Exercise-induced AMPK activation does not interfere with muscle hypertrophy in response to resistance training in men

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Lundberg TR, Fernandez-Gonzalo R, Tesch PA. Exercise-induced AMPK activation does not interfere with muscle hypertrophy in response to resistance training in men. *J Appl Physiol* 116: 611–620, 2014. First published January 9, 2014; doi:10.1152/japplphysiol.01082.2013.—As aerobic exercise (AE) may interfere with adaptations to resistance exercise (RE), this study explored acute and chronic responses to consecutive AE (~45 min cycling) and RE (4 × 7 maximal knee extensions) vs. RE only. Ten men performed acute unilateral AE + RE interspersed by 15 min recovery. The contralateral leg was subjected to RE. This exercise paradigm was then implemented in a 5-wk training program. Protein phosphorylation, gene expression, and glycogen content were assessed in biopsies obtained from the vastus lateralis muscle of both legs immediately before and 3 h after acute RE. Quadriceps muscle size and in vivo torque were measured, and muscle samples were analyzed for citrate synthase activity and glycogen concentration, before and after training. Acute AE reduced glycogen content (32%; $P < 0.05$) and increased ($P < 0.05$) phosphorylation of AMPK (1.5-fold) and rpS6 (1.3-fold). Phosphorylation of p70S6K and 4E-BP1 remained unchanged. Myostatin gene expression was downregulated after acute AE + RE but not RE. Muscle size showed greater ($P < 0.05$) increase after AE + RE (6%) than RE (3%) training. Citrate synthase activity (18%) and endurance performance (22%) increased ($P < 0.05$) after AE + RE but not RE. While training increased ($P < 0.05$) in vivo muscle strength in both legs, normalized and concentric torque increased after RE only. Thus AE activates AMPK, reduces glycogen stores, and impairs the progression of concentric force, yet muscle hypertrophic responses to chronic RE training appear not to be compromised.

aerobic exercise; gene expression; human skeletal muscle signaling; muscle strength and power

IF PRECEDED BY AEROBIC EXERCISE (AE), typical outcomes of resistance exercise (RE) training, e.g., increased strength, power, and muscle size, may be compromised (30, 37). While the requisites and associated mechanisms of such interference have not been elucidated, residual fatigue and antagonistic molecular responses derived from previous exercise have been put forth as tentative causes to such an effect (27, 40). However, when allowing for 6 h recovery between bouts, we recently reported that 5 wk concurrent AE + RE training produced similar improvements in muscle strength and power, and even greater increases in muscle size, as RE alone (42). Thus time allowed for recovery between bouts may be vital in optimizing muscle adaptations to AE + RE training.

It is well founded that acute AE reduces muscle glycogen content (13, 29) and may compromise neuromuscular function

(5, 36). Thus undertaking RE immediately after AE may impair muscle strength and power (39). Apart from interfering with in vivo function, decreased glycogen availability may also directly or indirectly impact aspects of muscle signaling following RE (17). Although this notion has been challenged (12), collectively, it appears that acute AE induces fatigue that could blunt the desired response to subsequent RE and hence attenuate muscle adaptations to chronic RE training.

At the cellular level, AE upregulates adenosine monophosphate-activated protein kinase (AMPK) to restore AMP/ATP balance and to trigger transcriptional activators, regulating mitochondrial biogenesis (14). RE increases protein synthesis through the mammalian target of rapamycin (mTOR) signaling pathway, and hence, cumulative RE favors contractile protein accretion (45). In support of this stereotypic mode-specific response, rat muscles subjected to either high- or low-frequency electrical stimulation purported to mimic RE and AE, respectively, showed increased mTOR phosphorylation after RE but not AE (3). Conversely, AE hyperphosphorylated AMPK and increased protein levels of the peroxisome proliferator-activated receptor- γ coactivator-1 (PGC-1 α) (3). While those findings imply that mode-specific signaling through AMPK and mTOR pathways dictates classical end-point adaptations to chronic AE and RE training, results of human studies are equivocal. For example, when strength- and endurance-trained athletes performed their habitual or nonhabitual exercises, robust anabolic muscle signaling was noted after unaccustomed exercise only (16). As reports also suggest AMPK increases in response to RE (20) and mTOR to be activated by AE (43), it appears important signaling routes dictating human skeletal muscle adaptations are shared for different exercise modes.

In the rat, contraction-induced mTOR signaling is inhibited by prior AMPK activation caused by endurance exercise (60). More specifically, translational signaling is compromised through attenuation of key downstream regulators such as eukaryotic initiation factor 4E-binding protein (4E-BP1) and p70S6 kinase (p70S6K) (60). This is in concert with the marked suppression of protein synthesis following AMPK activation (8). Collectively, this scenario offers reasonable mechanistic support for myofiber protein accretion to be blunted, as noted after concurrent AE and RE training (27).

In human subjects who performed AE prior to RE (15), the muscle anabolic response was somewhat attenuated compared with exercise performed in the reversed order. Indeed, performing RE followed by AE shows neither molecular interference (2) nor altered rate of protein synthesis (19) compared with RE alone. Our recent studies, examining responses to AE followed by RE, showed uncompromised translational signal-

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ing and muscle hypertrophy when recovery to restore muscle function between bouts was allowed (41, 42). Given that acute AE transiently impairs muscle function (5), reduces glycogen stores (36), and activates AMPK (11), it may be that back-to-back AE + RE interferes with translational signaling and hence attenuates muscle hypertrophy following chronic training.

Therefore, the aim of the current study was to investigate acute and chronic outcomes of consecutive AE + RE and training compared with RE alone. It was hypothesized that 1) acute AE would reduce muscle glycogen stores and activate AMPK signaling, 2) AE would compromise RE-induced mTOR signaling, and 3) acute and chronic AE + RE would impair in vivo contractile function and blunt muscle hypertrophy compared with RE alone.

METHODS

General design. Ten men performed unilateral consecutive bouts of knee extensor AE (~45 min) and RE (4 sets of 7 reps) interspersed by 15 min recovery. The contralateral limb was subjected to RE only. Analysis of vastus lateralis muscle biopsies, obtained in both legs before (PRE) and 3 h after (POST) RE (Fig. 1), measured glycogen concentration, gene expression, and protein phosphorylation. These two exercise regimens were subsequently implemented into a 5-wk training program. Before and after training, muscle strength and power were assessed and quadriceps femoris (QF) muscle volume, cross-sectional area (CSA), and signal intensity (SI) determined by means of magnetic resonance imaging (MRI). Resting muscle biopsies assessed training-induced changes in citrate synthase (CS) activity and glycogen content.

Subjects. Ten healthy male volunteers (26 ± 5 yr, 183 ± 7 cm, and 77 ± 9 kg) completed the study protocol. Subjects were moderately trained college students performing recreational exercise ~3 days/wk. While most subjects had practiced RE training at some time, none had performed structured training in the past year prior to the study. The study experiments and procedures were explained before subjects gave their written informed consent to participate. The study was approved by the Regional Ethical Review Board in Stockholm.

Exercise equipment. AE was performed using a modified one-legged cycle ergometer (model 828E, Monark Exercise AB, Varberg, Sweden) as previously described (1, 42). Open-chain concentric (CON) knee extensions were performed at a target cadence of 60 rpm. Power and cadence were sampled at 2 Hz (SRM GmbH, Jülich, Germany). RE was performed using a seated knee extension ergometer (YoYo Technology, Stockholm, Sweden) equipped with a flywheel offering inertial resistance (0.11 kg/m^2) during coupled CON and eccentric (ECC) muscle actions (59). Peak torque and power were calculated from force (MuscleLab, Langesund, Norway) and speed (SmartCoach, Stockholm, Sweden) measurements sampled at 100 Hz. The coefficient of variation (CV%) for assessing flywheel peak power across two different sessions within 1 wk was 5%. Maximal isometric

and isokinetic torque were assessed using a Cybex II (Lumex, New York) dynamometer employing protocols described elsewhere (42). For all apparatuses used, individual settings were maintained throughout the study. Subjects completed three familiarization sessions in the course of 2 wk prior to the study. Standardized warm-ups preceded any exercise test or training session.

Acute exercise bouts. The acute exercise experiment was performed 1 wk prior to commencing 5 wk training. First, one randomly chosen limb completed 40-min one-legged AE at ~70% of maximal workload ($W_{\max}$) using a cadence of 60 rpm. To ensure strenuous efforts, ratings of perceived exertion (RPE; central and local) were obtained using the 6–20 Borg scale (9). At 40 min, the workload was increased by ~20 W and subjects were requested to continue until failure. Heart rate was recorded continuously (Polar Electro, Kempele, Finland). Fifteen minutes after completing AE, unilateral RE (4×7 maximal repetitions; 2 min rest between sets) was executed for each leg in a random order on the knee extension ergometer. Subjects were verbally encouraged and requested to perform each repetition at maximal effort. Peak power was measured in each repetition.

Training protocols. The AE + RE training regimen was dedicated to the leg performing AE + RE during the acute experiments. The other limb served as control and performed RE only. Subjects completed 15 AE sessions (3 nonconsecutive days/wk) and 12 unilateral RE sessions for both limbs (2 days/wk during weeks 1, 3, and 5 and 3 days/wk during weeks 2 and 4). Thus any RE session (i.e., 4×7 knee extensions for each leg) was preceded by AE for the intervention leg, and allowed for 15 min recovery between bouts. All AE and RE training sessions were supervised.

Muscle biopsies and diet control. Muscle biopsies were obtained from the mid portion of vastus lateralis under local anesthesia immediately before (PRE) and 3 h after (POST) the acute RE bout of either leg (Fig. 1). The 3 h time point was chosen to accommodate for changes in both protein phosphorylation and gene expression. In addition, resting muscle samples from either leg were collected 72 h after the last training session. Five millimeter Bergström-needles (7) with suction applied were used to obtain ~150 mg tissue samples that were cleansed of excess blood, fat, and connective tissue before being frozen in liquid nitrogen-precooled isopentane and stored at -80°C . Subsequent biopsies were obtained from separate incisions, moving in direction distal to proximal. A standardized dinner (pasta, tomato sauce, and juice) consisting of 2.21 g carbohydrates/kg body wt (bw), 0.22 g protein/kg bw, and 0.04 g fat/kg bw was provided the night before experiments. On days of scheduled biopsies, a liquid formula (1.01 g carbohydrates/kg bw, 0.31 g protein/kg bw, and 0.24 g fat/kg bw) was provided as breakfast 2 h prior to the first biopsy. The liquid formulas contained 6.3 g protein (0.55 g leucine), 20.2 g carbohydrates, and 4.9 g fat per 100 ml (Ensure Plus, Abbott Laboratories, Maidenhead, UK).

Pre- and posttesting. MRI scans (see below) were scheduled prior to any test or biopsy. Three or four days after acute experiments, maximal isometric and isokinetic strength, knee extension torque and power, and one-legged endurance performance were assessed over 2 days. Muscle strength and power for the right leg and endurance for the left leg were measured on day 1. The order of tests was reversed on day 2. Measures of peak torque were obtained at constant velocities of 0.52, 1.05, 2.09, 3.14, 3.67, and 4.19 rad/s using the Cybex II dynamometer. Subjects performed 2–3 attempts (30 s rest) at each velocity and the best result represented peak torque. Maximal isometric torque was measured at knee angle 120° . Subjects were instructed to push with maximal effort for ~5 s. The best score (two trials) in a 1 s window defined peak isometric torque. Subsequently, peak torque and power were assessed on the flywheel knee extension ergometer. Subjects performed 2×7 repetitions (2 min rest between sets) under strong verbal encouragement. Peak values were averaged across sets and repetitions, and normalized torque ($\text{N}\cdot\text{m}/\text{cm}^2$) was calculated as the ratio between peak knee extension torque and average muscle CSA. Finally, the one-legged ergometer incremental test assessed

Fig. 1. Schematic overview of the study protocol. AE, aerobic exercise; RE, resistance exercise; B, muscle biopsy.

$W_{\max}$ and endurance performance. Resistance was increased by 2.5 N every 2nd minute until failure to maintain cadence. $W_{\max}$ was defined as the last successfully completed workload. Heart rate was recorded continuously throughout the test. RPE was obtained every 2nd minute and at exhaustion. Postperformance tests were completed identical to the pretests at the same time of the day (± 2 h). Subjects were blind to any test result to ensure nonbiased efforts. The post-MRI scans were obtained 48–72 h after the last training session. Resting muscle biopsies were obtained within 2 days after the MRI scans. Throughout the study, subjects were instructed to maintain ordinary daily routines, yet to refrain from strenuous activities involving the lower limbs.

Magnetic resonance imaging. Subjects rested in the supine position for 1 h prior to any MRI scan (6). Cross-sectional T2 weighted images were obtained using a 1.5-Tesla Philips MR Systems Intera (Best, The Netherlands) unit as previously described (42). During each scan, 50 images with 10-mm slice thickness were obtained. Anatomical landmarks and standardized procedures ensured that the same segment was scanned before and after training. CSA and SI (mean gray value; MGv) of each individual QF muscle [vastus lateralis (VL), vastus intermedius (VI), vastus medialis (VM), and rectus femoris (RF)] were analyzed from the image where gluteus maximus was no longer visible, to the last image in which RF appeared. Every third image was analyzed to quantify CSA and SI using ImageJ software (National Institutes of Health, Bethesda, MD). As an additional control, SI of the biceps femoris (BF) muscle was analyzed in the third image of each subject. Mean CSA was multiplied by slice thickness to obtain muscle volume. In our laboratory, the CV for interindividual assessment of muscle volume amounts to 1%.

CS activity and glycogen content. Freeze-dried muscle samples (~ 3 mg) were homogenized in phosphate buffer with 0.5% BSA. CS activity and glycogen content were subsequently determined in duplicates through fluorometric assays as described elsewhere (42).

RNA isolation, reverse transcription, and real-time PCR. Gene expression of established markers of muscle adaptations to AE and RE were analyzed. Twenty-milligram muscle samples were homogenized using TRIzol and total RNA was extracted. One microgram of total RNA from each sample was used for reverse transcription into cDNA for a final volume of 20 μ l (High Capacity Reverse Transcription Kit, Applied Biosystems, Foster City, CA). Real-time PCR (ABI-PRISMA 7700 Sequence Detector, Perkin-Elmer Applied Biosystems) procedures were employed to determine mRNA expression. Probes and primers (TaqMan) for atrogen-1 (Hs00369714_m1), Muscle RING-finger protein-1 (MuRF-1; Hs00822397_m1), myostatin (Hs00193363_m1), PGC-1 α (Hs01016724_m1), and vascular endothelial growth factor (VEGF; Hs99999070_m1) were purchased from Applied Biosystems. GAPDH (Hs99999905_m1) and 18S (Hs01375212_g1) were used as reference genes. The expression of reference genes did not differ across time points and the GAPDH/18S ratio did not change. Reaction and amplification mixes (10 μ l) consisted of the diluted (1:100) cDNA (4.5 μ l), TaqMan Fast Universal PCR Master Mix (5.0 μ l), and specific primers (0.5 μ l). Subsequent cycling protocols 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. Target gene expression was reported as a ratio to the average of the two reference genes using the $2^{-\Delta CT}$ formula.

Protein extraction and western blotting. About 30 mg muscle tissue was manually homogenized in RIPA buffer, and proteins were recovered as previously described (41). Protein concentrations were subsequently determined using the Bradford technique. Thirty micrograms of protein per sample were loaded on 10% SDS precast gels (Bio-Rad) and separated through electrophoresis together with a protein ladder. Gels were transferred to PVDF-membranes using the Trans-Blot Turbo Transfer System from Bio-Rad. Blocking was completed using fluorescent blocking buffer (Millipore, Billerica, MA) during 60 min at room temperature (RT). Membranes were incubated overnight at 4°C with primary antibodies (1:1,000) for phospho-P70S6K (Thr389), phospho-rpS6 (Ser235/236), phospho-4E-BP1 (Thr37/46), and phos-

pho-AMPK α (Thr172). All antibodies were from Cell Signaling Technology (Beverly, MA), except for the antibody against p70S6K which was from Santa Cruz Biotechnology (Santa Cruz, CA). After the overnight incubation, membranes were washed (4×5 min) in PBST (0.1%) and incubated with IRDye secondary antibody (LI-COR Biosciences, Cambridge, UK) for 60 min at RT. A final series of washes were then performed before scanning the membranes (Odyssey SA Infrared Imaging System, LI-COR Bioscience). The blots were subsequently quantified using ImageJ. To control for loading error, phosphorylated proteins were expressed relative to total α -tubulin abundance (1:20,000; Sigma-Aldrich, St. Louis, MO).

Data analysis. Dependent variables were analyzed by two-way repeated-measures ANOVA (factors: time and leg). When CON and ECC muscle actions were analyzed separately, three-way ANOVAs were employed. CS activity was analyzed by one-way ANOVA over time. Data skewness was assessed through histograms and the Shapiro-Wilk test. Positively skewed variables (PGC-1 α and VEGF) were log-transformed. When two-way interaction was found significant, a priori planned simple effect comparisons within each level were performed. The false discovery rate (FDR) procedure was employed to adjust for these comparisons (18). Significance was accepted at the 5% level ($P < 0.05$). Data are presented as means $\pm$ SD.

RESULTS

Acute exercise experiment. Average power during 40 min AE was 37 ± 6 W. Power increased to 60 ± 10 W during the final increment to exhaustion, which lasted $2 \text{ min} \pm 12 \text{ s}$. RPE rose in a linear fashion, to attain 13 (central) and 15 (local) during the final 10 min of exercise, and 14 and 18 at exercise completion. Average HR during AE was 118 ± 18 beats/min. HR amounted to 148 ± 16 beats/min during the final exercise stage, and peaked at 161 ± 14 beats/min. In the subsequent RE, the leg that had completed AE showed 10% lower (351 ± 97 W; $P = 0.020$) peak power than the leg subjected to RE only (387 ± 88 W).

Aerobic exercise training. Average power across the 15 AE sessions was 46 ± 13 W. Average power amounted to 39 ± 7 W in the first and increased ($P = 0.004$) to 53 ± 15 W during the last session. At the final incremental step to exhaustion ($2 \text{ min} \pm 25 \text{ s}$), power averaged 66 ± 18 W. RPE (local) increased over the course of exercise to attain near maximal values (19 ± 1) at exercise completion. HR averaged 120 ± 17 beats/min during the 40-min sessions and increased to 146 ± 21 beats/min during the final exercise stage. Average peak HR across training sessions was 160 ± 18 beats/min.

Resistance exercise training. Changes in peak power for CON and ECC muscle actions were similar over the 5-wk training; hence CON-ECC data were merged. There was no leg $\times$ time interaction for peak power. Thus the progression in power across RE training sessions (main effect of time, $P < 0.0005$; Fig. 2) was comparable for the different legs. However, the leg subjected to AE + RE performed $\sim 20\%$ lower peak power across the 5-wk training (main effect of leg, $P = 0.001$).

Endurance performance. Time to exhaustion in the one-legged incremental test increased 22% ($P = 0.001$) after AE + RE and was unchanged after RE (interaction: $P = 0.002$, Table 1). Peak HR was similar across time and leg (Table 1). At failure, RPE (local) amounted to 19 ± 1 for both legs at pre- and posttests.

Strength performance. Flywheel knee extension peak torque increased after training (Table 1). However, while peak ECC torque increased equally across legs, peak CON torque in-

Fig. 2. Peak power measured in the flywheel ergometer during 12 resistance exercise sessions with (AE + RE) or without (RE) preceding aerobic exercise. Means $\pm$ SD. Significant effect ($P < 0.05$): b = leg; c = time.

creased after RE (10%, $P = 0.004$), yet remained unchanged after AE + RE ($P = 0.237$). Consequently, CON torque normalized to muscle CSA was compromised after AE + RE training (interaction $P = 0.010$). Maximal isometric strength was unaltered by training (Table 1). The isokinetic tests showed large variations in individual responses, resulting in no differences across time or legs (all $P > 0.05$, Table 1).

Muscle volume, CSA, and signal intensity. Total QF volume and CSA increased after either intervention (Table 2). However, the increase was greater after AE + RE (6%) than RE (3%, Fig. 3; interaction QF volume: $F = 38.5$, $P < 0.0005$). Likewise, the increased volume of the four individual QF muscles was greater after AE + RE than RE (Table 2). SI increased 7% after AE + RE ($P = 0.009$), yet was unaltered by

RE. This effect was consistent across the four individual QF muscles (Table 2). BF of either leg showed no change in SI.

Glycogen content. There was a leg $\times$ time interaction for glycogen content ($F = 19.5$, $P = 0.001$). Thus at PRE, the leg that had completed AE showed 32% lower ($P < 0.0005$) glycogen concentration than the rested leg (Fig. 4). This effect was still evident 3 h after the acute RE bout ($P < 0.0005$). In the rested state after training, the leg subjected to AE + RE showed greater glycogen content than the RE leg ($P = 0.003$) and compared with basal values ($P = 0.004$).

CS activity. CS activity increased (18%; $P = 0.001$) in AE + RE (from 44.8 to 53.1 mmol $\cdot$ kg $^{-1}\cdot$ min $^{-1}$), but not in RE (43.3 mmol $\cdot$ kg $^{-1}\cdot$ min $^{-1}$).

Gene expression. There was a leg $\times$ time interaction for PGC-1 α expression ($F = 116.6$, $P < 0.0005$; Fig. 5). Thus the increased expression from pre to post was greater after AE + RE (10.3-fold, $P < 0.0005$), than RE (2.0-fold, $P = 0.001$). Likewise, there was an interaction effect for VEGF expression ($F = 28.4$, $P < 0.0005$), due to a greater increase after AE + RE (2.5-fold, $P < 0.0005$) vs. RE (1.2-fold, $P < 0.042$). Myostatin expression showed a tendency for interaction ($P = 0.086$) because the downregulation was greater after AE + RE (65%; $P < 0.0005$) than RE (31%; not significant after FDR procedures). MuRF-1 expression increased after AE + RE (2.9-fold, $P = 0.003$) and was unchanged after RE (interaction: $F = 20.4$, $P = 0.001$). Atrogin-1 showed interaction ($F = 42.0$, $P < 0.0005$), as mRNA levels decreased after RE (43%, $P = 0.004$) and tended to increase after AE + RE (1.3-fold, $P = 0.101$).

Protein phosphorylation. AMPK phosphorylation showed a leg $\times$ time interaction ($F = 11.4$, $P = 0.008$); values at PRE were greater (1.5-fold, $P = 0.034$) in the leg that had performed AE, compared with the “rested” (RE) leg (Fig. 6). Similarly, rpS6 signaling showed interaction ($F = 14.4$, $P = 0.004$) such that phosphorylation was elevated (1.3-fold, $P =$

Table 1. Selected performance measures pre and post resistance training with (AE + RE) or without (RE) preceding aerobic exercise

	AE + RE			RE		
	PRE	POST	$\Delta\%$	PRE	POST	$\Delta\%$
Endurance performance ^{a,b,c} , s	632 $\pm$ 105	770 $\pm$ 145*#	22	624 $\pm$ 151	654 $\pm$ 142	5
$W_{\max}$ ^{a,b,c} , W	57 $\pm$ 15	77 $\pm$ 19*#	35	56 $\pm$ 21	62 $\pm$ 18	11
Peak heart rate at $W_{\max}$ ^b , beats/min	163 $\pm$ 7	168 $\pm$ 16	3	160 $\pm$ 10	159 $\pm$ 15	-1
Flywheel merged peak power ^c , W	428 $\pm$ 110	496 $\pm$ 129*	16	435 $\pm$ 80	515 $\pm$ 109*	18
Flywheel peak CON power ^c , W	425 $\pm$ 105	480 $\pm$ 126*	13	427 $\pm$ 76	501 $\pm$ 104*	17
Flywheel peak ECC power ^c , W	432 $\pm$ 116	512 $\pm$ 135*	19	443 $\pm$ 88	529 $\pm$ 118*	19
Flywheel merged peak torque ^c , N·m	249 $\pm$ 37	274 $\pm$ 50*	10	251 $\pm$ 34	280 $\pm$ 39*	12
Normalized merged peak torque, N·m/cm ²	3.12 $\pm$ 0.40	3.23 $\pm$ 0.47	4	3.08 $\pm$ 0.37	3.36 $\pm$ 0.51	9
Flywheel peak CON torque ^{a,c} , N·m	236 $\pm$ 33	247 $\pm$ 43	5	234 $\pm$ 26	258 $\pm$ 35*	10
Normalized CON torque ^a , N·m/cm ²	2.95 $\pm$ 0.28	2.90 $\pm$ 0.34#	-2	2.87 $\pm$ 0.26	3.08 $\pm$ 0.39*	7
Flywheel peak ECC torque ^c , N·m	263 $\pm$ 44	301 $\pm$ 63*	14	268 $\pm$ 46	303 $\pm$ 51*	13
Normalized ECC torque, N·m/cm ²	3.29 $\pm$ 0.55	3.56 $\pm$ 0.66*	8	3.29 $\pm$ 0.54	3.64 $\pm$ 0.71*	11
Maximal isometric torque, N·m	323 $\pm$ 84	305 $\pm$ 52	-6	297 $\pm$ 52	314 $\pm$ 68	6
Isometric torque, N·m						
at 0.52 rad/s	300 $\pm$ 58	293 $\pm$ 55	-2	291 $\pm$ 46	296 $\pm$ 56	2
at 1.05 rad/s	265 $\pm$ 57	254 $\pm$ 46	-4	250 $\pm$ 45	254 $\pm$ 54	2
at 2.09 rad/s	218 $\pm$ 51	201 $\pm$ 44	-8	204 $\pm$ 35	206 $\pm$ 49	1
at 3.14 rad/s	178 $\pm$ 35	168 $\pm$ 37	-6	173 $\pm$ 29	173 $\pm$ 41	0
at 3.67 rad/s	164 $\pm$ 39	148 $\pm$ 28	-10	156 $\pm$ 30	156 $\pm$ 40	0
at 4.19 rad/s	140 $\pm$ 35	128 $\pm$ 31	-9	140 $\pm$ 26	141 $\pm$ 37	1

Values are means $\pm$ SD. $W_{\max}$, maximal workload; CON, concentric; ECC, eccentric. Significant main effects ($P < 0.05$): a = interaction, b = leg, c = time. Significant simple effects ($P < 0.05$): *time (POST vs. PRE); #leg (AE + RE vs. RE).

Table 2. Selected MRI measures pre and post resistance training with (AE + RE) or without (RE) preceding aerobic exercise

	AE + RE			RE		
	PRE	POST	$\Delta\%$	PRE	POST	$\Delta\%$
QF muscle volume ^{a,c} , cm ³	1,217 ± 246	1,286 ± 247*	6	1,239 ± 231	1,273 ± 237*	3
VL muscle volume ^{a,c} , cm ³	403 ± 95	425 ± 95*	6	411 ± 96	421 ± 96*	2
VI muscle volume ^{a,c} , cm ³	376 ± 77	389 ± 79*	3	390 ± 67	395 ± 71	1
VM muscle volume ^{a,c} , cm ³	322 ± 70	336 ± 69*#	4	318 ± 64	325 ± 64*	2
RF muscle volume ^{a,c} , cm ³	117 ± 26	135 ± 28*	15	120 ± 33	131 ± 34*	9
QF mean CSA ^{a,c} , cm ²	80.7 ± 13.2	85.2 ± 12.9*	6	82.2 ± 12.5	84.4 ± 12.5*	3
QF signal intensity ^a , MGv	48.0 ± 5.9	51.5 ± 7.6*	7	47.3 ± 5.8	46.8 ± 6.1	-1
VL signal intensity ^{a,b,c} , MGv	47.3 ± 4.5	51.1 ± 5.7*#	8	46.6 ± 4.2	46.6 ± 4.6	0
VI signal intensity ^a , MGv	47.4 ± 4.3	49.8 ± 4.8*#	5	46.5 ± 4.6	45.5 ± 4.4	-2
VM signal intensity ^a , MGv	50.4 ± 5.9	51.9 ± 7.0	3	49.6 ± 7.4	48.2 ± 7.3	-3
RF signal intensity ^{a,c} , MGv	47.0 ± 11.9	53.2 ± 15.4*	13	46.6 ± 9.5	46.8 ± 10.5	0
BF signal intensity, MGv	41.2 ± 5.7	41.6 ± 5.8	1	39.7 ± 4.2	39.9 ± 5.1	1

Values are means ± SD. CSA, cross-sectional area; MGv, mean gray value; QF, quadriceps femoris; VL, vastus lateralis; VI, vastus intermedius; VM, vastus medialis; RF, rectus femoris; BF, biceps femoris. Significant main effects ($P < 0.05$): a = interaction, b = leg, c = time. Significant simple effects ($P < 0.05$): *time (PRE vs. POST); #leg (AE + RE vs. RE).

0.021) in AE + RE at PRE. Although 4E-BP1 signaling was similar across legs, there was a trend toward a main effect of time ($P = 0.070$) due to a slight increase from PRE to POST. There were no changes across time or legs for p70S6K phosphorylation. Representative blots are shown in Fig. 7.

DISCUSSION

The current study scrutinized the proposed negative effect of AE-induced AMPK activation on subsequent muscle signaling and hypertrophic responses to RE training. Our novel results show that AMPK activation prompted by AE does not compromise hypertrophy in human muscle subjected to RE. Indeed, concurrent AE + RE rather produced greater increase in muscle size than RE. Thus, while we refute the purported incompatibility between AMPK signaling and muscle hypertrophic responses, the results reinforce that AE could obstruct the progression of in vivo muscle function to subsequent cumulative RE. If employed in competitive athletes or executed over extended time, there are obvious reasons to suspect this effect would have adverse impact on performance.

Fig. 3. Individual and mean increase in quadriceps muscle volume after resistance training with (AE + RE) or without (RE) concurrent aerobic exercise. *Greater increase after AE + RE.

AMPK activation induced by AE has been held responsible for interfering with muscle growth to subsequent RE training (4, 27). Indeed, animal and in vitro studies suggest AMPK signaling blocks mTOR activity (34), impairs protein synthesis (8, 60), and provokes myofibrillar protein degradation (51). In stark contrast, and somewhat as a surprise, we recently reported robust muscle hypertrophy after RE preceded by AE, and allowing for 6 h recovery, that overshadowed that produced by RE only (42). Collectively these findings spurred us to hypothesize that recovery between exercise bouts is a prerequisite to optimize functional and cellular responses to concurrent AE + RE training.

In the current investigation, and similar to the findings of our previous study (42), AE + RE produced more substantial increases in muscle size than RE. Thus inevitably, back-to-back AE + RE did not induce AMPK-mediated blunting of muscle hypertrophy. This may be because suppressed protein synthesis is associated with increased AMPK signaling only during and immediately after acute exercise (20). In fact, there

Fig. 4. Muscle glycogen concentration before (PRE) and 3 h after (POST) acute resistance exercise with (AE + RE) or without (RE) preceding aerobic exercise and in the rested state (BASAL) after 5 wk training. Significant effect ($P < 0.05$): a = interaction, b = leg, c = time. Significant differences ($P < 0.05$) vs. #opposite leg.

Fig. 5. A–E: PGC-1 α , VEGF, MuRF-1, atrogin-1, and myostatin mRNA levels before (PRE) and 3 h after (POST) resistance exercise with (AE + RE) or without (RE) preceding aerobic exercise. Significant effects ($P < 0.05$): a = interaction, b = leg, c = time. Significant differences ($P < 0.05$) vs. #opposite leg, *same leg at PRE.

is sound support for this presumption. First, increased AMPK activity is typically evident ≤ 1 h postexercise (11, 20, 46) returning to baseline shortly thereafter (20, 62), and cumulative exercise may even offset this response (46). Second, when RE was executed subsequent to AE (15), the only inhibitory effect occurred at the IGF-1 mRNA level while mTOR signaling was not impacted. Third, muscle protein fractional synthetic rate, assessed 1–4 h postexercise, was comparable across AE + RE and RE (13). Taken together, and given that protein synthesis may be elevated 48–72 h after acute RE (38), the very short-lived AMPK activation induced by AE most likely evokes minute, if any, impact on the net protein balance accumulated between exercise sessions.

While the lack of enhanced mTOR signaling after RE was unexpected, rpS6 phosphorylation was elevated after AE. This increase occurred in parallel with increased AMPK phosphorylation following AE, yet was unaccompanied by increases in other mTOR effectors. This response seems at odds with the current understanding of enhanced translation initiation and protein synthesis after acute exercise. However, increases in

muscle protein synthesis and rpS6 phosphorylation may occur in the absence of increased mTOR signaling (19). Further, our gene expression records support that AE + RE served a more potent stimulus for tissue remodeling than RE. More specifically, while RE alone failed to downregulate myostatin and increase MuRF-1 expression, these genes showed robust responses after AE + RE. Myostatin suppression has been associated with muscle hypertrophy and decreased specific force (47), and MuRF-1 expression appears crucial for loading-induced growth to occur (33). These findings largely also corroborate with changes in expression of other genes (i.e., PGC-1 α , VEGF and atrogin-1), which showed augmented mRNA response in the leg that was subjected to concurrent exercise. These results are intriguing, because apart from controlling mitochondrial biogenesis induced by AE, PGC-1 α induction may also protect against muscle protein breakdown (10) and elicit hypertrophy in response to loading (57). Further, it is noteworthy our results accord with the exaggerated gene expression response to AE + RE with 6 h between bouts (22, 41). Thus, at least during the initial training phase and regard-

Fig. 6. A–D: phosphorylation of phospho-P70S6K (Thr389), phospho-rpS6 (Ser235/236), phospho-4E-BP1 (Thr37/46), and phospho-AMPK α (Thr172) relative to total α -tubulin before (PRE) and 3 h after (POST) resistance exercise with (AE + RE) or without (RE) preceding aerobic exercise. Significant effects ($P < 0.05$): a = interaction. Significant differences ($P < 0.05$) vs. #opposite leg.

less of recovery, it appears the more voluminous AE + RE paradigm induced a more favorable cellular environment for muscular, vascular, and mitochondrial protein turnover.

It is well established that AE reduces glycogen stores, and commencing exercise in a low-glycogen state may evoke different metabolic and molecular responses compared with muscles displaying normal or supranormal glycogen levels (17, 28). It has been postulated that glycogen depletion, and/or associated changes in muscle energy balance, could contribute to the incompatibility of mixed-mode training (40, 50). In the current study, however, glycogen utilization induced by AE was not accompanied by altered mTOR signaling 3 h post RE.

Similarly, translational signaling and protein synthetic responses to RE were not compromised in glycogen-voided skeletal muscle at onset of exercise (12). Taken together, it appears human muscle commencing RE at $\sim 30\%$ reduced glycogen stores and $\sim 20\%$ compromised function possesses undiminished ability to undergo hypertrophy.

Evidently AE + RE exaggerated the increased muscle size shown with RE only. These findings were very consistent and the hierarchical response across individual QF muscles was identical to what we have reported earlier (42, 52). Moreover, SI of the RE leg and the nonused BF muscle serving as control was unaltered, providing further evidence that reported differences across legs were specific to the QF muscle group and the intervention imposed. However, given the established relationship between muscle anatomical CSA and in vivo force (44), the finding of greater muscle hypertrophy, but not force, after AE + RE than RE suggests that the increase in size was not entirely “functional.” At first, and consistent with the notion of increased SI of MRIs (26, 42), it would be tempting to attribute the reduced specific force to edema or swelling due to the preceding exercise. However, it should be recalled both legs executed the high-load ECC component of RE, and none of the subjects reported delayed onset of muscle soreness, associated with ECC exercise (23). It also remains that the acute molecular response implied greater anabolic environment after AE + RE compared with RE. Likewise, muscle samples obtained before and after 5 wk AE + RE or RE (42) showed unaltered protein concentrations despite the robust hypertrophy (unpublished observation). Altogether, these findings suggest that the increased SI was caused by events unrelated to cell swelling. As mitochondria constitute 4–6% of muscle tissue (32) and our proxy markers (CS activity; endurance performance) showed substantial increases after AE + RE, it may be that

Fig. 7. Representative blots of phospho-P70S6K (Thr389), phospho-rpS6 (Ser235/236), phospho-4E-BP1 (Thr37/46) and phospho-AMPK α (Thr172) with corresponding α -tubulin bands.

noncontractile protein constituents had subtle, yet significant impact on size measures. In fact, increases in mitochondrial volume and glycogen stores after short-term endurance training have been estimated to account for 3–4% of the increase in muscle CSA (53, 54). While it remains to establish a credible explanation for the more ample increase in muscle volume after AE + RE than RE, evidently increases in both myofibrillar and mitochondrial/sarcoplasmic protein pools occur in parallel.

Albeit the AE insult impaired succeeding RE performance throughout the training period (Fig. 2), force and power increased after both AE + RE and RE in the current investigation. However, RE-induced improvements in normalized and absolute CON torque were blunted by AE + RE. Previous research also suggests that concurrent training may interfere with the progression of in vivo muscle function (40). Such interference appears most evident for explosive strength and power (21, 24, 48, 55) and is not necessarily accompanied by compromised muscle hypertrophy (25, 35, 48). Regardless of mechanism(s) involved, it is imperative that certain unidentified events responsible for functional shortcomings brought about by the previous AE, must be normalized prior to RE. In support, high- but not low-intensity RE produced strength gains, whereas muscle size increased regardless of intensity (31). Further, back-to-back AE + RE impeded progression of strength, but not muscle size (58). Concurrent exercise allowing for 6 h recovery between exercise modes did not (42). Altogether, it is apparent that restored muscle function between exercise bouts is a prerequisite for attaining optimal gains in muscle function in response to AE + RE training. This contrasts the muscle hypertrophic response, which seems to occur independent of recovery.

The one-legged AE model employed here promotes early endurance adaptations (42, 49, 56). This was manifested in marked increases in PGC-1 α and VEGF expression after acute AE, and enhanced CS activity and endurance performance after AE + RE training. The current RE protocol increased muscle size and strength at rates comparable to what has been shown elsewhere (52, 61). While we have no apparent explanation for the somewhat less pronounced RE-induced increases in muscle size and strength than reported by us recently using the same RE paradigm (42), this observation should not distract from the mutual finding of significantly different response across AE + RE and RE in the two studies.

It should be acknowledged that in the current study, total work performed differed across legs. Such a response is inherent in any experimental design aimed at examining the effect of AE on responses prompted by RE. Given the particular question posted here, this effect presents no drawback in interpreting our results. The unique loading feature of the RE methodology used here allows for execution of maximal voluntary force or power through the entire range of motion in each CON action of each set, as well as ECC overload (59). Inevitably, the preceding AE, which consisted of CON knee extensions only, compromised peak power during this task (Fig. 2). In this context it should be appreciated that employing an AE mode other than the current cycling model, e.g., running comprising ECC actions and characterized by different loading history, may have produced different results. While being outside the scope of the present study, a recent meta-analysis, quantifying

a total of 422 effect sizes, reported that the interference effect is exacerbated with running compared with cycling (63).

In conclusion, consecutive bouts of AE + RE performed over 5 wk exaggerated the increase in muscle size shown with RE alone. This occurred despite increased AMPK phosphorylation, reduced glycogen content, and attenuated muscle function elicited by AE. Thus we demonstrate that AMPK activation induced by AE does not interfere with muscle growth produced by concurrent RE training. However, AE + RE blunt the progression of important aspects of in vivo muscle function. Employing this approach may therefore be counterproductive for athletes and individuals aiming at developing maximal strength and power.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: T.R.L., R.F.-G., and P.A.T. conception and design of research; T.R.L. and R.F.-G. performed experiments; T.R.L. and R.F.-G. analyzed data; T.R.L., R.F.-G., and P.A.T. interpreted results of experiments; T.R.L. and P.A.T. drafted manuscript; T.R.L., R.F.-G., and P.A.T. edited and revised manuscript; T.R.L., R.F.-G., and P.A.T. approved final version of manuscript; R.F.-G. prepared figures.

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