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Original Research Article

Aquatic reduced exertion high-intensity interval training (REHIT) increases cardiorespiratory fitness in untrained young adults compared with a land-based protocol

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ABSTRACT

Purpose: This randomized control trial compared cardiometabolic, performance, and subjective outcomes following an 8-week reduced exertion high intensity interval training (REHIT) program in land (LAND) vs. aquatic (AQUA) exercise matched for relative intensity. **Methods:** Young, untrained adults were randomized to LAND or AQUA groups and followed a REHIT exercise protocol (3 min warm up, 20 second maximal effort, 3 min active recovery, 20 second maximal effort, 3 min cool down) matched for exercise intensity 3x/wk for 8 consecutive weeks on a cycle ergometer (LAND) or with maximal jump squats in the pool (AQUA). Pre and post measures included height, body mass, BMI, resting blood pressure and heart rate, fasting glucose, estimated VO_{2max} test, Wingate Test, vertical jump, and the Physical Activity Enjoyment Questionnaire. **Results:** Neither the AQUA or LAND training influenced weight, BMI, or cardiometabolic variables ($p>0.05$). The AQUA group (Pre: 36.9 ± 7.7 vs Post: 43.9 ± 8.8 mL/kg/min), but not LAND group (Pre: 33.1 ± 6.9 vs Post: 34.9 ± 6.1 mL/kg/min), increased estimated VO_{2max} ($p<0.05$). The LAND group increased both absolute (Pre: 644.6 ± 210.7 vs Post: 794.1 ± 208.1 Watts) and relative maximal power (Pre: 8.6 ± 3.0 vs 10.7 ± 3.1 W/kg) ($p<0.05$) as assessed by the Wingate test; however, there was no difference in the AQUA group in absolute (Pre: 633.8 ± 172.5 vs Post: 683.8 ± 158.8 Watts) or relative maximal power (Pre: 9.2 ± 2.3 vs Post: 9.9 ± 1.9 W/kg) ($p>0.05$). There was no difference in vertical jump height or average change in response on the questionnaire in either group ($p>0.05$). **Conclusion:** Compared with a land-based protocol matched for relative intensity, an 8-week aquatic REHIT program increased estimated cardiorespiratory fitness, but not maximal power production, in young, untrained adults. Aquatic REHIT can be recommended as a time-efficient exercise program for those just starting exercise that need or prefer an alternate to land-based exercise. This study was registered on clinicaltrials.gov (NCT05573087) on Oct. 10, 2022.

KEY WORDS: Fitness, Physical Activity, Water Exercise, Untrained.

INTRODUCTION

The American College of Sports Medicine (ACSM) recommends that most adults

participate in at least 30 minutes of moderate-intensity continuous exercise most days of the week (≥ 5 days), vigorous-

intensity exercise ≥ 20 minutes for ≥ 3 days per week, or a combination of the two in order to achieve sufficient exercise volume¹. While this position stand is widely accepted and regarded as an evidence-based preventive strategy for many chronic diseases, adherence to exercise programming meeting this criteria continues to be a challenge as only 23% of U.S. adults meet these exercise recommendations². A perceived lack of time is the most frequently cited barrier for poor exercise adherence³⁻⁴. By utilizing very intense bouts of exercise paired with recovery, high-intensity interval training (HIIT) has been proposed as a more time efficient means of meeting exercise recommendations⁵⁻⁷.

The health and fitness benefits associated with HIIT include improvement in cardiometabolic factors, reduced subcutaneous fat deposition, and increased cardiorespiratory fitness, muscle mass, and energy expenditure⁸⁻⁹. Additionally, HIIT has $\geq 80\%$ adherence rate and positive psychological responses (i.e., reduced anxiety and depression)⁹⁻¹⁰. While this is promising, classic HIIT lasts 30 minutes per training session¹¹. Instead, reduced exertion high-intensity interval training (REHIT) is structured to last only 10 minutes with two or three sprinting bouts that last no more than 20 seconds followed by short recover periods¹²⁻¹³. The intention of this protocol is to be less time consuming (to alleviate the “lack of time barrier”), while still eliciting positive health benefits¹⁴. Indeed, compared with moderate-intensity continuous

exercise and HIIT, REHIT exercise has shown comparable changes to cardiometabolic, performance, and subjective outcomes, including enjoyment and adherence to exercise¹⁵⁻¹⁸.

Individuals that are beginning an exercise program may experience further barriers beyond that of time constraints (e.g., discomfort, pain, or fear) and the aquatic environment provides a forgiving setting that may be preferred to those initiating an exercise program. Upon immersion, the body experiences a variety of innate aquatic properties, or hydrostatic principles, that differ compared with land. Buoyancy, changes in hydrostatic pressure, and increased viscosity of water compared with air¹⁹ aid in accessibility and acclimation to exercise while limiting associated discomfort, pain, or fear that some individuals new to exercise may experience. Moreover, introducing exercise to untrained individuals via aquatic-based training may improve exercise tolerance and adherence²⁰. The physiological effects of aquatic immersion paired with multimodal exercise improves strength, cardiorespiratory fitness, and balance in healthy populations²¹. Further, for clinical populations, including those in which land-based training is difficult, painful, or poses safety concerns, aquatic exercise elicits favorable improvements in balance, pain, flexibility, strength, cardiorespiratory fitness, anthropometry, and quality of life²¹. Incorporating HIIT with aquatic exercise has also demonstrated advantageous results in

both healthy and compromised populations²².

Despite the growing popularity of REHIT and the beneficial effects of aquatic exercise, no study has yet compared an aquatic REHIT protocol to a land-based REHIT protocol. Therefore, the purpose of this randomized control trial was to compare cardiometabolic, performance, and subjective outcomes following an 8-week REHIT program in land (LAND) vs. aquatic (AQUA) exercise matched for relative intensity in young adults not meeting exercise recommendations. We hypothesized that the AQUA REHIT would elicit changes comparable to a LAND REHIT program matched for exercise intensity.

METHODS

Participants

English speaking, healthy (free of chronic illness), untrained individuals of both sexes ages 18-30 were included. Untrained was defined as less than 150 minutes of exercise/week. Vulnerable populations were excluded including adults unable to consent/cognitively impaired, individuals who are not yet adults, pregnant or nursing women, and prisoners or other detained individuals.

The sample size calculation for the primary outcome measure, estimated maximal oxygen uptake relative (VO_{2max}), a measure of cardiorespiratory fitness (CRF), assumed a power of 0.8 and an alpha-error probability of 0.05. This calculation determined a

required sample size of at least 16 subjects in each group to detect a significant effect between groups on CRF, such as a difference of $10\% \pm 10\%$. Participants were randomized using Excel.

Experimental design

This randomized control trial (land-based intervention versus aquatic intervention) was approved by the institutional review board of West Virginia University (IRB#: 1811776700), is registered at clinicaltrials.gov (NCT05573087) on Oct. 10, 2022 and data collection followed procedures in accordance with the ethical standards of the Helsinki Declaration. The investigators (exercise physiology faculty members at West Virginia University), assisted by trained undergraduate exercise physiology student interns, developed and implemented a randomized control trial comparing a land and aquatic REHIT program in previously untrained, but otherwise healthy young adults. Cardiometabolic, performance and survey results were collected before and after the 8-week exercise intervention. Post assessments were conducted within one week after completing the exercise intervention. Enrollment into the research project was voluntary and written and verbal informed consent were obtained from all participants.

Previous REHIT interventions have been conducted on cycle ergometers that elicit maximal power production. To match exercise intensity between the aquatic and

land protocols for the randomized control trial, a pilot project compared exercise intensity between a land-based cycle ergometer and exercise in an aquatic environment. Participants (n=10) completed two trials to assess maximal effort in an aquatic environment: 20 second maximal effort jump squats standing at a depth of where the water reaches the navel with or without ankle resistive devices (+R vs. w/oR). The order of trials was randomized. Peak heart rate and RPE were assessed to determine greatest effort and participants rated which protocol felt “hardest”.

A two-tailed student T-test found adding resistance devices to the ankles elicited higher peak heart rate (+R 160 ± 20 vs. w/oR 149 ± 12 bpm, $p = 0.03$) and RPE (+R 13 ± 3 vs. w/oR 11 ± 2 , $p = 0.06$) (both reported as means \pm SD). From these findings, the aquatic training protocol was developed for the randomized control trial: maximal jump squats with ankle resistance devices standing in the pool at a depth of where the water reached the navel.

Baseline Screening Procedures

Before arriving to the laboratory, all participants completed a health history questionnaire to assess information related to disease risk, medication use, and lifestyle behaviors (e.g., physical activity, nutrition, stress). Upon arrival to the laboratory, a resting 3-lead electrocardiogram (ECG) was performed by a trained technician and reviewed by a clinical exercise physiologist for evidence of irregular myocardial

electrical conductivity or arrhythmia. A technician measured resting, seated brachial blood pressure of the right arm using a stethoscope and sphygmomanometer. A fasting capillary blood glucose sample was taken using a lancet and measured with a glucometer and glucose testing strips (Contour Next Blood Glucose Monitoring System, Ascensia Diabetes Care, NJ, USA). A clinical exercise physiologist reviewed the health history questionnaire and assessment results. The American College of Sports Medicine safety guidelines were used to determine whether it was safe for participants to exercise²³. Any evidence of risk required the participant follow up with their physician before continuing with pre-assessments or starting the exercise program.

Following resting measures, participants completed a battery of assessments including anthropometrics, maximal graded exercise test, power measures (Wingate Test and Vertical Jump), and surveys.

Anthropometrics

Standing height (± 0.1 cm) was measured with a stadiometer and body weight (± 0.1 lb) was measured with a calibrated body mass scale with the participant barefoot and in light clothing. Body mass index (BMI) was calculated from height and weight (kg/m^2).

Maximal graded exercise test

A Bruce graded maximal treadmill test was administered by trained technicians and supervised by a clinical exercise physiologist.

Participants completed the staged protocol until 85% of heart rate reserve or volitional fatigue was reached. For the safety of the participant, heart rate, blood pressure and rate of perceived exertion were measured at each stage. Despite the design of the initial protocol, due to the COVID-19 pandemic, gas analysis was not permitted; therefore, VO_{2max} was estimated using established equations²³.

$$\text{Men: } VO_{2max} = 14.8 - (1.379 \times T) + (0.451 \times T) - (0.012 \times T)$$

$$\text{Women: } VO_{2max} = 4.38 \times T - 3.9$$

Where T represents the total time on the treadmill and is written as a fraction of minutes and seconds (e.g., 8 minutes and 45 seconds would be 8.75).

Wingate test

A five-minute warm-up was completed at a self-selected cadence and a resistance of 2% percent of the participant's body weight. During the warm-up participants completed three short sprints lasting five seconds at the second, third and fourth minutes with a resistance of 4.1% of the participants body weight. The five-minute warm up was followed by a three-minute warm-up recovery with no resistance. A thirty second Wingate test was then completed. Participants were instructed to pedal as fast as possible for the entire thirty seconds with the resistance at 7.5% of their body weight. A three-minute cool-down at no resistance was then completed.

Vertical jump test

Reach height was measured as participants walked underneath the Vertec and hit as many flags as possible without coming up onto their toes. Jump height was measured as participants completed a standing vertical jump (without stepping into the jump) and hitting as many flags as possible. Participants were asked to complete the vertical jump three times and the highest value reached was recorded. Vertical jump height was calculated by subtracting reach height from the jump height.

Surveys

Participants completed the self-administered Physical Activity Enjoyment Questionnaire, an 18-item questionnaire that rates how the participant feels at that moment about the physical activity that they have been doing²⁴. For each item, participants rate on a scale of 1 – 7, on how they feel about the physical activity.

Intervention

Participants followed a REHIT exercise protocol 3 times per week for 8 consecutive weeks under the supervision of trained research assistants while wearing a chest strap heart rate monitor (Polar Electro, Finland). Heart rate was collected continuously during each training session and the average of all data was reported as average heart rate. Peak heart rate and RPE were recorded during the maximum efforts.

The land based (LAND) protocol was completed on a Monark 894 E bike. To match

exercise intensity between the aquatic and land environments, the cycle ergometer was set to a resistance of 2.5 kg/kg body mass for the maximal sprint efforts. The exercise intervention included a three-minute unloaded warm-up on the cycle ergometer, followed by a 20 second maximum effort, a three-minute unloaded active recovery, a final 20 second maximum effort, and a three-minute unloaded cool-down.

The aquatic based (AQUA) protocol was performed at the university pool (85° F). Participants wore ankle resistance fin trainers (Speedo International Limited, UK) in a pool standing at a depth where the water line reached the navel. The exercise intervention included a three-minute warm-up of multidirectional movement (e.g., star lunges) and easy jumping, followed by a 20 second maximum effort repeated jump squats, a three-minute active recovery of easy multidirectional movements, a final 20 second maximum effort repeated jump squats, and a three-minute cool-down of mobility focused movements. Target jumps were not set, but participants were encouraged to go as fast as possible and accomplish as many jumps within the 20 seconds effort.

Statistical Analysis

Descriptive statistics are reported as Mean \pm SE. Between-group differences in outcome variables including descriptive characteristics, oxygen consumption testing, Wingate testing, and vertical jump testing were determined by two-way repeated

measures ANOVA (group \times time). Least Significant Difference post hoc comparisons were performed for all significant main effects ($\alpha=0.05$). The level of significance was set *a priori* at $\alpha=0.05$. Pre-post differences in group responses to survey data were compared with Mann Whitney U.

RESULTS

There were no adverse events reported during data collection.

At baseline there was no difference between AQUA (n=12) and LAND (n=11) groups in age, height, body mass, BMI, or cardiometabolic variables at baseline ($p>0.05$) (See Table 1.). Neither the AQUA nor LAND training influenced weight, BMI, or cardiometabolic variables pre vs post training. ($p>0.05$) (See Table 1.).

Absolute average heart rate across the training sessions was lower in AQUA vs LAND ($p<0.05$). Absolute peak heart rate across the training sessions was similar between AQUA vs LAND ($p>0.05$). Relative average and peak heart rates across training sessions were not different between AQUA and Land groups ($p>0.05$). See Table 2. for heart rate comparisons.

There was no difference in absolute or relative maximal power at baseline between the AQUA and LAND groups ($p>0.05$). Following training, the LAND group increased both absolute (Pre: 644.6 ± 210.7 vs Post: 794.1 ± 208.1 Watts) and relative maximal power (Pre: 8.6 ± 3.0 vs 10.7 ± 3.1

W/kg) ($p < 0.05$) as assessed by the Wingate test; however, there was no difference in the AQUA group in absolute (Pre: 633.8 ± 172.5 vs Post: 683.8 ± 158.8 Watts) or relative maximal power (Pre: 9.2 ± 2.3 vs Post: 9.9 ± 1.9 W/kg) ($p > 0.05$). See Figure 1. There was no difference in vertical jump height following either AQUA or LAND training, and no differences between groups at baseline or post-training ($p > 0.05$). See Figure 2.

There was no difference in estimated VO_{2max} at baseline between the AQUA and LAND groups ($p > 0.05$). The AQUA group (Pre: 36.9 ± 7.7 vs Post: 43.9 ± 8.8 mL/kg/min), but not

LAND group (Pre: 33.1 ± 6.9 vs Post: 34.9 ± 6.1 mL/kg/min), increased estimated VO_{2max} ($p < 0.05$). (See Figure 3). Training had no effect on maximal heart rate AQUA (Pre: 188 ± 3 vs Post: 192 ± 3 bpm) or LAND (Pre: 191 ± 3 vs Post: 193 ± 2 bpm) groups ($p > 0.05$). Additionally, RPE at the end of the treadmill test was not different pre vs post training in either the AQUA or LAND groups ($p > 0.05$).

There were no differences between groups in the average change in response on the Physical Activity Enjoyment Questionnaire (AQUA: -0.1 ± 4.4 vs. LAND -3.7 ± 7.9 , $p > 0.05$).

Table 1. Descriptive and cardiometabolic characteristics of AQUA and LAND groups pre vs post training.

	Pre		Post	
	AQUA	LAND	AQUA	LAND
Sex (% male)	30%	41%		
Age (year)	20.3 ± 2.2	21.8 ± 3.0		
Height (cm)	166 ± 2.9	166 ± 2.2	164 ± 2.3	166 ± 2.2
Body Mass (kg)	70 ± 5.3	75 ± 4.5	71 ± 5.5	74 ± 4.4
Body Mass Index ($kg \cdot m^{-2}$)	25.4 ± 1.5	27.3 ± 1.4	25.5 ± 1.7	27.1 ± 1.5
Fasting Blood Glucose (mg/dl)	87 ± 4	99 ± 4	94 ± 4	97 ± 4
Systolic Blood Pressure (mmHg)	110 ± 3	117 ± 3	112 ± 3	116 ± 3
Diastolic Blood Pressure (mmHg)	71 ± 3	72 ± 3	72 ± 3	72 ± 2
Resting Heart Rate (bpm)	87 ± 3	89 ± 5	88 ± 3	95 ± 5

Table 2. Heart rate during training in AQUA and LAND groups.

	AQUA	LAND
Average Heart Rate (bpm) during the session	124 ± 2	$136 \pm 4^*$
Average Heart Rate (%peak) during the session	67 ± 2	70 ± 2
Peak Heart Rate (bpm) during maximal effort	168 ± 3	176 ± 4
Peak Heart Rate (%peak) during maximal effort	90 ± 2	91 ± 2

* $p < 0.05$.

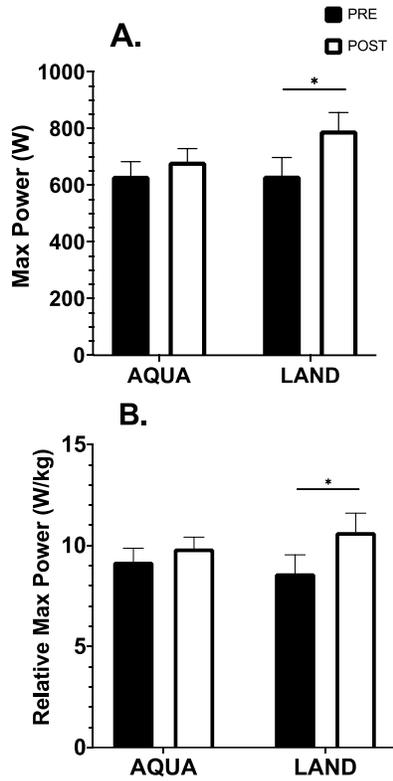


Figure 1. A) Absolute and B) relative max power during a Wingate Anaerobic Test; *p<0.05.

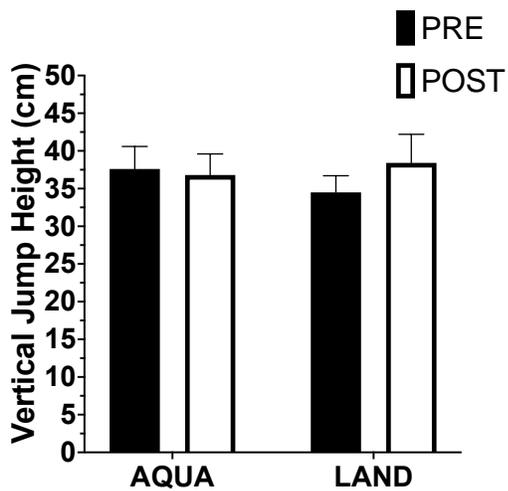


Figure 2. Vertical jump height pre vs post training in the AQUA and LAND groups.

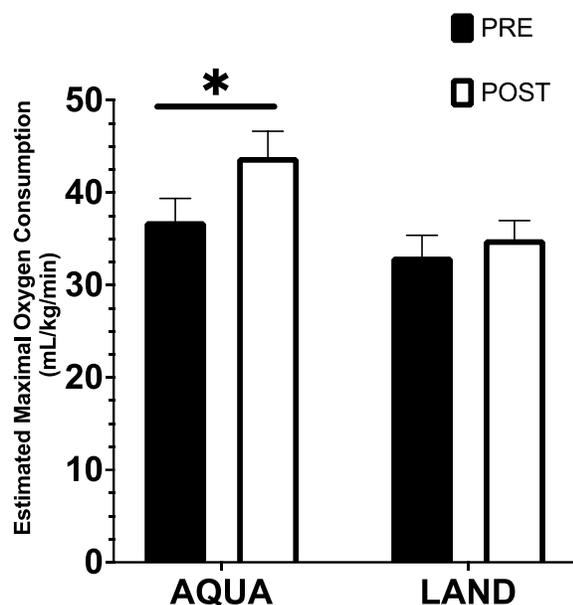


Figure 3. Pre vs post differences in estimated maximal oxygen consumption in both the AQUA and LAND groups; * $p < 0.05$.

DISCUSSION

The purpose of this randomized control trial was to compare cardiometabolic, performance, and subjective outcomes following an 8-week REHIT program in land (LAND) vs. aquatic (AQUA) exercise matched for relative intensity in young adults not meeting exercise recommendations. Our hypothesis that the AQUA REHIT would elicit changes in cardiometabolic, performance, and subjective measures comparable to a LAND REHIT program matched for exercise intensity was disproved. Following training, only the AQUA group increased estimated VO_{2max} , the primary outcome. While neither group improved vertical jump height following training, the LAND group increased both absolute and relative maximal power, assessed by the Wingate Test. There were no changes in either group for cardiometabolic or subjective measures.

In the present study, exercise intensities were matched between groups, as confirmed by no differences in average heart rates (%peak) during training. The lower average heart rate (bpm) in water is an expected and well-established consequence of the hydrostatic principles influencing heart rate. Hydrostatic pressure redistributes blood and lymph²⁵⁻²⁶ while buoyancy offsets the effects of gravity allowing for increased venous return²⁷. The result of these fluid shifts is an increase in central volume, cardiac volume, mean stroke volume and cardiac output which contribute to a net reduction in heart rate while immersed²⁸⁻³⁰. While heart rate reduction can be highly variable from person-to-person, a deduction of 17 BPM is commonly used in aquatic exercise and is similar (~12 bpm) to the difference in

average heart rate between land and aquatic in the present study.

Because exercise training programs were matched for relative intensity, we expected comparable increases in estimated VO_{2max} following training in both groups; however, only the AQUA group increased estimated VO_{2max} . Previous research has confirmed increases in estimated VO_{2max} following REHIT on the cycle ergometer: 8 weeks of REHIT elicited greater improvements in cardiorespiratory fitness compared with traditional moderate intensity continuous training¹⁴ and a 6-week REHIT program improved cardiorespiratory fitness in both male and female sedentary, healthy adults³¹. However, one of these studies set the sprint resistance to 7.5% of bodyweight³¹ and the other used a self-learning algorithm that adapted the resistance level as participants got stronger and fitter¹⁴. For our aquatic protocol, the buoyancy of water reduced stress and impact even at maximal intensities. Therefore, to match exercise intensity between the aquatic and land environments, the cycle ergometer was set to a resistance of 2.5 kg/bodyweight for the maximal sprints. The lower resistance likely elicited a lower training stress, which could explain the failure of the LAND group to achieve increases in estimated VO_{2max} following training.

Only the LAND group increased both absolute and maximal power on the Wingate Test and there were no between or within group differences in vertical jump height.

According to the principles of specificity of training, maximal power production increases specific to the training method. Since AQUA participants were performing maximal jump squats and LAND participants were performing maximal cycling sprints, both groups performed maximal power assessments on cycling sprints (Wingate Test) and jumping (Vertical Jump). Interestingly, the low sprint resistance on the cycle ergometer did not elicit increases in estimated VO_{2max} , but did increase power production as assessed by the Wingate. Due to the low cross-over between exercise modalities and estimated VO_{2max} ³², it is possible the lack of improvement in estimated VO_{2max} following training was a result of differences in modalities used in training (e.g., cycling/aquatic) and testing (i.e., treadmill). However, the improvement in Wingate performance in the LAND group is supported by prior research in which as little as 4-second cycling sprint bouts has been shown to increase maximal neuromuscular power³³.

Prior studies have found increases in vertical jump height following plyometric training in aquatic environments. Therefore, in the present study, we expected AQUA, but not LAND to increase jump height following training, yet, neither group increased jump height as assessed by vertical jump. The majority of prior studies testing plyometric training in aquatic environments has done so in athletic populations³⁴. Therefore, it is possible that pre-intervention training status of our participants may have restricted their

potential improvements in vertical jump height, as prior studies suggest that high relative strength may be necessary to maximize the effects of plyometric/jump training³⁵. Furthermore, the REHIT protocol used in the present study was not designed to elicit maximal neuromuscular power and therefore improve vertical jump height, rather the protocol was designed to elicit improvements in maximal aerobic power.

There were no differences within or between groups following training for cardiometabolic outcomes. This is in agreement with previous REHIT research which found 8 weeks of REHIT on a cycle ergometer did not elicit changes in weight, diastolic blood pressure, or fasting glucose¹⁴; however, this previous work did find reductions in systolic blood pressure, likely because the participants had higher SBP at baseline (130 ± 9 mmHg) compared with the present study (110 ± 3 mmHg AQUA, 112 ± 3 mmHg LAND). This caused a “basement effect” whereby the well-known hypotensive effects of exercise were unable to elicit changes in already low blood pressures at baseline. Similarly, there were likely no changes to resting heart rate, fasting glucose, or diastolic BP because the present cohorts presented with optimal or normal values at baseline.

There were no differences between groups in the average change in response to the Physical Activity Enjoyment Questionnaire. This survey assesses how the responder feels about the physical activity with lower scores

indicating more favorable attitudes towards physical activity. While previous work has found participants enjoy aquatic programming³⁶ and therapeutic activity in the water can improve mood and facilitate positive emotions³⁷, in the present study there was no difference in changes in enjoyment between groups, which is in agreement with previous literature comparing enjoyment of land and aquatic exercise³⁸.

This study is the first to suggest aquatic REHIT exercise can increase cardiorespiratory fitness but is not without limitations. Importantly, the COVID-19 pandemic had significant consequences on this study. First, direct measurement of VO_{2max} via gas analysis was prohibited to limit exposure of technicians to respiratory droplets so VO_{2max} was estimated indirectly using validated equations which might have introduced error to the results. Secondly, COVID limited within group socialization which may have negatively impacted the enjoyment of physical activity. Finally, the sample size was less than expected due to voluntary enrollment during the pandemic and findings should be interpreted accordingly. Future research could further examine the relationship between heart rate responses and VO_{2max} between aquatic and land-based exercise. Additional future work could examine the impact of aquatic REHIT on clinical populations that might prefer aquatic exercise due to limitations in balance, strength, and joint function (e.g., osteoarthritis).

CONCLUSIONS

In conclusion, compared with a land-based protocol matched for relative intensity, an 8-week aquatic REHIT exercise training program increased estimated cardiorespiratory fitness, but not maximal power production, in young, untrained, but otherwise healthy adults. These results suggest that an aquatic REHIT program can be recommended as a time-efficient exercise program for those just starting exercise that need or prefer an alternate to land-based exercise.

ADDRESS FOR CORRESPONDENCE

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DECLARATIONS

Ethics approval and consent to publish: Enrollment into the research project was voluntary and written and verbal informed consent were obtained from all

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participants. Data collection followed procedures in accordance with the ethical standards of the Helsinki Declaration. This randomized control trial (land-based intervention versus aquatic intervention) was approved by the institutional review board of West Virginia University (IRB#: 1811776700).

Availability of data and materials: All data are available from corresponding author upon reasonable request.

COMPETING INTERESTS

The authors have no competing interests as defined by IJREP, or other interests that might be perceived to influence the results and/or discussion reported in this paper.

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AUTHORS CONTRIBUTIONS

LS, BL, ER, and ML designed the study; all authors assisted with data collection; BL and ML analyzed the results; LS, BL, ER, and ML interpreted the results; LS, BL, ER, and ML drafted the manuscript; all authors reviewed and approved the final manuscript.

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