. Purpose: This study assessed the influence of an acute aerobic exercise bout on molecular responses to subsequent resistance exercise (RE). Methods: Nine physically active men performed a 45-min one-legged cycle ergometry exercise and 4 × 7 maximal concentric–eccentric knee extensions for each leg 6 h later. Thus, one limb was subjected to aerobic and resistance exercise (AE+RE), and the contralateral limb to resistance exercise (RE) only. Knee extensor peak power was determined. Biopsies were obtained from the m vastus lateralis before (PRE) and 15 min (POST1) and 3 h after RE. Analysis determined glycogen content, mRNA levels (vascular endothelial growth factor, peroxisome proliferator-activated receptor-γ coactivator-1, muscle RING-finger protein-1, atrogin-1, myostatin), and phosphorylated proteins (mammalian target of rapamycin, p70S6 kinase, ribosomal protein S6, eukaryotic elongation factor 2). Results: Peak power was similar in AE + RE and RE. After RE, the time course of glycogen utilization and protein signaling was similar across legs. However, phosphorylation of mammalian target of rapamycin and p70S6 kinase was elevated in AE + RE versus RE (main effect, P < 0.05). Vascular endothelial growth factor and peroxisome proliferator-activated receptor-γ coactivator-1 were higher in AE + RE than in RE at PRE and POST1 (P < 0.05). Myostatin was lower in AE + RE versus RE at PRE and POST1 (P < 0.05) and downregulated after resistance exercise only. Atrogin-1 was higher in AE + RE than in RE at PRE and POST1 (P < 0.05) and decreased after RE in AE + RE. MuscleRING-finger protein-1 was similar across legs. No difference for any marker was evident 3 h after RE. Conclusions: These results suggest that acute aerobic exercise alters molecular events regulating muscle protein turnover during the early recovery period from subsequent RE. Key Words: ENDURANCE, HUMAN SKELETAL MUSCLE, mTOR, MUSCLE POWER, PGC-1α, P70S6K

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any sports calling for speed and power require athletes to incorporate both aerobic and resistance exercise (RE) into their training regimen. However, inconclusive research suggests that favorable skeletal muscle adaptations, produced by resistance training, may be hampered if preceded by aerobic exercise (19,23). More specifically, it has been inferred that increases in speed, strength, power, and muscle size may be reduced with concurrent training (19,21,23). Although it is reasonable to assume that the origin of such an interference effect occurs in the muscle, the specific cellular mechanisms and signaling routes involved remain to be further elucidated.

Chronic aerobic exercise induces morphological and metabolic changes of skeletal muscle promoting improved endurance and fatigue resistance, i.e., increased mitochondrial density, oxidative, and β-oxidation capacity and capillarization (20). It is generally held that RE but not aerobic exercise augments myofibrillar protein accretion (21,35). Thus, long-term resistance training increases muscle fiber size, with concomitantly diminished oxidative enzyme content and capillary and mitochondrial density (35). Although it seems such exercise-specific changes contrast each other, it should be acknowledged that muscle fiber type or myosin heavy chain composition transformation occurs in the same direction regardless of exercise modality (21). Likewise, resistance training regimens relying on large volume (i.e., bodybuilders) may induce capillary proliferation (32) and increased oxidative enzyme activity (37). These adaptations are certainly less robust than changes that are produced with ultimate aerobic exercise programs but indicate that important signaling routes are shared. In support, a recent investigation reported that of 263 genes examined in the rested state, only 21 were differentially expressed in endurance- and strength-trained athletes (33). Nevertheless and given that chronic aerobic exercise and RE aim at and apparently result in divergent skeletal muscle profiles, these two exercise modalities may not be compatible at the cellular level.

Long-term adaptations to exercise training are the consequence of the cumulative effect of repeated exercise bouts leading to changed levels of specific muscle proteins, such
that each bout of contractile activity activates or inhibits molecular events controlling protein synthesis and degradation (30). The activity of p70S6 kinase (p70S6K), the most well-defined effector in the mammalian target of rapamycin (mTOR) signaling pathway, correlates with increased protein synthesis and muscle mass in both animals (3) and humans (34). The peroxisome proliferator–activated receptor-γ coactivator-1 (PGC-1α) is purported as the main regulator of mitochondrial content because it activates key transcription factors involved in mitochondrial biogenesis (25,31). Consequently, it has been put forth that activation of PGC-1α and mTOR signaling pathways are responsible for the specific adaptive responses shown after aerobic and resistance exercise (2). Although it may be that these signaling pathways operate in a selective “switch manner” (2), no clear distinction in exercise-specific signaling pathways has been noted in human skeletal muscle (10,39). Hence, the general and simplified idea of very selective signaling routes operating in response to aerobic exercise or RE should be viewed with caution.

Given the equivocal results regarding mode-specific muscle signaling, knowledge about the effects of acute aerobic exercise on the molecular response to RE is scarce. Rare reports showed molecular interferences when RE was performed near aerobic exercise (8) or repeated sprints (7). Thus, it seemed that consecutive bouts of aerobic and resistance exercise were incompatible with the desired molecular responses resulting from resistance exercise only. Because these studies (7,8) examined cellular responses to concurrent exercise with essentially no recovery between exercise bouts, it is uncertain whether the reported interference was due to the diverse contrac-
tile activity per se or fatigue and incomplete recovery.

Therefore, the purpose of the current study was to explore the effects of aerobic exercise on the acute molecular response supposed to control exercise-specific muscle adaptations to resistance exercise. By subjecting one leg to aerobic and resistance exercise, allowing for restored muscle function between bouts, and the contralateral limb to resistance exercise only, we hypothesized that aerobic exercise would interfere with the molecular response induced by subsequent resistance exercise.

**METHODS**

**General design.** After a 2-wk period of familiarization and baseline testing, the experimental day (Fig. 1) comprised a 45-min one-legged aerobic exercise in the morning (a.m.) and four sets of RE for each leg 6 h later (p.m.). Hence, legs randomly chosen in a counter-balanced manner were subjected to either AE + RE or RE only. Muscle biopsies were obtained from each leg before (PRE) and 15 min (POST1) and 3 h (POST2) after the RE bout. Tissue samples were subsequently analyzed to determine molecular responses.

**Subjects.** Nine healthy male volunteers complied with the study protocol. Subjects (mean ± SD age, height, and body mass: 23 ± 2 yr, 181 ± 6 cm, and 75 ± 6 kg, respectively) were physically active college students who had been involved in recreational aerobic exercise two to three times per week and/or habitual RE one to two times per week for more than a year. They reported a rigid training history and had not been competing at the elite level in any sport, nor had they performed advanced weight training before study commencement. The experimental procedures and potential risks and discomforts associated with participation were explained to the subjects before written informed consent was obtained. The study protocol was approved by the regional ethical review board in Umeå.

**Exercise equipment.** One-legged aerobic exercise was carried out on a cycle ergometer (Fig. 2; model 828E; Monark Exercise AB, Varberg, Sweden) described elsewhere (1,5). This particular exercise device emphasizes and allows for isolated quadriceps femoris muscle use in the concentric mode of knee extension (1). While seated, an adjustable bar linking the ergometer crank and a custom-made lightweight plastic boot worn by the subjects was set individually to allow for knee extensions from about 90° to 175° at completion of the movement. The flexion phase of the action was passive such that the inherent flywheel momentum repositioned the leg. Power and cadence (rpm) was sampled at 2 Hz using a wireless SRM Training System (SRM GmbH, Jülich, Germany) and analyzed using associated software. Resistance exercise, i.e., the seated leg press and knee extension, emphasizing quadriceps muscle use, used non–gravity-dependent devices (YoYo Technology, Inc., Stockholm, Sweden) described elsewhere (36). Briefly, these exercise apparatuses...
use the inertia of a spinning flywheel (\( \approx 4.2 \text{ kg, moment inertia} = 0.11 \text{ kg m}^{-2} \)) offering unlimited resistance during coupled concentric and eccentric actions. Subjects were seated and slightly reclined (hip angle \( \approx 90^\circ \)). The range of motion was from \(-90^\circ\) knee angle to near \(180^\circ\) (full extension). Machine settings, accommodated individually for each subject during familiarization, were maintained through subsequent sessions. By knowing the inertia used, the peak concentric and eccentric power of each repetition was calculated by measuring rotational velocity with aid of a SmartCoach™ encoder and SmartCoach™ data acquisition system (SmartCoach Europe AB, Stockholm, Sweden) with associated Smartcoach™ software (v3.1.3.0). RE protocols using machines prescribed here have shown efficacy in promoting muscle hypertrophy (36). Peak power assessed during 3 d within a week showed a coefficient of variation of 7.3% for both knee extension and the leg press.

**Familiarization and baseline testing.** Subjects reported to the laboratory six times during 2 wk before the actual experiments. The objective of the initial two sessions was to familiarize subjects with the exercises used and customize individual machine settings. The third and fourth sessions comprised baseline tests to assess muscle power and one-legged endurance performance, respectively. After a standardized warm-up, two sets of seven repetitions for each leg and RE mode were performed, beginning with the leg press. Each set began with the AE + RE leg (rest between sets = 90 s). Strong verbal encouragement was used for each repetition calling for maximal effort. The one-legged ergometer incremental test, performed on the fourth visit, determined maximal workload (\( W_{\text{max}} \)). Resistance was increased by 2.5 N every second minute until subject failure to maintain the prescribed cadence of 60 rpm. The last successfully completed load was defined as \( W_{\text{max}} \). Capillary blood (20 \( \mu \text{L} \)) was sampled from the earlobe at rest, every second minute into exercise, and 1 and 3 min after exercise. Samples were placed in a 1-mL hemolyzing solution and subsequently analyzed for lactate (EKF Diagnostics GmbH, Magdeburg, Germany). HR was recorded (Polar Electro Oy, Kempele, Finland) throughout the test and subsequently analyzed using compatible software (Polar ProTrainer 5). RPE (central and local) was obtained every second minute and at exhaustion using the original 6–20 Borg scale. Sessions 5 and 6 used the protocol, i.e., the two RE preceded (6 h) by one-legged cycle ergometer exercise, conducted on the experimental day.

**Aerobic exercise.** In the morning of the experimental day, subjects performed a 40-min continuous one-legged cycle ergometer exercise. The target load was 70% of the \( W_{\text{max}} \) (cadence \( \approx 60 \text{ rpm} \)). After 40 min, workload was increased by \( \approx 20 \text{ W} \), and subjects were requested to continue until failure to maintain the prescribed cranking cadence, which typically occurred within 1–4 min (2 min 43 s). Subjects received real-time feedback via a computer monitor displaying trails or digital numbers of power and cadence. Capillary blood was sampled, and HR and RPE were monitored by using techniques and equipment used to assess \( W_{\text{max}} \).

Blood lactate concentration was analyzed in samples obtained at rest, every 10th minute, and 1 and 3 min after exercise. RPE was obtained every 10th minute and at termination of exercise.

**RE.** In the afternoon, i.e., 6 h after one-legged cycling, unilateral RE, in the order of leg press then knee extension, was performed. For each leg, two sets of seven repetitions (90-s rest between sets) were performed for each exercise, beginning with the AE + RE leg. Subjects were supervised and instructed to perform each repetition with maximal effort and verbally encouraged throughout each set.

**Diet/exercise control.** Subjects were requested to refrain from any physical activity using the lower limbs for a minimum of 24 h before the baseline tests and throughout the study. A standardized meal (pasta, tomato sauce, and juice) consisting of 2.21 g CHO kg\(^{-1}\) body weight (bw), 0.22 g protein kg\(^{-1}\) bw, and 0.04 g fat kg\(^{-1}\) bw was provided at \(-8:00 \text{ p.m.} \) the night before the experimental day. Subjects also had a standardized breakfast (1.01 g CHO kg\(^{-1}\) bw, 0.31 g protein kg\(^{-1}\) bw, and 0.24 g fat kg\(^{-1}\) bw) 1 h before the aerobic exercise session and lunch (2.02 g CHO kg\(^{-1}\) bw, 0.62 g protein kg\(^{-1}\) bw, and 0.49 g fat kg\(^{-1}\) bw) consumed \(-3 \text{ h} \) before RE. These meals consisted of commercial energy drinks (Ensure Plus®; Abbott Laboratories BV, Zwolle, The Netherlands). Water was allowed ad libitum at any time during the intervention.

**Muscle biopsies.** Using the percutaneous needle biopsy technique, muscle samples (~200 mg) were obtained from the m vastus lateralis of each leg (beginning with the AE + RE leg) at PRE, POST1, and POST2. After local anesthesia administered to the skin and muscle fascia, biopsies were taken from the midportion of the muscle through three separate incisions, 20 mm apart moving distal to proximal using the 5-mm Bergström needle with suction applied. Samples were visually inspected, and excess blood, fat, and connective tissue were removed before being frozen in liquid nitrogen and stored at \(-80^\circ\text{C} \) for subsequent analysis.

**Glycogen.** One aliquot of freeze-dried powdered muscle (~3 mg) was digested in 0.5 M of KOH-ethanol and cysteine (5 mM) at 60°C and neutralized with HCl. Glycogen was hydrolyzed enzymatically to free glucose and assayed by fluorometry (18).

**RNA isolation, reverse transcription, and real-time polymerase chain reaction.** About 20 mg of frozen muscle tissue was homogenized using TRIzol® (Invitrogen Life Technologies, Carlsbad, CA) and total RNA was subsequently extracted. Reverse transcription into cDNA was performed on 1 \( \mu \text{g} \) of total RNA from each sample using the High Capacity Reverse Transcription Kit (Applied Biosystems, Foster City, CA) in a total volume of 20 \( \mu \text{L} \). Subsequently, real-time polymerase chain reaction (PCR) was performed on the ABI-PRISMA 7700 Sequence Detector System (Perkin-Elmer Applied Biosystems, Foster City, CA). The PCR reaction mix for all genes of interest consisted of 4.5 \( \mu \text{L} \) of the diluted (1:100) complementary DNA template, 5 \( \mu \text{L} \) of the 2× TaqMan® PCR Master Mix (Life
Technologies, Invitrogen, Carlsbad, CA), and 0.5 μL of a gene-specific primer/probe set. The cycling parameters were 2 min at 50°C and 10 min at 90°C followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. TaqMan® primers and probes for atrogin-1 (Hs00369714_m1), muscle RING-finger protein-1 (MuRF-1, Hs00822397_m1), myostatin (Hs00193363_m1), PGC-1α (Hs01016724_m1), and vascular endothelial growth factor (VEGF, Hs99999070_m1) were derived from the TaqMan® Gene Expression Assays from Applied Biosystems. No systematic changes occurred in the housekeeping gene (GAPDH, Hs99999905_m1). Samples for each individual were analyzed in a single-run assay. Relative changes in gene expression levels were determined using the 2-ΔΔCt method, which provides a ratio of the number of the target gene to the housekeeping gene.

**Protein extraction and Western blot.** For each sample, 30 mg of frozen muscle was homogenized in 1 M of HEPES (pH 7.4) buffer containing 20% Triton X-100 (Sigma, St. Louis, MO), 100 mM of EDTA, 500 mM of EGTA, 1 M of MgCl2, 500 mM of β-glycerophosphate, 0.1 M of DTT, 200 mM of PMSF, 1 mM of Na3VO4, leupeptin (10 mg·mL−1), aprotimein (10 mg·mL−1), and 1× complete protease inhibitor cocktail (Roche Diagnostics GmbH, Mannheim, Germany). The homogenate was gently rotated at 4°C for 60 min followed by centrifugation at 4°C for 10 min (15,000g). The supernatant was collected, and protein concentrations were subsequently determined by the Bradford protein assay. The samples (40 μg of protein) and a fluorescent protein ladder were run on 7.5% or 10% SDS gels. All concentrations were subsequently determined by the Bradford protein assay. The samples (40 μg of protein) and a fluorescent protein ladder were run on 7.5% or 10% SDS gels. All samples from any subject were run on the same gel. After electrophoresis, gels were equilibrated in transfer buffer for 30 min, and proteins were transferred (90 V for 90 min) to PVDF membranes. The membranes were blocked at room temperature for 30 min using a fluorescent blocking buffer (Millipore, Billerica, MA). Incubation with primary antibodies was performed overnight at 4°C. The primary antibodies used were phospho-mTOR (Ser2448, 1:500), phospho-ribosomal protein S6 (rpS6) (Ser235/236, 1:2000), and phospho-eukaryotic elongation factor 2 (eEF2) (Thr56, 1:1500) from Cell Signaling Technology (Beverly, MA) and phospho-p70S6K (Thr389, 1:1000) from Santa Cruz Biotechnology (Santa Cruz, CA). Membranes were then washed 4 × 5 min in PBST (0.1%) before incubation with IRDye® secondary antibody (LI-COR Biosciences, Cambridge, United Kingdom) for 60 min in room temperature in 1:10,000 or 1:20,000 concentration. A final wash (4 × 5 min in PBST and 2 × 5 min in PBS) was performed before membranes were scanned with the Odyssey® Sa Infrared Imaging System (LI-COR Biosciences) and blots quantified using a public domain software (ImageJ, National Institutes of Health, Bethesda, MD). Phosphorylated proteins were expressed relative to total α-tubulin abundance (1:20,000; Cell Signaling Technology) to ensure equal protein loading.

**Data analysis.** Peak power for AE + RE versus RE, averaged across sets and muscle actions, was analyzed using paired t-tests. Glycogen content, gene expression, and protein phosphorylation were examined using two-way ANOVA (leg × time). Significant interactions and main effects were further examined with simple effect tests and pairwise comparisons when applicable. Data distribution was visually examined on histograms, and analyses on some positively skewed variables were done using log-transformed data. The false discovery rate procedure was used to correct the critical significance level for multiple post hoc comparisons (12). The level of significance was set at 5% (P < 0.05). All statistical analyses were performed using SPSS version 18 (SPSS, Inc., Chicago, IL). Data are presented as means ± SD.

**RESULTS**

**Baseline Testing**

Time to exhaustion in the Wmax test for one-legged cycling averaged 10 min 12 s (±1 min 40 s). The average power during the last completed workload (Wmax) was 53 ± 14 W. At completion of the test, HR averaged 162 ± 16 bpm, blood lactate concentration was 5.7 ± 1.6 mmol·L−1, and muscle exertion was maximal. Peak power performance during the baseline test (Table 1) showed no difference across legs (P > 0.05).

**Aerobic Exercise**

HR during 40-min one-legged cycling averaged 124 ± 17 and increased to 153 ± 17 bpm during the final increment. At exercise completion, HR was 164 ± 16 bpm. Blood lactate concentration during exercise averaged 3.2 ± 1.1 mmol·L−1. One and three minutes after exercise, blood lactate was 4.6 ± 1.4 and 4.5 ± 1.4 mmol·L−1, respectively. RPE rose in a linear fashion throughout the 40-min exercise. After 10 min, RPE averaged 11 ± 2 (central) and 14 ± 2 (local). The ratings were 14 ± 1 and 17 ± 1, respectively, after 40-min exercise and 17 ± 2 and 20 ± 0 at exercise completion.

**Peak Power**

Peak power for both knee extension and leg press was similar (P > 0.05) for AE + RE and RE (Table 1).

**Muscle Glycogen**

There was no leg × time interaction for glycogen content. Thus, glycogen decreased in response to RE in both legs (F = 20.4, P < 0.0005), with similar rates of utilization (79 vs 82 mmol·kg−1 dry weight from PRE to POST1 in AE + RE and RE, respectively). However, glycogen content (Table 2)

**TABLE 1.** Unilateral peak concentric (CON) and eccentric (ECC) power (W) in knee extension and leg press at baseline and during the experimental bout.

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<tr>
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<th>Baseline</th>
<th>Experimental Bout</th>
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<tr>
<td></td>
<td>CON</td>
<td>ECC</td>
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<tr>
<td><strong>Knee extension</strong></td>
<td></td>
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<tr>
<td>AE + RE</td>
<td>315 ± 70</td>
<td>321 ± 70</td>
</tr>
<tr>
<td>RE</td>
<td>336 ± 63</td>
<td>352 ± 62</td>
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<tr>
<td><strong>Leg press</strong></td>
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</tr>
<tr>
<td>AE + RE</td>
<td>221 ± 36</td>
<td>250 ± 31</td>
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<tr>
<td>RE</td>
<td>235 ± 50</td>
<td>256 ± 43</td>
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Values denote means ± SD in legs subjected to AE + RE and RE only.
was overall lower in AE + RE compared with RE (main effect: $F = 6.5, P = 0.034$).

**Gene Expression**

**PGC-1α.** There was a leg $\times$ time interaction ($F = 18.1, P < 0.0005$) in PGC-1α expression. Levels were higher in AE + RE at PRE (4.3-fold) and POST1 (2.8-fold) compared with RE ($P < 0.01$; Fig 3A). There was no change over time in AE + RE. The RE leg showed a 2.5-fold increase in PGC-1α from POST1 to POST2 ($P = 0.01$).

**VEGF.** There was a leg $\times$ time interaction ($F = 31.6, P < 0.0005$) in VEGF expression. Levels were higher in AE + RE at PRE (2.0-fold) and POST1 (1.6-fold) compared with RE ($P < 0.0005$; Fig. 3B). The AE + RE leg showed a 1.4-fold decrease in VEGF from POST1 to POST2 ($P = 0.004$). No change over time occurred in RE.

**MuRF-1.** Expression of MuRF-1 over time was similar in AE + RE and RE (interaction: $P = 0.077$), with a modest decrease over time (main effect: $F = 4.0, P = 0.038$) evident (Fig. 3C).

**Atrogin-1.** There was a leg $\times$ time interaction ($F = 5.7, P = 0.013$) in atrogin-1 expression. Levels were higher in AE + RE at PRE (1.3-fold) and POST1 (1.4-fold) compared with RE ($P < 0.05$; Fig. 3D). Within the AE + RE leg, atrogin-1 decreased over time ($P = 0.001$), ~60% from PRE to POST2. No change over time occurred in RE.

**Myostatin.** There was a leg $\times$ time interaction ($F = 5.3, P = 0.017$) in myostatin expression. Levels were lower in

| TABLE 2. Glycogen content (mmol kg$^{-1}$ dry weight) at PRE, POST1, and POST2 of resistance exercise with (AE + RE) or without (RE) preceding aerobic exercise. |
|-----------------|----------------|----------------|
|                 | PRE            | POST1          |
| AE + RE*        | 365 ± 95       | 286 ± 95       |
| RE**            | 405 ± 42       | 323 ± 66       |
| POST2           | 300 ± 51       | 340 ± 56       |

Data are means ± SD.  
* Significant effects ($P < 0.05$) for leg.  
** Significant effects ($P < 0.05$) for time.
AE + RE at PRE (2.9-fold) and POST1 (2.4-fold) compared with RE ($P < 0.01$; Fig. 3E). No change over time occurred in AE + RE. Within the RE leg, myostatin decreased over time ($P = 0.02$) ~ 60% from PRE to POST2.

**Protein Phosphorylation**

**mTOR.** There was no leg × time interaction in mTOR phosphorylation (Fig. 4A). However, signaling was greater in AE + RE compared with RE (main effect: $F = 13.7, P = 0.006$), but no pairwise differences were detected after post hoc comparisons. There was no significant change over time.

**p70S6K.** There was no leg × time interaction in p70S6K signaling (Fig. 4B). However, phosphorylation was greater in AE + RE compared with RE (main effect: $F = 8.1, P = 0.022$). Yet, post hoc tests showed no pairwise differences. There was a trend toward a main effect of time ($P = 0.093$) because of the increase of p70S6K phosphorylation after RE in both legs (range = 1.4- to 1.8-fold).

**rpS6.** Phosphorylation of rpS6 remained unchanged over time, and there was no interaction with exercise condition. However, there was a trend ($P = 0.075$) toward greater phosphorylation in the AE + RE leg (Fig. 4C).

**eEF2.** Phosphorylation of eEF2 was unchanged over time for both legs, and no difference across legs was evident (Fig. 4D).

**DISCUSSION**

The interest in this novel study arose from earlier observations inferring that aerobic exercise may hamper established skeletal muscle responses prompted by RE. We hypothesized that acute muscle signaling and gene expression, associated with myogenic or proteolytic activity, would be compromised after RE if preceded by aerobic exercise. As it turned out, concurrent exercise elicited greater mTOR and p70S6K phosphorylation compared with RE. Although these differences were modest, if anything, they indicate that translational capacity was reinforced rather than compromised by the AE + RE intervention. In parallel, myostatin was suppressed for longer time in AE + RE, with no obvious sign of exacerbated protein degradation. Thus, in contrast to the posted hypothesis, it seems that concurrent AE + RE may enhance skeletal muscle anabolic environment.

To provide an aerobic stimulus, we used unilateral knee extensor exercise (1). Although it should be acknowledged...
that this one-legged insult was not intended to maximize cardiovascular stress (i.e., HR averaged ~120–130 bpm to attain ~80% of age-predicted maximal HR at exhaustion), we believe this particular exercise paradigm presents an aerobic stimulus realized in athletic sports training by offering a highly intense and prolonged challenge at the muscle cell level. This is supported by the marked increase in plasma lactate concentration, despite the relatively small muscle mass involved, and strong locally perceived exertion during exercise. Along with these manifestations of muscle metabolic stress and in support of previous works (16,31), PGC-1α and VEGF showed increased expression after recovery. Not less importantly, the current model allows for intraindividual comparisons. Previously, protein turnover was reported unaltered in the rested leg after aerobic exercise performed by the contralateral limb (29). Similarly, unilateral RE increased p70S6K signaling but had no effect on the nonexercised limb (17). Therefore, we are confident that any difference demonstrated across legs truly reflects a response imposed by the current intervention.

RE, as conducted here, offers unlimited resistance with coupled eccentric–eccentric actions while providing brief eccentric overload. Short-term training interventions using this approach result in robust muscle hypertrophy (36). Exercise using eccentric muscle actions also produces a more prominent gene expression pattern (15) and enhanced anabolic signaling (14) than exercise using concentric actions. Consequently, the RE protocol prescribed here should have provoked the desired alterations in the cellular milieu of the muscle under study. In an attempt to further optimize the anabolic response, we examined subjects in the fed rather than the fasted state and allowed for 6-h recovery before the RE session. Collectively, the paradigm, which aimed at examining quadriceps-specific muscle adaptations to concurrent AE + RE, is believed to mimic the conditions experienced in real-life athletic sports training.

Enhanced translational capacity mediates RE-induced increase in muscle protein synthesis. In particular, the activation of mTOR signaling and its downstream effectors p70S6K and rpS6 control translational efficiency. Indeed, RE was shown to activate this pathway (13,28) to boost muscle protein synthesis and eventually lead to increased muscle size (34). Also, eEF2 may be deactivated during recovery from RE (13) to favor enhanced translation elongation and protein turnover. Both mTOR and p70S6K showed greater phosphorylation in response to AE + RE than RE. Although the time course for changes in these proteins was similar across legs, the significant main effect of leg indicated that the activity was sustained at least during 3-h recovery from RE. This contrasts findings by Coffey et al., reporting compromised molecular events when aerobic (8) or sprint (7) exercise bouts preceded RE. Given that only 15-min recovery was implemented between the different modes, the hampered molecular response could have been attributed to residual fatigue (7). Using a protocol with an equally short recovery, no difference in muscle protein synthesis was reported after RE with or without preceding aerobic exercise (5). Given the ambiguity, it is tempting to propose that complete recovery before RE may be critical for prolonged enhanced signaling favoring increased muscle protein synthesis.

At the transcriptional level, increased expression of PGC-1α (31) and VEGF (16), reflecting mitochondrial biogenesis and angiogenesis, respectively, occurs in response to aerobic exercise. Commensurate with these findings is the increased PGC-1α and VEGF mRNA in AE + RE shown here. However, in contrast to the notion that PGC-1α expression responds exclusively to endurance-type exercise (2,9), it was also increased 3 h after RE. This marked increase of PGC-1α in response to no more than 28 forceful muscle actions is a secondary yet intriguing and novel finding of the current study. Although this would designate PGC-1α as a master regulator of several cell processes involved in exercise-induced adaptations (26), it is worth noting that VEGF abundance responded to aerobic exercise only.

There is compelling evidence that the ubiquitin ligase proteins MuRF-1 and atrogin-1 regulate muscle protein breakdown. Atrogin-1 expression either decreased (27,28) or showed no change [current study, (9,40)] in response to RE, whereas aerobic exercise seemed to increase atrogin-1 expression in both trained (9) and moderately trained (27) subjects. In AE + RE, atrogin-1 abundance was greater compared with RE. As a consequence, atrogin-1 expression was downregulated after RE in AE + RE but not in RE. It therefore remains open for speculation if the responses to AE + RE infer greater induction of protein degradation or simply reflect events necessary for tissue remodeling. Albeit MuRF-1 expression increases after acute RE (27,28,40), it seems that only a few exercise bouts attenuate this increase (28). In fact, trained subjects showed unaltered MuRF-1 expression 3 h after RE. Thus, the modest decrease in MuRF-1 expression reported here is consistent with these findings. Collectively, the current investigation offers little or no support for accentuated proteasome-dependent protein breakdown.

Myostatin inhibits muscle hypertrophy, mainly by suppressing skeletal muscle protein synthesis rather than promoting protein breakdown (38). The current study suggests that both aerobic exercise and RE downregulate myostatin expression, with no apparent additive effect resulting from combining the two exercise modalities. Indeed, both aerobic exercise (27) and RE (27,28) may diminish myostatin expression. Thus, contractile activity per se, rather than any specific mode of exercise, seems to stimulate downregulation of myostatin. However, within the window of adaptation, it could be argued that the prolonged time of myostatin suppression in the AE + RE leg would benefit molecular events eventually leading to positive muscle protein turnover balance.

The present data suggest that aerobic exercise can precede RE on the same day without compromising in vivo muscle power. This contrasts other studies reporting impaired ability to produce maximal force of previously exercised muscles (4,22). Certainly, the time course for recovery is critical to avoid residual fatigue and allow for glycogen repletion and
normalization of other metabolic changes resulting from previous exercise. Thus, when using rest ranging from 8 to 32 h, muscle function could be restored (24). Apparently, the 6-h recovery with nutritional provision was sufficient to restore in vivo muscle power in the current study. Thus, although low glycogen content may blunt the anabolic signaling responses to acute RE (11), it seems the protocol and design used here ensured functional (average peak power) recovery from aerobic exercise. Hence, basal glycogen content and the rate of glycogen utilization during RE were similar in AE + RE and RE. This provides evidence for almost comparable energy status and muscle use in the two conditions.

In summary, the current study examined the influence of acute aerobic exercise on the adaptive skeletal muscle molecular responses resulting from subsequent RE. By measuring in vivo muscle function, glycogen utilization, and cellular markers for protein turnover, we addressed important issues pertinent to concurrent exercise. A novel finding was that the scheduling of RE 6 h after aerobic exercise did not compromise signaling of mTOR-related proteins. This was accompanied by altered gene expression levels, indicating favorable protein turnover during the early recovery period. Thus, it seems the diverse exercise modes used in the current study can successfully be scheduled on the same day without compromising performance or vital molecular responses controlling protein turnover. However, it remains to be shown if cumulative exercise training, using the current paradigm, modifies chronic skeletal muscle adaptations, i.e., muscle size and strength and power-related performance, compared with resistance training.

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