Document Date: 2/05/2020

The Impacts of Aquatic vs Land Walking on Vascular Health and Exercise Tolerance in Patients with Peripheral Artery Disease

NCT#03849300

Full study protocol (Control vs. Aquatic walking exercise group 1)

Participants

Participants were recruited with flyers and clinic referrals in a metropolitan city in South Korea at multiple community health centers where the members were primarily female. Study allocation was determined randomly with a computerized random number generator **(Figure 1)**. All participants were female and classified as Fontaine stage I or II PAD with an ABI of 0.7 – 0.9. All participants were sedentary, defined as participating in under 1 hour of regular exercise participation per week within the previous year. Exclusion criteria included current smoker (smoking within ≤ 6 months), psychiatric conditions, as well as pulmonary, renal, and thyroid diseases. These exclusion criteria for these diseases were included to avoid any secondary disease effects on the target population used in the present study. All procedures were conducted in accordance with the protocols approved by the Institutional Review Board designated by Ministry of Health and Welfare (PNU IRB-2016_92_HR) and carried out in accordance with the Declaration of Helsinki, and all subjects provided written, informed consent prior to experimental measures. All protocols and lab procedures took place at the community centers. This study was registered with clinicaltrials.gov (NCT03849300).

Study design

This study used a 2-armed parallel experimental design. Arterial stiffness, resting HR, BP, ABI, VO_2max, 6MWD, physical function scores, hand grip strength, leg strength, anthropometrics, RMR, and flexibility were assessed at baseline and after the 12 weeks. All measurements were performed at the same time of day (8:00 AM, ±1 h) after a 12-hour fast. The VO_2max test and 6-minute walk test were separated by a period of ~24 hours to prevent confounding effects of fatigue. Following baseline measurements, participants were randomly allocated to either the aquatic

walking training group (AQ, n = 35) or the control group (CON, n = 37). The AQ group participated in a supervised aquatic walking training program for 12 weeks that included a warm-up, main exercise session, and a cool-down. The CON group did not participate in any exercise program or perform any additional exercise. The CON group participants were present and supervised in the laboratory at the same frequency and time of day as the AQ group (10:00AM, ±1h) and performed sedentary activities such as reading books, listening to music, or learning new computer skills, which are among some of the activities these participants normally perform when attending the community center daily. The AQ and CON groups were advised to refrain from changing any dietary habits throughout the study period, and diet logs were given to researchers weekly to monitor caloric intake to ensure there were no changes in diet throughout the intervention. If researchers noticed differences in caloric intake, they advised participants to maintain their normal diet. Lunch and dinner were provided to participants at the community centers. All participants were supervised by qualified trainers and measurements were taken by experienced researchers, and researchers who performed the laboratory measurements were blinded to the randomization of subjects.

Exercise program

Participants in the AQ group participated in an aquatic walking exercise training program for 12 weeks, 4 days per week, for 60 minutes per day **(Table 1)**, and this was adapted from previous literature for older individuals and patients with PAD (3, 47). Sessions were conducted in waist-to-chest-deep water (28-30°C) in a group setting (3). Participants were allowed to use flotation devices as needed for safety purposes. Participant heart rate (HR) was monitored using a wearable Polar HR monitor (Electro, Oy, Kempele, Finland) during all exercise sessions to maintain the appropriate training intensity. The aquatic walking exercise training program

intensity was established using heart rate reserve (HRR) and Borg's revised rating of perceived exertion scale (RPE, 0 to 10 scale). HRR was calculated as (HRR = [% intensity desired * (HR_{max} – HR_{rest})] + HR_{rest}). The intensity of the program increased every 4 weeks: weeks 1-4 were at 50-60% HRR and 6-8 RPE, weeks 5-8 were at 60-70% HRR and 6-8 RPE, and weeks 9-12 were at 70-85% HRR and 6-8 RPE. Each training session was supervised, and each trainer was assigned to work with 10 participants during the sessions. HR monitors and RPE were checked every 5 minutes during the sessions. If the HR or RPE were too low or too high according to the intensity level, the participants were encouraged to either increase or decrease their effort during the exercise training session.

The program was divided into 3 sections including a warm-up (10 min), main exercise component (40 min), and a cool-down (10 min) **(Table 1)**. Both the warm-up and cool-down included underwater stretching that emphasized lower limb muscles (quadriceps, hamstrings, hip abductors/adductors, and hip flexors) and low-intensity gait training (forward, backward, and lateral movement) (3). The main exercises were performed for a total of 40 minutes. The first 10 minutes of main exercises included simple movement patterns such as hip flexion-extension, hip abduction-adduction, and knee flexion-extension (3). The next 30 minutes of the main exercise session consisted of water walking exercises (forward, backward, lateral) (3).

Arterial stiffness, HR, BP, and ABI

Participants rested in a supine position for 5 minutes. Femoral-to-ankle pulse wave velocity (legPWV), a measurement of peripheral arterial stiffness, was measured using applanation tonometry (TU-100 and VP-2000, OMRON Healthcare, Kyoto, Japan). A pulse wave sensor was placed on the femoral artery, while the cuffs with pulse wave sensors on the ankles and EKG electrodes remained in place from the ABI measurement. BP, EKG, and pulse waveforms were

simultaneously recorded for 10-30 seconds. Data analysis was performed according to the Clinical Application of Arterial Stiffness, Task Force III (69).

Participants rested in a seated position for 5 minutes. Resting HR, systolic BP, and diastolic BP were measured in duplicate using radial artery palpation and an automated sphygmomanometer (BP-200, OMRON, Kyoto, Japan), respectively, before and after 12 weeks. The average of the two measurements was recorded as the resting values. If the measurements differed by >5 mmHg, an additional measurement was taken and the two closest readings (differed by ≤2 mmHg) were averaged (50). The interclass correlation coefficient in our laboratory for systolic and diastolic BP calculated on two measurements is ≈0.97.

Participants rested in a supine position for 5 minutes. ABI was measured using the VP-2000 (OMRON, Kyoto, Japan). Electrocardiogram (EKG) electrodes were placed on the forearms. BP cuffs with pulse wave sensors were wrapped around both arms (brachial artery) and ankles (posterior tibial artery). Measurements were recorded for 10-30 seconds.

Cardiorespiratory capacity and 6-minute walking distance

The Cornell modified Bruce treadmill test was used to determine the maximal volume of O_2 consumption (VO_2max) (32). This Bruce protocol modification has been proven to be valid and reproducible (48) and has been used in disease populations (76). The modified Bruce consists of 11, 2-minute stages that progress from 1.7 mph and 0% grade to 5 mph and 18% grade (48). The treadmill test was ended when the participants reached volitional fatigue. Expired gases were measured with a metabolic cart (OxyCon Pro, Viasys Healthcare, Conshohocken, PA).

The 6-minute walk test was used to determine maximal walking distance, or exercise tolerance. Participants were asked to walk as many laps as possible around a 100-meter track for 6 minutes.

Physical function

Physical function was assessed collectively using physical function questionnaires from the Medical Outcomes Study Short-Form 36 General Health Survey (MOS SF-36) (73). Other parameters (i.e. not physical function domain) were not assessed using the MOS-SF 36. Physical function questionnaires of the MOS SF-36 are valid and reliable surveys that are often used for assessing physical function in patients with PAD (22, 24, 66, 73).

Muscular strength

Upper body muscular strength was determined using maximal isometric handgrip strength with a standard handgrip dynamometer (JAMAR, Bolingbrook, IL). Measurements of the dominant hand were taken 3 times, and the best of the 3 trials was recorded as the measurement.

Lower body muscular strength was assessed using one-repetition maximum (1RM) on a leg extension machine (Cybex 6000; Lumex, Albertson, NY). The 1RM was measured with the dominant leg, and the 1RM was achieved within 5 or fewer attempts. The highest weight lifted using the proper form was recorded as the 1RM.

Anthropometrics

Anthropometric measurements, including height, total body mass, body mass index (BMI), and body composition were measured before and after 12 weeks. Height was measured using a standard stadiometer to the nearest 0.1 cm. Body composition was estimated using bioelectrical impedance analysis (BIA) (InBody 230, Biospace, Seoul, Korea), which simultaneously recorded total body mass (nearest 0.1 kg), and body fat (nearest 0.1%) (60). BMI was calculated as body mass divided by the height squared (kg/m^2).

Resting metabolic rate

Resting metabolic rate was estimated using a metabolic cart (OxyCon Pro, Viasys Healthcare, Conshohocken, PA). Subjects remained in the supine position and were instructed to breathe normally and to not fall asleep during the measurement, and the measurements were performed for 30 minutes.

Flexibility

Flexibility was measured using the YMCA sit-and-reach test. Each participant was given 3 attempts to reach as far as possible with proper form, and the best of the 3 trials was recorded as the score.

Statistical analysis plan

The Shaprio-Wilk test was used to determine data normality. Independent *t* tests were used to determine any baseline differences between the AQ and CON groups. A two-way analysis of variance (ANOVA) with repeated measures [group (AQ and CON) x time (pre- and post-12 weeks)] was used to determine the difference of changes between pre- and post-aquatic walking training program within and between groups on the dependent variables. When a significant interaction was noted, paired *t* tests were used for post-hoc comparisons. All analyses were performed using SPSS 24 (SPSS Inc. Chicago, IL, USA). Data are presented as Mean ± SD. Statistical significance was set to $p < 0.05$. Additionally, correlations between variables were assessed with Pearson product-moment correlations. A power analysis calculation was used to determine a minimum sample size of 64 (32 each group) would allow for the observation of a difference of 3-5% between groups (AQ vs. CON) for PWV with a power of 90% (4, 47).

Full study protocol (Aquatic walking exercise group 2 vs. Land-based walking exercise group)

Participants (mean age 65±10, n = 53) were recruited with flyers, referrals, and newspaper advertisements in a metropolitan city in South Korea at multiple community health centers where the membership population was primarily female. Study allocation was determined randomly using a computerized random number generator **(Figure 1)** to the HWET group or LET group. All participants were postmenopausal females (cessation of menses for at least 12 months), sedentary, and classified as Fontaine stage II or III PAD with an ABI of 0.6 – 0.8. Sedentary lifestyle was termed as taking part in <1 hour of regular exercise per week within the previous year. Exclusion criteria included stage IV PAD (tissue loss/gangrene), previous or pending revascularization procedure, hormone replacement therapy, current smoker (smoking within the previous 6 months), psychiatric conditions, as well as pulmonary, renal, and thyroid diseases. These exclusion criteria were incorporated to avoid potential secondary disease effects on the target population examined in this study. All exercise and experimental methods were conducted in accordance with approved protocols by the Institutional Review Board. All participants provided written, informed consent and all procedures were performed in accordance with the Declaration of Helsinki. This study was registered with clinicaltrials.gov (NCT03849300).

Study design

A 2-armed parallel experimental design was used **(Figure 2)**. Anthropometrics, vascular function, resting metabolic rate, walking capacity, COT, and muscular strength were assessed at baseline and after the 12 weeks. All lab assessments were performed at the same time of day (8:00 AM, ±1 h) following an overnight fast. All participants were asked to abstain from medications for at least 24 hours prior to the baseline and post-exercise therapy measurements but continued taking medications throughout the exercise therapy protocols according to their physician

instructions. After baseline measurements, participants were allocated randomly to the HWET group (n = 28) or the LBET group (n = 25). The HWET group participated in a supervised heated-water exercise therapy program for 12 weeks. The LBET group participated in supervised treadmill therapy program for the same period of time. Both exercise groups were asked to refrain from altering dietary habits throughout the study period, and researchers obtained weekly diet logs from each participant to audit caloric intake. The exercise therapy sessions and experimental measures took place at the community centers. All participants were supervised by qualified trainers during exercise therapy sessions, and measurements were taken by experienced researchers who were blinded to participant randomization.

Exercise programs

The HWET group participated in a supervised heated-water exercise therapy program **(Table 1)** for 12 weeks, 4 days per week, for 60 minutes per day. The 12-week duration, exercise frequency, and intensity levels were programmed to be similar with previous water-based and land-based studies that demonstrated improvements in vascular function for older individuals and patients with PAD (3, 45, 54, 56, 87). Exercise sessions were performed in waist-to-chest-deep water (30-31°C). HR was monitored using a standard chest strap Polar HR monitor (Electro, Oy, Kempele, Finland) during each exercise session to manage the appropriate training intensity level. Intensity was programmed using heart rate reserve (HRR) and Borg's revised rating of perceived exertion scale (RPE, 0 to 10 scale). HRR was calculated as (HRR = [% intensity desired * (HR_{max} – HR_{rest})] + HR_{rest}). Exercise intensity was increased every 4 weeks: weeks 1-4 were at 50-60% HRR and 6-8 RPE, weeks 5-8 were at 60-70% HRR and 6-8 RPE, and weeks 9-12 were at 70-85% HRR and 6-8 RPE. Exercise sessions were monitored by qualified trainers. Chest HR monitor readings and RPE were checked every 5 minutes during the sessions. To maintain the appropriate

intensity, if the HR or RPE were too low or too high according to the exercise program, the trainer encouraged participants to increase or decrease their effort during the session. The HWET program was divided into 3 components including a warm-up, main exercise section, and a cool-down. The warm-up and cool-down included underwater stretching that emphasized major muscle groups and low-intensity gait training (forward, backward, and lateral movement) (3). The main exercises were performed for a total of 40 minutes. For the first 10 minutes of the main section, participants performed lower limb movement patterns including hip flexion-extension, hip abduction-adduction, and knee flexion-extension (3). The next 30 minutes of the main exercise session included water walking (3).

The LBET group performed a supervised walking exercise program on a treadmill. The warm-up and cool-down components included stretches similar to those in the HWET group, and the therapy program also lasted 12 weeks, 4 times per week, for 60 minutes per session **(Table 2)**. The main exercise component consisted of simple movement patterns for 10 minutes that included low-intensity forward, backward, and lateral side-stepping movements on flat ground. The next 30 minutes included treadmill walking at the same intensity (HRR and RPE values) as in the HWET group. Target HRR and RPE were achieved by adjusting the treadmill speed and incline grade. Participants with leg symptoms were encouraged to exercise to near maximal leg symptoms until failure of walking.

Anthropometrics

Measurements of height, total body mass, and body composition were measured before and after 12 weeks for the LBET and HWET groups. Height was measured using a standard stadiometer to the nearest 0.1 cm. Body composition was assessed using bioelectrical impedance analysis (BIA) (InBody 230, Biospace, Seoul, Korea). Total body mass (nearest 0.1 kg) and body

fat (nearest 0.1%) were recorded. Body mass index (BMI) was calculated as body mass divided by the square of height (kg/m^2).

Vascular function

With participants in the supine position, ankle-brachial index (ABI) was recorded using the VP-2000 (OMRON, Kyoto, Japan). Electrocardiogram (EKG) electrodes were placed on the forearms. BP cuffs with pulse wave sensors were wrapped around both arms (brachial artery) and ankles (posterior tibial artery). Measurements were recorded for 10-30 seconds. Brachial-to-ankle pulse-wave velocity (baPWV) was also recorded using the VP-2000 as previously described (89).

Femoral-to-ankle pulse-wave velocity (legPWV), a measurement of peripheral arterial stiffness, was measured using applanation tonometry (TU-100 and VP-2000, OMRON Healthcare, Kyoto, Japan) (56). A tonometer was placed on the femoral artery, while the pulse-wave sensor BP cuffs on the ankles and EKG electrodes remained in place from the ABI measurement. BP, EKG, and pulse waveforms were simultaneously recorded for 10-30 seconds. Data analysis was performed according to the Clinical Application of Arterial Stiffness, Task Force III (77).

Participants were then moved to a resting seated position. After 5 minutes of quiet sitting the resting HR, systolic BP, and diastolic BP were measured in duplicate using radial artery palpation and an automated sphygmomanometer (BP-200, OMRON, Kyoto, Japan), respectively, before and after 12 weeks of LBET or HWET. The average of the two measurements was recorded as the resting values. If the BP measurements differed by more than 5 mmHg, an additional measurement was taken and the two readings that were the closest (differed by $\leq$2 mmHg) were averaged and recorded as the resting BP (58). The interclass correlation coefficient in our laboratory for systolic and diastolic BP calculated on two measurements is $\approx$0.97.

Resting metabolic rate

Resting metabolic rate (RMR) was calculated using a metabolic cart (OxyCon Pro, Viasys Healthcare, Conshohocken, PA). Subjects rested in the supine position on a padded table and were asked to breathe normally and to not fall asleep during the assessment. Measurements with the metabolic cart were performed for 30 minutes.

Exercise tolerance and claudication assessment

The 6-minute walk test was used to determine 6-minute walking distance, or exercise tolerance. Participants were asked to walk as many laps as possible around a 60-meter track for 6 minutes. COT was measured during the 6-minute walk test. Once the participants experienced any leg and/or foot pain during the walking test, they reported the pain to the trainers.

Muscular strength

Upper body muscular strength was quantified using a standard handgrip dynamometer to assess maximal isometric handgrip (JAMAR, Bolingbrook, IL). Measurements of the dominant hand were taken 3 times. The best value of the 3 trials was recorded as the hand grip strength value. Lower body muscular strength was quantified using leg extension one-repetition maximum (1RM) (Cybex 6000; Lumex, Albertson, NY). The 1RM was assessed using the dominant leg, which was attained within 5 or fewer attempts. The highest weight lifted with proper form and full range of motion was recorded as the 1RM.

Physical function

Physical function was assessed collectively using physical function questionnaires from the Medical Outcomes Study Short-Form 36 General Health Survey (MOS SF-36) (83). This is a valid and reliable survey that is often used for patients with PAD (27, 30, 75, 83). Other parameters (i.e. not physical function domain) were not assessed using the MOS-SF 36.

Statistical analysis plan

The Shaprio-Wilk test was used to assess normality of the data. Independent *t* tests were used to determine any baseline differences between the LBET and HWET groups. A two-way repeated measure analysis of variance (ANOVA) [group (LBET and HWET) x time (pre- and post-12 weeks)] was used to assess changes between pre- and post- LBET vs. HWET within and between groups on the dependent variables. When a significant interaction was found, paired *t* tests were used for post-hoc comparisons. All analyses were performed using SPSS 24 (SPSS Inc. Chicago, IL, USA). Statistical significance was set to $p < 0.05$. Data are presented as Mean ± SD. A power analysis calculation was used to determine the minimum sample size of 50 (25 in each group) would allow for the observation of a change of 3-5% between groups (LBET vs. HWET) for legPWV with a power of 90% (4, 54).