

# Aerobic exercise does not compromise muscle hypertrophy response to short-term resistance training

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<sup>1</sup>Department of Health Sciences, Mid Sweden University, Östersund, Sweden; <sup>2</sup>Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden; and <sup>3</sup>Department of Laboratory Medicine, Section for Clinical Physiology, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden

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**Lundberg TR, Fernandez-Gonzalo R, Gustafsson T, Tesch PA.** Aerobic exercise does not compromise muscle hypertrophy response to short-term resistance training. *J Appl Physiol* 114: 81–89, 2013. First published October 25, 2012; doi:10.1152/jappphysiol.01013.2012.— This study tested the hypothesis that chronic aerobic and resistance exercise (AE+RE) would elicit greater muscle hypertrophy than resistance exercise only (RE). Ten men ( $25 \pm 4$  yr) performed 5 wk unilateral knee extensor AE+RE. The opposing limb was subjected to RE. AE completed 6 hr prior to RE consisted of ~45 min one-legged cycle ergometry. RE comprised  $4 \times 7$  maximal concentric-eccentric knee extensions. Various indexes of in vivo knee extensor function were measured before and after training. Magnetic resonance imaging (MRI) assessed m. quadriceps femoris (QF) cross-sectional area (CSA), volume, and signal intensity (SI). Biopsies obtained from m. vastus lateralis determined fiber CSA, enzyme levels, and gene expression of myostatin, atrogen-1, MuRF-1, PGC-1 $\alpha$ , and VEGF. Increases ( $P < 0.05$ ) in isometric strength and peak power, respectively, were comparable in AE+RE (9 and 29%) and RE (11 and 24%). AE+RE showed greater increase (14%;  $P < 0.05$ ) in QF volume than RE (8%). Muscle fiber CSA increased 17% after AE+RE ( $P < 0.05$ ) and 9% after RE ( $P > 0.05$ ). QF SI increased (12%;  $P < 0.05$ ) after AE+RE, but not RE. Neither AE+RE nor RE showed altered mRNA levels. Citrate synthase activity increased ( $P < 0.05$ ) after AE+RE. The results suggest that the increased aerobic capacity shown with AE+RE was accompanied by a more robust increase in muscle size compared with RE. Although this response was not carried over to greater improvement in muscle function, it remains that intense AE can be executed prior to RE without compromising performance outcome.

endurance; gene expression; muscle cross-sectional area; muscle power and strength

IT IS GENERALLY BELIEVED that aerobic exercise (AE) compromises increases in muscle strength, power and size induced by resistance exercise (RE) training (18, 22, 28, 44). Only recently (12, 13) have human experiments offered support that this interference effect could be due to incompatibility between cellular pathways controlling protein turnover. Intrigued by these compelling findings, we investigated the acute molecular response to unilateral knee extensor RE performed 6 h after a single bout one-legged cycle ergometry (32). Surprisingly, our AE+RE paradigm prompted greater mammalian target of rapamycin (mTOR) and p70S6 kinase (p70S6K) phosphorylation than RE only. In further support of enhanced protein synthesis, gene expression of myostatin, a negative regulator of muscle hypertrophy, was suppressed following concurrent ex-

ercise. Thus, and in contrast to the general view, it appeared AE performed prior to RE boosted muscle anabolic milieu.

Activation of the mTOR signaling pathway is held as a necessity for increased protein synthesis. Indeed, reports suggest p70S6K phosphorylation correlates with muscle hypertrophy in both humans (49) and rodents (5). If this holds true, our recent findings (32) would indicate that AE may act synergistically with RE to promote muscle hypertrophy. Although this hypothesis is controversial, it remains to be shown whether the greater anabolic response, inferred after acute AE+RE rather than RE, correlates with associated changes in muscle size resulting from chronic training.

Because acute AE and RE appear to activate different cellular pathways (4), it was proposed that these signaling routes, assumed to operate in a selective switch manner, would govern mitochondrial biogenesis and myofibrillar protein synthesis, respectively. Although this seems to have become an established dogma, it should be appreciated that human skeletal muscle shows minor signaling specificity in the untrained state (53). Likewise, cumulative exercise training alters cellular responses to single exercise bouts (33, 53). Thus, although acute exercise experiments exploring skeletal muscle molecular responses may provide important mechanistic insights, results from such studies must be cautiously interpreted. Hence, studies investigating functional and skeletal muscle adaptations to chronic training to justify the significance of altered signaling induced by acute exercise are warranted.

Typically, RE training produces increased muscle strength, power, and size (50). Such effects depict classical end points resulting from cumulative training and serve as markers assessing efficacy of any RE program, but could also be employed to validate the predictive value of snapshot molecular markers. Several reports have investigated the effects of single AE bouts on cellular responses to RE (6, 12, 13, 32). Yet no human study has examined whether acute molecular responses to concurrent AE+RE correlate with related muscle adaptations resulting from chronic training.

These classical end-point markers may also be accompanied by altered basal levels of intracellular markers, increasing or decreasing the responsiveness to subsequent exercise bouts (15). For example, steady-state gene expression levels were altered in athletes following either chronic AE or RE (40, 45, 48). Although our previous study showed that acute AE may alter gene expression response to subsequent RE (32), it remains to be studied if this applies to basal mRNA levels after chronic RE with or without AE.

To address these issues, we implemented an AE+RE regimen favoring knee extensor muscle use (32) into a 5-wk training program and determined established end-point mea-

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tures of skeletal muscle adaptations. One leg was assigned to concurrent AE+RE, whereas the opposing limb was subjected to RE only. In vivo muscle function, muscle size, enzyme content or activity, and gene expression were measured before and after training. To challenge the findings of our recent investigation reporting on the acute responses to this concurrent exercise insult (32), we hypothesized that AE+RE training would manifest in greater muscle hypertrophy compared with RE.

## METHODS

**General design.** Ten moderately trained men performed 5 wk unilateral knee extensor AE+RE while the contralateral limb was subjected to RE only. Subjects completed 15 AE and 12 RE sessions such that RE was scheduled 6 h after AE. Maximal strength and power were determined before and after training, and peak power was measured during all sessions. M. quadriceps femoris (QF) volume, cross-sectional area (CSA), and signal intensity (SI) were assessed by magnetic resonance imaging (MRI). Analysis of muscle biopsies, obtained at rest before and after training, determined fiber type-specific CSA, enzyme and glycogen levels, and gene expression.

**Subjects.** Ten healthy men ( $25 \pm 4$  yr,  $184 \pm 6$  cm, and  $83 \pm 13$  kg) volunteered and showed 100% compliance to the study protocol. Hence all subjects completed the scheduled exercise sessions and pre- and posttests. Subjects were moderately trained college students engaged in recreational activities, such as skiing and team sports, on a regular basis (2 days/wk). They had modest experience to weight training before the study and had performed no regular or structured RE training in the past year. The study experiments and procedures including risks and discomforts were explained before subjects gave their written informed consent to participate. The study protocol was approved by the Regional Ethical Review Board in Umeå.

**Exercise equipment and familiarization.** AE was performed using a one-legged cycle ergometer (3) (model 828E, Monark Exercise, Varberg, Sweden) as previously described (32). This ergometer allows for isolated concentric (CON) knee extensions in a range of motion from about 90 to 175°. Power and cadence were sampled at 2 Hz using the SRM training system (SRM, Jülich, Germany). RE was performed using a nongravity-dependent seated knee extensor ergometer (YoYo Technology, Stockholm, Sweden) equipped with a 4.2-kg (moment inertia 0.11 kg/m<sup>2</sup>) flywheel to provide inertial resistance during coupled CON and eccentric (ECC) actions (51). Peak CON and

ECC torque and power, averaged across sets, was calculated from measures of force (MuscleLab, Langesund, Norway) and rotational velocity (SmartCoach, Stockholm, Sweden). Knee extensor maximal isometric and isokinetic torque were further assessed using a Cybex II (Lumex, New York) dynamometer, calibrated before each test. Thigh, hip, and chest were stabilized to the dynamometer chair using straps. The ankle was strapped to the dynamometer lever arm that was aligned with the axis of rotation of the knee joint. Torque was sampled at 100 Hz using MuscleLab and peak values averaged across sets were used for data analysis. For all apparatuses used, individual settings were maintained throughout the study. To customize machine settings and familiarize subjects with the exercises employed during training and testing, subjects reported to the laboratory three times in the course of 2 wk prior to the study.

**Pre- and posttesting.** Identical test protocols were employed before and after the 5-wk training period. These tests were scheduled on four different days within ~1 wk. First, muscle volume and SI were assessed through MRI scans (described below). During the second visit, muscles biopsies (details below) were obtained from the RE leg before (PRE) and from both legs (AE+RE and RE) 72 h after the last training session. To limit the total number of biopsies, we refrained from obtaining biopsies from both legs before training. Given our previous finding of no difference in protein concentration across randomized legs and because muscle volume and function were very similar before training (Table 1), we are confident that the sole prebiopsy accurately represents the untrained condition of either leg.

Three or four days after the biopsy procedure, maximal isometric and isokinetic strength, flywheel ergometer torque and power, and one-legged endurance were assessed. These performance tests were scheduled on 2 days interspersed by 48 h rest, such that *day 1* comprised strength and power related measures for the right leg and an endurance test for the left leg. The order was reversed on *day 2*. Maximal isokinetic strength was assessed using isokinetic dynamometry. 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. Subjects performed two maximal actions (30 s rest) at each velocity and the best result represented peak torque. A third attempt was allowed if peak torque differed >5%. Maximal isometric torque was measured at knee angle 120°. Subjects were instructed to push with maximal effort for ~5 s. Three trials with 60 s rest between attempts were allowed. The best score in a 1-s window defined peak isometric torque.

After 5 min rest, peak torque and power (averaged across sets and repetitions) were assessed using the knee extension flywheel ergom-

Table 1. Selected outcome measures pre- and postresistance training with (AE+RE) or without (RE) concurrent aerobic exercise

	AE+RE			RE		
	PRE	POST	Δ%	PRE	POST	Δ%
Endurance performance, s <sup>b,c</sup>	590 ± 104	752 ± 129*	29	553 ± 98	659 ± 109*	19
W <sub>max</sub> , W <sup>b,c</sup>	50 ± 12	72 ± 19*	44	49 ± 12	62 ± 15*	27
Peak heart rate at W <sub>max</sub> , beats/min <sup>b,c</sup>	158 ± 18	169 ± 12	7	153 ± 17	161 ± 17	5
Lactate 3 min post W <sub>max</sub> , mmol/l <sup>b,c</sup>	6.7 ± 1.5	8.0 ± 1.1*	19	6.1 ± 1.0	7.2 ± 1.2*	18
Flywheel mean peak power, W <sup>c</sup>	400 ± 137	514 ± 129*	29	406 ± 120	502 ± 126*	24
Flywheel mean peak torque, Nm <sup>c</sup>	218 ± 31	279 ± 59*	28	217 ± 35	280 ± 61*	29
Maximal isometric torque, Nm <sup>c</sup>	287 ± 53	312 ± 86	9	276 ± 49	307 ± 73	11
QF muscle volume, cm <sup>3</sup> <sup>a,b,c</sup>	1,147 ± 249	1,303 ± 276*†	14	1,111 ± 262	1,198 ± 273*	8
QF mean CSA, cm <sup>2</sup> <sup>a,b,c</sup>	79 ± 10	90 ± 10*†	14	78 ± 12	84 ± 12*	8
QF greatest CSA, cm <sup>2</sup> <sup>a,b,c</sup>	89 ± 11	101 ± 12*†	13	87 ± 13	94 ± 14*	8
Normalized torque, Nm/cm <sup>2</sup> <sup>c</sup>	2.77 ± 0.37	3.10 ± 0.52*	12	2.81 ± 0.36	3.36 ± 0.69*	19
Normalized power, W/cm <sup>2</sup> <sup>c</sup>	5.03 ± 1.16	5.67 ± 1.16*	13	5.22 ± 0.98	6.00 ± 1.29*	15
QF signal intensity, MGV <sup>a,c</sup>	52 ± 7	58 ± 8*	12	52 ± 7	52 ± 9	0
VL signal intensity, MGV <sup>a,b,c</sup>	53 ± 8	58 ± 8*†	9	52 ± 8	51 ± 8	-2
BF signal intensity, MGV	40 ± 7	41 ± 6	2	41 ± 10	40 ± 10	-2

Values are means ± SD. Significant effects ( $P < 0.05$ ); a, interaction; b, leg; c, time. Significant post hoc differences ( $P < 0.05$ ): \*within leg vs. PRE; †vs. RE at POST. W<sub>max</sub>, maximal workload; CSA, cross-sectional area; MGV, mean gray value; QF, quadriceps femoris; VL, vastus lateralis; BF, biceps femoris.

eter. Subjects performed  $2 \times 7$  repetitions with 2 min rest between sets. Strong verbal encouragement was used to call for maximal effort. Normalized torque and power were further calculated as the ratio between peak knee extension torque/power and average muscle CSA ( $\text{Nm}/\text{cm}^2$ ). Fifteen minutes later, the one-legged ergometer incremental test was performed for the opposing limb to assess maximal workload ( $W_{\text{max}}$ ) and endurance performance. Resistance was increased by 2.5 N every 2nd min until failure to maintain the prescribed cadence of 60 rpm. When this occurred, time to exhaustion was noted and  $W_{\text{max}}$  was defined as the last successfully completed workload. Capillary blood (20  $\mu\text{l}$ ) was sampled from the ear lobe at rest, every 2nd min into exercise and 1 and 3 min postexercise. Samples were subsequently placed in a 1-ml hemolyzing solution and analyzed for lactate concentration (EKF-diagnostic, Magdeburg, Germany). Heart rate was recorded (Polar Electro OY, Kempele, Finland) continuously throughout the test and subsequently analyzed (Polar ProTrainer 5). Rate of perceived exertion (RPE; central and local) was obtained every 2nd min and at exhaustion using the 6–20 “Borg scale” (9). Subjects were blind to any test result to ensure nonbiased efforts during pre- and posttests.

**Training protocols.** During the 5-wk training intervention, subjects completed 15 unilateral 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). Hence, one limb performed concurrent AE+RE whereas the other limb was subjected to RE only. Legs chosen for the AE+RE intervention were randomized in a counterbalanced manner. The particular RE hardware and regimen have proven success in earlier studies (41, 51) and RE for a particular muscle group should not be prescribed more frequently. To justify the AE protocol chosen, previous research showed marked increases in peak oxygen uptake and skeletal muscle oxidative capacity after employing a 3 times/wk 30-min one-legged exercise (23, 24). These protocols are also commensurate with ACSM guidelines. AE was scheduled in the morning and RE 6 h after completion of AE on the same day. Subjects were requested to have a nonstandardized lunch between sessions. A standardized warm-up at submaximal effort preceded each exercise prescribed. Subjects were supervised during all training sessions.

AE comprised 40-min continuous one-legged cycle ergometer exercise. The initial target load was 70% of the  $W_{\text{max}}$  at a fixed cadence of 60 rpm. However, RPE (central and local) was obtained every 10th min to customize the workload such that a very strenuous effort was achieved during each exercise bout. After 40 min, the workload was increased by  $\sim 20$  W, and subjects were requested to continue until failure, which occurred within 1–5 min (average 2 min 30 s). Subjects received real-time visual feedback of power and cadence via a computer monitor. Heart rate was recorded continuously during one randomly selected session each week. Capillary blood, for subsequent analysis of lactate concentration, was sampled at rest, every 10th min and 1 and 3 min after exercise in the same session.

RE was carried out 6 h after one-legged cycling had been terminated. Each leg (beginning with the right) performed four sets of seven CON-ECC knee extensions (2 min rest between sets) in the flywheel ergometer. Subjects were requested to perform each repetition with maximal effort and were verbally encouraged throughout each set. Peak power for each repetition was measured during all sessions.

**Diet/exercise control.** Subjects refrained from strenuous physical activity and alcohol for a minimum of 48 h prior to any pre- and posttest day. A standardized meal (pasta, tomato sauce, and juice) consisting of 2.21 g carbohydrates (CHO)/kg body weight (BW), 0.22 g protein/kg BW, and 0.04 g fat/kg BW was provided at  $\sim 8:00$  PM on the night before the biopsies were obtained. The following morning, subjects had a standardized breakfast (1.01 g CHO/kg BW, 0.31 g protein/kg BW, and 0.24 g fat/kg BW)  $\sim 2$  h prior to the biopsy procedure. This meal consisted of commercial energy drinks (Ensure Plus, Abbott Laboratories B.V. Zwolle, The Netherlands). Water was

allowed ad libitum at any time. Subjects were requested to record food intake on pretesting days and replicate the same diet regimen on posttesting days. The individual testing schedule was very similar ( $\pm 2$  h) on pre- and posttest days. Throughout the study, subjects were instructed to maintain ordinary daily activities and routines and to refrain from strenuous activities involving the lower limbs.

**Magnetic resonance imaging.** Cross-sectional images were obtained using a 1.5-Tesla Philips MR Systems Intera (Best, The Netherlands) unit; Turbo spin echo, T2 weighted, TE 110 ms, TR 5723 ms, NSA 3, FOV 48.5 cm, scan time 13 min 10 s and voxel size  $0.95 \times 0.95 \times 10$  mm. For each subject, 50 continuous images with 10-mm slice thickness were obtained. To minimize the influence of fluid shift on muscle volume, subjects were resting in the supine position for 1 h prior to any scan (7). A custom-made foot-restraint device ensured no compression of thigh muscles and fixed limb position. Scout images were obtained to confirm identical positioning in pre- and postscans. The top of caput femoris was used as an anatomical landmark to ensure that the same segment was scanned before and after training. Stacks of images were created in TIF format with a scale of 10.887 pixels/ $\text{cm}^2$  and deidentified and subsequently coded. Thus, although pre-post images were analyzed in parallel, the individual who performed the analysis was blind to any intervention. CSA and SI [i.e., mean gray value (MGV)] of each individual QF muscle in order vastus lateralis (VL), vastus intermedius (VI), vastus medialis (VM), and rectus femoris (RF) were analyzed from the first image not displaying m. gluteus maximus and ending with the last image in which RF appeared. Within this segment (range 11–17 images), every third image was analyzed by manually encircling the muscle (1) using public domain software (Image J, National Institutes of Health, Bethesda, MD). Two sample sets failed to accurately display all individual muscle borders. Hence total QF volume only was measured in these cases. The average value of at least three circumscriptions showing less than 1.5% difference between the highest and lowest values was multiplied by slice thickness to obtain muscle volume. The individual muscle's MGV was averaged to determine SI for QF and for VL specifically. As an additional control, SI of m. biceps femoris (BF) was analyzed in the third image of each subject.

**Muscle biopsies.** Percutaneous muscle biopsies (8) were obtained from VL at rest before (RE leg) and 72 h after (both legs) the training period. Local anesthesia was administered to the skin and muscle fascia, and tissue samples ( $\sim 180$  mg) were subsequently obtained through incisions 20 mm apart (distal to proximal) using a 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 precooled isopentane and stored at  $-80^\circ\text{C}$  until processing.

**Immunohistochemical analysis.** A small ( $\sim 20$  mg) portion of each muscle biopsy was oriented for transverse sectioning and 5  $\mu\text{m}$  cross sections were cut in a cryostat at  $-22^\circ\text{C}$  and mounted on glass slides. Sections were subsequently stained using monoclonal antibodies (mAbs) against slow myosin heavy chain (mAb A4.840 from Developmental Studies Hybridoma Bank, Iowa City, IA) and mAb Merosin IgG1 (Novocastra Laboratories Ltd) against the laminin  $\alpha 2$ -chain for detection of cell borders. There were no stainings performed to identify type II A/X fibers. The vast majority of each muscle section was captured (5–7 images) using a microscope camera for fluorescence detection (DFC360 FX Leica Microsystems, Wetzlar, Germany). To determine the frequency of fibers expressing slow myosin heavy chain (type I fibers) and to measure fiber CSA using custom-developed semi-automated analysis software, an average of 177 fibers (range 101 to 250) from each sample was analyzed.

**Enzyme activity, glycogen, and water content.** Freeze-dried muscle samples (2–3 mg) were homogenized in phosphate buffer with 0.5% BSA. Citrate synthase (CS) and phosphofructokinase (PFK) enzyme activity and lactate dehydrogenase (LDH) content were subsequently determined by  $\text{NAD}^+$ -NADH-coupled reactions using fluorometry (31) (CS and PFK) or spectrophotometry (LDH). Glycogen was



hydrolyzed enzymatically to free glucose and assayed by fluorometry (20). Muscle water content was estimated by relating ~10 mg wet muscle sample to dry weight, as measured on a precision microbalance at standardized temperature and humidity, and expressed as a percentage of initial wet weight.

**RNA isolation, reverse transcription, and real-time PCR.** Exercise-induced changes in the basal muscle molecular environment were assessed by analyzing expression of key genes (11) regulating angiogenesis [vascular endothelial growth factor (VEGF)], mitochondrial biogenesis [peroxisome proliferator-activated receptor- $\gamma$  coactivator-1 (PGC-1 $\alpha$ )], and protein turnover [myostatin, atrogin-1 and muscle RING-finger protein-1 (MuRF-1)]. One aliquot of ~20 mg frozen muscle tissue was homogenized using TRIzol (Invitrogen Life Technologies, Carlsbad, CA), and total RNA was extracted. One microgram of total RNA was subsequently reverse transcribed into cDNA using high capacity reverse transcription kit (Applied Biosystems, Foster City, CA) in a total volume of 20  $\mu$ l. Real-time PCR was performed on ABI-PRISMA 7700 Sequence Detector System (Perkin-Elmer Applied Biosystems, Foster City, CA). The reaction mix consisted of 4.5  $\mu$ l of the diluted (1:100) cDNA template, 5  $\mu$ l of the 2  $\times$  TaqMan PCR Mastermix, and 0.5  $\mu$ l gene specific primers. The cycling procedures 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 for atrogin-1 (Hs00369714\_m1), MuRF-1 (Hs00822397\_m1), myostatin (Hs00193363\_m1), PGC-1 $\alpha$  (Hs01016724\_m1), and VEGF (Hs99999070\_m1) were derived from the TaqMan Gene Expression Assays (Applied Biosystems). Samples from each individual were assayed on the same plate. GAPDH (Hs99999905\_m1) was used as the housekeeping gene. For further control, 18S (Hs01375212\_g1) was analyzed as an additional reference gene. The results were almost identical with 18S or GAPDH as housekeeping genes. Hence the GAPDH/18S ratio did not change across time points. Gene expression levels were determined using the  $2^{-\Delta\Delta C_T}$  method (29), relating mRNA changes as a ratio to the housekeeping gene.

**Data analysis.** Muscle volume, CSA and SI, isometric and normalized torque/power, and mean peak torque and power were analyzed using two-way repeated measures ANOVA with factors time (pre and post)  $\times$  leg (AE+RE and RE). Average peak power across the training period was examined using a two-way ANOVA (sessions  $\times$  leg). The torque-velocity relationship was examined using a three-way ANOVA (time  $\times$  leg  $\times$  velocity). Differences in basal enzyme levels, glycogen and water content, gene expression and fiber type, and CSA were assessed using one-way repeated-measures ANOVA. Analyses on some positively skewed variables (VEGF and atrogin-1) were done using log-transformed data. Significant interactions were further examined with simple effect tests, and the false discovery rate (FDR) procedure was employed after pairwise post hoc comparisons (14). The level of significance was set at 5% ( $P < 0.05$ ). All statistical analyses were performed using SPSS version 18 (SPSS, Chicago, IL). Data are presented as means  $\pm$  SD.

## RESULTS

**Aerobic exercise training.** The average power during one-legged cycling across the 5 wk was  $43 \pm 8$  W. It increased ( $P < 0.05$ ) from  $37 \pm 5$  (session 1) to  $49 \pm 10$  W (session 15). Power at the final stage averaged  $65 \pm 13$  W. Heart rate averaged  $124 \pm 14$  beats/min across the 5 wk to peak at  $153 \pm 17$  beats/min during the final increment. At exercise completion, peak heart rate was  $163 \pm 16$  beats/min. Blood lactate concentration during exercise averaged  $4.5 \pm 1.0$  mmol/l. Lactate concentration 1 and 3 min after exhaustion was  $6.2 \pm 1.4$  and  $6.3 \pm 1.6$  mmol/l, respectively. RPE rose in a linear fashion during exercise and amounted to 15 (central) and 17

(local), respectively, after 40 min. Local muscle exertion was maximal at exercise completion.

**Resistance exercise training.** Average peak power across muscle actions rose almost linearly (main effect of time  $P < 0.0005$ ) during the 12 RE exercise sessions (Fig. 1). The increase from the 1st to the 12th session was 27% ( $384 \pm 105$  vs.  $488 \pm 142$  W) for AE+RE and 28% ( $395 \pm 112$  vs.  $507 \pm 154$  W) for RE with no difference across legs ( $P > 0.05$ ).

**Endurance performance.** Although both legs showed improved endurance (time to exhaustion) after training, the increase tended to be more prominent (interaction time  $\times$  leg,  $P = 0.052$ ) after AE+RE than RE (Table 1). Similarly,  $W_{\max}$  increased after both AE+RE and RE. The average heart rate and lactate concentration at exhaustion increased (main effects of time) from pre- to posttests, with no interaction effect (Table 1).

**Strength and power performance.** Increases in maximal isometric torque (main effect of time  $P < 0.0005$ ) were comparable for AE+RE and RE (Table 1). Likewise, flywheel peak torque increased 28% after AE+RE for both CON (196 to 251 Nm) and ECC (240 to 307 Nm) muscle actions. The corresponding increases after RE amounted to 30% for CON (193 to 251 Nm) and 27% for ECC (242 to 308 Nm). There were no differences across legs (Table 1). Isokinetic peak torque increased on average 11% across velocities for both legs from pre- to posttraining (main effect of time  $P = 0.007$ ). However, there was a time  $\times$  leg  $\times$  velocity interaction ( $P = 0.017$ ). Simple effect tests revealed that this was due to a leg  $\times$  velocity interaction before training (Fig. 2;  $P = 0.02$ ). Normalized torque showed a trend ( $P = 0.077$ ) toward a time  $\times$  leg interaction due to a greater increase for RE (19%) vs. AE+RE (12%; Table 1).

**Muscle volume, CSA, and signal intensity.** Total QF volume showed a time  $\times$  leg interaction ( $F = 52.3$ ;  $P < 0.0005$ ). Values were similar between AE+RE and RE at baseline, but differed after training (Table 1). The increase in muscle volume averaged 13.6% ( $P < 0.0005$ ) after AE+RE and 7.8% ( $P < 0.0005$ ) after RE. The response was very consistent across all 10 subjects (Fig. 3 and 4). Likewise, the increase in

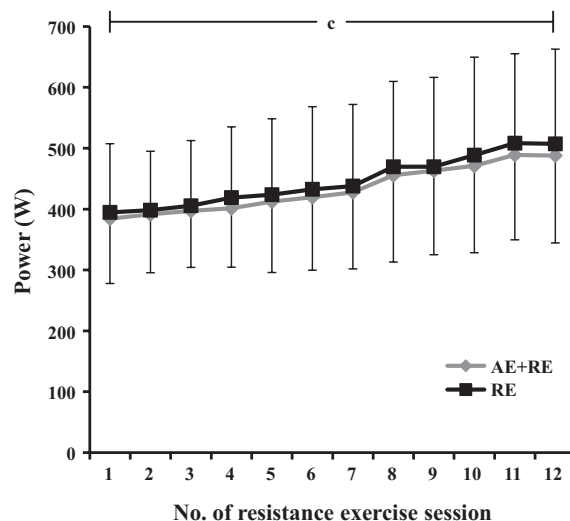


Fig. 1. Peak power measured in the flywheel knee extension ergometer during 12 resistance exercise sessions with (AE+RE) or without (RE) preceding aerobic exercise. Means  $\pm$  SD. Significant effect ( $P < 0.05$ ); c = time.

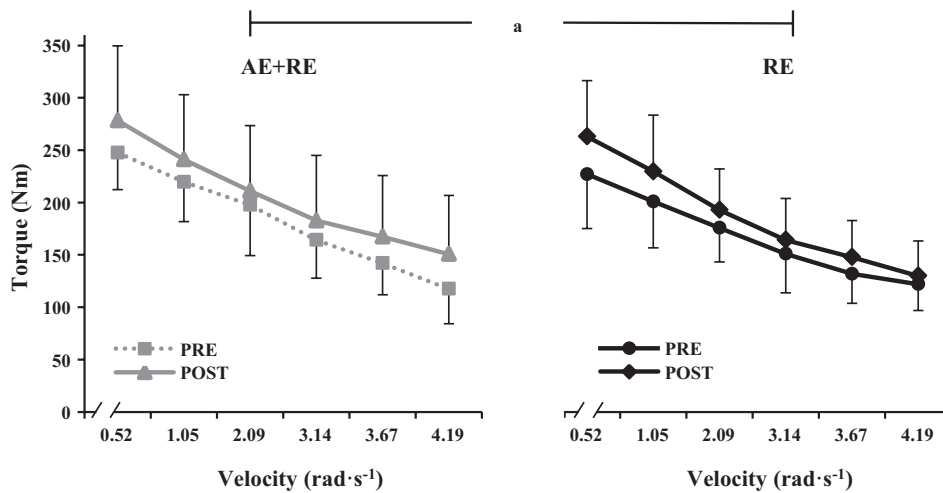


Fig. 2. Knee extensor torque-velocity relationship pre- and post-aerobic and resistance (AE+RE) exercise compared with resistance exercise (RE). Means  $\pm$  SD. Significant effect ( $P < 0.05$ ); a = interaction (time  $\times$  leg  $\times$  velocity).

CSA was greater after AE+RE than RE (Table 1). Analysis of individual muscles showed the following increases in volume after training (AE+RE vs. RE): VL 16.6 vs. 7.6%; VI 11.0 vs. 6.4%; VM 11.8 vs. 6.4%, and RF 25.8 vs. 16.4%. The SI of both QF and VL was similar across legs before training and increased after AE+RE, but not RE (Table 1; QF interaction

time  $\times$  leg:  $P < 0.0005$ ). SI of BF showed no differences over time or across legs.

**Fiber type and CSA.** Type I and type II fiber percentage did not change after training (Table 2). Mean fiber CSA increased 17% following AE+RE ( $P = 0.015$ ) compared with a 9% increase ( $P = 0.200$ ) after RE. The more robust increase

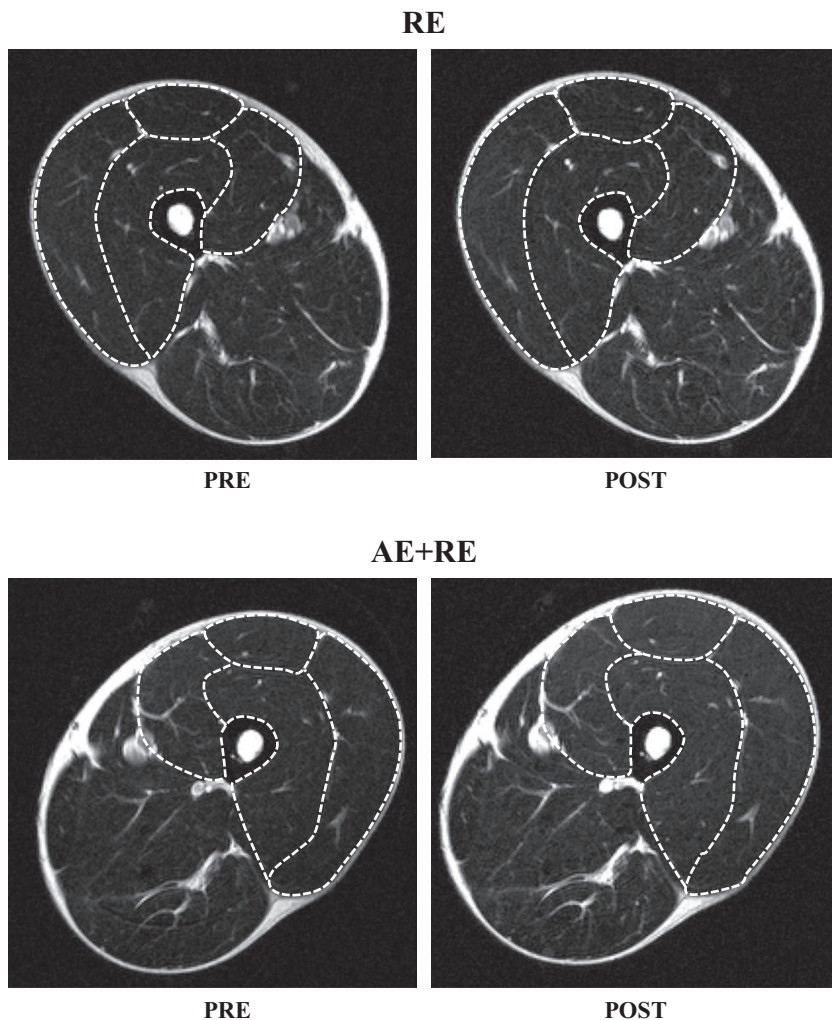


Fig. 3. Magnetic resonance imaging images depicting thigh muscle cross-section obtained in 1 representative individual pre- and postresistance training with (AE+RE) or without (RE) concurrent aerobic exercise. Slices shown are 27 cm distal to the top of caput femoris.

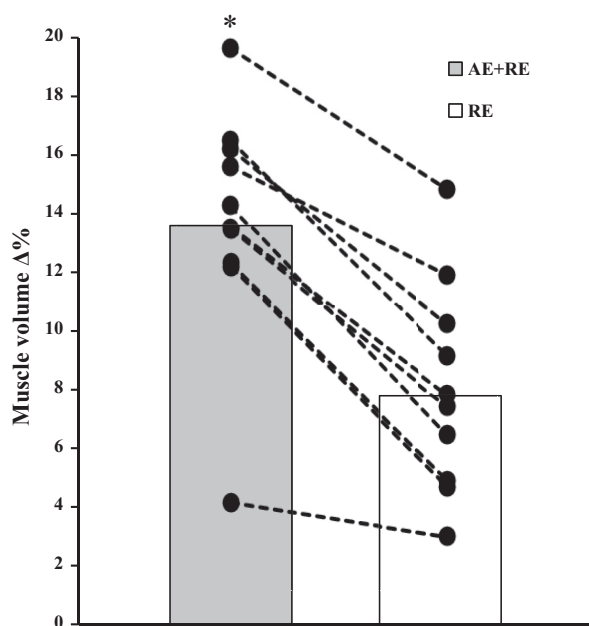


Fig. 4. Individual and group mean increase (%) in m. quadricep muscle volume following resistance training with (AE+RE) or without (RE) concurrent aerobic exercise. \*Greater increase after AE+RE.

following AE+RE appeared to be due to a relatively greater increase in type I fiber CSA (Table 2). Type II fiber CSA increased 19% after AE+RE ( $P = 0.025$ ) and tended to increase (16%) after RE ( $P = 0.052$ ).

**Enzyme activity, glycogen, LDH, and water content.** CS activity increased 19% following AE+RE ( $P = 0.006$ ; Table 2). PFK activity or LDH content showed no changes ( $P > 0.05$ ). Glycogen content was greater ( $P = 0.001$ ) following AE+RE than RE (Table 2). Estimated muscle water content was similar across legs and showed no change over time.

**Gene expression.** There was no change in basal expression levels for any of the genes investigated (Fig. 5).

## DISCUSSION

The interest in this study arose from our recent intriguing finding of greater skeletal muscle anabolic response to acute concurrent AE+RE compared with RE (32). The current study design employed this particular exercise paradigm to determine skeletal muscle adaptations resulting from 5 wk RE training

with or without preceding exhaustive AE. Given our earlier observation, we hypothesized that concurrent AE+RE would elicit greater increase in muscle size than RE. Indeed, while in vivo muscle strength and power showed comparable improvements across legs, the increase in muscle size was more evident following AE+RE. These novel results suggest that AE could offer a synergistic hypertrophic stimulus to RE training without compromising the progress in in vivo muscle function resulting from RE.

The main finding of the current study was the remarkable increase in QF muscle volume following AE+RE. The gained muscle CSA and volume was accompanied by increased muscle fiber CSA that was greater after AE+RE than RE and appeared to be ascribed to a more substantial, yet not significant, type I fiber hypertrophy. At first, it would be tempting to attribute this effect to accretion of contractile material. However, it must be acknowledged that QF SI was enhanced after concurrent exercise, but not after RE. Thus, and given that m. biceps femoris of either leg showed unaltered SI, it seems unlikely this effect was systemic or the finding random. We rather believe this obscured effect resulted from adaptations specific to this particular exercise regimen. Similar to us, a recent report (19) noted increased SI of muscles undergoing hypertrophy following AE training. Augmented SI shown after exercise is typically attributed to increased muscle water/hydrogen content (35) due to very transient osmotic fluid shifts or as a result of edema from muscle damage after, e.g., ECC exercise (16). None of these explanations could account for the increased SI because MRI scans were obtained at least 48 h after completing the final exercise session, and no subject reported delayed onset of muscle soreness at this time. Interestingly, Harber and associates (19) observed increased muscle water content accompanied by increased sarcoplasmic-to-myofibrillar protein content ratio after training. This contrasts earlier reports suggesting unaltered myofibrillar packing and myosin actin filament ratio after cumulative RE (10). Similarly, concentrations of cytoplasmic and contractile protein pools were unchanged in both trained and untrained men (2) and in individuals subjected to long-term bed rest or unloading with or without resistance exercise (21). Albeit our crude estimate showed unchanged muscle water content, it cannot be precluded that the robust muscle hypertrophy in part could have been due to expanded sarcoplasmic or interstitial fluids. Regardless, future studies disclosing the underpinnings of exercise-induced muscle hypertrophy are warranted.

Table 2. Selected outcome measures pre- and postresistance training with (AE+RE) or without (RE) concurrent aerobic exercise

	PRE	AE+RE	RE
CS activity, $\text{mmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ c	$34 \pm 6$	$41 \pm 8^{*†}$	$35 \pm 7$
PFK activity, $\text{mmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$	$87 \pm 24$	$93 \pm 24$	$86 \pm 18$
LDH content, $\mu\text{katal/l}$	$12.0 \pm 4.3$	$11.9 \pm 3.8$	$13.2 \pm 5.8$
Glycogen content, $\text{mmol/kg dry wt}^c$	$550 \pm 143$	$682 \pm 143^{\dagger}$	$453 \pm 96^*$
Estimated muscle water content, %	$74.0 \pm 2.0$	$73.8 \pm 2.5$	$72.0 \pm 2.8$
Type I fibers, %	$50 \pm 15$	$51 \pm 18$	$51 \pm 13$
Type II fibers, %	$50 \pm 15$	$49 \pm 18$	$49 \pm 13$
Mean fiber CSA, $\mu\text{m}^c$	$4,601 \pm 1,097$	$5,361 \pm 781^*$	$5,033 \pm 767$
Type I fiber CSA, $\mu\text{m}$	$4,055 \pm 911$	$4,547 \pm 660$	$4,259 \pm 932$
Type II fiber CSA, $\mu\text{m}^c$	$5,046 \pm 1,439$	$5,995 \pm 1,010^*$	$5,829 \pm 999$

Values are means  $\pm$  SD. Significant effects ( $P < 0.05$ ); c, condition. Significant post hoc differences ( $P < 0.05$ ): \*vs. PRE;  $^{\dagger}$ vs. RE. CS, citrate synthase; PFK, phosphofructokinase; LDH, lactate dehydrogenase.



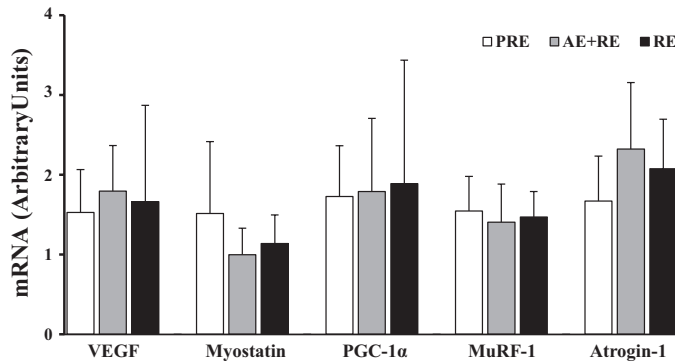


Fig. 5. Basal gene expression levels of VEGF, myostatin, PGC-1 $\alpha$ , MuRF-1, and atrogin-1 pre- and postresistance training with (AE+RE) or without (RE) concurrent aerobic exercise.

Given that both anatomical and physiological muscle CSA correlate with muscle force (34, 38), one could argue that the more pronounced increase in muscle volume after AE+RE would have been accompanied by greater strength and power gains than RE only. Evidently this was not the case. In fact, the increase in normalized torque ( $\sim$ specific force) tended to be greater after RE than AE+RE, suggesting the muscle hypertrophy established after AE+RE dissociated from the increase in muscle strength or power.

By employing the current 5-wk RE paradigm, which favors “eccentric overload” using flywheel ergometry, QF volume showed a 6–8% increase (41, 51). In concert, QF muscle size increased by 7.8% in the present study of RE. Considering that power and hence workload during RE sessions were very similar across legs over time, the corresponding (13.6%) increase noted after AE+RE is rather remarkable and exceeds the rate typically reported in response to short-term RE training (42, 52). This would imply that repetitive concentric low-force actions as employed here act synergistically with high-force RE training to govern increased skeletal muscle size at a rate far greater than shown with highly effective RE. This is in frank contrast to the fairly established view that AE prompts no or minute muscle hypertrophy (25). In support of our findings however, one-legged cycling performed 5 day/wk for 5 wk increased muscle size by 7% in recreationally active men (37), and low-force actions, performed until failure with or without vascular occlusion, induced hypertrophy similar to traditional high-force RE training (26, 36). Although we acknowledge these collective findings remain controversial, it may be that low-force actions repeated until failure ultimately promote muscle hypertrophy. Notwithstanding, and given the lack of any obvious impact on in vivo muscle function, we are currently not capable of providing a feasible explanation in regards to what (1) particular stimulus triggered the more robust hypertrophy and associated increase in SI, (2) constituents were responsible for the increased muscle size, and (3) is the physiological significance of the more robust increase in muscle size observed after concurrent training.

To present an AE challenge, we employed the one-legged cycle exercise model introduced and validated by Andersen et al. (3). This exercise model isolates the QF muscle group during repeated CON actions. Oxygen uptake ( $\dot{V}O_2$ ) rises linearly with increased external work, and at maximal work rate, aerobic metabolic rate of active muscles increases more

than  $\sim$ 100-fold and  $\dot{V}O_2$  attains 1.2 l/min (3). The blood flow and a-v  $O_2$  diff of the exercising limb is comparable to what has been reported during maximal cycle exercise (37). During the  $\sim$ 45-min exercise bouts performed in the current study,  $\sim$ 2,500 CON muscle actions were executed at an average work rate of 70% of  $W_{max}$ . To accomplish 45 min at 60 rpm on a standard cycle ergometer would require 2,700 contractions per limb. Thus, although this exercise modality does not resemble classical endurance training involving much larger muscle mass (e.g., treadmill running, cross-country skiing), it remains that stress at the muscle cellular level certainly is most “aerobic” in nature. This is justified by findings of parallel increases in work capacity, muscle capillary supply, and oxidative enzyme activity following chronic one-legged cycling (23, 24, 47). Furthermore, employing training 5 day/wk for 5 wk increases in peak work rate, peak  $\dot{V}O_2$ , thigh blood flow, and  $O_2$  delivery amounted to 17–24% (37), and 4 wks training increased CS activity by 22% (43). The current model allows for strictly controlled intrasubject comparisons, such that adaptations of a single “isolated” muscle group can be explored in response to, e.g., various exercise programs. In contrast, treadmill running or rowing would call for involvement of additional muscle groups, which could not be quantified or controlled for.

The improved muscle endurance following AE performed three times weekly was paralleled by enhanced skeletal muscle aerobic capacity as reflected in increased CS activity. Thus, although the AE stimulus per se was highly effective, the increases in in vivo muscle strength and power as result of training were very similar across legs and hence exercise modes. Perhaps most convincingly, day-by-day power and total work and thus exercise stimulus during each of the 12 RE sessions were almost identical across legs. It is therefore concluded that there was no interference from the previous AE bouts compromising performance outcome in response to short-term (i.e.,  $\leq$ 5 wk) RE training. More likely, program design features, e.g., duration, intensity, volume, and rest between exercise sessions, are to dictate any potential interference with RE performance and the desired adaptations (27, 54).

Selected genes may display altered basal mRNA levels after chronic training (40, 48). Yet microarray data showed that only 12 genes were differently expressed in young adults subjected to 12 wk RE training (45). The present study investigated genes that acutely respond to either AE (17, 39) or RE (30, 33) and also show responsiveness to training (11). The results infer basal gene expression was unaltered regardless of training modality. In contrast, myostatin expression was downregulated 48–72 h after completion of 8–9 wk RE training (26, 46). Yet myostatin and atrogin-1 levels might be downregulated 48 h after acute exercise (33) to suggest that muscle samples taken at or before this time point may simply reflect the response to the very last exercise session rather than the cumulative effect. Our results regarding PGC-1 $\alpha$  concord with the findings of Pilegaard et al. (43), who reported similar basal mRNA levels across trained and untrained limbs. Collectively, it seems plausible that with regard to the genes chosen here, the protein levels are regulated independent of basal transcriptional changes. Resting expression levels of these particular genes may therefore be poor markers reflecting training-induced muscle adaptations.

In conclusion, the concurrent 5-wk AE+RE paradigm resulted in robust increased in vivo muscle strength and power. Although this effect appeared to be evoked by the RE stimulus alone, the combined approach was accompanied by more substantial muscle hypertrophy, which was not carried over to greater improvement in muscle function. It remains that intense AE can be executed prior to RE without compromising performance outcome.

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## DISCLOSURES

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

## AUTHOR CONTRIBUTIONS

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

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