

Anabolic responses to acute and chronic resistance exercise are enhanced when combined with aquatic treadmill exercise

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Submitted 19 December 2013; accepted in final form 18 November 2014

Lambert BS, Shimkus KL, Fluckey JD, Riechman SE, Greene NP, Cardin JM, Crouse SF. Anabolic responses to acute and chronic resistance exercise are enhanced when combined with aquatic treadmill exercise. *Am J Physiol Endocrinol Metab* 308: E192–E200, 2015. First published November 25, 2014; doi:10.1152/ajpendo.00689.2013.—Aquatic treadmill (ATM) running may simultaneously promote aerobic fitness and enhance muscle growth when combined with resistance training (RT) compared with land-treadmill (LTM) running. Therefore, we examined acute and chronic physiological responses to RT, concurrent RT-LTM, and concurrent RT-ATM. Forty-seven untrained volunteers (men: $n = 23$, 37 ± 11 yr, 29.6 ± 4.6 kg/m²; women: $n = 24$, 38 ± 12 yr, 27.53 ± 6.4 kg/m²) from the general population were tested for $\dot{V}O_{2\max}$, body composition, and strength before and after training. All groups performed 12 wk of RT (2 wk, 3×8 –12 sets at 60 to approximately 80% 1-repetition maximum). The RT-LTM and RT-ATM groups also performed 12 wk of LTM or ATM training (2 wk immediately post-RT and 1 wk in isolation, 60–85% $\dot{V}O_{2\max}$, 250–500 kcal/session). Additionally, 25 subjects volunteered for muscle biopsy prior to and 24 h post-acute exercise before and after training. Stable isotope labeling (70% ²H₂O, 3 ml/kg) was utilized to quantify 24 h post-exercise myofibrillar fractional synthesis rates (myoFSR). Mixed-model ANOVA revealed that RT-ATM but not RT-LTM training produced greater chronic increases in lean mass than RT alone ($P < 0.05$). RT-LTM training was found to elicit the greatest decreases in percent body fat (-2.79% , $P < 0.05$). In the untrained state, acute RT-ATM exercise elicited higher 24-h myoFSRs compared with RT ($+5.68\%$ /day, $P < 0.01$) and RT-LTM ($+4.08\%$ /day, $P < 0.05$). Concurrent RT-ATM exercise and training elicit greater skeletal muscle anabolism than RT alone or RT-LTM.

concurrent training; aquatic exercise; skeletal muscle; aquatic treadmill; protein metabolism

IN RECENT YEARS, AQUATIC EXERCISE HAS GROWN in popularity in the general, overweight, elderly, and athletic populations as a mode of therapeutic or rehabilitative exercise (43, 66). Aquatic-based running exercises such as deep water running and aquatic treadmill (ATM) running (Fig. 1) have also been shown to be effective alternatives to land-based aerobic exercises for promoting increases in aerobic fitness (11, 18, 38). Recently, we compared the efficacy of ATM training with traditional land treadmill (LTM) training using a training prescription similar to that recommended by the American College of Sports Medicine (ACSM) (38). Following training,

increases in aerobic capacity and decreases in fat mass were similar regardless of training mode. However, leg lean mass [measured with dual-energy X-ray absorptiometry (DEXA)] was increased significantly following ATM training, twice that of the LTM group. We suspected that because vertical load, lateral resistance, and skeletal muscle activation have been shown to differ between ATM exercise and traditional LTM exercise (67), chronic ATM training may also elicit unique mode-specific adaptive responses compared with LTM training.

Although no measures of strength were obtained in our original investigation (38), the gains in lean mass observed following training led us to consider a potential role for ATM exercise in a concurrent aerobic and resistance training model. Previous investigators (4, 9, 40, 44) have reported that aerobic training may interfere with skeletal muscle hypertrophy and strength development when performed concurrently with resistance training compared with performing resistance training in isolation. Primary adaptations to aerobic exercise include reductions in muscle fiber fatigue ability and increases in aerobic capacity, oxidative metabolism, and mitochondrial density (42, 71). On the other hand, primary adaptations to traditional resistance exercise typically include skeletal muscle hypertrophy, increases in strength, and an increase in glycolytic metabolism (13, 16, 53, 56). Because the principle of training specificity states that physiological adaptations to training are specific to the types of training performed (6, 16), some (3, 20, 39, 44, 46, 47) have presented hypotheses that link concurrent training interference to diverging adaptive intracellular responses to acute exercise, exercise mode-specific contractile characteristics, and overtraining. However, published findings are inconclusive since investigations that both support (19, 20, 34, 39, 40, 44, 46) and refute (1, 22, 25, 48, 58) these hypotheses exist. Regardless, the identification of low-impact exercise training regimens that simultaneously promote cardiovascular adaptation, skeletal muscle hypertrophy, strength, and improvements in body composition is of importance to the general public as well as clinical populations suffering from obesity, arthritis, type 2 diabetes, joint injury, or age-related illness.

In the present study, we expanded on our previous findings (38) and examined the exercise-induced adaptations to 12 wk of concurrent resistance and ATM exercise training (RT-ATM), concurrent RT-LTM training, and resistance training (RT) alone in previously untrained subjects ($n = 47$) recruited from the surrounding general population. Additionally, we

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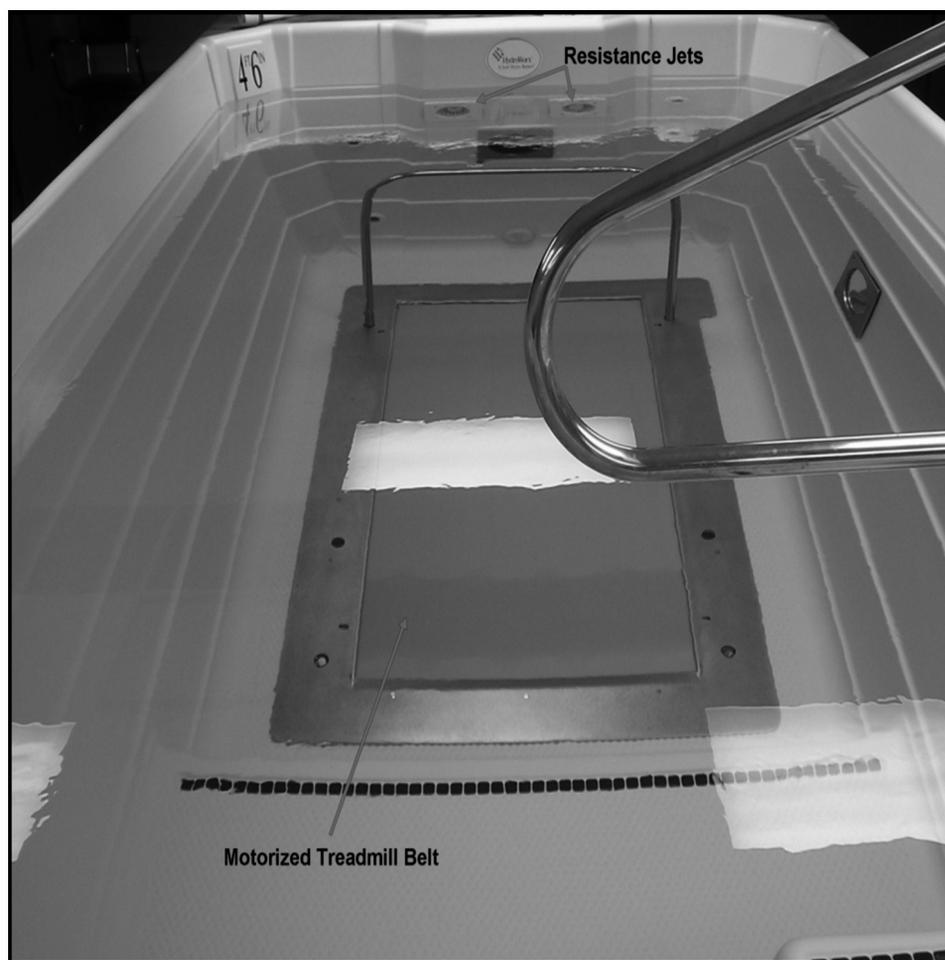


Fig. 1. Aquatic treadmill: HydroWorx 1000i series aquatic treadmill as used in the current study, with markers denoting motorized belt and frontal resistance jets. The variable-speed motorized treadmill from 0 to 200 ml/min/j (0–7.5 mph) allows for precise control of running velocity; in addition, variable-force resistance jets are configured to push against the subject, providing resistance to forward ambulation.

utilized isotope labeling to determine the acute effects of RT-ATM, RT-LTM, and RT exercise on myofibrillar fractional synthesis rates (myoFSRs) measured for 24 h following acute exercise before and after training in a subset of subjects who volunteered to undergo muscle biopsy sampling ($n = 25$). We hypothesized that acute RT-ATM exercise would yield greater 24-h myoFSRs than RT-LTM or RT and that 12 wk of concurrent RT-ATM training would enhance rather than interfere with gains in lean mass and strength compared with 12 wk of RT-LTM or RT alone. We also hypothesized that RT-ATM and RT-LTM training would elicit similar gains in aerobic capacity and reductions in fat mass but that LTM exercise would interfere with strength and lean mass development when performed concurrently with RT.

METHODS

All methods and procedures were approved by the Texas A & M University Institutional Review Board for Human Subjects in Research. Prior to participation, all subjects provided informed consent. Sixty-eight untrained volunteers were recruited from Texas A & M University and the College Station, TX, communities to participate in the study. A goal for recruitment was to select subjects who were untrained but otherwise healthy that represented physiologically “average” men and women from the surrounding area. Potential subjects were recruited through informational flyers and e-mail and by word of mouth. Volunteers were screened to ensure that they had not regularly performed planned exercise (>1 bout/wk) for the previous 3 mo. Prior

to participation, all subjects were screened to ensure that they were healthy enough for exercise (<2 cardiovascular risk factors) (63). Screening was based on ACSM risk stratification criteria to exclude subjects with contraindications to exercise or those who were taking medications known to affect metabolism or blood clotting. For subjects with two or more risk factors for cardiovascular disease, examination and clearance from a cardiologist was required prior to participation. Of the 68 volunteers who were recruited, 47 (men: $n = 23$, 37 ± 11 yr, 182.7 ± 6.7 cm, 98.9 ± 16.1 kg; women: $n = 24$, 38 ± 12 yr, 165.6 ± 4.8 cm, 82.1 ± 19.1 kg) completed all required aspects of the study which included all subject testing and completion of $\geq 85\%$ of all programmed training sessions. Additionally, rescheduled exercise sessions due to any unforeseen absences were required to be completed during the week of the missed session. Baseline demographics for subjects who completed this investigation are shown in Table 1.

Prior to training, participants were asked whether they would be willing to volunteer for an additional portion of the study that involved acute exercise and muscle sampling before and after training (methods to follow). Subjects were informed of all details related to participation and compensation prior to consent. Of the original 47 subjects, 25 volunteers (men: $n = 16$, 40 ± 4 yr, 182.2 ± 1.7 cm, 93.8 ± 5.5 kg; women: $n = 9$, 38 ± 4 yr, 166.3 ± 2.0 cm, 73.1 ± 4.0 kg) elected to participate in acute exercise testing and muscle sampling.

General study protocol. Physiological and demographic assessments were completed on the second visit to the laboratory (methods to follow). Subjects were then selectively randomized into one of the three training groups (RT, RT-LTM, and RT-ATM). After the com-

Table 1. Pretraining subject demographics

	RT			RT-LTM			RT-ATM		
	Men	Women	Total	Men	Women	Total	Men	Women	Total
No.	7	8	15	8	8	16	8	8	16
Age, yr	37 ± 5	37 ± 4	37 ± 3	37 ± 4	40 ± 4	38 ± 3	36 ± 3	40 ± 5	38 ± 3
Weight, kg	96.1 ± 6.4	88.4 ± 7.4	92.0 ± 4.9	96.1 ± 6.7	79.0 ± 4.5	87.3 ± 4.5	104.2 ± 4.4	79.6 ± 8.1	91.9 ± 5.5
BMI, kg/m ⁻²	28.3 ± 1.5	31.9 ± 2.1	30.2 ± 1.4	28.4 ± 1.9	28.2 ± 2.0	28.3 ± 1.4	31.9 ± 1.3	28.5 ± 2.7	30.2 ± 1.5
%Body fat	31.8 ± 2.7	49.3 ± 3.0	41.1 ± 3.1	31.7 ± 3.1	45.4 ± 2.2	38.5 ± 2.6	35.1 ± 2.4	43.0 ± 2.9	38.8 ± 2.1

Values are presented as means ± SE. RT, resistance training; RT-LTM, resistance and land treadmill exercise training; RT-ATM, resistance and aquatic treadmill exercise training.

pletion of training, all physiological and demographic testing procedures were repeated within 4 days after the final exercise training session.

Diet and activity logs. Subjects were instructed to maintain their accustomed dietary and activity habits throughout the course of the study. No attempt was made to modify diet or activity outside of the study protocol. To verify compliance with these instructions, dietary and activity habits were assessed on two occasions coinciding with the beginning and end of exercise training using previously described methods (10). Briefly, subjects were instructed to complete dietary and physical activity records on days that best represented their normal daily habits. On both occasions, dietary records were recorded for 3 consecutive days, including 1 weekend day. The 3-day dietary records were analyzed for total caloric intake and for carbohydrate, fat, and protein composition using commercially available computer software (Nutribase; Cybersoft, Phoenix, AZ). Activity records were recorded for 7 consecutive days and were analyzed for total energy expenditure. Because activity records were used to compare the energy expenditure of daily living before and after training, exercise participation in the study was excluded from the final activity logs.

Physiological assessments. Body composition, including whole body percent fat, fat mass, and lean body mass, was assessed using DEXA. An incremental maximal graded exercise test (GXT) was conducted on a motor-driven treadmill according to the Bruce protocol (17). Oxygen consumption during exercise was assessed using a calibrated metabolic gas analysis system (Ultima; Medical Graphics, Minneapolis, MN). $\dot{V}O_{2max}$ was taken as the highest 15-s average oxygen uptake achieved during the exercise test. Heart rate (HR) and rhythm were monitored continuously from a 12-lead electrocardiogram. Ratings of perceived exertion (70) using a Borg 15-point scale ranging from 6 to 20 (14) and manual blood pressures were obtained during the last 30 s of each treadmill stage and at maximal exercise.

At least two of the following criteria were required for the maximal exercise test to be considered valid: 1) achievement of maximum HR within 10 beats/min of the age-predicted maximum; 2) rating of perceived exertion ≥ 18 ; 3) respiratory exchange ratio >1.1 at maximal exertion; or 4) O₂ uptake plateau despite further increases in workload. The same skilled laboratory personnel consistently performed all physiological measurements.

Muscular strength was assessed using Keiser resistance training equipment (Keiser, Fresno, CA). Prior to the strength assessment, subjects performed a standardized warmup protocol involving 3 min of light cycling followed by a series of standardized stretching exercises. After completion of warmup, maximal strength was assessed for the following exercises in this order: leg press, chest press, leg curl, lat pull, leg extension, triceps pushdown, and biceps curl. Procedures for assessing maximal strength were adapted from Baechle and Earle (5).

Exercise training. Subjects in the RT-LTM and RT-ATM groups performed resistance exercise that was immediately followed by LTM or ATM twice/wk, separated by one session of either LTM or ATM performed in isolation (total of 3 sessions/wk). Subjects in the RT group performed resistance exercise in isolation twice/wk. The same resistance exercise training program was performed by all groups. Resistance exercise consisted of the same exercises and exercise sequence used during strength assessment. The RT-ATM and RT-LTM groups trained at equivalent caloric expenditures and relative intensities (Table 2).

ATM training was conducted using a HydroWorx 1000 series treadmill (HydroWorx International, Middletown, PA). LTM training was conducted on a standard motor-driven treadmill (Quinton TM65; Quinton, Bothell, WA). Treadmill velocity and grade/jet resistance were adjusted as necessary during the training session to attain the HR and rate of perceived exertion (RPE) that matched the prescribed

Table 2. Training progression: resistance training progression performed equally by all groups

	Resistance Exercise (All Groups)	Endurance Exercise (RT-LTM and RT-ATM Only)
Exercises	Leg Press, Chest Press, Leg Curl, Lat Pull, Leg Extension, Triceps Pushdown, Biceps Curl	ATM or LTM
Frequency	2/Wk	3/Wk, 2/wk following resistance exercise, 1/wk in isolation
Progression (weeks 1–6)	Warmup set: 1 × 12 at 50% 1-RM; working sets: 3 × 12 at 60% 1-RM; rest period: ~1.5 min between sets (↑ 5% of baseline 1-RM if all working sets were completed with 12 repetitions for a given exercise)	Week 1: 250 kcal, 60% baseline $\dot{V}O_{2max}$ (↑ 50 kcal and 5% of baseline $\dot{V}O_{2max}$ each week); week 6: 500 kcal, 85% baseline $\dot{V}O_{2max}$ (kcal indicate total expenditure per session which included resistance exercise on concurrent training days).
Week 7	Midpoint physiological assessments; warmup Set - 1 × 12 at 60% 1-RM; Working Sets - 3 × 8 at 75% 1-RM; rest period ~1.5 min between sets (↑ 5% of midpoint 1-RM if all working sets were completed with 8 repetitions for a given exercise)	500 kcal, 85% midpoint $\dot{V}O_{2max}$ (kcal indicate total expenditure per session which included resistance exercise on concurrent training days)

1-RM, repetition maximum. The aerobic training progression was additionally performed by the RT-LTM and RT-ATM groups twice/wk immediately following resistance training and once/wk in isolation. For concurrent training groups, the volume of aerobic exercise on concurrent training days was determined by subtracting the kcal expenditure of resistance training from the total prescribed kcal expenditure for the day. Resistance exercise kcal expenditure was approximated by the following proprietary equation: kcal = [2.251 × height cm] + [0.140 × lean mass kg] + [1.263 × $\dot{V}O_{2max}$ ml·kg⁻¹·min⁻¹] + [0.002 × (total volume sets × reps × resistance)]. ↑ Increase.

intensity. Each individual's exercise prescription was adjusted for increases in $\dot{V}O_{2\max}$ during *week 6* such that the prescribed intensity and duration were maintained throughout the study. During aerobic exercise (LTM or ATM), individual energy costs (kcal/min) were estimated as the product of $\dot{V}O_2$ (l/min) and the respiratory exchange ratio energy/oxygen equivalent (kcal/LO₂) measured during the GXT at each respective intensity of interest. Using this relationship, the exercise duration required to expend the required kilocalories of energy per exercise session was calculated for each subject (8). HR and RPE were recorded during each exercise session as a means of tracking intensity. As an additional precaution, a metabolic cart was used to sample $\dot{V}O_2$ from each subject during a training session to ensure that correct exercise intensities and volumes were being achieved (*weeks 2, 4, 8, and 10*). Caloric expenditure of resistance exercise was approximated using the following proprietary equation: resistance exercise kcal expenditure = $[2.251 \times \text{height cm}] + [0.140 \times \text{lean mass kg}] + [1.263 \times \dot{V}O_{2\max} \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}] + [0.002 \times (\text{total volume}^{\text{sets} \times \text{reps} \times \text{resistance}})]$.

The prescribed exercise progression was such that by *week 6* subjects expended ~1,500 kcal/wk in exercise training. On concurrent training days, the prescribed caloric expenditure was met by following resistance training with the appropriate volume of aerobic exercise to achieve the prescribed total for a given week. On days where aerobic exercise was performed in isolation, the total prescribed caloric expenditure was performed aerobically (Table 2). Although time of day was not controlled for subject training, sessions were separated evenly throughout each training week to avoid back-to-back sessions. Each session was also monitored one-on-one for each subject by a trained member of the laboratory staff.

Acute exercise blood and muscle sampling. Methods for tissue collection have been published previously (31, 36). The number of subjects in each group who elected to participate in muscle biopsy sampling was as follows: RT, $n = 7$; RT-LTM, $n = 7$; RT-ATM, $n = 11$. Blood samples were obtained on six occasions and muscle samples on four occasions. *Muscle sample no. 1* and *blood sample no. 1* were obtained 3 days prior to acute exercise (resting/untrained state). Resting samples were taken after ≥ 72 h without strenuous activity. For blood and muscle sampling, each subject reported to the laboratory [time of day controlled (between 5 and 11 AM)] after a 12-h fast (water allowed ad libitum). Prior to sample collection, subjects completed a form reporting their physical activity and dietary adherence over the previous 24 h and the time of their last meal. Blood samples were drawn without stasis from an antecubital vein, with the subject seated at quiet rest, into Vacutainer tubes containing 10.5 mg of Na-EDTA for plasma collection. Plasma samples were immediately isolated by centrifugation at 1,500 g for 30 min at 4°C. Aliquots of plasma were stored at -80°C for later analysis. Biopsies were taken from the vastus lateralis under local anesthesia (1% xylocaine HCl) using a 5-mm needle. All muscle samples were cleaned of visible fat, connective tissue, and blood. Muscle samples were immediately frozen in liquid nitrogen (-190°C), and then stored at -80°C until they were analyzed.

On the morning of acute exercise, subjects arrived to the laboratory and performed a standardized warmup, which was followed by a

single bout of resistance exercise. The intensity and caloric volume of the prescribed acute exercise bouts were matched to *weeks 1* (1st acute session/untrained) and *12* of training (2nd acute session/trained) (Table 3). The rationale for this was to analyze the response of each group to exercise in the context of the volumes and intensities that might be prescribed when untrained or trained. Resistance exercises occurred in the same order as listed for strength assessment. HR and oxygen consumption were measured continuously using a metabolic cart (CPX Express; Medical Graphics, Minneapolis, MN), and caloric expenditure was determined using indirect calorimetry. Additionally, subjects in the RT-LTM and RT-ATM groups performed either LTM or ATM exercise immediately following resistance exercise. Acute LTM exercise protocols began with a 3-min warmup period at 53.6 m/min at a 0% grade (RT-LTM group). Acute ATM exercise protocols began with a 3-min warmup period at 53.6 m/min at a 0% jet resistance (RT-ATM group). The duration of each acute exercise session was defined as the time required to expend the remaining volume of kcals need to reach a total of 250 kcal (resistance exercise expenditure) at 60% of $\dot{V}O_{2\max}$ (1st acute session/untrained) or 500 kcal at 85% of $\dot{V}O_{2\max}$ based on the most recently acquired $\dot{V}O_{2\max}$ (2nd acute session/trained). HR, RPE, and $\dot{V}O_2$ were measured every 5 min at the beginning of exercise to adjust treadmill velocity and grade until the required $\dot{V}O_2$ was achieved; thereafter, $\dot{V}O_2$ measurements were taken every 5 min until the cessation of exercise, and minor adjustments were made as necessary to the treadmill velocity and grade to maintain the required $\dot{V}O_2$ as well as exercise time to ensure the correct kilocalorie expenditure.

Deuterium administration and isotope tracing. The following methods have been published previously (31). Immediately following acute exercise, subjects received their second blood draw and their first of four boluses of 70% ²H₂O (3 ml/kg) (Cambridge Isotopes, Andover, MA) to achieve ~0.4 to 0.8% ²H-labeling of body water. The remaining boluses were given 2, 7.5, and 9.5 h following acute exercise. Thirty minutes following exercise, participants received their first of five meals (Boost Nutritional Energy Shake; Novartis Medical Nutrition, Fremont, IN), which supplied the subjects with 8,037 kJ, 52% carbohydrate, 20% protein, and 28% fat. The remaining four meals (Boost) were provided at 3, 5.5, 8.5, and 11 h following acute exercise. Total supplemented energy intake was matched to each subject's baseline dietary analysis. Subjects returned to the laboratory 24 h following cessation of exercise for the third blood draw and second vastus lateralis muscle biopsy. This process was again repeated following 12 wk of training and 5 days following the completion of final physiological assessments.

Analysis of myoFSR. Analysis of ²H labeling of body water and protein-bound alanine was determined as described previously (31–33). Briefly, 20 μ l of plasma was reacted with 2.0 μ l of 10 N NaOH and 4.0 μ l of a 5% (vol/vol) solution of acetone in acetonitrile for 24 h. Acetone was removed by the addition of 0.6 ml of chloroform and 0.5 g of Na₂SO₄. To determine myoFSR, ~40 mg of wet muscle was homogenized on ice in 0.4 ml of 1× Norris buffer (25 mmol/l Tris-HCl, 5 mmol/l β -glycerophosphate, 2 mmol/l dithiothreitol, 0.1 mmol/l Na₃VO₄, and 10 mmol/l MgCl₂; Cell Signaling Technology, Danvers, MA) and 0.01% Triton. Homogenates were centrifuged at

Table 3. Acute exercise: sessions matched to weeks 1 (untrained) and 12 (trained) of training

	Acute Exercise: Untrained Matched to <i>Week 1</i> Exercise Prescription Prior to Training	Acute Exercise: Trained Matched to <i>Week 12</i> Exercise Prescription Following Training
RT	Warmup set: 1 × 12 at 50% baseline 1-RM; working sets: 3 × 12 at 60% baseline 1-RM; total kcal expenditure = 174 ± 15	Warmup set: 1 × 12 at ~70% midpoint 1-RM; working sets: 3 × 8 at ~85% midpoint 1-RM; total kcal expenditure = 235 ± 22
RT-ATM and RT-LTM	The RT-ATM and RT-LTM groups performed the same resistance exercise session as listed above followed by either ATM or LTM endurance exercise; intensity = 60% baseline $\dot{V}O_{2\max}$ Total Kcal Expenditure = 250 (resistance + aerobic exercise)	The RT-ATM and RT-LTM groups performed the same resistance exercise session as listed above followed by either ATM or LTM endurance exercise; intensity = 85% midpoint $\dot{V}O_{2\max}$ Total kcal expenditure = 500 (resistance + aerobic exercise)

Resistance exercise kcal expenditure was approximated by the following proprietary equation: kcal = $[2.251 \times \text{height cm}] + [0.140 \times \text{lean mass kg}] + [1.263 \times \dot{V}O_{2\max} \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}] + [0.002 \times (\text{total volume}^{\text{sets} \times \text{reps} \times \text{resistance}})]$.

14,000 rpm at 4°C for 30 min. An aliquot (100 μ l) of the hydrolysate was dried, and a 3:2:1 ratio (0.1 ml) of *N,N*-dimethylformamide dimethyl acetal (methyl-8 reagent; Pierce, Rockford, IL), methanol, and acetonitrile was added to the residue to determine the ^2H labeling of alanine on its methyl-8 derivative. All samples were analyzed using an Agilent 5973N-MSD (Agilent Technologies, Santa Clara, CA) equipped with an Agilent 6890 GC system and a DB17-MS capillary column (30 m \times 0.25 mm \times 0.25 μ m). For quantification of 24-h post-exercise myoFSR following training, residual background enrichment of ^2H labeling of body water and protein-bound alanine was determined using the posttraining/unexercised biopsy samples (*muscle biopsy no. 3*). Residual enrichment of ^2H -labeled alanine found only in the myofibrillar fraction was then subtracted prior to calculation of 24-h post-exercise myoFSR in the trained state using *muscle biopsy no. 4*.

Statistical analysis. A 3 (group) \times 2 (time) \times 2 (sex) mixed-model ANCOVA (covariate = baseline measures) repeated across training was used to detect group \times time interactions for maximal strength, $\dot{V}\text{O}_{2\text{max}}$, body composition, dietary recall, and daily energy expenditure before and after training. A 1 \times 3 mixed-model ANOVA was used to analyze changes in the above variables following training: change = (posttraining value) – (pretraining value). A mixed-model ANOVA was used to compare 24-h myoFSR between groups following acute exercise before (untrained state) and after (trained state) training. The comparison-wise error rate, α , was set at 0.05 for all statistical tests. Where significant *F* ratios were found, a Tukey's post hoc analysis was performed to determine difference among groups. All data were analyzed using the SAS Enterprise Guide (version 4.3; SAS, Cary, NC).

RESULTS

Pretraining physiological characteristics of subjects in each group by sex are shown in Table 1. No significant differences in any of these characteristics were found between training groups at the beginning of exercise training. Our statistical analyses showed that there were no differential effects of sex difference on exercise training outcomes (i.e., no interaction due to sex difference); therefore, all exercise training data were collapsed across sex difference for subsequent analysis and for the presentation of results that follow. No significant within- or between-group interactions were found for dietary intake or daily energy expenditure.

Body composition, strength, and aerobic capacity. Statistical analysis of pre- and posttraining values revealed significant group \times time interactions for body composition, strength, and aerobic capacity ($P < 0.05$). Total lean mass (1.05–2.62 kg) and leg lean mass (0.43–0.87 kg) were increased significantly following training in all groups. The highest gains were observed in the RT-ATM group, which were found to be significantly greater than in the RT group (Fig. 2, A and B). A significant increase in trunk lean mass was observed in the RT-ATM group only (Fig. 2C). Percent body fat (%BF: –1.71 to 2.79%) and fat mass (–1.10 to 1.70 kg) were reduced after exercise training in the RT-LTM and RT-ATM groups, with the greatest decrease found in the RT-LTM group for %BF

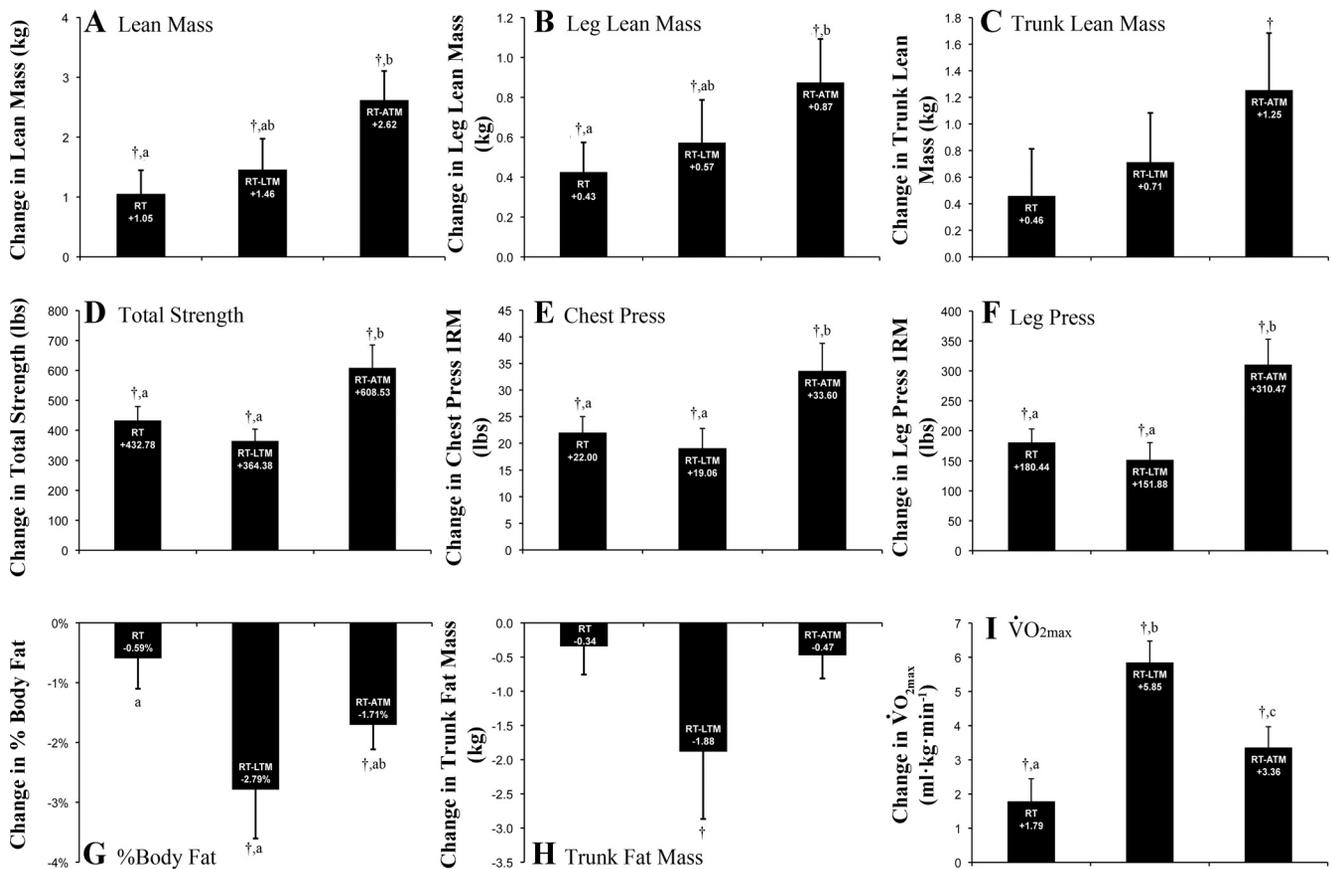


Fig. 2. Physiological adaptations to chronic training. Values are shown as means \pm SE for change from baseline for body composition, strength, and $\dot{V}\text{O}_{2\text{max}}$ following 12 wk of resistance training (RT), concurrent resistance and land treadmill training (RT-LTM), or concurrent resistance and aquatic treadmill training (RT-ATM). \dagger Significantly different from baseline. Letters indicate between-group interaction, with like letters indicating no significant differences between groups. Type I error set at $\alpha = 0.05$.

(Fig. 2G). Additionally, a significant decrease in trunk fat mass (-1.88 kg) was observed in the RT-LTM group only (Fig. 2H).

Significant increases in strength were observed in all groups for all exercises; however, significantly greater increases in total strength (sum of predicted 1-repetition maximum values for all lifts), leg press, and chest press strength were observed in the RT-ATM group compared with RT and RT-LTM groups (Fig. 2, D–F). No differences were observed between groups for gains in leg curl, lat pull, leg extension, or biceps curl.

$\dot{V}O_{2\max}$ ($+1.79$ – 5.85 ml·kg $^{-1}$ ·min $^{-1}$) and time to exhaustion measured during maximal exercise treadmill testing (GXT) were increased in all groups following exercise training (Fig. 2I). The RT-LTM group was found to have significantly greater increases in both variables compared with the RT or RT-ATM groups, although the RT-ATM group did demonstrate a greater increase in $\dot{V}O_{2\max}$ (ml·kg $^{-1}$ ·min $^{-1}$) than the RT group.

Acute exercise: 24-h myoFSRs. In the untrained state (prior to training), acute RT-ATM exercise was found to elicit higher 24-h myoFSRs compared with RT ($+5.68\%$ /day, $P < 0.01$) and RT-LTM ($+4.08\%$ /day, $P < 0.05$) (Fig. 3A). Following training, acute RT-ATM resulted in the highest mean myoFSR values on average, but no differences between groups were observed (Fig. 3B). No differences in myoFSR were observed between acute RT and RT-LTM exercise.

DISCUSSION

Body composition, strength, and aerobic capacity. In previously untrained men and women, RT-ATM exercise elicited a greater anabolic response to acute exercise in the untrained state and greater chronic hypertrophy compared with acute RT-LTM exercise and RT alone. Although this was found to be the case statistically only in the untrained state, the findings coincided with enhanced skeletal muscle growth and strength following chronic training (Figs. 2 and 3). Strength measurements and regional body composition measurements made from DEXA analysis also revealed that augmentation of muscle growth and strength was not limited to the legs alone. These findings suggest that the novel addition of low-impact ATM exercise following resistance exercise in a concurrent training program could potentially benefit elderly populations, those

who have previously been on bed rest, those recovering from injury, and athletic populations (52, 62).

During this investigation, both the RT-LTM and RT-ATM groups performed at equivalent training volumes, intensities, and frequencies. Because the RT group performed only resistance exercise twice/wk, overall training volume was lower compared with the concurrent training groups (*weeks 6–12*: RT, $<1,000$ kcal/wk; RT-LTM and RT-ATM, $1,500$ kcal/wk). Although it was expected that both the RT-ATM and RT-LTM would demonstrate greater decreases in %BF and fat mass than the RT group, the RT-LTM group experienced significantly greater reductions in %BF than the RT-ATM group (Fig. 2). DEXA analysis further revealed that this decrease in %BF was driven primarily by decreases in fat mass and, more specifically, trunk fat mass, which was found to be significantly decreased in the RT-LTM group alone. In our previous investigation (38), 12 wk of ATM and LTM were found to elicit similar reductions in %BF and fat mass. Therefore, whether or not the combination of RT and either ATM or LTM may have contributed to our present findings remains unclear. Analysis of pre- and posttraining dietary and daily activity records revealed no statistical interactions regarding daily activity or nutritional intake, as all groups were regularly given nutrition and activity instructions to maintain their normal diets and activities. It is possible that a difference in substrate utilization could account for some of these differences in body fat reduction between modes. Of note is that Svedenhag and Seger (60) reported elevated respiratory exchange ratio (RER) and blood lactate concentrations during deep water running compared with land-based exercise at similar aerobic workloads. Michaud et al. (49) and Broman et al. (15) reported similar findings. Together, these data indicate the possibility of elevated carbohydrate oxidation and decreased lipid oxidation during aquatic running compared with running on land. However, these findings are contrary to those by our laboratory (35) and others (21, 59, 67) who reported no difference in RER or blood lactate concentration between submaximal running exercise performed on land vs. in water. Therefore, further comparative research is needed between ATM and LTM running to determine whether any mode-specific differences in fuel substrate utilization exist during exercise and recovery.

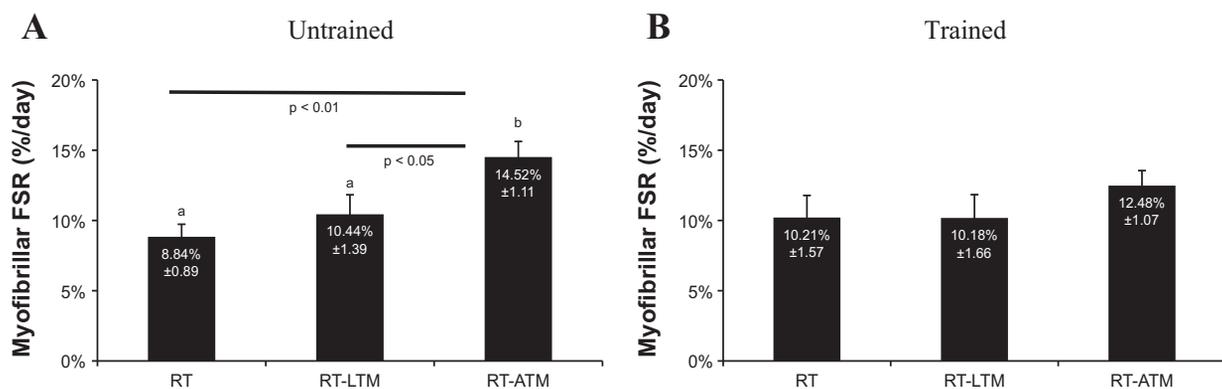


Fig. 3. Effects of acute RT, RT-LTM, and RT-ATM exercise on 24-h myofibrillar fractional synthesis rate (myoFSR). Values are presented as means \pm SE for myoFSR-measured fed state for 24 h following acute RT ($n = 7$), RT-LTM ($n = 7$), or RT-ATM ($n = 11$) exercise in the untrained state before training (A) and in the trained state (B). Letters indicate a significant between-group interaction, with like letters indicating no significant differences between groups ($P < 0.05$).

In addition to greater decreases in fat mass, the RT-LTM group demonstrated greater improvements in aerobic capacity and time to exhaustion than the other groups (Fig. 2). However, we suspect that the RT-LTM group may have had an advantage over the other groups in that they were trained on standard land-based treadmills that were identical to the treadmills on which they were tested. Previous research indicates that training specificity can impact maximal aerobic performance, depending on familiarization to the specific mode of exercise testing (29, 54). Therefore, although our results indicate that concurrent RT-LTM exercise may be more effective in increasing aerobic capacity, we caution that factors related to training specificity may have affected these outcomes. This was an unforeseen limitation, as our previous investigation resulted in no statistical difference between ATM or LTM exercise training performed in isolation (38). Future investigations comparing ATM and LTM exercise may avoid this limitation by also testing subjects using a cycle ergometer to overcome training specificity factors.

Skeletal muscle responses to acute exercise and training. We are the first to examine the acute effects of concurrent RT-ATM exercise on myoFSR and to compare 24-h myoFSR following acute resistance exercise and concurrent exercise before and after chronic training. Consistent with the findings of Donges et al. 2012 (25), the addition of aerobic exercise following a bout of resistance exercise did not result in suppression of myoFSR compared with resistance exercise alone in previously untrained subjects (Fig. 3). Furthermore, the addition of ATM exercise following RT exercise was found to be additive in stimulating myoFSR in the untrained state. Consequently, these data are at odds with hypothesis that reductions in anabolism occur following concurrent exercise compared with resistance exercise in isolation (3, 50). However, we acknowledge that the specific population used, exercise modes selected, exercise intensities, frequencies, and volumes may have been a factor in the lack of interference observed in this investigation (69) compared with previous investigations using trained or athletic populations (19, 44).

Contrary to previous investigations (51, 61), we did not observe a reduction in the stimulatory effects of acute exercise following chronic training. However, it should be noted that relative exercise intensities and exercise volumes were not matched between untrained acute exercise and trained acute exercise in this study. Instead, they performed the prescribed exercise intensity and volume for *week 1* of training when subjects were untrained and *week 12* when they were trained (Table 3). Therefore, we find it likely that differences in exercise intensity and volume between the acute exercise sessions performed prior to and following training may have produced the findings observed here. We acknowledge that this may not have been the case had relative exercise workloads been matched in a fashion similar to previous investigations involving acute exercise measures before and after chronic training (51, 61).

A recent study from our laboratory (44a) may provide some insight with regard to the observed outcomes in this study. Briefly, we compared acute hemodynamic responses to acute exercise stress following 12 wk of either ATM or LTM training. The aerobic training prescription was identical to the present investigation with regard to intensity and frequency. In conjunction with significant reductions in blood pressure, skel-

etal muscle endothelial nitric oxide synthase (eNOS) expression was increased following ATM training compared with LTM training. Because of the role of eNOS in the regulation of endothelial-mediated vasodilation (65), those results suggested that ATM exercise may potentially enhance skeletal muscle blood flow. Recent findings by Timmerman et al. (64) have shown that any compromises in blood flow will have deleterious effects on skeletal muscle anabolism, perhaps as a result of diminished nutrient delivery. Therefore, future investigations will be needed to determine whether skeletal muscle blood flow is enhanced following ATM or RT-ATM training. The possibility that our ATM protocol enhances blood flow, even in the rested state, may optimize FSR over the course of the 24-h period by facilitating nutrient delivery. Those results are consistent with the heightened and similar myoFSR responses in the RT-ATM group compared with other groups.

ATM exercise provides a unique exercise stimulus in that it is performed with reduced vertical load, increased lateral resistance, and an increase in lower body positive pressure as opposed to running on land. As a result, lower ground reaction forces, reduced joint compression, reduced stride frequency, and differences in skeletal muscle activation patterns have also been reported compared with LTM exercise (7, 23, 55, 67). Because skeletal muscle adaptation has been shown to be highly dependent on both contraction frequency and intensity (2, 53), we find it probable that ambulation through water at chest depth as opposed to air may have been a factor in producing the observed outcomes. The low-impact nature of ATM exercise, along with our past and present results, also serves as an impetus for further investigation into inflammatory and endocrine responses to ATM exercise, which have been shown to effect skeletal muscle adaptation (41, 44).

In summary, we observed that the novel use of ATM exercise augments skeletal muscle growth and strength when performed concurrently with resistance exercise. Importantly, these findings are of clinical significance for those who may benefit from muscle mass and strength preservation or development while simultaneously improving cardiovascular capacity. Neither concurrent training group (RT-LTM or RT-ATM) experienced acute or chronic anabolic interference. Therefore, our results challenge the view that training for strength is universally incompatible with training for endurance. Accordingly, future investigations should seek to determine the time course of cell signaling responses following acute exercise that govern acute and chronic adaptation in skeletal muscle. With differing cell signaling outcomes being observed previously between resistance and endurance exercise, such data may provide some explanation regarding the outcomes observed in this investigation (2, 20). Additional findings observed in this investigation provide further evidence of differential mode-specific adaptations to training. First, trained at the same weekly caloric expenditure from exercise, RT-LTM training was found to result in greater decreases in fat mass compared with RT-ATM training, which may indicate differences in energy substrate utilization between ATM and LTM training. Second, RT-LTM training was found to increase aerobic capacity to a greater extent than RT (expected) and RT-ATM (not expected). Although testing specificity may have been a factor, future investigations should also prioritize determination of mitochondrial FSR and function to gain information on potential changes in the oxidative capacity of skeletal muscle (42,

68, 71, 72). Nonetheless, in light of our present results, the combination of moderate-resistance training and low-impact ATM running may serve as an effective stimulus for simultaneously promoting increases in skeletal muscle mass, strength, and aerobic fitness.

ACKNOWLEDGMENTS

We thank the dedicated students of the Applied Exercise Science, Human Countermeasures, and Muscle Biology Laboratories of Texas A & M University, without whom none of these studies would have been possible. In particular, we thank Aaron Carbuhn, Dustin Joubert, Alex Carradine, Justin Dobson, and Drs. Jonathan Oliver, Heath Gasier, Steve Martin, John Green, and Mats Nilsson for their aid in the study design, data collection, and data analysis. We also thank Dr. J. P. Bramhall for oversight during muscle sampling and Dr. Janet Parker for additional contributions to this project.

GRANTS

This research was funded in part by HydroWorx International, the Sydney and J. L. Huffines Institute for Sports Medicine and Human Performance, and the National Strength and Conditioning Association.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

B.S.L., J.D.F., S.E.R., N.P.G., and S.F.C. conception and design of research; B.S.L., J.D.F., S.E.R., and N.P.G. performed experiments; B.S.L., K.L.S., J.D.F., N.P.G., and J.M.C. analyzed data; B.S.L., K.L.S., and J.D.F. interpreted results of experiments; B.S.L. prepared figures; B.S.L. drafted manuscript; B.S.L., K.L.S., J.D.F., S.E.R., N.P.G., J.M.C., and S.F.C. edited and revised manuscript; B.S.L., K.L.S., J.D.F., S.E.R., N.P.G., J.M.C., and S.F.C. approved final version of manuscript.

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