Aquatic Treadmill Training Reduces Blood Pressure Reactivity to Physical Stress

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ABSTRACT

LAMBERT, B. S., N. P. GREENE, A. T. CARRADINE, D. P. JOUBERT, J. D. FLUCKEY, S. E. RIECHMAN, and S. F. CROUSE. Aquatic Treadmill Training Reduces Blood Pressure Reactivity to Physical Stress. *Med. Sci. Sports Exerc.*, Vol. 46, No. 4, pp. 809–816, 2014. **Purpose**: Endurance exercise may reduce blood pressure and improve vasodilatory capacity, thereby blunting the hypertensive response to stress. Therefore, we sought to test the efficacy of a novel model of low-impact endurance training, the aquatic treadmill (ATM), to improve blood pressure (BP) parameters. **Methods**: Sixty sedentary adults were randomized to 12-wk of either ATM (n = 36 [19 males and 17 females], 41 ± 2 yr, 173.58 ± 1.58 cm, 93.19 ± 3.15 kg) or land-based treadmill (LTM, n = 24 [11 males, 13 females], 42 ± 2 yr, 170.39 ± 1.94 cm, 88.14 ± 3.6 kg) training, three sessions per week, progressing to 500 kcal per session, 85% $\dot{V}O_{2max}$. The maximal Bruce treadmill test protocol was performed before and after training with BP measured before, at the end of each stage, and for 5 min after exercise testing. Twelve subjects (five ATM and seven LTM) volunteered for biopsies of the vastus lateralis before and after training, and muscle samples were assessed for endothelial nitric oxide synthase content. Data collected during exercise testing were analyzed using group by training ANCOVA repeated across training, α = 0.05. **Results**: ATM but not LTM training significantly reduced resting diastolic BP (−3.2 mm Hg), exercise systolic BP (range 9–18.2 mm Hg lower for each exercise stage), diastolic BP (3.2–8.1 mm Hg), mean arterial pressure (4.8–8.3 mm Hg, lower than LTM posttraining), and pulse pressure (7.5–15 mm Hg) during stages of exercise stress and recovery ($P < 0.05$). In addition, an increase (+31%) in skeletal muscle endothelial nitric oxide synthase content after training ($P < 0.05$) occurred in only the ATM group. Body mass (−1.27 kg) and $\dot{V}O_{2max}$ (+3.6 $mL \cdot kg^{-1} \cdot min^{-1}$) changes were significant for both groups ($P < 0.001$). **Conclusion**: ATM training can reduce BP reactivity to physical stress. **Key Words:** AEROBIC EXERCISE, HEMODYNAMIC, eNOS, HYPERTENSION

The prevalence of chronic diseases such as cardiovascular disease, obesity, diabetes, and hypertension has risen to epidemic levels (5). Therefore, the identification of new therapies to combat and prevent these diseases is paramount. Previous reports have demonstrated the link of heightened cardiovascular reactivity, such as an exaggerated stress-induced hypertensive response, to the development of essential hypertension (13,27), early atherosclerosis (28), and stroke (14). More specifically, exaggerated blood pressure responses to exercise are predictive of future hypertension (29).

Exercise is recommended as a therapy for many chronic diseases, including hypertension (35). The American College of Sports Medicine Position Stand on exercise and hypertension suggests that exercise training may result in a reduction of systolic blood pressure (SBP) between 3 and 7 mm Hg, depending on resting, ambulatory, and exercise measures as well as the population studied (hypertensive vs nonhypertensive), with hypertensives typically showing the greatest reduction (35). However, these reductions are not always observed (30,31), and the conditions in which exercise training may reduce blood pressure hyperreactivity to stress are not fully elucidated. Many factors may play into such contrasting findings, including the model used (e.g., human vs rodent, hypertensive vs normotensive); the frequency, intensity, and duration of training; the length of the training protocol; and the mode of exercise. Common training modes used in previous studies have included walking, jogging, running, cycling, and resistance training (15,23,24). Most findings indicate some exercise-induced protection against hypertension when endurance training is used (15,23,24), whereas findings for resistance training are mixed (10,22). However, because of other variations in study design (training duration, frequency, and intensity), it is difficult to ascertain any inherent differences in the efficacy of different endurance training modes. Therefore, the

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optimal exercise-induced stimulus or stimuli for blood pressure reduction are still in question.

Recently, we investigated the efficacy of aquatic treadmill (ATM) exercise, a novel modality of low-impact aerobic exercise, compared with more conventional land-based treadmill (LTM) exercise with regard to improvements in physical fitness and changes in body composition and blood lipids after 12 wk of exercise training (19,20). After training, it was observed that ATM exercise provided similar benefits to LTM exercise. However, our previous findings (18) and those of others (38) demonstrate that acute cardiovascular responses to exercise in water differ from standard land-based exercise. In fact, previous findings indicate that swim training may diminish stress-induced hypertension in rat (8), suggesting additional benefit of aquatic-based exercise. Swim training has also been shown to reduce resting blood pressure parameters in both prehypertensive and hypertensive humans (33,41), suggesting the potential efficacy of aquatic-based training in improving blood pressures.

Therefore, the purpose of the current study was to examine the efficacy of ATM training to reduce blood pressure responses to exercise-induced stress, thereby acting as a novel therapy in the prevention and treatment of hypertension. Herein, we demonstrate that individuals performing ATM training have a blunted blood pressure response during exercise-induced stress after training, which was not seen with LTM. Therefore, ATM training can reduce blood pressure reactivity to physical stress and may as a result prevent the hypertensive response to physical stress and reduce cardiovascular risk.

METHODS

All methods and procedures were approved by the Texas A&M University Institutional Review Board for Human Subjects in Research, and the general protocol and study design have been previously reported (19,20). Before participation, all subjects were informed of all details related to the study and provided informed consent. In brief, 83 physically inactive men and women were recruited from the communities in Texas A&M University, College Station, TX, to participate in the study. Potential volunteers were recruited through informational flyers, e-mail announcements, and word of mouth. Among the inclusion criteria, volunteers were selected who were not currently taking medications that are known to alter blood pressure and who had not participated in regular physical activity for the previous 3 months (physically inactive). Exclusion criteria included current regular physical activity outside of the study, medications known to affect blood pressure, and any physical limitation that prevented subjects from performing vigorous exercise. All subjects were screened for contraindications to exercise according to the American College of Sports Medicine guidelines and protocols (1). Subjects were matched for age, sex, and BMI and then randomized into ATM or LTM training groups. Of the 83 initial subjects that were recruited, 60 subjects completed the investigation. Data from the remaining 23 subjects were excluded for the following reasons: positive cardiac stress test on the entry physical, failure to complete the required 85% minimal attendance to training sessions, and dropout due to injury or personal time constraints. Preliminary physiological characteristics of the 60 subjects completing the study are presented in Table 1. The primary investigators were blinded to all postintervention measures at time of collection. To make certain that all changes in outcome variables were a result of the prescribed exercise intervention, subjects were instructed to maintain their accustomed dietary and physical activity habits throughout the course of the study. Compliance with these instructions was monitored by the investigators as previously described (19).

Maximal-graded exercise stress testing and body composition assessment. All graded exercise stress tests (GXT) were performed 1–2 wk before initiating exercise training and repeated 72–96 h after the final session of exercise training. Subjects underwent body composition assessment via dual energy x-ray absorptiometry (DEXA; Lunar Prodigy, GE®, Fairfield, CN) immediately before completing an incremental maximal GXT on a motor-driven treadmill according to the Bruce protocol (4). Oxygen consumption ($\dot{V}O_2$), HR and rhythm, and RPE, using a Borg 15-point scale (3), were assessed during the GXT as previously described (17,19,20).

TABLE 1. Physiological baseline demographics and training adaptations to 12 wk of exercise training.

	All Subjects (n = 60)		ATM (n = 36)		LTM (n = 24)	
Sex	**M = 29, F = 30**		**M = 19, F = 17**		**M = 11, F = 13**	
Age (yr)	41 ± 1		41 ± 2		42 ± 2	
Height (cm)	172.3 ± 1.2		173.5 ± 1.51		170.3 ± 1.9	
Training adaptations	Pre-Tr	Pos-Tr	Pre-Tr	Post-Tr	Pre-Tr	Post-Tr
Body mass (kg)	91.1 ± 2.4	89.9 ± 2.2*	93.9 ± 3.1	91.8 ± 3.0	88.14 ± 3.6	86.98 ± 3.4
Body mass index ($kg \cdot m^{-2}$)	30.6 ± 0.7	30.2 ± 0.7*	30.9 ± 0.9	30.4 ± 0.9	30.33 ± 1.1	29.92 ± 1.1
% body fat	38.5 ± 1.2	37.2 ± 1.2*	38.9 ± 1.6	37.6 ± 1.7	37.98 ± 1.9	36.55 ± 1.9
Fat-free mass (kg)	55.1 ± 1.5	55.6 ± 1.6	56.2 ± 2.1	56.4 ± 2.0	53.51 ± 2.3	54.34 ± 2.5
Fat mass (kg)	35.5 ± 1.6	33.8 ± 1.6*	36.7 ± 2.1	35.0 ± 2.2	33.79 ± 2.5	31.97 ± 2.2
$\dot{V}O_{2max}$ ($L \cdot min^{-1}$)	2.5 ± 0.1	2.9 ± 0.1*	2.6 ± 0.1	2.9 ± 0.1	2.46 ± 0.1	2.81 ± 0.1
$\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	28.6 ± 0.8	32.2 ± 0.9*	28.6 ± 1.2	32.2 ± 1.3	27.96 ± 1.0	32.28 ± 1.2

Body masses from DEXA scan. Data are presented as mean ± SEM collapsed across sex.
*$P < 0.05$ significant main effects of exercise training without interactions indicate that both groups responded to training.

Assessment of supine and exercising blood pressure. Serial blood pressure measurements were obtained using standard sphygmomanometric procedures with subjects resting in the supine position during the last 30 s of each 3-min stage of the GXT and at 1, 3, and 5 min during recovery (methods previously described by Pickering et al. [36]). Resting blood pressure was defined in this study as those recorded with the subject in the supine position just before exercise testing to get a better reflection of true resting blood pressure. Recovery measures were recorded during active recovery on the treadmill at zero grade, 1.7 mph. Measurements were taken by trained laboratory staff whose measurements were first validated using sample subjects against measurements taken by experienced senior laboratory personnel. During the test, peak blood pressure was defined as the highest SBP measurement recorded during exercise.

Exercise training. The exercise prescription and training progression for this study have previously been reported (19,20). Briefly, exercise training included three sessions per week for 12 wk using either LTM or ATM modalities, with sessions progressively increasing from 250 kcal per session, 60% $\dot{V}O_{2max}$ during the first week, to 500 kcal per session, 85% $\dot{V}O_{2max}$ during weeks 6–12. ATM velocity and resistance were estimated using a previously developed metabolic equation for ATM (18). To ensure that subjects were training at the correct metabolic workloads, oxygen consumption during exercise was periodically measured throughout training. Data from these oxygen consumption measurements were used to assess workloads, and readjust as necessary, to ensure that all subjects from both groups were training at the prescribed intensity and for the time required to expend the required calories. In this way, we were able to make certain that all subjects from both ATM and LTM groups were exercising at the same intensity and volume. ATM water depth was standardized to the level of the suprasternal notch (chest depth), and water temperature was 33°C to maintain thermoneutrality during exercise (6,9).

Assessment of endothelial nitric oxide synthase expression. As part of a previously reported aim (17), a subset of subjects (n = 12, ATM = 5, LTM = 7) had volunteered for muscle biopsy. After our initial training observations, which showed ATM lowered exercise blood pressures, we elected to assess endothelial nitric oxide synthase (eNOS) content in the biopsy samples in an attempt to gain insight into potential mechanisms by which ATM exerts its effects. These additions to the study protocol have been previously described (17,20). Briefly, willing volunteers performed an additional two bouts of exercise on the land treadmill for the duration required to expend 400 kcal, one each before the onset of training and after the conclusion of training. Three muscle biopsies were obtained from these participants: one before any exercise and one each 24 h after the additional exercise bouts. Muscle biopsy was performed as described (17) and is a modification of Bergstrom's (2) technique, as described by Evans et al. (12). Muscle biopsies were taken from the vastus lateralis under local anesthetic (1% Xylocaine HCl) using a 5-mm needle. Muscle samples were cleaned of visible fat, connective tissue, and blood, immediately frozen in liquid nitrogen, and stored at −80°C until analyzed.

Biopsy samples were analyzed using Western blot methods as previously described with minor modifications (11,17). Tissue was weighed, powdered at the temperature of liquid nitrogen, and homogenized in cold buffer (25 mM HEPES, 4 mM EDTA, 25 mM benzamidine, 1 μM leupeptin, 1 μM pepstatin, 0.15 μM aprotinin, 2 mM phenylmethylsulfonyl fluoride, pH 7.4). The homogenate was then centrifuged (10,000g for 30 min at 4°C). Protein concentration of the supernatant was determined as described by Smith et al. (39). An aliquot of the supernatant was diluted in an equal volume of buffer (125 mM Tris, pH 6.8, 4% SDS, 20% glycerol, 200 mM DTT, and 0.002% bromophenol blue). Protein was then separated by SDS–PAGE and transferred to nitrocellulose membrane (Amersham Biosciences, Piscataway, NJ). Membranes were incubated in 5% nonfat dried milk in Tris-buffered saline for 1 h at room temperature. After blocking, membranes were incubated overnight with mouse anti-eNOS (BD Transduction Laboratories, 610297, BD Biosciences, San Jose, CA) primary antibody. Membranes were then washed and incubated for 1 h with fluorescent-tagged anti-Mouse IgG (Sigma-Aldrich, St. Louis, MO) and developed using Li-COR imaging software (Li-COR Bioscience, Lincoln, NE). All samples from each subject were represented on the same blot, and both groups were represented on all blots for qualitative comparisons. Band density was corrected for total protein content using β-actin (Cell Signaling, 4967).

Statistical analysis. A 2 × 2 mixed model (group × time) ANOVA repeated across exercise training was used as the analysis for $\dot{V}O_{2max}$, body composition, and body weight measured before and after training. A 2 × 2 mixed model (group × time) ANCOVA repeated across exercise training was used to analyze DBP, SBP, mean arterial pressure (MAP), rate pressure product (RPP), and pulse pressure (PP) before, during each stage of stress testing, and after acute exercise stress testing. A 2 × 3 (group × time) ANOVA was used to compare skeletal muscle eNOS concentrations in our subject volunteers (muscle biopsy samples taken at the aforementioned time points). The comparisonwise error rate, α, was set at 0.05 for all statistical tests. When significant F-ratios were found, a Tukey *post hoc* analysis was used to distinguish differences among means. All data were analyzed using SAS (version 9.2; SAS Institute Inc., Cary, NC) and expressed as means ± SEM.

RESULTS

Physical characteristics before and after 12 wk of exercise training. Physical characteristics of the subjects are shown in Table 1. Subjects were nonhypertensive and categorized as overweight/obese based on their BMI (30.6 ± 0.7) at the onset of the study. Body mass (−1.27 kg,

$P < 0.001$), BMI (-0.42 kg·m^{-2}, $P < 0.001$), %body fat (-1.38%, $P < 0.001$), and fat mass (-1.71 kg, $P < 0.001$) were all reduced, and $\dot{V}O_{2max}$ increased ($+0.31$ L·min^{-1}, $+3.6$ mL·kg^{-1}·min^{-1}, $P < 0.0001$) after training in all subjects regardless of mode of exercise training. Fat-free mass was not significantly altered in either group after training.

ATM training ameliorates blood pressure response to GXT. ATM training was found to significantly decrease resting DBP (-3.2 mm Hg, $P < 0.05$) compared with LTM. Under exercise stress, the ATM group, but not LTM, demonstrated reductions in HR (range 6.5–7.9 bpm lower for each stage, $P < 0.05$), SBP (-9.0 to 18.2 mm Hg, $P < 0.05$), MAP (-4.8 to 8.3 mm Hg, $P < 0.05$), PP (-7.5 to 15 mm Hg, $P < 0.05$), and RPP (-1.8 to 3.9 bpm·mm Hg·10^3, $P < 0.05$) at equivalent workloads after exercise training. Reductions in HR (-6.8 to 7 bpm, $P < 0.05$) and RPP (-2 bpm·mm Hg·10^3, $P < 0.05$) were found during exercise stress in the LTM group (Fig. 1 and Table 2).

eNOS content increases after ATM training. To investigate potential mechanisms by which ATM training selectively reduced the blood pressure response during stress, we used skeletal muscle biopsy samples obtained from a subset of our participants. As noted in the methods, muscle biopsy samples were obtained before any exercise or training, and again 24 h after a single bout of exercise completed by each subject in both the untrained and trained

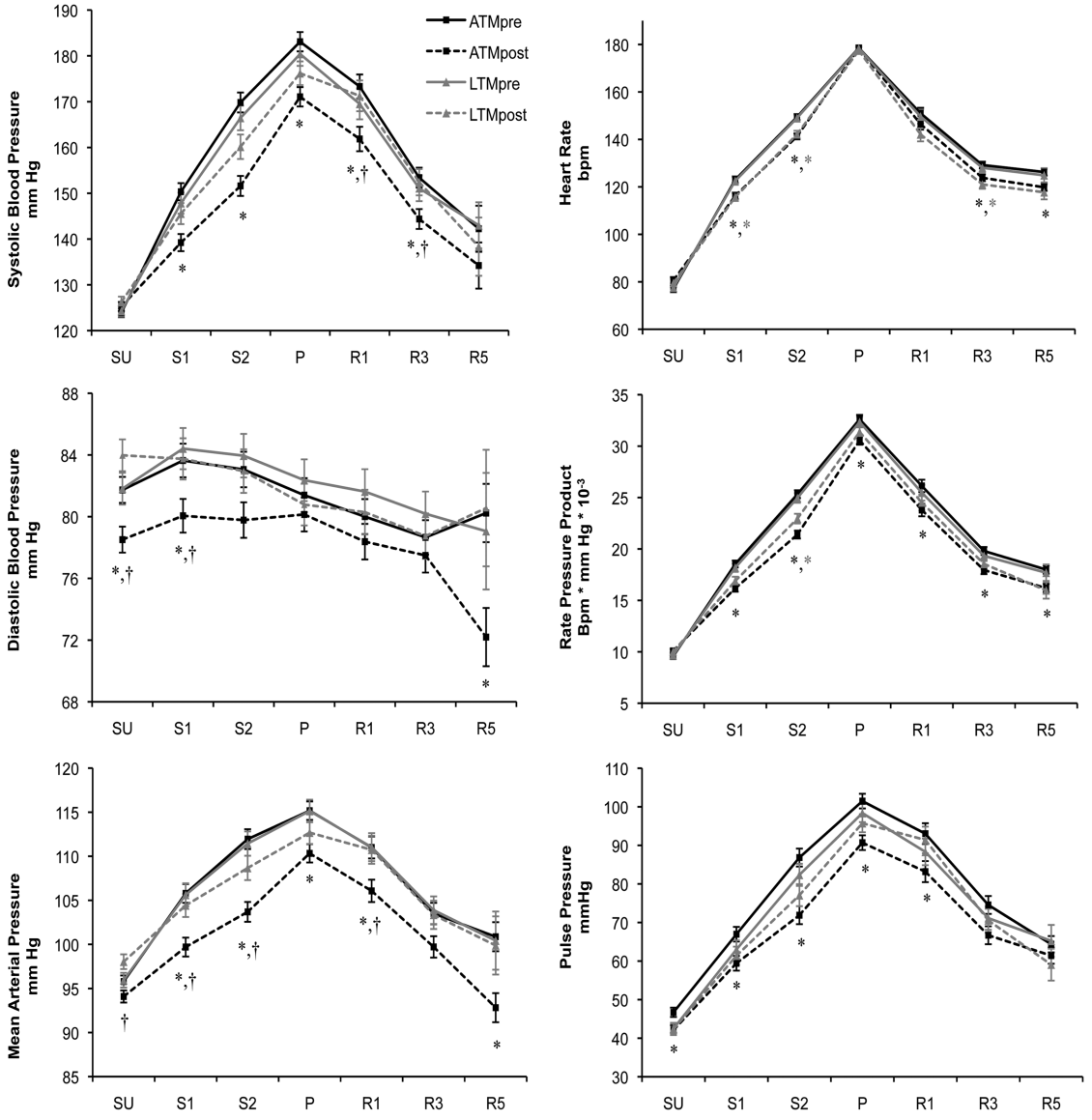

FIGURE 1—Blood pressure responses before and during the Bruce protocol exercise stress test before and after 12 wk of exercise training using either aquatic (ATM) or land-based (LTM) treadmills. *$P < 0.05$ compared with pretraining value in same group. †$P < 0.05$ between groups at same measurement time point. Data are presented as adjusted means ± SEM. Pre, pretraining; Post, posttraining. Testing stages: SU, supine; S1, stage 1; S2, stage 2; P, peak exercise. R1, R3, and R5 indicate recovery at 1, 3, and 5 min, respectively.

TABLE 2. Blood pressure adaptations to exercise training at rest and during exercise stress.

Independent Variable	Group	Training	Supine	Stage 1	Stage 2	Peak Exercise	Recovery 1	Recovery 3	Recovery 5
Systolic blood pressure (mm Hg)	ATM	Pre-Tr	123.9 ± 1.0	150.3 ± 1.9	169.8 ± 2.2	183.1 ± 2.1	173.3 ± 2.6	153.4 ± 2.2	142.3 ± 2.3
		Post-Tr	125.3 ± 1.0	139.2 ± 1.9[a]	151.6 ± 2.2[a]	171.1 ± 2.1[a]	161.9 ± 2.7[a]	144.4 ± 2.2[a]	134.2 ± 2.3
	LTM	Pre-Tr	124.3 ± 1.2	147.8 ± 2.3	166.4 ± 2.7	180.4 ± 2.6	169.5 ± 3.4	151.1 ± 2.8	143.00 ± 4.30
		Post-Tr	126.2 ± 1.2	145.6 ± 2.3	160.2 ± 2.7	176.2 ± 2.6	171.3 ± 3.4[b]	152.4 ± 2.9[b]	141.00 ± 3.34
Diastolic blood pressure (mm Hg)	ATM	Pre-Tr	81.7 ± 0.8	83.6 ± 1.1	83.1 ± 1.2	81.4 ± 1.1	80.0 ± 1.1	78.7 ± 1.1	80.2 ± 1.9
		Post-Tr	78.5 ± 0.8[a]	80.1 ± 1.1[a]	79.8 ± 1.2	80.1 ± 1.1	78.4 ± 1.2	77.5 ± 1.1	72.2 ± 1.9[a]
	LTM	Pre-Tr	81.8 ± 1.0	84.4 ± 1.3	84.0 ± 1.4	82.4 ± 1.4	81.6 ± 1.5	80.2 ± 1.4	79.1 ± 3.8
		Post-Tr	84.0 ± 1.0[b]	83.7 ± 1.3[b]	83.0 ± 1.4	80.8 ± 1.4	80.3 ± 1.5	78.7 ± 1.5	80.6 ± 3.8
Mean arterial pressure (mm Hg)	ATM	Pre-Tr	95.8 ± 0.7	105.8 ± 1.1	111.9 ± 1.1	115.2 ± 1.0	111.0 ± 1.2	103.5 ± 1.2	100.9 ± 1.6
		Post-Tr	94.1 ± 0.7	99.7 ± 1.1[a]	103.7 ± 1.1[a]	110.3 ± 1.0[a]	106.1 ± 1.3[a]	99.7 ± 1.2	92.8 ± 1.6[a]
	LTM	Pre-Tr	95.9 ± 0.8	105.6 ± 1.3	111.4 ± 1.4	115.1 ± 1.3	111.0 ± 1.6	103.9 ± 1.5	100.4 ± 3.3
		Post-Tr	98.0 ± 0.8[b]	104.4 ± 1.3[b]	108.7 ± 1.4[b]	112.7 ± 1.3	110.7 ± 1.6[b]	103.4 ± 1.6	99.9 ± 3.3
Pulse pressure (mm Hg)	ATM	Pre-Tr	46.7 ± 1.2	66.9 ± 1.9	86.8 ± 2.3	101.4 ± 2.3	93.0 ± 2.7	74.6 ± 2.3	64.4 ± 2.1
		Post-Tr	42.1 ± 1.2[a]	59.4 ± 1.9[a]	71.9 ± 2.3[a]	90.7 ± 2.3[a]	83.2 ± 2.7[a]	66.7 ± 2.3	61.4 ± 2.1
	LTM	Pre-Tr	42.5 ± 1.5	63.0 ± 2.3	82.3 ± 2.9	98.4 ± 2.9	88.3 ± 3.5	71.1 ± 3.0	65.2 ± 4.1
		Post-Tr	42.3 ± 1.5	61.4 ± 2.3	77.1 ± 2.9	95.7 ± 2.9	91.4 ± 3.5	70.6 ± 3.0	59.1 ± 4.1
Rate pressure product (bpm·mm Hg·10^3)	ATM	Pre-Tr	9.5 ± 0.2	18.5 ± 0.3	25.3 ± 0.4	32.6 ± 0.4	26.1 ± 0.6	19.8 ± 0.4	17.9 ± 0.4
		Post-Tr	10.0 ± 0.2	16.1 ± 0.3[a]	21.4 ± 0.4[a]	30.6 ± 0.4[a]	24.60 ± 0.6[a]	17.9 ± 0.4[a]	16.2 ± 0.4[a]
	LTM	Pre-Tr	9.6 ± 0.2	18.1 ± 0.4	24.9 ± 0.5	32.3 ± 0.5	25.5 ± 0.7	19.3 ± 0.5	17.6 ± 0.8
		Post-Tr	10.0 ± 0.2	16.9 ± 0.4	22.9 ± 0.5[a]	31.4 ± 0.5	24.5 ± 0.7	18.6 ± 0.5	16.0 ± 0.8
Heart rate (bpm)	ATM	Pre-Tr	77.0 ± 1.4	123.0 ± 1.3	149.2 ± 1.2	178.5 ± 1.0	151.0 ± 2.3	129.2 ± 1.4	126.2 ± 1.5
		Post-Tr	80.6 ± 1.4	116.2 ± 1.3[a]	141.3 ± 1.2[a]	178.4 ± 1.0	146.5 ± 2.3	123.8 ± 1.4[a]	119.8 ± 1.5[a]
	LTM	Pre-Tr	77.5 ± 1.7	122.5 ± 1.6	149.0 ± 1.5	178.4 ± 1.2	149.7 ± 2.9	128.1 ± 1.8	124.9 ± 2.9
		Post-Tr	79.5 ± 1.7	115.7 ± 1.6[a]	142.1 ± 1.5[a]	177.4 ± 1.2	142.1 ± 2.9	121.1 ± 1.8[a]	117.7 ± 2.9

Data are presented as adjusted mean ± SEM collapsed across sex.
[a]$P < 0.05$ significant effect of exercise training within group.
[b]Significantly different from ATM group at the same measurement time point.

conditions. Analysis of vastus lateralis muscle revealed an increase in eNOS content (+31%, $P < 0.05$) after ATM but not LTM training (Fig. 2).

DISCUSSION

In the present study, our purpose was to determine whether ATM training results in a reduced blood pressure response to exercise-induced stress, thus acting as a potential antihypertensive therapy. Here, we demonstrate that subjects performing exercise training using the ATM modality had reduced blood pressure reactivity to GXT after training, which was not seen in subjects who trained using the more traditional LTM mode of training. These findings in normotensive adults demonstrate reduced blood pressure reactivity to physical stress after ATM training. These findings suggest a therapeutic utility for this mode of exercise in the treatment and prevention of hypertension (13,27), stroke (14), and atherosclerosis (28). We further examined skeletal muscle biopsy samples from a subset of our subjects and observed that ATM trained subjects, but not LTM trained, showed increased eNOS content after training. This finding provides a possible mechanistic explanation for ATM-induced improvements in blood pressure parameters.

We observed that SBP, DBP, and MAP reactivity to exercise stress were reduced after training with ATM, but not LTM. Importantly, resting DBP was also reduced after ATM training. Exaggerated SBP and MAP during exercise may be indicative of failure to reduce total peripheral resistance and of early structural changes in the vasculature, which can lead to hypertension (34,40,44). Our measures of reduced SBP and MAP strongly suggest improved vessel compliance and a resultant reduced risk of future hypertension and related diseases in ATM trained subjects.

We also observed a reduced reactivity to exercise stress in measures of both PP and RPP. Pulse pressure is proportional to stroke volume and widening of PP may serve as a marker

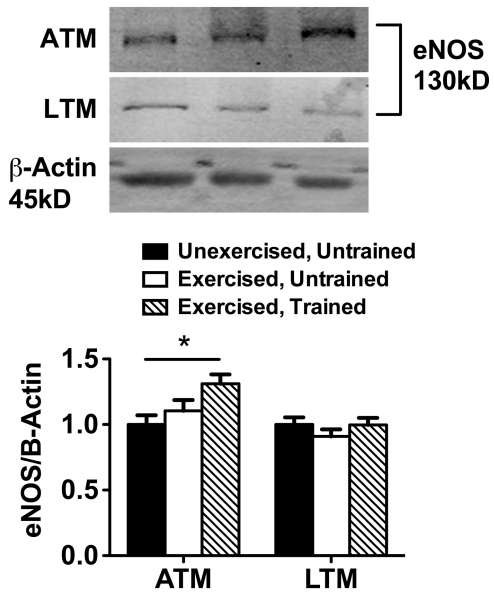

FIGURE 2—Influence of exercise and exercise training on eNOS content in ATM compared with LTM-trained individuals from vastus lateralis biopsies. Upper panel: sample immunoblots of eNOS for both training groups and β-actin loading control; samples organized from left to right are unexercised/untrained (UU), exercised/untrained (EU), and exercised/trained (ET). Lower panel: quantitation of the relative abundance of eNOS normalized to β-actin control; densitometry for each subject is normalized to their own unexercised/untrained sample. *$P < 0.05$ compared with resting, untrained. Data are presented as mean ± SEM.

of lost compliance of the vessel wall (28). The observed blunted increases in PP during exercise stress after ATM training may indicate improved vessel compliance. RPP serves as an indicator of myocardial work/oxygen consumption (16), therefore reduced RPP during similar exercise stress is indicative of a reduced workload on the heart to perform equivalent total body work. Taken together, these findings in previously inactive, normotensive adults demonstrate reduced blood pressure reactivity to physical stress after ATM training, which may have application in the treatment and prevention of hypertension. At the current time, we can only speculate as to how ATM training may affect these measures in a hypertensive population. However, previous data in more traditional exercise training modalities suggest that exercise is more likely to improve such measures in the hypertensive population (35). It would therefore be of great clinical interest to determine whether ATM training may enhance such benefits in this population.

At this time, we cannot be certain as to why ATM training may provide these potentially antihypertensive benefits. Acute hemodynamic changes in response to both water immersion (of various depths) and exercise in water have been observed compared with land exercise (6,7,21,32,37). More specifically, previous investigations (6,7) have observed greater increases in pulmonary arterial pressure, end-diastolic volume, stroke volume, and ejection fraction and lower HR during cycle ergometer exercise using similar water depth and temperatures as in our current study (i.e., chest depth) compared with similar land-based exercise. These observations are consistent with our own previous findings, which demonstrated lower HR at maximal exercise during ATM exercise compared with LTM exercise in healthy subjects, with no difference in $\dot{V}O_{2max}$ (18). These previous findings indicate the possibility of assisted venous return during aquatic exercise caused by the positive lower body pressures as a result of being partially submerged in water at chest depth. Given the current data, the vasodilatory response to exercise may be enhanced as a result of an attempt to overcome the change in positive pressure on the lower body. Decreased levels of circulating catecholamines resulting from possible differences in baroreceptor stimulation have also been observed during exercise in water compared with land (38). It is possible that these variations in hemodynamics in response to the single bout of aquatic exercise contribute to the training adaptations we observed here in ATM trained subjects.

Because hemodynamic responses differ between land-based and water-based training, it is reasonable to speculate that ATM training elicited different adaptive responses to cope with these altered stressors compared with LTM training. In agreement with our findings, previous evidence suggests the efficacy of swim training to reduce resting blood pressures in both prehypertensive and hypertensive subjects (33,41). These previous findings appear to be the result of improved baroreflex sensitivity and flow-mediated dilation after swim training (33). These findings appear to agree with those in our current study; however, we cannot be certain as to the similarity of the dynamics of immersion between swimming and ATM exercise. Therefore, in an effort to identify possible mechanisms that may contribute to the ameliorated blood pressure responses observed in ATM trainers in our study, we examined skeletal muscle biopsy samples (vastus lateralis) obtained from a subset of our participants in three conditions of exercise: unexercised and untrained, exercised and untrained, and exercised and trained as previously described (17). Our analysis reveals that eNOS content was enhanced only after exercise training in the ATM group with no change observed in the LTM group (Fig. 2). eNOS is the primary means by which nitric oxide is produced to invoke a vasodilatory response from smooth muscle, which would result in reduced blood pressure, and may also influence the baroreceptor reflex (26,43). At this time, we cannot say if this increase is the result of greater eNOS content in the endothelium or is a by-product of greater angiogenesis in the ATM group. Future investigations will be needed to better elucidate the role of eNOS in the reduced blood pressure reactivity seen here. Interestingly, eNOS content has been reported to be both enhanced, which may be dependent on the vessel of interest (25), and unchanged (42) with exercise training. We can only speculate at this time why such an adaptation would occur after ATM training but not LTM. A study by Trott et al. (42) similarly showed no increase in eNOS content with LTM-type training in rat. However, we must caution that those studies were carried out in isolated vessels, which may show different dynamics than that in the whole skeletal muscle lysate examined here. Considering the previous evidence that swim training may attenuate stress-induced hypertension in rats (8), it seems reasonable that such a change in eNOS may be training mode specific. Finally, we have elected to focus our efforts on the differential regulation of eNOS as a potential mechanism behind improved blood pressure reactivity in ATM training. The observed effects on ATM training on whole muscle eNOS expression may relate to similar improvements in flow-mediated dilation as seen with swim training (33). However, we acknowledge that nNOS is also a major source of nitric oxide in skeletal muscle, which may also have profound effects on the phenotype seen in our current study. In addition, it is highly likely that mechanisms related to the hemodynamic response to acute exercise and chronic exercise training are interrelated; as such, we cannot at this time exclude the possibility of improved baroreceptor sensitivity with ATM training.

In the current investigation, we have shown that ATM exercise preferentially ameliorates blood pressure reactivity to exercise stress, which would then be predicted to prevent or delay the onset of chronic diseases such as essential hypertension. In addition, we observed that this effect was accompanied by a mode-specific increase in eNOS expression in skeletal muscle biopsy samples after ATM training, indicating that these adaptations may be due to enhanced

ability to induce endothelium dependent vasodilation. Our study may lead to new and effective strategies to prevent and reduce hypertension.

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REFERENCES

1. American College of Sports Medicine. G*uidelines for Exercise Testing and Prescription*. Philadelphia: Lippincott Williams & Wilkins; 2006.
2. Bergstrom J. Muscle electrolytes in man, determined by neutron activation analysis on needle biopsy specimens—study on normal subjects, kidney patients, and patients with chronic diarrhoea. *Scand J Clin Lab Invest*. 1962;14:1.
3. Borg G. Perceived exertion as an indicator of somatic stress. *Scand J Rehabil Med*. 1970;2:92–8.
4. Bruce RA, Kusumi F, Hosmer D. Maximal oxygen intake and nomographic assessment of functional aerobic impairment in cardiovascular disease. *Am Heart J*. 1973;85:546–62.
5. Centers for Disease Control and Prevention. *Behavioral Risk Factor Surveillance System Survey Data*. Atlanta (GA): U.S. Department of Health and Human Services, Centers for Disease Control and Prevention; 2012.
6. Christie JL, Sheldahl LM, Tristani FE, et al. Cardiovascular regulation during head-out water immersion exercise. *J Appl Physiol*. 1990;69:657–64.
7. Connelly TP, Sheldahl LM, Tristani FE, et al. Effect of increased central blood volume with water immersion on plasma catecholamines during exercise. *J Appl Physiol*. 1990;69:651–6.
8. Cox RH, Hubbard JW, Lawler JE, Sanders BJ, Mitchell VP. Exercise training attenuates stress-induced hypertension in the rat. *Hypertension*. 1985;7:747–51.
9. Craig AB, Dvorak M. Thermal regulation of man exercising during water immersion. *J Appl Physiol*. 1968;25:28–35.
10. Dunstan DW, Puddey IB, Beilin LJ, Burke V, Morton AR, Stanton KG. Effects of a short-term circuit weight training program on glycaemic control in NIDDM. *Diabetes Res Clin Pract*. 1998;40: 53–61.
11. Dupont-Versteegden EE, Strotman BA, Gurley CM, et al. Nuclear translocation of endog at the initiation of disuse muscle atrophy and apoptosis is specific to myonuclei. *Am J Physiol Regul Integr Comp Physiol*. 2006;291:R1730–40.
12. Evans WJ, Phinney SD, Young VR. Suction applied to a muscle biopsy maximizes sample-size. *Med Sci Sports Exerc*. 1982;14: 101–2.
13. Everson SA, Kaplan GA, Goldberg DE, Salonen JT. Anticipatory blood pressure response to exercise predicts future high blood pressure in middle-aged men. *Hypertension*. 1996;27:1059–64.
14. Everson SA, Lynch JW, Kaplan GA, et al. Stress-induced blood pressure reactivity and incident stroke in middle-aged men. Editorial comment: something old and something new. *Stroke*. 2001; 32:1263–70.
15. Fagard RH. Exercise characteristics and the blood pressure response to dynamic physical training. *Med Sci Sports Exerc*. 2001; 33:S484–92.
16. Gobel F, Norstrom L, Nelson R, Jorgensen C, Wang Y. The rate-pressure product as an index of myocardial oxygen consumption during exercise in patients with angina pectoris. *Circulation*. 1978; 57:549–56.
17. Greene NP, Fluckey JD, Lambert BS, Greene ES, Riechman SE, Crouse SF. Regulators of blood lipids and lipoproteins? PPARδ and AMPK, induced by exercise, are correlated with lipids and lipoproteins in overweight/obese men and women. *Am J Physiol Endocrinol Metab*. 2012;303:E1212–21.
18. Greene NP, Greene ES, Carbuhn AF, Green JS, Crouse SF. $\dot{V}O_2$ prediction and cardiorespiratory responses during underwater treadmill exercise. *Res Q Exerc Sport*. 2011;82:264–73.
19. Greene NP, Lambert BS, Greene ES, Carbuhn AF, Green JS, Crouse SF. Comparative efficacy of water and land treadmill training for overweight or obese adults. *Med Sci Sports Exerc*. 2009;41:1808–15.
20. Greene NP, Martin SE, Crouse SF. Acute exercise and training alter blood lipid and lipoprotein profiles differently in overweight and obese men and women. *Obesity*. 2012;20:1618–27.
21. Hall J, Bisson D, O'hare P. The physiology of immersion. *Physiotherapy*. 1990;76:517–21.
22. Harris KA, Holly RG. Physiological response to circuit weight training in borderline hypertensive subjects. *Med Sci Sports Exerc*. 1987;19:246–52.
23. Kelley G, Mcclellan P. Antihypertensive effects of aerobic exercise: a brief meta-analytic review of randomized controlled trials. *Am J Hypertens*. 1994;7:115–9.
24. Kelley GA, Kelley KA, Vu Tran Z. Aerobic exercise and resting blood pressure: a meta-analytic review of randomized, controlled trials. *Prev Cardiol*. 2001;4:73–80.
25. Laughlin MH, Pollock JS, Amann JF, Hollis ML, Woodman CR, Price EM. Training induces nonuniform increases in enos content along the coronary arterial tree. *J Appl Physiol*. 2001;90:501–10.
26. Matsuda T, Bates JN, Lewis SJ, Abboud FM, Chapleau MW. Modulation of baroreceptor activity by nitric oxide and S-nitrosocysteine. *Circ Res*. 1995;76:426–33.
27. Matthews KA, Katholi CR, Mccreath H, et al. Blood pressure reactivity to psychological stress predicts hypertension in the CARDIA study. *Circulation*. 2004;110:74–8.
28. Matthews KA, Owens JF, Kuller LH, Sutton-Tyrrell K, Lassila HC, Wolfson SK. Stress-induced pulse pressure change predicts women's carotid atherosclerosis. *Stroke*. 1998;29:1525–30.
29. Miyai N, Arita M, Miyashita K, Morioka I, Shiraishi T, Nishio I. Blood pressure response to heart rate during exercise test and risk of future hypertension. *Hypertension*. 2002;39:761–6.
30. Murphy MH, Hardman AE. Training effects of short and long bouts of brisk walking in sedentary women. *Med Sci Sports Exerc*. 1998;30:152–7.
31. Murtagh EM, Boreham CAG, Nevill A, Hare LG, Murphy MH. The effects of 60 minutes of brisk walking per week, accumulated in two different patterns, on cardiovascular risk. *Prev Med*. 2005;41:92–7.
32. Norsk P, Bonde-Petersen F, Christensen NJ. Catecholamines, circulation, and the kidney during water immersion in humans. *J Appl Physiol*. 1990;69:479–84.
33. Nualnim N, Parkhurst K, Dhindsa M, Tarumi T, Vavrek J, Tanaka H. Effects of swimming training on blood pressure and vascular function in adults >50 years of age. *Am J Cardiol*. 2012;109:1005–10.
34. Palatini P. Exaggerated blood pressure response to exercise: pathophysiologic mechanisms and clinical relevance. *J Sports Med Phys Fit*. 1998;38:1–9.
35. Pescatello LS, Franklin BA, Fagard R, et al. Exercise and hypertension. *Med Sci Sports Exerc*. 2004;36:533–53.

36. Pickering TG, Hall JE, Appel LJ, et al. Recommendations for blood pressure measurement in humans and experimental animals. *Circulation*. 2005;111:697–716.
37. Pump B, Shiraishi M, Gabrielsen A, Bie P, Christensen NJ, Norsk P. Cardiovascular effects of static carotid baroreceptor stimulation during water immersion in humans. *Am J Physiol Heart Circ Physiol*. 2001;280:H2607–15.
38. Reilly T, Dowzer CN, Cable NT. The physiology of deep-water running. *J Sports Sci*. 2003;21:959–72.
39. Smith PK, Krohn RI, Hermanson GT, et al. Measurement of protein using bicinchoninic acid. *Anal Biochem*. 1985;150:76–85.
40. Stewart KJ, Sung J, Silber HA, et al. Exaggerated exercise blood pressure is related to impaired endothelial vasodilator function. *Am J Hypertens*. 2004;17:314–20.
41. Tanaka H, Bassett DRJ, Howley ET, Thompson DL, Ashraf M, Rawson FL. Swimming training lowers the resting blood pressure in individuals with hypertension. *J Hypertens*. 1997;15:651–7.
42. Trott DW, Gunduz F, Laughlin MH, Woodman CR. Exercise training reverses age-related decrements in endothelium-dependent dilation in skeletal muscle feed arteries. *J Appl Physiol*. 2009;106:1925–34.
43. Waki H, Murphy D, St Yao, Kasparov S, Paton JFR. Endothelial no synthase activity in nucleus tractus solitarii contributes to hypertension in spontaneously hypertensive rats. *Hypertension*. 2006;48:644–50.
44. Wilson MF, Sung BH, Pincomb GA, Lovallo WR. Exaggerated pressure response to exercise in men at risk for systemic hypertension. *Am J Cardiol*. 1990;66:731–6.