Water-based continuous and interval training in older women: Cardiorespiratory and Neuromuscular Outcomes (WATER study)

Luana Siqueira Andrade[a]

Stephanie Santana Pinto[a]

Mariana Ribeiro Silva[a]

Gustavo Zaccaria Schaun[a]

Elisa Gouvêa Portella[a]

Gabriela Neves Nunes[a]

Gabriela Barreto David[a]

Eurico Nestor Wilhelm[b]

Cristine Lima Alberton[a]

[a] Physical Education School, Federal University of Pelotas, RS, Brazil.

[b] Physical Education School, Federal University of Rio Grande of Sul, RS, Brazil.

Corresponding author: Luana Siqueira Andrade

Luís de Camões Street, nº 625 - Pelotas, RS – Brazil, telephone number: +55 (53) 3273-2752;

andradelu94@gmail.com

Abstract

The purpose of this study was to investigate the effects of two water-based aerobic programs on cardiorespiratory and neuromuscular outcomes in older women. Forty-one women (60 to 75 years old) volunteered to participate in the study. Participants were randomized into a water-based continuous (CTG; n = 21; 63.9 ± 2.5 years) or an interval (ITG; n = 20; 64.8 ± 3.6 years) aerobic training group. Both training programs were performed for 12 weeks (45-min sessions twice a week), with exercise intensity based on rating of perceived exertion (Borg's RPE 6-20 Scale). Pre and post training assessments of cardiorespiratory and neuromuscular outcomes were performed. Data analyses were conducted using Generalized Estimating Equations and Bonferroni *post-hoc* test (α = 0.05). After the intervention, the CTG and the ITG displayed similar improvements in time to exhaustion (8% vs. 11%), peak oxygen uptake (9% vs. 7%), maximal dynamic knee extension strength (5% vs. 6%), dynamic muscular endurance of knee extensors (10% vs. 11%), maximal vastus lateralis electromyographic signal amplitude (13% vs. 35%), as well as an increase in muscle thickness (5% vs. 6%) and decrease in muscle echo intensity (-2% vs. -3%) of the quadriceps femoris. In conclusion, older women benefited from water-based exercise training prescribed based on participants' RPE, with both the interval and the continuous training programs resulting in similar increases in the cardiorespiratory and neuromuscular parameters.

Keywords: aerobic training, aquatic exercise, aging, interval exercise, muscle strength, aerobic capacity, muscle thickness, muscle echo intensity.

1. Introduction

Aging is characterized by several physiological processes, including cardiorespiratory and muscular deconditioning, which are generally related to muscle wasting and negatively affect the health of older individuals (Aagaard et al., 2010; Izquierdo et al., 2001). Although biological aging is inexorable, regular practice of physical exercise can counteract some of the deleterious effects, leading to a healthy aging phenotype (American College of Sports Medicine et al., 2009).

Exercise performed in the aquatic environment promotes several health-related benefits, due to the drag force created by the water (Torres-Ronda and Schelling i del Alcázar, 2014). The effectiveness of water-based training programs to improve cardiorespiratory capacity (Bocalini et al., 2008; Kanitz et al., 2015; Meredith-Jones et al., 2009; Takeshima et al., 2002) and neuromuscular function (Bento et al., 2012; Kanitz et al., 2015; Meredith-Jones et al., 2009; Takeshima et al., 2002; Tsourlou et al., 2006) of older adults has been reported in a number of investigations. Nevertheless, the majority of studies investigated both cardiorespiratory and neuromuscular adaptations as a result of water-based, combined training programs (i.e., aerobic and resistance exercises; Bento et al., 2012; Kanitz et al., 2015; Katsura et al., 2010; Meredith-Jones et al., 2009; Takeshima et al., 2002; Tsourlou et al., 2006).

Recent findings suggest that the drag force generated during water-based aerobic exercises may create enough resistive load to bring about improvements not only in cardiorespiratory parameters but also in neuromuscular outcomes (Costa et al., 2018; Kanitz et al., 2015). Studies comparing neuromuscular adaptations to aerobic training to combined (Kanitz et al., 2015) or resistance (Costa et al., 2018) water-based training programs revealed similar strength gains between training routines, with superior cardiorespiratory adaptations after water-based aerobic training. To the best of the authors' knowledge, studies on the effects of water-based aerobic training on physical fitness are scarce (Broman et al. 2006; Kanitz et al., 2015; Pasetti et al., 2012; Reichert et al., 2016), especially in a shallow water pool (Bergamin et al. 2013; Bocalini et al. 2008; 2010; Costa et al., 2018; Rica et al., 2013). Although the neuromuscular adaptations to water-based aerobic training programs in older individuals have been described (Costa et al., 2018; Kanitz et al., 2015), the benefits of different training routines (e.g., interval or continuous training) upon cardiorespiratory and neuromuscular outcomes are largely unknown.

Movement velocity exerts great influence on water drag force (Alexander, 1977), so increasing exercise velocity during water-based aerobic exercises also increases exercise resistance. As such, greater force output and muscle activation are necessary to overcome water resistance during high velocity movements (Alberton et al., 2011; Pinto et al., 2011). Accordingly, interval training performed with high intensity efforts, interspaced by active recovery, could create a greater stimulus for neuromuscular adaptations than continuous exercise performed at a moderate intensity, but this hypothesis remains speculative. Therefore, the purpose of the present study was to investigate the effects of 12 weeks of water-based continuous and interval aerobic training programs on cardiorespiratory and neuromuscular parameters in older women. It was hypothesized that (a) both training programs would result in positive cardiorespiratory and neuromuscular adaptations; (b) but superior improvements would be observed after the interval training program because of the greater water drag force and resistance created during this exercise routine.

2. Methods

Experimental design and approach to the problem

The study, Effects of Two **W**ater-based **A**erobic **T**raining Programs in **E**lde**r**ly Women (WATER), is characterized as a randomized clinical trial, registered in

ClinicalTrials.gov (NCT03289091). To compare the effects of water-based continuous and interval aerobic training programs on cardiorespiratory and neuromuscular outcomes in older women, both training programs were performed for 12 weeks, with a frequency of two 45-min sessions per week. Forty-one individuals were evaluated at baseline and one week after the intervention period (i.e. weeks 0 and 13). A sub-sample of twelve participants (63.9 ± 3.7 years) was assessed twice prior to the beginning of the intervention as a control period (weeks -4 and 0). In each of the aforementioned time points, all participants completed the assessments within a week and each test was conducted by the same investigator blinded to the participants' group assignment, using the same equipment and procedures. Four to six days of recovery were given between the last training session and the post-training assessment period. In addition, participants were instructed to maintain their usual eating habits throughout the study period.

Participants

Sample size calculation was performed in the GPower version 3.1 software, adopting the data corresponding to peak oxygen uptake (VO_{2peak}) and maximal dynamic knee extension strength from the study by Kanitz et al. (2015). Assuming alpha level of 5%, and an effect size of 0.34 for VO_{2peak} and 0.28 dor dynamic strength, a sample size of 15 participants for each water-based training group was required to identify differences between groups with a power of 80%. Assuming a drop-out rate of about 30%, 41 participants were included in the study.

Older women (from 60 to 75 years old) were recruited to take part in the study from March to July 2017. Participants had not been engaged in any systematic training program in the previous six months. Exclusion criteria included the presence of any history of cardiovascular disease (except hypertension controlled by medications) and/or osteoarticular limitations for practice of exercise. Volunteers meeting the criteria signed a written informed consent form and were informed regarding the details of the study and research procedures. The study was approved by the Local Research Ethics Committee (CAAE: 69931817.5.0000.5313) and was conducted in accordance with the Declaration of Helsinki.

Participants were randomly allocated (1:1 ratio) to either the continuous training group (CTG) or the interval training group (ITG) after baseline assessments by a researcher who was not engaged in the recruitment and assessment procedures. Participant allocation was performed using a randomization list with block sizes of 6 to 12, based on participants' baseline VO_{2peak} (classified into three categories: <23 $ml \cdot kg^{-1} \cdot min^{-1}$; between 23-28 $ml \cdot kg^{-1} \cdot min^{-1}$; and >28 $ml \cdot kg^{-1} \cdot min^{-1}$).

Antropomethrical measurements

An initial session was performed with each participant for familiarization with tests and research procedures, as well as to take anthropometric measurements. Participants' body mass and height were measured using a digital scale with a stadiometer (WELMY, Santa Bárbara d'Oeste – São Paulo, Brazil). A 7-skinfold thickness protocol was used to estimate body density (Jackson et al., 1980) using a skinfold caliper (CESCORF, Porto Alegre, Brazil), and body fat was then calculated using Siri's equation (Siri, 1993).

Cardiorespiratory capacity

A maximal incremental treadmill exercise test (Arktus, Santa Tereza do Oeste, Brasil) was performed for cardiorespiratory fitness assessment. Participants rested during 5 min for resting heart rate (HR_{rest}) assessment, and then performed a 3 min warm-up with gradual velocity increments up to 3 $km \cdot h^{-1}$. The test started at 3 $km \cdot h^{-1}$ with sequential increments of

0.5 km·h^{-1} every minute, and a 1% increase in grade every 2 min until maximum effort. The test was finished when the participant was no longer able to exercise at a given workload and indicated exhaustion. All tests were supervised by a medical doctor. Oxygen uptake was recorded during the incremental protocol using a mixing-box-type portable gas analyzer (VO2000, MedGraphics; Ann Arbor, USA) for determination of participants' peak oxygen uptake (VO_{2peak}), oxygen uptake at the first (VO_{2VT1}) and second ventilatory threshold (VO_{2VT2}). Data were acquired as an average of three breaths using the Aerograph software (MedGraphics; Ann Arbor, USA). Heart rate (HR) was obtained every 30 s using a heart rate monitor (FT1, Polar, Kempele, Finland), and participants' rating of perceived exertion (RPE) was assessed at the end of each stage with the Borg's RPE 6-20 scale. Tests were considered valid when at least two of the following criteria were met: a) a plateau in oxygen uptake with an increase in the protocol stage; b) when an estimated maximal HR was reached (220 – age); c) a respiratory exchange ratio ≥ 1.15; or d) a RPE ≥ 18 (Howley et al., 1995).

The individual VO_{2peak} was determined as the higher 15 s mean oxygen uptake value in the last test stage (Schaun, 2017). The VT_1 and VT_2 were determined based on the ventilation by test stage plot and confirmed by the ventilatory equivalent of oxygen (V_E/VO_2) and carbon dioxide (V_E/VCO_2), respectively (Wasserman et al., 1973). Three experienced physiologists independently detected VT_1 and VT_2 through visual inspection while blinded to the participants' experimental group. When there was no agreement among them, the median value was used for analysis. Time to exhaustion was considered as the total test time in minutes, including the warm-up.

Muscle thickness and muscle quality

Participants' right quadriceps femoris muscle thickness and echo intensity were assessed by B-mode ultrasonography (Tosbee, Toshiba, Japan), with a 7.5 MHz linear array probe. Each participant rested for 15 min in supine position with legs extended and relaxed and then transversal images of the four portions of the quadriceps femoris were recorded. Images of the vastus lateralis (VL), rectus femoris (RF) and vastus intermedius (VI) muscles were obtained at the midpoint between the anterosuperior iliac spine and upper edge of the patella, whereas the vastus medialis (VM) was assessed at 30% of the distance between the lateral condyle and the greater trochanter of the femur, as adapted from previous studies (Korhonen et al., 2009; Kumagai et al., 2000). To ensure similar probe position in subsequent tests, the assessment site of each muscle was marked on transparent paper and used for probe repositioning (Narici et al., 1989). All images were analyzed using the software ImageJ (National Institutes of Health, USA, version 1.37).

Muscle thickness was assessed as the distance from the superior and inferior muscle aponeurosis for each muscle (Abe et al., 2000), and overall quadriceps femoris muscle thickness was calculated as the sum of each individual muscle thickness (i.e. RF+VL+VM+VI). Muscle quality was determined by the echo intensity (EI) values, which were calculated from gray-scale analysis using the standard histogram function in ImageJ (National Institute of Health, USA, version 1.37). A region of interest was selected in each muscle including as much of the muscle as possible while avoiding surrounding fascia, and the EI within the region of interest was calculated and expressed in values between 0 and 255 (0 = black; 255 = white; Radaelli et al., 2013; Wilhelm et al., 2014). The EI of quadriceps femoris was calculated as the mean EI of the four individual quadriceps femoris muscles ((RF+VL+VM+VI)/4). Based on data from our control period (weeks -4 and 0), muscle thickness and EI intraclass correlation coefficients (ICC) were 0.97 and 0.95, respectively.

Neuromuscular activity

The neuromuscular activity of the right vastus lateralis (VL) muscle was assessed using surface electromyography (EMG) during knee extension maximal isometric voluntary contractions (MVC). Briefly, after shaving and cleaning the skin with alcohol, bipolar electrodes were positioned on the muscle according to the SENIAM project (www.seniam.org). The position of each electrode was recorded on transparent paper, which was used in the post tests to ensure a similar electrode positioning between pre and post intervention period (Narici et al., 1989). A reference electrode was fixed on the participant's anterior crest of the tibia. After the electrode positioning, participants warmed-up for 5 min on a cycle ergometer, and then seated with their hips and thighs firmly strapped (hip angle at 90°, and knee angle at 60°, considering 0° as the anatomic position). Participants were instructed to perform isometric knee extensions, exerting maximal force as fast as possible against a fixed resistance, while receiving strong verbal encouragement. Three 5-s MVC attempts with 2 min of rest intervals were performed, and the raw EMG signal was acquired at 2000 Hz per channel using an EMG acquisition system (Miotool, Miotec®, Porto Alegre, Brazil). Signals were then band-pass filtered by a fifth order Butterworth filter using a cutoff frequency of 20–500 Hz, and the root mean square (RMS) value was determined during the greatest stable 1-s period at the force-time signal. Force was measured using a load cell (Miotec®, Porto Alegre, Brazil) with 200 kgf capacity and 2000 Hz sampling rate. The VL EMG signal obtained during the three MVCs were recorded, and the MVC attempt with highest 1-s stable force signal was used to analyze the maximal voluntary muscle activation. Based on data from our control period, ICC for the VL EMG signal was 0.78.

Maximal dynamic strength

A bilateral knee extension one repetition maximum (1RM) test was performed using a knee extension machine (New Fitness, São Paulo, Brazil) for maximal dynamic strength assessment. The 1RM value was considered the greatest load that the participant could lift for one complete repetition (i.e., concentric and eccentric phase) following a predetermined cadence (i.e., approximately 2 s per phase). Participants performed a 5-min warm-up on a cycle ergometer and a specific warm-up (i.e. 10 submaximal repetitions on the bilateral knee extension machine). Participants started the test at ≈90° of knee flexion and moved to full knee extension, which was individualized for each participant and controlled by a range of motion custom-build device. The 1RM of each participant was determined within five attempts, and at least 3 min of rest interval was given between trials. When the participant could perform more than one complete repetition, a new load was estimated following the Lombardi's coefficient (Lombardi, 1989) for the next trial. We highlight that during baseline the 1RM test was performed twice, with a 48-h interval period between tests (ICC = 0.95). In addition, based on the control period data, 1RM ICC was 0.96.

Dynamic muscular endurance

The same knee extension machine was used for the assessment of dynamic muscular endurance. To do so, participants completed the maximal number of bilateral knee extension repetitions at 60% of their 1RM. The test cadence (2 s for each contraction phase) and range of motion was the same used for the 1RM test, and the post-intervention assessment was performed using the same absolute load employed at baseline (i.e. 60% of baseline 1RM). Based on the control period data, dynamic muscular endurance ICC was 0.93.

Training Programs

The water-based aerobic continuous and interval training programs were performed during 12 weeks, two times per week on non-consecutive days. Before the beginning of the training programs, participants performed two water-based exercise sessions to become familiarized with the exercises and the Borg's RPE 6-20 Scale (Borg, 1990). Sessions for both the CTG and ITG lasted 44 min (4 min of warm-up, followed by 36 min of exercise, and 4 min of stretching) throughout the intervention period. Each training session was composed by the same exercise sequence as follows: stationary running, frontal kick and cross-country skiing. The training intensity was based on the Borg's RPE 6-20 Scale, with a progressive increase in intensity as presented in Table 1. For CTG the target intensity was constant throughout the entire session, while for ITG an effort to rest ratio of 1:1 was employed. The study by Alberton et al. (2016) showed water-based aerobic exercise RPE of 12.1–12.7 corresponded to 50–59%VO_{2peak}; 13.7–14.8 to 60–69%VO_{2peak}; 15.8–16.4 to 70–79%VO_{2peak}; 17.3–18.1 to 80–89%VO_{2peak}; and 18.5–18.9 to 90–99% VO_{2peak} in active women. Therefore, small changes in the RPE values (≤2 points) may represent different training zones during water-based aerobic exercises.

All training sessions were supervised by two experienced instructors trained to deliver the water-based protocols employed in the present study. Furthermore, participants received constant feedback regarding exercise intensity and participants reported achieving their target RPE in each training session. The water temperature was maintained between 30 e 32°C and the depth immersion between the participant's xiphoid process and the shoulders.

Table 1. Description of the aerobic training programs.

Weeks	Total time	Intensity (Borg's RPE 6-20 Scale)	
		Continuous training	Interval training
1-4	36 min	RPE 13	9x (2 min RPE 16 + 2 min RPE 11)
5-8	36 min	RPE 14	12x (1.5 min RPE 17 + 1.5 min RPE 11)
9-10	36 min	RPE 15	18x (1 min RPE 18 + 1 min RPE 11)
11-12	36 min	RPE 16	18x (1 min RPE 18 + 1 min RPE 11)

RPE = rating of perceived exertion.

Statistical Analysis

Results are presented as mean ± standard deviation (SD). Data distribution was tested using the Shapiro-Wilk test, and paired samples t-tests were used to compare the outcomes between time points in the control period (weeks -4 and 0). Independent t-tests were used for comparing sample characteristics data between groups after testing normality and homogeneity data using Shapiro-Wilk and Levene tests. *Generalized Estimating Equations* (GEE) and Bonferroni *post-hoc* tests were used for comparison between time points (baseline and post-training) and groups (CTG and ITG) for both per protocol and intention to treat analysis. A GEE was chosen in the present study as it is appropriate for analysis of continuous variables in longitudinal models, even for data displaying violation of sphericity and presenting nonparametric distribution (Liang and Zeger, 1986). Furthermore, the GEE can cope with missing data (Liu et al., 2006), and according to Ma et al. (2012), for the same statistical power, it requires a smaller sample size compared to factorial ANOVA to identify a similar effect size. The intention to treat analysis included all randomized participants whereas per protocol analysis excluded those who missed more than two subsequent sessions or presented a total attendance lower than 80% throughout the training period. All tests were processed in the SPSS version 20.0 software adopting an alpha level equal to 5%.

3. Results

The study flowchart is presented in Figure 1. One hundred ninety-six women were contacted. From these 126 did not meet the eligibility criteria and 29 refused to take part in the study. Therefore, 41 older women were selected and randomized for the CTG or the ITG. Seven participants dropped out during the intervention period due to health-related issues not related to the study (3 from CTG and 4 from ITG) and 2 of participants from the CTG did not complete post intervention measurements. As such, a total of 32 participants (16 from CTG and 16 from ITG) concluded the interventions and were assessed for intention to treat analysis. For per protocol analysis, however, 10 participants (3 from CTG and 7 from ITG) were not included because of low attendance rate (i.e. < 80%).

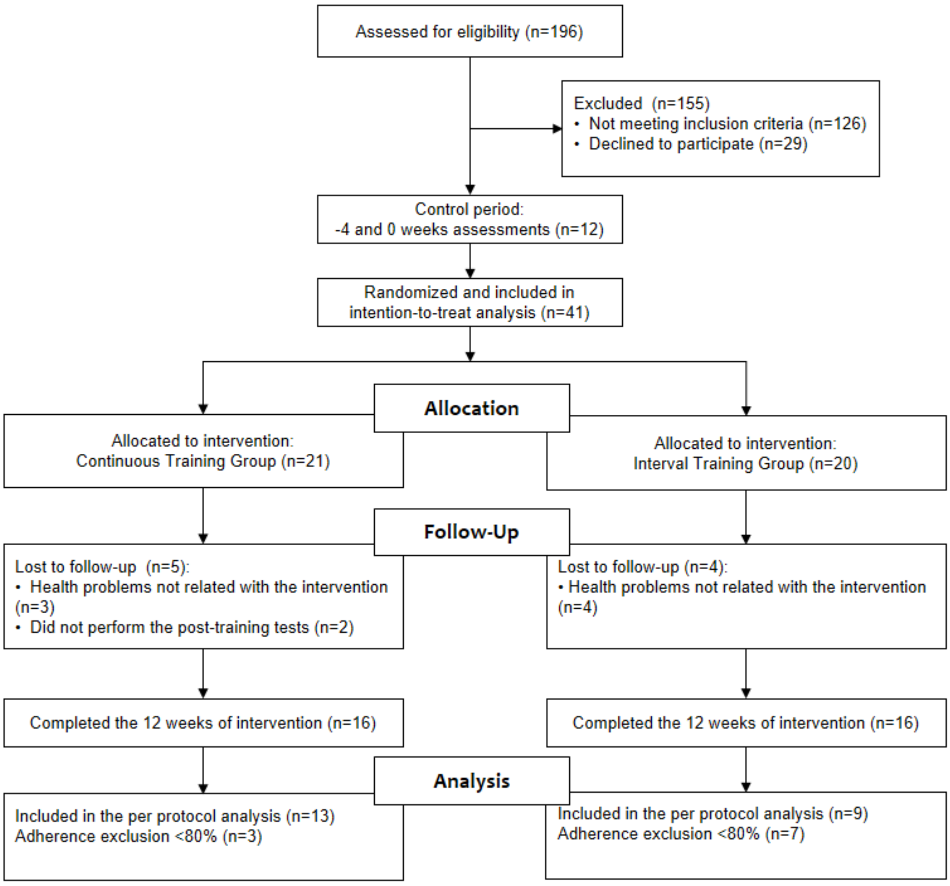

Figure 1. Participants' flowchart.

The attendance of participants who completed the intervention was similar between groups (CTG = 82.6 ± 2.7%; ITG = 82.2 ± 3.5%; p = 0.941). No injuries or adverse events were reported in either study group. Age, height, body mass, body mass index, body fat and VO_{2peak} were also similar between groups at the baseline (Table 2).

Table 2. Participants' characteristics at baseline (mean ± SD).

Variables	Continuous group (n=21)	Interval group (n=20)	P
Age (years)	63.9 ± 2.5	64.8 ± 3.6	0.389

Height (cm)	153.4 ± 4.3	155.1 ± 5.8	0.293
Body mass (kg)	74.4 ± 14.7	73.1 ± 13.9	0.777
Body mass index ($kg \cdot m^{-2}$)	31.7 ± 6.4	30.2 ± 4.6	0.416
Body fat percentage (%)	42.9 ± 5.2	43.1 ± 5.5	0.881

No differences in the study outcomes were observed between time points in the control period (weeks -4 and 0), with the exception of quadriceps femoris EI and maximal neuromuscular activity of the VL muscle, which increased from week -4 to week 0 ($p < 0.05$; Table 3).

Table 3. Cardiorespiratory and neuromuscular parameters during the control period (−4 and 0 weeks; mean ± SD).

Variables	n=12		
	Week -4	Week 0	P
HR_{rest} (bpm)	78 ± 13	75 ± 13	0.262
VO_{2peak} ($ml.kg^{-1}.min^{-1}$)	26.24 ± 4.28	26.13 ± 4.92	0.890
VO_{2VT1} ($ml.kg^{-1}.min^{-1}$)	14.67 ± 2.60	14.40 ± 2.27	0.746
VO_{2VT2} ($ml.kg^{-1}.min^{-1}$)	20.04 ± 2.96	20.16 ± 3.11	0.896
TTE (min)	13.62 ± 2.63	13.99 ± 2.57	0.238
KE 1RM (kg)	29.50 ± 6.04	29.25 ± 6.25	0.714
KE DME (repetitions)	11.00 ± 1.80	10.92 ± 1.78	0.754
VL EMG (µV)	118.58 ± 65.23	167.96 ± 103.22	0.039*
QF MT (cm)	6.41 ± 0.82	6.46 ± 0.72	0.508
QF EI (a.u.)	118.51 ± 7.80	121.45 ± 9.15	0.015*

HR_{rest} = resting heart rate; VO_{2peak} = peak oxygen uptake; VO_{2VT1} = oxygen uptake in the first ventilatory threshold; VO_{2VT2} = oxygen uptake in the second ventilatory threshold; TTE = time to exhaustion; KE = knee extension; 1RM = maximal dynamic strength; DME = dynamic muscular endurance; EMG = neuromuscular activity during KE maximal isometric voluntary contractions; VL = vastus lateralis; QF = quadriceps femoris; MT = muscle thickness; EI = echo intensity. * Significant difference between weeks −4 and 0 ($p < 0.05$).

The cardiorespiratory outcomes analyzed per protocol and by intention to treat revealed similar results. Based on per protocol analysis, a significant reduction in HR_{rest} (CTG: -6.8 ± 15.4%; ITG: -6.2 ± 11.8%) and a significant increase in VO_{2peak} (CTG: 9.2 ± 10.3%; ITG: 6.6 ± 16.5%) and time to exhaustion (CTG: 7.5 ± 5.6%; ITG: 11.0 ± 13.5%)

were observed in both groups ($p < 0.05$), with similar results between them ($p > 0.05$; Table 4).

Table 4. Cardiorespiratory parameters before and after training (mean ± SD).

Variables	Continuous group		Interval group		Group	Time	Group*Time
	Pre	Post	Pre	Post	P	P	P
HR_{rest} (bpm)	83 ± 21	75 ± 11	80 ± 14	75 ± 14	0.847	0.009*	0.611
VO_{2peak} ($ml.kg^{-1}.min^{-1}$)	26.30 ± 3.68	28.76 ± 5.05	24.07 ± 4.10	26.01 ± 7.95	0.352	0.030*	0.799
VO_{2VT1} ($ml.kg^{-1}.min^{-1}$)	16.34 ± 2.87	15.47 ± 1.46	16.52 ± 3.35	15.21 ± 2.85	0.964	0.201	0.797
VO_{2VT2} ($ml.kg^{-1}.min^{-1}$)	20.27 ± 3.34	21.47 ± 2.35	20.31 ± 2.38	19.75 ± 3.87	0.416	0.722	0.330
TTE (min)	13.08 ± 1.91	14.02 ± 1.88	12.75 ± 1.45	14.04 ± 1.40	0.818	<0.001*	0.526

HR_{rest} = resting heart rate; VO_{2peak} = peak oxygen uptake; VO_{2VT1} = oxygen uptake in the first ventilatory threshold; VO_{2VT2} = oxygen uptake in the second ventilatory threshold; TTE = time to exhaustion. *Significant difference between pre and post-training in both groups.

The neuromuscular outcomes analyzed per protocol and by intention to treat revealed similar results. Based on per protocol analysis, a significant increase in maximal dynamic strength (CTG: 5.0 ± 5.9%; ITG: 6.3 ± 12.5%), dynamic muscular endurance (CTG: 10.1 ± 15.3%; ITG: 10.6 ± 6.7%) and maximal neuromuscular activity of the VL (CTG: 12.8 ± 38.4%; ITG: 35.3 ± 47.7%) was found in both groups after training ($P < 0.05$), with no difference between groups ($P > 0.05$). As for the morphological outcomes, similar increases in quadriceps femoris muscle thickness (CTG: 4.5 ± 4.8%; ITG: 5.5 ± 3.9%) and reductions in EI (CTG: -1.7 ± 5.4%; ITG: -2.9 ± 4.3%) were observed in both groups after the training period ($p < 0.05$; Table 5).

Table 5. Neuromuscular parameters before and after training (mean ± SD).

Variables	Continuous group		Interval group		Group	Time	Group*Time
	Pre	Post	Pre	Post	P	P	P
KE 1RM (kg)	30.92 ± 6.29	32.42 ± 6.42	28.00 ± 5.68	29.50 ± 5.21	0.230	0.020*	0.999
KE DME (repetitions)	11.83 ± 2.21	13.08 ± 3.48	12.63 ± 2.07	14.00 ± 2.62	0.425	<0.001*	0.851
VL EMG (μV)	94.16 ± 40.82	102.30 ± 45.26	121.00 ± 67.62	151.13 ± 72.62	0.117	0.005*	0.105
QF MT (cm)	6.07 ± 0.79	6.35 ± 0.87	6.26 ± 0.98	6.62 ± 1.11	0.589	<0.001*	0.534
QF EI (a.u.)	115.43 ± 6.17	113.50 ± 8.79	121.48 ± 9.33	117.91 ± 9.42	0.136	0.021*	0.489

KE = knee extension; 1RM = maximal dynamic strength; DME = dynamic muscular endurance; EMG = neuromuscular activity during KE maximal isometric voluntary contractions; VL = vastus lateralis; QF = quadriceps femoris; MT = muscle thickness; EI = echo intensity. *Significant difference between pre and post-training in both groups.

4. Discussion

The present study shows that periodized water-based continuous or interval aerobic programs can improve both cardiorespiratory and neuromuscular parameters in older women, irrespective of the training pattern employed. In contrast to our initial hypothesis, the interval training did not bring about superior neuromuscular gains compared to the continuous water-based exercise routine in older women, even though water drag, and thus resistance to movement, was supposedly higher in the ITG.

According to the general fluid equation ($F_d = 0.5 \cdot \rho \cdot A \cdot V^2 \cdot C_d$), fluid density ($\rho$), projected area (A), velocity of motion (V) and drag coefficient (C_d) are directly proportional to the drag force (F_d; Alexander, 1977). Since movement velocity is squared, it becomes a main influential factor during water-based exercises. Therefore, as movement velocity is increased during water-based exercise a greater force is necessary to overcome the resistance imposed by the water and, thus, training intensity and RPE increases (David et al., 2017). In contrast to our hypothesis, no additional increment in the cardiorespiratory conditioning and muscle strength were observed for ITG. Although the exercise intensity stimulus (i.e., RPE 16-18) for water-based ITG was greater in comparison to CTG (i.e., RPE 13-16) during exertion throughout the 12 weeks, the mean session RPE (i.e., exertion + recovery vs. exertion) and the similar duration (i.e., 36 min) may have resulted in equivalent total exercise volume in both groups. For example, during weeks 1-4, the ITG performed 3,888 RPE·min of exercise, whereas the CTG performed 3,744 RPE·min of exercise; during weeks 5-8, both groups performed 4,032 RPE·min of exercise; and during weeks 9-12, the ITG group performed 4,176 RPE·min and CTG performed 4,464 RPE·min of exercise.

In the present study, the CTG and ITG presented similar improvements in VO_{2peak}, time to exhaustion, and HR_{rest}, all of which reflect cardiorespiratory adaptations to exercise training. These results are in accordance to previous studies in which positive cardiorespiratory adjustments were also observed in other water-based training programs with varying duration (8-12 weeks), performed by older individuals (Bocalini et al., 2008; Broman et al., 2006; Costa et al., 2018; Kanitz et al., 2015; Meredith-Jones et al., 2009; Takeshima et al., 2002). However, the literature is scarce regarding cardiorespiratory adaptations to water-based training programs employing only aerobic exercises (Bocalini et al., 2008; Broman et al., 2006; Costa et al., 2018; Kanitz et al., 2015). The improvements in VO_{2peak} after water-based aerobic training programs reported in the literature ranged between 11-42% (Bocalini et al., 2008; Broman et al., 2006; Costa et al., 2018; Kanitz et al., 2015) in older individuals. Such findings suggest that the higher increase in VO_2-related outcomes (i.e., VO_{2peak}, VO_{2VT2} and VO_{2VT1}) may relate to either greater weekly training frequencies or longer training sessions. In addition, the improvement in time to exhaustion observed for both groups in the present study (8-11%) was similar to those reported by Costa et al. (2018; 10%), which may be explained by the increase in both cardiorespiratory capacity and lower limbs muscular strength.

The improvement in VO_{2peak} in response to exercise training generally relates to central and peripheral adaptations, as evidenced by increases in maximal stroke volume, skeletal muscle capillarization, mitochondrial biogenesis and activity of oxidative enzymes in trained muscles (Wilmore et al., 2015). Although we could not investigate whether such adaptations took place in the current study, the increase in cardiorespiratory function suggests that some improvement in the oxygen carrying capacity and/or skeletal muscle oxidative capacity took place in response to the water-based exercise programs performed in the present study. The current findings are relevant for the older population, since aging is associated with a progressive decline in VO_{2max}, which may lead to impairments in the functional capacity and performance of daily living activities of older adults (Fleg and Lakatta, 1988).

Our results show that both types of water-based endurance training employed in the present study are useful to counteract this aging-related decrement in cardiorespiratory capacity.

The present findings regarding the reduction in HR_{rest} corroborate with previous studies investigating cardiorespiratory adaptations to water-based programs (Bocalini et al., 2008; Broman et al., 2006; Kanitz et al., 2015). Although the mechanisms underlying the reduction in HR_{rest} were not investigated, this response generally reflects an improvement in the heart pumping efficiency. For example, endurance-type exercise training leads to intermittent volume overload and can bring about physiological (non-pathological) cardiac hypertrophy resulting in increased ventricular filling and contracting capacity (Wilmore et al., 2015). An increase in parasympathetic activity of the heart, associated with a reduction in sympathetic activity may also explain the reduction in basal heart rate observed after aerobic training (Wilmore et al., 2015). Since elevated HR_{rest} has been associated with an increased risk of cardiovascular disease, cancer, and all-cause mortality (Aune et al., 2017), the present findings from water-based exercise may have positive clinical implication for older women.

Regarding neuromuscular outcomes, the present study found an increase in the maximal dynamic strength and dynamic muscular endurance corresponding to 5% and 10% after CTG and 6% and 11% after ITG, respectively. Such similar muscle strength improvement between intervention groups was unexpected, since the ITG exercised against greater water resistance (i.e. drag), which was hypothesized to produce a superior stimulus for neuromuscular adaptation due to the more strength-like nature of the interval training routine. Previous studies have shown that water-based training programs can improve neuromuscular parameters in older individuals, however, the most of them investigated the effects of a combined training program (i.e., aerobic and resistance exercises; Bento et al., 2012; Kanitz et al., 2015; Katsura et al., 2010; Meredith-Jones et al., 2009; Takeshima et al., 2002; Tsourlou et al., 2006). Therefore, the observed improvements probably resulted not only from aerobic exercises, but also from specific resistance exercises applied in these studies. Nevertheless, the present findings support the evidence that training employing only water-based aerobic exercises can bring about improvements in muscle strength of sedentary older individuals (Costa et al., 2018; Kanitz et al., 2015). It should be highlighted, however, that these previous experiments employed interval protocols throughout the aerobic training program and, therefore, the present study adds to the current body of knowledge by showing that a continuous water-based protocol can result in similar increase in muscle strength in previously sedentary older women.

The lack of difference in strength gains is supported by the similar neural and morphological adaptations observed in the participants of both training groups. For example, the maximal neuromuscular activity of the VL muscle increased similarly after both interventions, and quadriceps femoris muscle thickness also increased similarly after training, indicating a greater neuromuscular capacity and hypertrophy of the quadriceps femoris muscle. In addition, quadriceps femoris EI reduced after training in both groups, which suggests a decrease in the content of non-contractile elements in the studied muscles (e.g. a reduction in fat and connective tissues; Pillen et al. 2009; Arts et al. 2010). The EI adaptation observed in the present study agrees with changes observed after resistance or combined training programs performed on dry land (Radaelli et al., 2013; Wilhelm et al., 2014). These neuromuscular adaptations are relevant since the older population is at greater risk of dynapenia and sarcopenia, which can lead to impairment in their functional capacity (Clark and Manini, 2008; Manini and Clark, 2012).

The present results, therefore, show that 12 weeks of water-based aerobic training, regardless of the continuous or interval-mode prescription, improved muscle strength of the lower limbs, leading to similar neural and morphological adaptations in sedentary older women. Moreover, the present study is the first reporting such positive adaptations with water-based exercise based on RPE, a method of training prescription that may have greater external validity and practical application for the population investigated.

This study has some limitations, such as the lack of individual record of RPE during training sessions to confirm the progression of subjective training intensity throughout the intervention programs. In addition, no control group was included in this study, although a control period was employed to minimize this issue. It should also be stressed that logistic issues hindered us from acquiring neuromuscular economy data, which is a variable registered at clinicaltrials.gov. In addition, the EMG signal from the rectus femoris muscle was also recorded during the MVIC, but its results were not included in the present manuscript because it displayed low reproducibility (ICC=0.08) based on data from our control period (weeks -4 and 0). Finally, it should be highlighted that our results apply specifically to previously sedentary older women.

5. Conclusion

In summary, both water-based training programs employed in the present study resulted in similar improvements in the cardiorespiratory capacity and lower limb muscular strength, which were supported by positive adaptations in terms of maximal neuromuscular activity, muscle mass and muscle quality in older women.

A water-based aerobic program performed in a continuous or interval model resulted in positive adaptations after 12 weeks of training in both cardiorespiratory and neuromuscular parameters, which were similar in nature. Therefore, employing 45-min water-based aerobic sessions twice a week can be considered an efficient strategy to counteract the negative impact of biological aging in sedentary older women. In addition, it should be highlighted that training prescription based on RPE is a simple method of easy applicability for group classes in the aquatic environment and can be used to bring about positive adaptations for relevant health parameters in this population. It is suggested that future studies analyze the effect of water-based CTG and ITG programs on cardiorespiratory and neuromuscular outcomes during longer training periods, in other populations and different training status.

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Referências

Aagaard, P., Suetta, C., Caserotti, P., Magnusson, S.P., Kjaer, M., 2010. Role of the nervous system in sarcopenia and muscle atrophy with aging: strength training as a countermeasure. Scand. J. Med. Sci. Sports 20, 49–64. https://doi.org/10.1111/j.1600-0838.2009.01084.x

Abe, T., DeHoyos, D. V, Pollock, M.L., Garzarella, L., 2000. Time course for strength and muscle thickness changes following upper and lower body resistance training in men and women. Eur. J. Appl. Physiol. Occup. Physiol. 81, 174-180. https://doi.org/10.1007/s004210050027

Alberton, C.L., Cadore, E.L., Pinto, S.S., Tartaruga, M.P., Da Silva, E.M., Kruel, L.F.M., 2011. Cardiorespiratory, neuromuscular and kinematic responses to stationary running performed in water and on dry land. Eur. J. Appl. Physiol. 111, 1157–1166. https://doi.org/10.1007/s00421-010-1747-5.

Alberton, C.L., Pinto, S.S., Gorski, T., Antunes, A.H., Finatto, P., Cadore, E.L., Bergamin, M., Kruel, L.F., 2016. Rating of perceived exertion in maximal incremental tests during head-out water-based aerobic exercises. J Sports Sci. 34, 1691-1698. https://doi.org/10.1080/02640414.2015.1134804.

Alexander, R., 1977. Mechanics and energetics of animal locomotion. London.

American College of Sports Medicine, Chodzko-Zajko, W.J., Proctor, D.N., Fiatarone Singh, M.A., Minson, C.T., Nigg, C.R., Salem, G.J., Skinner, J.S., 2009. American College of Sports Medicine position stand. Exercise and physical activity for older adults. Med. Sci. Sports Exerc. 41, 1510–30. https://doi.org/10.1249/MSS.0b013e3181a0c95c

Arts, I.M.P., Pillen, S., Schelhaas, H.J., Overeem, S., Zwarts, M.J., 2010. Normal values for quantitative muscle ultrasonography in adults. Muscle Nerve 41, 32–41. https://doi.org/10.1002/mus.21458

Aune, D., Sen, A., Ó'Hartaigh, B., Janszky, I., Romundstad, P.R., Tonstad, S., Vatten, L.J., 2017. Resting heart rate and the risk of cardiovascular disease, total cancer, and all-cause mortality – A systematic review and dose–response meta-analysis of prospective studies. Nutr. Metab. Cardiovasc. Dis. 27, 504–517. https://doi.org/10.1016/j.numecd.2017.04.004

Bergamin, M., Ermolao, A., Tolomio, S., Berton, L., Sergi, G., Zaccaria, M. 2013. Water-versus land-based exercise in elderly subjects: effects on physical performance and body composition. Clin Interv Aging. 8:1109–1117. https://doi.org/10.2147/CIA.S44198

Bento, P.C.B., Pereira, G., Ugrinowitsch, C., Rodacki, A.L.F., 2012. The Effects of a Water-Based Exercise Program on Strength and Functionality of Older Adults. J. Aging Phys. Act. 20, 469–483. https://doi.org/10.1123/japa.20.4.469

Bocalini, D.S., Serra, A.J., Murad, N., Levy, R.F., 2008. Water- versus land-based exercise effects on physical fitness in older women. Geriatr. Gerontol. Int. 8, 265–271. https://doi.org/10.1111/j.1447-0594.2008.00485.x

Bocalini, D.S., Serra, A.J., Rica, R.L., Dos Santos, L. 2010. Repercussions of training and detraining by water-based exercise on functional fitness and quality of life: a short-term follow-up in healthy older women. Clinics 65, 1305-1309. https://doi.org/10.1590/S1807-59322010001200013

Borg, G., 1990. Psychophysical scaling with applications in physical work and the perception of exertion. Scand. J. Work. Environ. Health 16, 55–58. https://doi.org/10.5271/sjweh.1815

Broman, G., Quintana, M., Lindberg, T., Jansson, E., Kaijser, L., 2006. High intensity deep water training can improve aerobic power in elderly women. Eur. J. Appl. Physiol. 98, 117–123. https://doi.org/10.1007/s00421-006-0237-2

Clark, B.C., Manini, T.M., 2008. Sarcopenia =/= dynapenia. J. Gerontol. A. Biol. Sci. Med. Sci. 63, 829–834.

Costa, R.R., Kanitz, A.C., Reichert, T., Prado, A.K.G., Coconcelli, L., Buttelli, A.C.K., Pereira, L.F., Masiero, M.P.B., Meinerz, A.P., Conceição, M.O., Sbeghen, I.L., Kruel, L.F.M., 2018. Water-based aerobic training improves strength parameters and cardiorespiratory outcomes in elderly women. Exp. Gerontol. 108, 231–239. https://doi.org/10.1016/j.exger.2018.04.022

David, G.B., Andrade, L.S., Schaun, G.Z., Alberton, C.L., 2017. HR, VO2, and RPE Relationships in an Aquatic Incremental Maximum Test Performed by Young Women. J.

Strength Cond. Res. 31, 2852–2858. https://doi.org/10.1519/JSC.0000000000001719

Fleg, J.L., Lakatta, E.G., 1988. Role of muscle loss in the age-associated reduction in VO2 max. J. Appl. Physiol. 65, 1147–1151. https://doi.org/10.1152/jappl.1988.65.3.1147

Howley, E.T., Bassett, D.R., Welch, H.G., 1995. Criteria for maximal oxygen uptake: review and commentary. Med. Sci. Sports Exerc. 27, 1292–1301.

Izquierdo, M., Häkkinen, K., Antón, A., Garrues, M., Ibañez, J., Ruesta, M., Gorostiaga, E.M., 2001. Maximal strength and power, endurance performance, and serum hormones in middle-aged and elderly men. Med. Sci. Sports Exerc. 33, 1577–1587. https://doi.org/10.1097/00005768-200109000-00022

Jackson, A.S., Pollock, M.L., Ward, A., 1980. Generalized equations for predicting body density of women. Med. Sci. Sports Exerc. 12, 175–181.

Kanitz, A.C., Delevatti, R.S., Reichert, T., Liedtke, G.V., Ferrari, R., Almada, B.P., Pinto, S.S., Alberton, C.L., Kruel, L.F.M., 2015. Effects of two deep water training programs on cardiorespiratory and muscular strength responses in older adults. Exp. Gerontol. 64, 55–61. https://doi.org/10.1016/j.exger.2015.02.013

Katsura, Y., Yoshikawa, T., Ueda, S.-Y., Usui, T., Sotobayashi, D., Nakao, H., Sakamoto, H., Okumoto, T., Fujimoto, S., 2010. Effects of aquatic exercise training using water-resistance equipment in elderly. Eur. J. Appl. Physiol. 108, 957–964. https://doi.org/10.1007/s00421-009-1306-0

Korhonen, M.T., Mero, A.A., Alén, M., Sipilä, S., Häkkinen, K., Liikavainio, T., Viitasalo, J.T., Haverinen, M.T., Suominen, H., 2009. Biomechanical and skeletal muscle determinants of maximum running speed with aging. Med. Sci. Sports Exerc. 41, 844–56. https://doi.org/10.1249/MSS.0b013e3181998366

Kumagai, K., Abe, T., Brechue, W.F., Ryushi, T., Takano, S., Mizuno, M., 2000. Sprint performance is related to muscle fascicle length in male 100-m sprinters. J. Appl. Physiol. 88, 811–816. https://doi.org/10.1152/jappl.2000.88.3.811

Liang, K-Y., Zeger, S.L., 1986. Longitudinal data analysis using generalized linear models. Biometrika. 73, 13-22. https://doi.org/10.1093/biomet/73.1.13

Liu, M., Wei, L., Zhang, J., 2006. Review of guidelines and literature for handling missing data in longitudinal clinical trials with a case study. Pharm Stat. 5, 7-18.

Lombardi, V.P., 1989. Beginning weight training: the safe and effective way. Dubuque.

Ma, Y., Mazumdar, M., Memtsoudis, S.G., 2012. Beyond repeated-measures analysis of variance: advanced statistical methods for the analysis of longitudinal data in anesthesia research. Reg Anesth Pain Med. 37, 99-105. https://doi.org/10.1097/AAP.0b013e31823ebc74.

Manini, T.M., Clark, B.C., 2012. Dynapenia and aging: an update. J. Gerontol. A. Biol. Sci. Med. Sci. 67, 28–40. https://doi.org/10.1093/gerona/glr010

Meredith-Jones, K., Legge, M., Jones, L.M., 2009. Circuit Based Deep Water Running Improves Cardiovascular Fitness, Strength and Abdominal Obesity in Older, Overweight Women Aquatic Exercise Intervention in Older Adults. Med. Sport. 13, 5–12. https://doi.org/10.2478/v10036-009-0002-9

Narici, M. V., Roi, G.S., Landoni, L., Minetti, A.E., Cerretelli, P., 1989. Changes in force, cross-sectional area and neural activation during strength training and detraining of the human quadriceps. Eur. J. Appl. Physiol. Occup. Physiol. 59, 310–319. https://doi.org/10.1007/BF02388334

Pasetti, S.R., Gonçalves, A., Padovani, C.R., 2012. Continuous training versus interval training in deep water running: health effects for obese women. Rev. Andaluza Med. del Deport. 5, 3–7. https://doi.org/10.1016/S1888-7546(12)70002-3

Pillen, S., Tak, R.O., Zwarts, M.J., Lammens, M.M.Y., Verrijp, K.N., Arts, I.M.P., van der Laak, J.A., Hoogerbrugge, P.M., van Engelen, B.G.M., Verrips, A., 2009. Skeletal Muscle Ultrasound: Correlation Between Fibrous Tissue and Echo Intensity. Ultrasound Med. Biol. 35, 443–446. https://doi.org/10.1016/j.ultrasmedbio.2008.09.016

Pinto, S.S., Cadore, E.L., Alberton, C.L., Silva, E.M., Kanitz, A.C., Tartaruga, M.P., Kruel, L.F.M., 2011. Cardiorespiratory and neuromuscular responses during water aerobics exercise performed with and without equipment. Int. J. Sports Med. 32, 916–923. https://doi.org/10.1055/s-0031-1283176

Radaelli, R., Wilhelm, E.N., Botton, C.E., Bottaro, M., Cadore, E.L., Brown, L.E., Pinto, R.S., Radaelli R, Wilhelm EN, Botton CE, Cadore EL, Brown LE, Pinto RS, 2013. Effect of two different strength training volumes on muscle hypertrophy and quality in elderly women. J. Sports Med. Phys. Fitness 53, 1–2.

Reichert, T., Kanitz, A.C., Delevatti, R.S., Bagatini, N.C., Barroso, B.M., Kruel, L.F.M., 2016. Continuous and interval training programs using deep water running improves functional fitness and blood pressure in the older adults. Age (Omaha). 38, 20. https://doi.org/10.1007/s11357-016-9882-5

Rica, R.L., Carneiro, R.M.M., Serra, A.J., Rodriguez, D., Pontes Junior, F.L., Bocalini, D.S., 2013. Effects of water-based exercise in obese older women: Impact of short-term follow-up study on anthropometric, functional fitness and quality of life parameters. Geriatr. Gerontol. Int. 13, 209–214. https://doi.org/10.1111/j.1447-0594.2012.00889.x

Schaun, G.Z., 2017. The Maximal Oxygen Uptake Verification Phase: a Light at the End of the Tunnel? Sport. Med. - open 3, 44. https://doi.org/10.1186/s40798-017-0112-1

Siri, W.E., 1993. Body composition from fluid spaces and density: analysis of methods. 1961. Nutrition 9, 480–91; discussion 480, 492.

Takeshima, N., Rogers, M.E., Watanabe, E., Brechue, W.F., Okada, A., Yamada, T., Islam, M.M., Hayano, J., 2002. Water-based exercise improves health-related aspects of fitness in older women. Med. Sci. Sports Exerc. 34, 544–551.

Torres-Ronda, L., Schelling i del Alcázar, X., 2014. The Properties of Water and their Applications for Training. J. Hum. Kinet. 44, 237–248. https://doi.org/10.2478/hukin-2014-0129

Tsourlou, T., Benik, A., Dipla, K., Zafeiridis, A., Kellis, S., 2006. The Effects of a Twenty-Four–Week Aquatic Training Program on Muscular Strength Performance in Healthy Elderly Women. J. Strength Cond. Res. 20, 811-818. https://doi.org/10.1519/R-18455.1

Wasserman, K., Whipp, B.J., Koyl, S.N., Beaver, W.L., 1973. Anaerobic threshold and respiratory gas exchange during exercise. J. Appl. Physiol. 35, 236–243. https://doi.org/10.1152/jappl.1973.35.2.236

Wilhelm, E.N., Rech, A., Minozzo, F., Botton, C.E., Radaelli, R., Teixeira, B.C., Reischak-Oliveira, A., Pinto, R.S., 2014. Concurrent strength and endurance training exercise sequence does not affect neuromuscular adaptations in older men. Exp. Gerontol. 60, 207–214. https://doi.org/10.1016/j.exger.2014.11.007

Wilmore, J.H., Costill, D.L., Kenney, W.L., 2015. Physiology of Sports and Exercise, 6a edition. ed. United States.