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Concurrent resistance and aerobic exercise stimulates both myofibrillar and mitochondrial protein synthesis in sedentary middle-aged men

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Concurrent resistance and aerobic exercise stimulates both myofibrillar and mitochondrial protein synthesis in sedentary middle-aged men. Eight subjects (age 53.3 ± 1.8 yr; body mass index 29.4 ± 1.4 kg·m⁻²) randomly completed 8 40 min cycling at 55% peak aerobic power output (AE), or consecutively 50% of the RE and AE trials (CE). Biopsies were obtained (during a primed, constant infusion of L-[ring-¹³C₅]phenylalanine) while fasted, and at 1 and 4 h following postexercise ingestion of 20 g of protein. All trials increased mitochondrial FSR above fasted rates (RE 1.3-fold; AE = 1.5; CE = 1.4; P < 0.05), although only CE (2.2) and RE (1.8) increased myofibrillar FSR (P < 0.05). At 1 h postexercise, phosphorylation of Akt on Ser473 (CE = 7.7; RE = 4.6) and Thr308 (CE = 4.4; RE = 2.9), and PRAS40 on Thr246 (CE = 3.8; AE = 2.5) increased (P < 0.05), with CE greater than AE for Akt Ser473-Thr308 (4.6) and Thr308 (3.8; AE = 2.1; P < 0.05) and 4 ring-¹³C₅phenylalanine) while fasted, and at 1 and 4 h following postexercise ingestion of 20 g of protein. All trials increased mitochondrial FSR above fasted rates (RE 1.3-fold; AE = 1.5; CE = 1.4; P < 0.05), although only CE (2.2) and RE (1.8) increased myofibrillar FSR (P < 0.05). At 1 h postexercise, phosphorylation of Akt on Ser473 (CE = 7.7; RE = 4.6) and Thr308 (CE = 4.4; RE = 2.9), and PRAS40 on Thr246 (CE = 3.8; AE = 2.5) increased (P < 0.05), with CE greater than AE for Akt Ser473-Thr308 (4.6) and greater than RE for PRAS40 (P < 0.05). Despite increased phosphorylation of Akt-PRAS40, phosphorylation of mammalian target of rapamycin (mTOR)Ser2448 was unchanged (P > 0.05), while rpS6 (Ser235/236) increased only in RE (10.4) (P < 0.05). CE and AE both resulted in increased peroxisome proliferator receptor-γ coactivator 1-α (PGC1α) expression at 1 h (CE = 2.9; AE = 2.8; P < 0.05) and 4 h (CE = 2.6; AE = 2.4) and PGC1β expression at 4 h (CE = 2.1; AE = 2.6; P < 0.05). These data suggest that CE-induced acute stimulation of myofibrillar and mitochondrial FSR, protein signaling, and mRNA expression are equivalent to either isolate mode (RE or AE). These results occurred without an interference effect on muscle protein subfractional synthesis rates, protein signaling, or mRNA expression.

untrained adults; muscle protein synthesis; anabolic protein signaling; gene expression

THE AGE-RELATED DECLINE IN skeletal muscle mass and strength may relate to a reduced intrinsic capacity for the synthesis of myofibrillar proteins (2). Additionally, there appears to be a reduction in mitochondrial protein synthesis rates in aging muscle (38), which may, in part, lead to a reduction in mitochondrial content. Thus the reduction in the turnover of specific muscle protein pools could ultimately lead to an age-related decline in muscle quality and function (17, 38). Such a reduction could promote the progression of subclinical abnormalities such as insulin resistance and atherosclerosis (3, 27). Exercise is an effective stimulus, irrespective of age, to increase myofibrillar and mitochondrial protein synthesis rates (6, 17, 43). Consequently, exercise is commonly recommended for the prevention of diabetes, cardiovascular disease, and age-related sarcopenia (27, 28).

The combination of resistance exercise (RE) and aerobic endurance exercise (AE), termed concurrent exercise (CE), is currently recommended to middle-aged individuals (~40–65 yr) who are at increased risk for these subclinical abnormalities and chronic diseases (21). However, it has been speculated that sequential performance of RE and AE during a single exercise session could result in “interference” of the molecular signals involved in the genetic and/or translational regulation of myofibrillar and mitochondrial protein synthesis (30, 34). Such signal antagonism could result in an attenuation of the hallmark adaptations of RE (e.g., muscle size) and AE (e.g., oxidative capacity) after independent training (30, 34). To date, measurement of the acute phenotypic responses of myofibrillar and mitochondrial protein synthesis rates, intramuscular protein signaling, and muscle mRNA expression has not been concurrently investigated in all three modes of exercise. Moreover, in sedentary middle-aged men, no study has substantiated whether CE offers lesser, equivalent, or even a greater acute adaptive molecular response compared with isolated bouts of RE or AE.

The purpose of the current study was to examine the acute effects of RE, AE, or CE on myofibrillar and mitochondrial protein synthesis rates, intramuscular signaling protein phosphorylation, and mRNA expression in sedentary middle-aged men. We hypothesized that exercise mode-specific responses would be present, including increased Akt-mammalian target of rapamycin (mTOR)-ribosomal protein S6 kinase (S6K) signaling, myogenic expression, and myofibrillar protein synthesis rates after RE and increased adenosine monophosphate-activated protein kinase (AMPK), p38 mitogen-activated protein kinase (MAPK) signaling, peroxisome proliferator recep-
tor-γ coactivator 1-α (PGC1α) expression, and mitochondrial protein synthesis after AE (14, 15). Based on previous research (14, 15), it was further hypothesized that myofibrillar and mitochondrial protein synthesis rates, intramuscular signaling protein phosphorylation, and muscle mRNA expression responses would be diminished in response to CE incumbent with a molecular interference compared with response to AE and RE alone.

**MATERIALS AND METHODS**

**Subjects.** Eight sedentary middle-aged men (Table 1) were recruited for this study. Sedentary status was defined as no planned or incidental regular pattern of physical activity or exercise involving more than one exercise session ≥30 min per week for the preceding year before study involvement. At baseline, subjects were prescreened by a physician and were free from known or diagnosed diabetes, cardiovascular disease, hepatic or renal disorders, and any other potentially confounding medical conditions. All subjects were free from orthopedic limitations and were given oral and written information regarding experimental procedures and potential risks before giving their informed consent to participate in the study. This study was approved by the University of Auckland Human Subjects Ethics Committee and conformed to standards for the use of human subjects in research as outlined in the fifth revision of the Declaration of Helsinki.

**Experimental protocol.** Following study recruitment and initial prescreening, all subjects underwent anthropometric measures (height, mass, waist, and hip girth) and a supine whole-body dual-energy X-ray absorptiometry scan (model DPX+ with software version 3.6y; GE-Lunar, Madison, WI) for estimation of absolute fat mass and fat-free mass. During this visit, subjects also completed familiarization procedures in the Exercise Science Laboratories, where all exercise testing and exercise trial procedures were explained, demonstrated, and practiced. One week later, subjects returned to the laboratory and underwent (in order; separated by ≥30 min) one repetition-maximum (1RM) strength testing of knee extensor quadriceps muscle group with machine-based leg extension exercise (Fitness Works, Auckland, New Zealand) and incremental graded exercise testing (GXT) on a cycle ergometer (Velotron; RacerMate, Seattle, WA). The GXT commenced at an initial exercise intensity of 2.0 W/kg body mass for 150 s, increasing by 50 W for 150 s for the second stage, and increasing by 25 W every 150 s for subsequent stages until volitional exhaustion to determine peak oxygen consumption (V\textsubscript{O}\textsubscript{2peak}) and the power output associated with V\textsubscript{O}\textsubscript{2peak} (29). Pulmonary gas exchange was measured by determining O\textsubscript{2} and CO\textsubscript{2} concentrations and ventilation to calculate V\textsubscript{O}\textsubscript{2} using a calibrated metabolic gas analysis system (Moxus modular oxygen uptake system; AEI Technologies, Pittsburgh, PA).

Following 1 wk of recovery, each subject returned to the laboratory to complete three exercise trials, each separated by 1 wk. In a randomized counterbalanced fashion, subjects completed an RE trial consisting of eight sets of eight repetitions of machine-based leg extension exercise at 70% of 1RM, with sets separated by 150-s rest; an AE trial involving 40 min of stationary ergometer cycling at 55% of peak aerobic power output; and a CE trial that comprised 50% of the RE and AE trials. For CE, subjects first completed four sets of eight repetitions of machine-based leg extension exercise at 70% of 1RM (with 150-s rest) and, immediately after the fourth set, completed 20-min stationary ergometer cycling at 55% of peak aerobic power output. Machine and ergometer settings documented during baseline testing were, respectively, standardized for the RE and CE trials (seat height position; seat backrest position, and lever arm positioning) and AE and CE trials (ergometer seat height and handlebar height). During cycling, heart rate (Vantage NV, Polar, Finland) was recorded every 5 min, and pulmonary gas exchange was measured at 5 and 15 min (Moxus modular oxygen uptake system; AEI Technologies). Rating of perceived exertion (RPE; CR10 scale) was recorded following each set of leg extension exercise, every 5 min during cycling exercise, and 10 min postsession (session RPE) for all trials.

For 3 days before the first exercise infusion trial, subjects refrained from physical activity. Dietary intake (both normal diet and additional carbohydrate) and timing of intake was documented and repeated before the remaining two exercise protocols. After arriving at the laboratory at ~0700, and following an ~10-h overnight fast (Fig. 1),
body mass was obtained and subjects rested on a bed while an 18-gauge catheter was inserted into a dorsal hand vein. A 0.9% saline drip was coupled to the catheter to permit repeated blood sampling, of which occurred while the hand was placed in a box heated at ~65°C to ensure the collection of arterialized-venous blood (16). Following the collection of a baseline blood sample, a second catheter was inserted in the contralateral arm for the primed constant infusion (PHD 2000; Harvard Apparatus, Natick, MA) of L-[ring-13C5]phenylalanine (prime: 2 μmol·kg⁻¹·h⁻¹; 0.05 μmol·kg⁻¹·min⁻¹; Fig. 1), which was passed through a 0.2-μm filter. During the first trial, a 3 h steady-state infusion period was observed before exercise initiation, while subjects rested on the bed and blood samples were drawn at 1, 2, and 3 h following infusion commencement (9). Subsequently, a single muscle biopsy was obtained (vastus lateralis) at 3 h to measure fasting rates of muscle protein synthesis (fast) and further served as a preexercise reference for changes of intramuscular protein signaling and gene expression (9).

After biopsy collection (~5–10 min) in trial 1, or 30 min after initiating the primed constant infusion (trials 2 and 3), subjects completed the randomized exercise trials. Immediately postexercise, a blood sample was drawn and subjects consumed a drink containing 20 g whey protein isolate (Fonterra Alacen-895-I, Auckland, New Zealand; Fig. 1) to ensure amino acid substrate did not become limiting and thus maximized the potential for detectable increases in exercise-mediated rates of muscle protein synthesis (32). To minimize disturbances in isotopic equilibrium, the drinks were enriched to 4% with tracer according to a measured phenylalanine content of 3.5% in the whey protein; this approach is explained in detail elsewhere (9). Following drink consumption, subjects rested on the beds while further blood samples were drawn at 30-min intervals to measure insulin and glucose responses. Additionally, muscle biopsies were collected at 1 and 4 h postexercise. We chose to collect the biopsy at 1 h postexercise as this time point has been reported to be ideal to capture peak phosphorylation (12) of many of the studied intramuscular signaling proteins examined within our current study. Muscle biopsies were blotted on filter paper, and any visible fat or connective tissue was removed before prompt immersion in liquid nitrogen and storage at ~80°C.

Blood analyses. Plasma insulin was measured in triplicate using a commercially available immunoassay kit (Milliplex Human Metabolic Hormone Panel; Millipore, Billerica, MA). Plasma glucose was measured in triplicate using a standard enzymatic-colorimetric assay (Stanbio Glucose LiquiColor, Stanbio Laboratory, Boerne, TX).

Muscle protein synthesis. A piece of wet muscle (~100 mg) was homogenized with a Dounce glass homogenizer on ice in an ice-cold homogenizing buffer (10 μl/mg; 1 M sucrose, 1 M Tris-HCl, 1 M KCl, and 1 M EDTA) supplemented with a Complete Mini, protease inhibitor and phosphatase cocktail tablets (PhosSTOP; Roche Applied Science, Mannheim, Germany) per 10 ml of buffer. The myofibrillar and mitochondrial enriched protein fractions were isolated as described in our previous work (6). Free amino acids from myofibrillar and mitochondrial enriched fractions were purified using cation-exchange chromatography (Dowex 50WX8–200 resin; Sigma-Aldrich) and converted to their heptafluorobutyric derivatives for analysis by gas chromatography combustion-isotope ratio mass spectrometry (GC-C-IRMS; Hewlett Packard 6890; IRMS model Delta Plus XP; Thermo Finnigan, Waltham, MA). Muscle intracellular amino acids (IC) were extracted from a separate piece of wet muscle (~20 mg) with ice-cold 0.6 M PCA as previously described (6). The muscle free amino acids (IC) were purified by cation-exchange chromatography and converted to their heptafluorobutyric derivatives before analysis by GC-MS (models 6890 GC and 5973 MS; Hewlett-Packard, Palo Alto, CA) as previously described (32).

Western blotting procedures. Muscle biopsy samples (~30 mg) were homogenized in ice-cold lysis buffer [1% (vol/vol) NP-40; 10% (vol/vol) glycerol; 137 mmol/l NaCl; 20 mmol/l Tris-HCl, pH 7.4; 4 μg/ml aprotinin; 4 μg/ml leupeptin; 1 μmol/l 4-(2-aminoethyl) benzenesulfonfluoride; 4 μg/ml pepstatin; 10 mmol/l EDTA; 1 μmol/l EGTA, pH 8.0; 20 mmol/l NaF; 1 mmol/l Na pyrophosphate; and 1 mmol/l vanadate]. Following homogenization, extracted proteins were quantified using a BCA protein assay kit (Pierce, Auckland, New Zealand) and 100 mg of protein were boiled and vortexed at 99°C for 7 min. Prepared lysates were then separated by SDS-PAGE and transferred to polyvinylidene difluoride membranes. After blocking for 60 min at room temperature in 5% (wt/vol) BSA/0.1% (vol/vol) Tween-20 in TBS, membranes were incubated overnight at 4°C on a rocker with polyclonal antibodies (1:1,000; Cell Signaling Technologies, Auckland, New Zealand) specific for phosphorylation of Akt on Ser473 and Thr308, PRAS40 on Thr246, mTOR on Ser2448, p70S6K on Thr389, AMPK on Thr172, MAPK on Thr180/Tyr182, rps6 on Ser235/236, and pS6K2 on Thr389/Ser405, detecting bands were quantified by densitometry (Multi-gauge v3.0; UVP Inc., Upland, CA) and enhanced chemiluminescence (ECL-Plus; Amsterdam Biosciences, Auckland, New Zealand). Detection was made using a phosphorimager (FLA 4000; Fujiﬁlm, Valhalla, NY), and detected bands were quantiﬁed by densitometry (Multi-gauge v3.0; Fujiﬁlm). The membranes were then stripped (2% (wt/vol) SDS, 100 mmol/l β-mercaptoethanol, and 50 mmol/l Tris, pH 6.8) for 30 min at 55°C and reprobed with polyclonal total antibodies for the respective proteins (Cell Signaling Technologies). Immunoreactivity was detected with ECL, and all blots were detected and quantiﬁed as per phosphorylated protein analysis. All prepared lysates (7 biopsy samples) related to each person were run on the same gel. All data are expressed as ratio between the phosphorylated to the total protein. Supplemental Fig. S3 contains representative blots for all the measured proteins (Supplemental material for this article and be found online at the J Appl Physiol web site).

Real-time quantitative PCR. Muscle biopsy samples (~20 mg) were homogenized with 1.0 ml of ice-cold TRIzol Plus Reagent according to manufacturer’s instructions (Invitrogen, Carlsbad, CA). Each homogenate was stored for 5 min at room temperature, and following this 0.2 ml of chloroform was added for each ml of TRIzol Plus incorporated. Each sample was covered and shaken vigorously for 15 s and allowed to stand at room temperature for 2–3 min, and following centrifugation at 12,000 g for 15 min at 4°C, each homogenate was separated into a lower (chloroform phase) and an upper aqueous phase. RNA in the upper aqueous phase was transferred to fresh ice-cooled tubes and mixed with 1 μl of 20 mg/ml glycogen in diethylpyrocarbonate-free H2O and 520 μl of 1-propanol to precipitate the RNA. After centrifugation and drying, precipitated RNA was dissolved in diethylpyrocarbonate-treated water, and the concentration and purity of extracted RNA were assessed with a NanoDrop 1000 UV-Vis Spectrophotometer (NanoDrop Technologies) using a 1.5-μl aliquot of total RNA solution. The size and distribution of extracted RNA molecules were then evaluated using an Agilent 2100 Expert Bioanalyzer with the RNA 6000 Nano LabChip kit (Agilent technologies, Palo Alto, California, CA). RNA integrity number (RIN) was determined using the RIN algorithm of the Agilent 2100 expert software, and RNA with a RIN ≥7.8 was included in the study (means ± SD of utilized RIN: 8.3 ± 0.4; range of RIN: 7.8–9.0).

Two micrograms of RNA were then treated with DNase I (Invitrogen) and reverse-transcribed using a TaqMan SuperScript VILO cDNA synthesis kit for real-time RT-PCR according to the protocol recommended by the manufacturer (Invitrogen). TaqMan Universal PCR Master Mix (Perkin-Elmer Applied Biosystems, Foster City, CA) and TaqMan Gene Expression assays (Perkin-Elmer Applied Biosystems) were used to analyze mRNA levels of the following: PGC1α (Hs01016722_m1); PGC1β (Hs00991677_m1); hexokinase (Hs00606086_m1); GLUT4 (Hs01689666_m1); MyoG (Hs00231167_m1); and MyoD (Hs0159528_m1). PCR was performed using a 7900HT Fast Real-Time PCR System and SDS 2.3 software (Perkin-Elmer Applied Biosystems). Expression data were normalized to the mRNA
level of GAPDH (Hs99999905_m1). For each individual, all samples were simultaneously analyzed in one assay run. All samples were analyzed in triplicate. Measurements of the relative distribution of each target gene were performed for each individual; a cycle threshold (Ct) value was obtained by subtracting GAPDH Ct values from respective target Ct values. The expression of each target was then evaluated by the ΔΔCt algorithm (36).

Calculations. The fractional synthetic rates of myofibrillar and mitochondrial enriched protein fractions were calculated using the muscle intracellular free pool and the standard precursor-product equation as described in Refs. 9, 10, 33, 42. The recruitment of “tracer-naïve” subjects allowed us to use the preinfusion blood sample, which has been measured as being equivalent in enrichment to a preinfusion biopsy in our laboratory (unpublished observations) and others (31), as the preinfusion baseline enrichment ($E_{01}$) for the calculation of resting muscle protein synthesis (fast). The single biopsy approach has been established to be reliable between means for all significant main effects and interactions. For all analyses, differences were considered significant at $P < 0.05$. All results are presented as means ± SE.

RESULTS

Exercise trial responses. Set and repetition completion in the RE (8 × 8) and CE (4 × 8) trials were 100%, with no difference in the per repetition load lifted between trials (70% 1RM; 52.7 ± 3.5 kg; $P > 0.05$). In the same way, no difference in relative (55%) or absolute (129 ± 11 W) power output ($P > 0.05$) was observed following the AE (40 min) or CE (20 min) trials. Heart rate (5, 10, 15, and 20 min) and $\dot{V}O_2$ (5 and 15 min; $P > 0.05$). In all three trials, there were increases in mitochondrial protein synthesis rates during the 1– 4 h postexercise period of 1.3- 1.8- and 2.2-fold increases above fasted rates ($P < 0.05$). The increase in myofibrillar protein synthesis rates by both CE and RE was significantly greater than the AE trial ($P < 0.05$), which remained unchanged postexercise ($P > 0.05$). In all three trials, there were increases in mitochondrial protein synthesis rates during the 1– 4 h postexercise period of 1.3-, 1.5-, and 1.4-fold increases above fasted rates ($P < 0.05$) for RE, AE, and CE, respectively. There were no differences between respective trials (Fig. 2).

Table 2. Heart rate, oxygen consumption, and rating of perceived exertion data

<table>
<thead>
<tr>
<th>Measure</th>
<th>RE Set, AE Time Point, and Corresponding CE Data</th>
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<tr>
<td></td>
<td>RE</td>
</tr>
<tr>
<td></td>
<td>RE</td>
</tr>
<tr>
<td>RPE</td>
<td>13.5 ± 1.0*</td>
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<tr>
<td>HR, beats/min</td>
<td></td>
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<tr>
<td>V̇O₂, ml·kg⁻¹·min⁻¹</td>
<td></td>
</tr>
<tr>
<td>RE</td>
<td></td>
</tr>
<tr>
<td>RPE</td>
<td>11.6 ± 1.1</td>
</tr>
<tr>
<td>V̇O₂, ml·kg⁻¹·min⁻¹</td>
<td>26.0 ± 1.7</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>131 ± 2</td>
</tr>
<tr>
<td>AE</td>
<td></td>
</tr>
<tr>
<td>RPE</td>
<td>12.1 ± 0.5</td>
</tr>
<tr>
<td>V̇O₂, ml·kg⁻¹·min⁻¹</td>
<td>—</td>
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<tr>
<td>HR, beats/min</td>
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Data are means ± SE ($n = 8$) rating of perceived exertion (RPE; 6–20 scale), volume of oxygen consumption (V̇O₂), and heart rate (HR) corresponding to set completion in resistance exercise (RE) or combined exercise (CE) and 5-min intervals in aerobic exercise (AE) or CE. *$P < 0.05$, different from AE.
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**Intramuscular protein signaling.** Changes of phosphorylation state and representative blot images for all analyzed proteins are respectively displayed in Figs. 3 and 4. The phosphorylation state of Akt on Ser473 was respectively increased 7.7- and 4.6-fold above fasting rates for CE and RE at 1 h postexercise (P < 0.05; Fig. 3). A similar pattern emerged for phospho-Akt on Thr642, whereby 4.4- and 2.9-fold respective increases were observed for CE and RE at 1 h postexercise (P < 0.05). Phospho-Akt on Ser2448 (Fig. 3) increased 2.5-fold for CE and AE at 1 h postexercise (P < 0.05), which was significantly greater than RE at 4 h, and all 1 h postexercise time points (P < 0.05). There was no change in GLUT4 mRNA expression following any exercise trial (P > 0.05). Hexokinase mRNA expression was increased 2.7-fold above fasting rates at 4 h postexercise following AE, which was significantly greater than RE at 4 h, and all 1 h postexercise time points (P < 0.05; Fig. 5). MyoG and MyoD mRNA expression were respectively increased by 4.0- and 3.3-fold at 4 h postexercise following RE (P < 0.05). The increase of MyoG mRNA expression was greater than AE and CE at 1 h and AE at 4 h postexercise (P < 0.05). Further, the increase of MyoD mRNA was greater than CE at 1 h and AE at 4 h postexercise (P < 0.05; Fig. 5).

**DISCUSSION**

Our study is the first to examine the respective acute effects of RE, AE, and CE on the stimulation of myofibrillar and mitochondrial protein synthesis rates, intramuscular protein signaling, and mRNA expression responses in sedentary middle-aged men. In contrast to our hypotheses of an transcription-and/or signaling-based antagonism between exercise modes, CE resulted in a stimulation of myofibrillar protein synthesis rates that were equivalent to RE, which is notable since this occurred despite the completion of 50% less resistance exercise volume than the RE trial. In addition, in the CE trial RE was followed by 20 min of AE, which has previously been hypothesized to be a confounding stimulus due to “opposing” signaling responses (30, 34). However, our finding is not entirely surprising since as little as three sets of resistance exercise can result in a robust stimulation of myofibrillar protein synthesis rates that are long lasting (8). In the same way, CE promoted a comparable mitochondrial protein synthetic response to AE, again with 50% less aerobic exercise volume. Interestingly, the RE trial also stimulated mitochondrial protein synthesis rates to a degree that was not different from that seen with AE and CE; however, this finding is in agreement with previous data (7, 43). Nonetheless, despite the proposed interference between AE and RE, the current muscle protein subfractional synthetic responses show that CE induced an acute stimulation of both the myofibrillar and mitochondrial protein fractions equivalent to performance of RE or AE alone. Thus the data from the present study indicate that the CE condition induced an equivalent stimulation of both the myofibrillar and mitochondrial protein pools despite a reduced volume compared with each of the isolate RE and AE exercise conditions.

In the current study, subjects were sedentary at baseline and not involved in exercise training in the prior year. As such, subjects were exposed to an unaccustomed contractile stimulus, which can affect the proteins that are synthesized after an acute exercise bout (43) and may influence the degree to which interference effects may occur (14). All exercise modes stimulated mitochondrial protein synthetic rates equivalently, and RE stimulated myofibrillar protein synthesis rates more so than AE. Recent evidence in younger men suggests that resistance exercise has the capacity to robustly stimulate mitochondrial protein synthesis rates; yet, a prolonged time under muscle...
tension has minimal impact on the stimulation of myofibrillar protein synthesis rates during acute exercise recovery (7). Thus there appears to be a shift toward the synthesis of mitochondrial and sarcoplasmic proteins after prolonged contractions. Wilkinson et al. (43) also observed responses in the mitochondrial and myofibrillar protein pools that were similar to those we report but that were altered with exercise training to be more specific to the contractile stimulus applied.

Examination of the acute postexercise molecular regulation of skeletal muscle to differing modes of exercise indicates a lack of mode-specific sensitivity in muscle protein synthesis, intramuscular signaling, and mRNA expression in response to unaccustomed contractile stimuli (14, 15, 43). In support of this notion, before a training period of RE there is a propensity to increase myofibrillar and mitochondrial protein synthesis rates to an equivalent extent, whereas myofibrillar protein synthesis rates are preferentially stimulated by RE posttraining (43). Moreover, in habitually RE- or AE-trained subjects that crossed-over to perform an unfamiliar exercise mode, a respective lack of AMPK or Akt-mTOR-S6K-rpS6 phosphorylation occurred in the habitual mode, whereas the unfamiliar mode stimulated significantly increased phosphorylation of the aforementioned proteins (15). Taken together, these data suggest that innate skeletal muscle molecular responses are influenced

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**Fig. 3.** Data are ratio of phosphorylated to total protein content of AktSer473 (A), AktThr308 (B), PRAS40Thr246 (C), mammalian target of rapamycin (mTORSer2448, D), AS160Thr642 (E), and rpS6Ser235/236 (F). Ratios are from rested fasted (fast), and 1 h and 4 h after RE, AE, or CE. Values are fold changes and are reported as means ± SE. *P < 0.05, different from fast; †P < 0.05, different from AE 1 h; ‡P < 0.05, different from AE 1-h and all 4-h trials; #P < 0.05, different from all trials.
by the level of practice of the specific contractile stimulus. In the present study, all modes were unfamiliar, although RE (or the RE component of CE) appeared to be the least familiar and induced the greatest myofibrillar protein synthetic response indicating some degree of early contractile specificity.

In the present study, phosphorylation status of Akt (Ser\textsuperscript{473} and Thr\textsuperscript{308}) peaked at 1 h after RE or CE exercise and returned to baseline levels by 4 h postexercise; this observation is consistent with other work (12, 14, 15, 24, 44). From an RE perspective, full acute activation of Akt results in the phosphorylation of downstream signaling proteins involved in the regulation of muscle protein synthesis (18). Limited data exist for CE or an AE-induced Akt response in middle-aged humans (40–65 yr); however, other studies in younger men have shown increased phosphorylation after AE (12, 15, 44) or CE (14), respectively. In the present study, there was no change in phosphorylation of Akt following AE; however, when RE was combined with AE, phosphorylation was increased more than RE alone. Given the similar phosphorylation patterns of Ser\textsuperscript{473} and Thr\textsuperscript{308} by CE and RE, it may be that the Akt signaling response is more indicative of a feeding-induced stimulation facilitated by RE, although why CE induced a greater Akt response with 50% less RE volume requires further investigation. Downstream, Akt substrate of 160 kDa (AS160) has been implicated to be important for GLUT4 translocation and eventual glucose uptake into skeletal muscle (45). Exercise, whether acutely resistive or aerobic in nature, has been reported to increase AS160 phosphorylation (18, 22); however, this is not always the case and likely relates to the timing of the muscle biopsies (12). Here, we observed a significant increase in AS160 phosphorylation only after the RE condition, which provides additional support that exercise resistance is capable of inducing AS160 phosphorylation during the acute postexercise recovery period (22).

Although an increase of mTOR phosphorylation was absent in the present study, previous investigations report that an increase is fundamental for the stimulation of muscle protein synthesis rates in humans, whether this be in the fed or fasted state (12, 20, 23, 25). Importantly, mTOR possesses a reported direct ability to phosphorylate S6K and 4E binding protein-1 (5), thus hypothetically explaining increases of muscle protein synthesis (23). In contrast, PRAS40 is highlighted as a potent inhibitor of mTOR and consequent downstream S6K and 4E binding protein-1 activity (39). In the present study, PRAS40 was phosphorylated on Thr\textsuperscript{246} at 1 h following CE and AE, with no effect from RE. Surprisingly, and as was the reverse case after RE, the phosphorylation state of Akt was not altered after AE, suggesting that PRAS40 may be regulated by other upstream kinases (39). Moreover, the phosphorylation state of mTOR (Ser\textsuperscript{2448}; Fig. 3), and S6K (Thr\textsuperscript{389}), and 4E-binding protein-1 (Thr\textsuperscript{3746}; figures not shown) remained unchanged in all conditions. As such, these data suggest that despite assumed inhibition of PRAS40 (reflected by increased phosphorylation), and whether mediated by Akt or not, there was no subsequent effect with respect to mTOR signaling. Yet despite these occurrences, the phosphorylation of rpS6, a key protein involved in translation initiation, was increased in RE only. Although others have suggested that concurrent increases of Ser\textsuperscript{2448} phosphorylation may necessitate muscle protein synthesis (23), in this study the signaling responses did not influence the observed equivalent increase in myofibrillar protein synthesis rates in response to CE or RE.

We observed, and as reported by others, increased expression of PGCl\textalpha{} and \textbeta{} mRNA following AE (15, 37) and CE (14). Increased PGCl\textalpha{} and \textbeta{} mRNA expression is indicative of an acute adaptive event preceding mitochondrial biogenesis (1, 46). Furthermore, a recent study (7) showed an increase in mitochondrial protein synthesis rates and PGCl\textalpha{} expression after RE. In response to exercise (increasing AMP: ATP ratio) and cellular stress, respectively, PGCl\textalpha{} mRNA expression may be increased due to phosphorylation by AMPK (or) p38 MAPK (26, 46). However, the phosphorylation status of AMPK on Thr\textsuperscript{172} and p38 MAPK on Thr\textsuperscript{180} (figures not shown) did not change in the current study, which could be explained by the timing of muscle collection (AMPK) and that subjects were fed immediately after exercise or possibly that the exercise did not impose an energetic stress of a sufficient magnitude to increase AMP levels (37, 41). No change in PGCl\textalpha{} or \textbeta{} expression occurred following RE in the current study; however, when combined with an AE stimulus (as in CE), expression increased comparably to AE.

The differentiation and maintenance of myofibrillar proteins are reliant upon complex interactions between muscular regul-

![Fig. 4. Representative blots of intramuscular signaling proteins measured before (fast) and at 1 and 4 h after RE, AE, or CE. All lysates for each subject were run on the same gel, and all bands for a particular antibody were obtained from a single Western blot. 4EBP1, 4E binding protein-1.](image-url)
MyoD is a master regulatory gene involved in the determination of skeletal muscle, whereas MyoG plays an important role in terminal differentiation and lineage maintenance of myofibers (35). In addition, myostatin upregulates inhibitory processes related to satellite cell proliferation, differentiation, and self-renewal, ultimately preventing increases of myofibrillar protein synthesis (13). In the current study, MyoD and MyoG expression was increased at 4 h after RE; however, there was no increase following either CE or AE (Fig. 4). Furthermore, there was no change of myostatin mRNA (figures not shown) after exercise in any mode, which in light of the MyoD/MyoG mRNA response, and the reported inhibitory effect of myostatin on hypertrophic myofibrillar processes (13), could be interpreted to suggest that myogenesis was not likely inhibited. The finding that RE stimulates MyoD/MyoG postexercise has been previously reported by other groups (4, 47), yet our results showed that while RE (8 sets × 8 repetitions) was sufficient to induce myogenesis, the RE component of CE (4 sets × 8 repetitions) was not. Thus this finding raises the possibility that either a dose-response relationship exists or that antagonism of the response after CE, potentially by the AE component, may have masked a myogenic response; further research is required to elucidate whether this was in fact the case.

In summary, in sedentary middle-aged men CE induced equivalent acute stimulation of myofibrillar and mitochondrial protein synthesis rates to RE and equivalent acute stimulation of mitochondrial protein synthesis rates to AE. Importantly, the increase of mitochondrial and myofibrillar protein synthesis rates after CE occurred despite the completion of only 50% of
the workload performed in each of the isolated RE and AE modes. Full activation of Akt was induced by CE and AE; however, this appeared to be inconsequential with respect to phosphorylation of downstream anabolic signaling proteins (mTOR-S6K-4EBP1). Postexercise mRNA expression related to mitochondrial biogenesis (PGC1α, PGC1β, and hexokinase) and myogenesis (MyoG and MyoD) presented in a manner that was consistent with their previously characterized roles. None-theless, despite the proposed interference between RE and AE the current study data indicates that CE is as effective as either isolated mode in stimulating acute myofibrillar and mitochondrial protein synthesis rates in sedentary middle-aged men.

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

AUTHOR CONTRIBUTIONS

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