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Neuromuscular adaptations to water-based concurrent training in postmenopausal women: effects of intrasession exercise sequence

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Abstract This study investigated the effects of different exercise sequences on the neuromuscular adaptations induced by water-based concurrent training in postmenopausal women. Twenty-one healthy postmenopausal women (57.14±2.43 years) were randomly placed into two water-based concurrent training groups: resistance training prior to (RA, $n=10$) or after (AR, $n=11$) aerobic training. Subjects performed resistance and aerobic training twice a week over 12 weeks, performing both exercise types in the same training session. Upper (elbow flexors) and lower-body (knee extensors) one-repetition maximal test (1RM) and peak torque (PT) (knee extensors) were evaluated. The muscle thickness (MT) of upper (*biceps brachii*) and lower-body (*vastus lateralis*) was determined by ultrasonography. Moreover, the maximal and submaximal (neuromuscular economy) electromyographic activity (EMG) of lower-body (*vastus lateralis* and *rectus femoris*) was measured. Both RA and AR groups increased the upper- and lower-body 1RM and PT, while the lower-body 1RM increases observed in the RA was greater than AR (34.62±13.51 vs. 14.16±13.68 %). RA and AR showed similar MT increases in upper- and lower-body muscles evaluated. In addition, significant improvements in the maximal and submaximal EMG of lower-body muscles in both RA and AR were found, with no differences between groups. Both exercise sequences in water-based concurrent training presented relevant improvements to promote health and physical fitness in postmenopausal women. However, the exercise sequence resistance–aerobic optimizes the strength gains in lower limbs.

Keywords Combined training · Aquatic exercise · Electromyography · Muscle thickness · Muscle strength · Aerobic exercise · Resistance exercise

Introduction

Aging results in a decline in the muscle mass, strength, and cardiorespiratory fitness (Fleg and Lakatta 1988;

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Izquierdo et al. 2001; Snijders et al. 2009). These declines are associated with an impairment in the functional capacity, which affects the performance of several independent daily living activities (Izquierdo et al. 2003; Christensen et al. 2009; Aagaard et al. 2010). In addition, in women, the incidence and development of these changes (e.g., increased body fat and a loss of fat-free mass) are more pronounced after menopause (Bemben and Bemben 2000). It has been postulated that the combination of resistance and aerobic training is an optimal strategy to reduce metabolic abnormalities and to improve the cardiorespiratory fitness, muscular force, and functional capacity in elderly (Cadore and Izquierdo 2013). Thereby, some studies have shown that the concurrent training (i.e., resistance associated with aerobic training) in elderly is effective to retard the functional capacity loss, since this type of training induces improvements not only in the neuromuscular but also in the cardiorespiratory function (Wood et al. 2001; Izquierdo et al. 2004; Cadore et al. 2011).

Some studies have reported that resistance training combined with the aerobic one results in lower force and power gains when compared to a resistance training performed alone. This phenomenon has been called in the literature "interference effect" (Kraemer et al. 1995; Bell et al. 1997, 2000; Häkkinen et al. 2003; Cadore et al. 2010), and it may occur due to the negative influence of the aerobic training on the neuromuscular adaptations induced by resistance training (Kraemer et al. 1995; Dolezal and Potteiger 1998). In addition, current evidences suggest that the intrasession exercise order during concurrent training in dry land environment may influence the magnitude of neuromuscular and cardiorespiratory adaptations in elderly men (Cadore et al. 2012, 2013; Cadore and Izquierdo 2013). In this way, it seems that the performance of resistance prior to aerobic exercise optimizes neuromuscular gains induced by concurrent training on dry land in elderly men (Cadore et al. 2013).

Regarding the aquatic environment, few studies have investigated the effects of resistance combined with aerobic training in water-based programs on neuromuscular and cardiorespiratory variables (Takeshima et al. 2002; Tsourlou et al. 2006). However, in these studies, only one intrasession exercise sequence was employed (i.e., aerobic followed by resistance exercises). Therefore, at the best of the authors' knowledge, no study has already investigated the effects of different intrasession exercise sequence during water-based programs in postmenopausal women.

During the water-based resistance training, the exercises are performed at maximal effort, speed, and amplitude in order to achieve a greater resistance of water. The aerobic water-based exercises are commonly performed at submaximal velocity of motion; however, the typical water-based exercises, even at submaximal velocity, may produce a peripheral fatigue of lower limb muscles (Pinto et al. 2014). Therefore, the performance of water-based aerobic exercises before resistance exercises may impair the performance of the last, which justifies the investigation of what the best intrasession exercise sequence during water-based concurrent training in postmenopausal women is.

Regular physical exercise may decrease a woman's risk of developing several medical illnesses, including osteoporosis, cardiovascular disease, obesity, and depression (Hagey and Warren 2008). In addition, due to the characteristics of water, the exercises performed in the aquatic environment are safe (Meredith-Jones et al. 2011; Alberton et al. 2013a) and effective to improve physical fitness of women in aging process (Takeshima et al. 2002; Bento et al. 2012). Despite the benefits of water-based programs reported in the literature, the optimal prescription of these programs is not entirely established in individuals in aging process (Bergamin et al. 2012). Thus, in order to broaden the knowledge related to the prescription in this modality, it is relevant to determine the most effective intrasession exercise sequence to promote greater neuromuscular and cardiorespiratory gains during concurrent training in the aquatic environment. Therefore, the purpose of the present study was to investigate the effects of different intrasession exercise sequences during water-based concurrent training on the neuromuscular adaptations in postmenopausal women. Our hypothesis was that performing resistance exercises before aerobic exercises would result in greater strength increases than in the opposite sequence (i.e., aerobic followed by resistance exercises).

Methods

Experimental design

To investigate the effects of the intrasession concurrent exercise sequence on neuromuscular parameters in postmenopausal women, two training groups performed a water-based concurrent training regime that has previously resulted in marked improvements in both aerobic and strength performance in young women (Pinto et al. 2014). Ten subjects (56.90±2.85 years) were evaluated twice before the start of training (weeks −4 and 0), and it served as control period. Both groups (i.e., resistance–aerobic and aerobic–resistance) trained during 12 weeks and each subject were evaluated before and after (weeks 0 and 13) the water-based concurrent training. The post-measurements started 72 h after the last training session, and the subjects completed all the evaluations within a week with an interval of 48 h between the tests. Different tests were conducted on different days to avoid fatigue. Each specific test at pre- and post-intervention was overseen by the same investigator, who was blinded to the training group of the subjects, and was conducted on the same equipment with identical subject/equipment positioning. Throughout the training period, the water temperature was maintained at 31.0±0.1 °C. In addition, the water depth in all subjects was fixed between the xiphoid process and shoulders.

Subjects

Twenty-one postmenopausal women, who were not engaged in any regular and systematic training program in the previous 6 months, volunteered for the study after completing an informed consent. All subjects were recruited from the city of Porto Alegre (Brazil) through sending flyers by e-mail to several postmenopausal women and announcements in a widely read local newspaper. Volunteer subjects were randomly assigned into two groups: resistance training prior to aerobic training (n=10) (mean±SD, age 57.20±2.53 years, body mass 66.78±9.08 kg, height 161.57±5.67 cm, percent fat 36.25±4.12 %) and aerobic training prior to resistance training (n=11) (mean±SD, age 57.09±2.47 years, body mass 73.05±13.65 kg, height 158.64±7.64 cm, percent fat 38.05±5.61 %). Exclusion criteria included any history of neuromuscular, metabolic, hormonal, and cardiovascular diseases. Subjects were not taking any medication, which could influence on hormonal and neuromuscular metabolism and were advised to not change their nutrition practices throughout the study. Medical evaluations were performed using clinical anamnesis and effort electrocardiograph test (ECG), to ensure subject suitability for the testing procedure. Body mass and height were measured using an Asimed analog scale (resolution of 0.1 kg) and an Asimed stadiometer (resolution of 1 mm), respectively. Body composition was assessed using the skinfold technique. A seven-site skinfold equation was used to estimate body density (Jackson et al. 1980), and body fat was subsequently calculated using the Siri equation (Siri 1993). Subjects were carefully informed about the design of the study and the possible risks and discomforts related to the measurements. The study was conducted according to the Declaration of Helsinki, and the protocol was approved by the local Institutional Review Board.

Maximal strength (1RM) and muscle thickness (MT)

Maximal dynamic strength was assessed using the one-repetition maximal test (1RM) on the bilateral knee extensors (World-Esculptor, Porto Alegre, Brazil) and free-weight barbell bilateral elbow flexors. The 1RM value was considered as the maximal load possible to exert at the concentric phase for a given exercise. One week prior to the test day, subjects were familiarized with all procedures in two sessions. On the test day, the subjects warmed up for 5 min on a cycle ergometer, stretched all major muscle groups, and performed specific movements for the exercise test. Each subject's maximal load was determined with no more than five attempts with a 4-min recovery between attempts. Performance time for each contraction (concentric and eccentric) was 2 s, controlled by an electronic metronome (MA-30, KORG, Japan). The test–retest reliability coefficient (intraclass correlation coefficient, ICC) was 0.83 for the knee extensors 1RM and 0.88 for the elbow flexors 1RM.

The muscle thickness (MT) was measured using B-mode ultrasound (Philips, VMI, MG, Brazil). A 7.5-MHz linear-array probe, with 38 mm, was placed on the skin perpendicular to the tissue interface, and the scanning head was coated with a water-soluble transmission gel to provide acoustic contact without depressing the dermal surface. The images were digitalized and after analyzed in software ImageJ (National Institutes of Health, USA, version 1.37). The subcutaneous adipose tissue–muscle interface and the muscle–bone interface were identified from the ultrasonic image, and the distance from the adipose tissue–muscle interface to the muscle–bone interface was defined as MT (Abe et al. 2000). The MT images were determined in the *vastus lateralis* (midway between the edge of the lateral condyle of the femur and the tip of the greater trochanter) (Miyatani et al. 2002) and *biceps brachii* (40 % of the distance from the lateral epicondyle to the acromion process of the scapula, starting at the lateral epicondyle) muscles (Fukunaga et al. 2001). To ensure the same probe position in subsequent tests, the right thigh and arm of each subject was mapped for the position of the electrodes moles and small angiomas by marking on transparent paper (Narici et al. 1989). Subjects were evaluated in supine position, after 15 min resting in lying position and after 72 h without any vigorous physical activity. The test–retest reliability coefficients (ICC) for the MT were 0.95 for the *vastus lateralis* and 0.86 for the *biceps brachii*.

Isometric PT and EMG

Maximal isometric peak torque (PT) was obtained using an isokinetic dynamometer (Biodex, New York, USA). Before the measurement session, the subjects were carefully familiarized with the testing procedure. The subjects were positioned seated with their hips and thighs firmly strapped to the seat of the dynamometer, with the hip angle at 85°. After that, subjects warmed up for ten knee extension repetitions at angular velocity of $90°s^{-1}$, performing a submaximal effort. The dynamometer was connected to an A/D converter (Dataq Instruments Inc., Akron, OH, USA), which made it possible to quantify the torque exerted when each subject executed the knee extension at the determined angle. After having their right leg positioned by the evaluators at an angle of 120° (Baroni et al. 2013) in the knee extension (180° represented the full extension), the subjects were instructed to exert maximal strength possible as fast as was possible when extending the right knee. The subjects had three attempts at obtaining the maximal voluntary contraction (MVC) of the knee extensors, each lasting 5 s. During all the maximal tests, the researchers provided verbal encouragement so that the subjects would feel motivated to produce their maximum force. Signal processing included filtering with a fifth-order low-pass Butterworth filter, with a cutoff frequency of 9 Hz. Maximal peak torque was defined as the highest value of the torque (N m) recorded during the unilateral knee extension. The test–retest reliability coefficient (ICC) was 0.96 for the knee extensors maximal isometric peak torque.

During the isometric test above mentioned, the maximal neuromuscular activity of agonist knee extensors muscles was evaluated using surface electromyography (RMS values) on the *vastus lateralis* (VL) and *rectus femoris* (RF). Electrodes were positioned on the muscular belly in a bipolar configuration (20-mm interelectrode distance) in parallel with the orientation of the muscle fibers, according to SENIAM project (www.seniam.org). Hair was shaved away from the site of electrode placement, and the skin was abraded and cleaned with alcohol to keep the inter-electrode resistance low (<3 kΩ). To ensure the same electrode position in subsequent tests, the right thigh of each subject was mapped for the position of the electrode moles and small angiomas by marking on transparent paper (Narici et al. 1989). The reference electrode was fixed on the anterior crest of the tibia to record the electromyography recording (EMG) signal of the knee extensors muscles. The raw EMG signal was acquired simultaneously with the MVC using an eight-channel electromyograph (AMT-8, Bortec Bio-medical Ltd., Canadá). The raw EMG was converted by an A/D converter DI-720 with 16-bits resolution (Dataq Instruments Inc. Akron, OH, USA), with a sampling frequency of 2000 Hz per channel, connected to a PC. Following acquisition of the signal, the data were exported to the SAD32 software, where they were filtered a fifth-order band-pass Butterworth filter, with cutoff frequencies between 20 and 500 Hz. After that, the EMG records were sliced exactly in 1 s when maximal value of stable force (1 s) was determined between the second and fourth second of the force–time curve, and the RMS values were calculated. After the maximal neuromuscular activity measure, the submaximal neuromuscular activity from the VL and RF was evaluated in order to determine the isometric neuromuscular economy. Thus, subjects had three 10-s attempts to exert 40 % of the pre-training isometric peak torque and maintain it for, at least, 3 s receiving a visual feedback of the torque values. The apparatus and the collection and analysis procedures were the same used to determine the maximal EMG signal. The EMG records were sliced exactly in 2 s when submaximal value of stable torque was determined of the torque–time curve, and the RMS values were calculated and normalized using the maximal RMS values obtained during the MVC in each muscle. The test–retest reliability coefficients (ICC values) were 0.76 for the EMG of the VL and 0.86 for the EMG of the RF.

Peak oxygen uptake and second ventilatory threshold

Subjects performed a Bruce protocol in order to determine the peak oxygen uptake (VO_{2peak}) and the oxygen uptake corresponding to the second ventilatory threshold (VO_{2VT2}). Each stage of the Bruce protocol consists of 3 min. The initial speed was 1.7 mph, and the inclination is 10 %. A 0.7–0.8-mph increase in speed and 2 % increase in the inclination are given for each consecutive stage. The test was halted when the subject indicated exhaustion by means of a hand signal. The expired gas was analyzed using a metabolic cart (CPX/D, Medical Graphics Corporation, St. Paul, MN, USA) breath by breath. The maximal VO_2 value (milligram per kilogram per minute) obtained close to exhaustion was considered the VO_{2peak}. The assessment was considered valid when some of the following criteria were met at the end of the test: Estimated maximal heart rate was reached (220 age), and a respiratory exchange ratio greater than 1.15 was reached and a maximal respiratory rate of at least 35 breaths per minute (Howley et al. 1995). The second ventilatory threshold was determined using the ventilation slope and confirmed through the slope of the ventilatory equivalent for CO_2 (V_E/VCO_2) (Wasserman et al. 1973). Two experienced and independent blind physiologists detected the thresholds through visual inspection according to the criteria previously described above. When the results were discordant, the graphs were assessed by a third physiologist (Hug et al. 2004). The test–retest reliability coefficient (ICC) was 0.87 for VO_{2peak} and 0.74 for VO_{2VT2}.

Water-based concurrent training programs

Subjects of the study trained both resistance and aerobic training in the same session, two times a week, on nonconsecutive days. Training groups were differentiated by their intrasession exercise order during the water-based concurrent training. One group trained resistance training prior to (RA) aerobic training and the other one trained aerobic training prior to (AR) resistance training. Before the start of the concurrent training, subjects completed two familiarization sessions in the water environment to practice the exercises they would further perform during the training period. In addition, in the familiarization sessions, the subjects also performed the resistance exercises at their perceived exertion of maximal effort. During the resistance training, the individuals were instructed to perform each repetition at maximal effort, velocity, and amplitude in order to achieve, a greater resistance. Verbal encouragement was provided by the same instructor during all resistance exercises.

The progression of the water-based resistance training was previously described in the study conducted by Pinto et al. (2014). The resistance exercises were separated into two blocks, and each block had one exercise for the upper limbs and one exercise for the lower limbs, starting from the anatomical position. The block 1 consisted of bilateral elbow flexion and extension and unilateral hip flexion and extension. Additionally, the block 2 consisted of bilateral shoulder flexion and extension and unilateral knee flexion and extension (starting from hip flexion at 90°). In the weeks 1–4, the subjects performed three sets of 20 s of each block. In the weeks 5–8, four sets of 15 s of each block were performed. In the last mesocycle (weeks 9–12), the subjects performed six sets of 10 s of each block with an active interval (5 min) after the performance of the three sets of blocks 1 and 2. The resistance training sessions lasted 13 min 20 s in the first mesocycle, 16 min 50 s in the second mesocycle, and 28 min 20 s in the third mesocycle.

The aerobic training program was performed using three water-based exercises performed at the heart rate (HR) corresponding to the second ventilatory threshold (VT_2). During the first 4 weeks, subjects performed two sets of 3 min with the following sequence, without interval between the sets: 3 min of stationary running, 3 min of cross-country skiing, and 3 min of frontal kick, totaling 18 min. In the weeks 5–8, subjects performed three sets of 3 min with the same sequence abovementioned, totaling 27 min, and in the last 4 weeks (9–12), subjects performed four sets of 3 min, totaling 36 min. The three water-based exercises are described in the study of Alberton et al. (2013a). During the sessions of aerobic training, all the subjects used a coded Polar monitor in order to control the HR corresponding to VT_2. Three experienced water-based trainers carefully supervised all training sessions.

The VT_2, used as a parameter to prescribe the intensity of aerobic training, was determined during a maximal progressive test at the water environment with the stationary running exercise, since Alberton et al. (2013b) have demonstrated that there were no differences in the HR corresponding to VT_2 among the stationary running, cross-country skiing, and frontal kick water-based exercises. Thus, the maximal test with stationary running was conducted with an initial cadence of 85 b min^{-1} for 3 min, with 15 b min^{-1} increases in cadence every 2 min until maximal effort was obtained. The cadences were set by a digital metronome (MA-30, KORG, Japan).

To evaluate the ventilatory data, a mixing-box-type portable gas analyzer (VO2000, MedGraphics, Ann Arbor, USA) was used and had been previously calibrated according to the manufacturer's specifications. The HR was measured using a Polar monitor (FS1, Shanghai, China). The sampling rate of the collected HR and ventilatory data was 10 s, and the data were acquired using the Aerograph software. The second ventilatory threshold analyses were the same described in the Bruce protocol. The VT_2, used to prescribe the intensity of aerobic training, corresponded to 78.50±10.30 % of the VO_{2peak}. The VT_2 was also evaluated in the week 6 in order to adjust the training intensity, and significant change was observed in this parameter compared to the week 0 (VT2: 83.55±7.37 % of the VO_{2peak}). The whole water-based concurrent training periodization is shown in Table 1.

Statistical analysis

Results are reported as mean±SD. Normal distribution and homogeneity parameters were checked with Shapiro–Wilk and Levene tests, respectively. Statistical comparisons in the control period (from weeks −4 to 0) were performed by using Student's paired *t* tests. In addition, the test–retest reliability for each dependent variable between the weeks −4 to 0 was determined using the intraclass correlation coefficient (ICC). The training-related effects were assessed using repeated measures two-way ANOVA (factors: group and time). When the interaction effect was significant, relative changes between groups were compared via one-way ANOVA. Significance was accepted when $\alpha=0.05$ and the SPSS statistical software package (version 20.0) was used to analyze all data. The observed statistical power was 97 and 99.9 % for elbow flexors and knee extensors maximal dynamic strength, respectively, 99.9 % for both *vastus lateralis* and *biceps brachii* muscle thickness, 99 % for knee extensors maximal isometric peak torque, 68 and 97 % for the maximal neuromuscular activity of *vastus lateralis* and *rectus femoris*, respectively, and 48 and 91 % for the submaximal neuromuscular activity of *vastus lateralis* and *rectus femoris*, respectively.

Results

During the control period (i.e., between weeks −4 and 0), no significant differences were observed in all variables analyzed, except the KEX 1RM variable that showed slight change between weeks −4 and 0 (Table 2).

No significant differences were observed in training compliance between RA and AR (96.25±5.36 vs. 96.97±7.47 %, respectively). In addition, there were no differences between groups before training in the age, body mass, height, and percent fat ($p>0.05$). After training, no significant increase was found in both groups RA and AR in the VO_{2peak}. However, there was a significant increase in both RA and AR in the VO_{2VT2} ($p=0.027$) with no differences between groups ($p=0.093$). This result showed that our aerobic training program was effective in improving cardiorespiratory fitness (RA 7.46±17.99 vs. AR 11.20±12.57 %).

Maximal strength (1RM) and muscle thickness (MT)

At baseline, there were no differences between groups in the elbow flexors and knee extensors 1RM (Table 3). After training, there was significant time vs. group interaction in the knee extensors 1RM. While both RA and AR increased the knee extensor 1RM values, the increase observed in the RA was significantly greater than AR (34.62±13.51 vs. 14.16±13.68 %, $p=0.005$, respectively). In the elbow flexor 1RM, there were

Table 1 Complete water-based concurrent training periodization

	Resistance training	Aerobic training					
Week	Sessions	Sets	Duration	Interval	Intensity	Volume	Intensity
1–4	2	3	20 s	2 min	Maximal effort	18 min	HR_{VT2}
5–8	2	4	15 s	2 min	Maximal effort	27 min	HR_{VT2}
9–12	2	6	10 s	2 min	Maximal effort	36 min	HR_{VT2}

HR_{VT2} heart rate corresponding to the second ventilatory threshold

Table 2 Pre-values and post-values during the control period (−4 and 0 weeks)

	Postmenopausal women, $n=10$				
	Week −4		Week 0		
	Mean	SD	Mean	SD	*p*
EFLE 1RM (kg)	16.75	±3.95	16.62	±3.16	0.844
KEX 1RM (kg)	54.25	±11.13	47.37	±10.97	0.012[a]
KEX PT (N m)	159.93	±38.30	157.72	±35.85	0.561
EMG VL (v)	0.08	±0.02	0.08	±0.04	0.770
EMG RF (v)	0.07	±0.04	0.06	±0.04	0.860
VL MT (mm)	18.06	±3.84	18.65	±3.66	0.058
BB MT (mm)	19.46	±4.50	19.19	±3.62	0.687

EFLE elbow flexors, *KEX 1RM* knee extensors one maximal repetition, *KEX PT* knee extensors maximal isometric peak torque, *EMG VL* and *EMG RF* maximal neuromuscular activity of *vastus lateralis* and *rectus femoris*, *VL MT* and *BB MT vastus lateralis* and *biceps brachii* muscle thickness

[a] Significant difference between weeks −4 and 0

significant increases in both RA and AR, with no difference between groups (RA 11.91±10.56 vs. AR 7.21 ±10.15 %).

Regarding the muscle thickness, at baseline, there were no differences between groups in the *biceps brachii* and *vastus lateralis* MT (Table 3). After training, there were significant increases in both RA and AR in the *biceps brachii* (5.04±4.16 vs. 6.67±3.83 %, respectively) and *vastus lateralis* (4.24±1.23 vs. 4.12±2.69 %, respectively) MT, with no difference between groups.

Isometric torque and EMG recordings

At baseline, there were no differences between groups in the knee extensors maximal isometric PT (Table 4). After training, there were significant increases in both RA and AR in the knee extensors maximal isometric PT (7.53±6.42 vs. 6.30±6.12 %, respectively), with no difference between groups.

Regarding the EMG recordings, at baseline, there were no differences between groups in the maximal and submaximal neuromuscular activity of *vastus lateralis* and *rectus femoris* (Table 4). After training, there were significant increases in both RA and AR in the maximal neuromuscular activity of *vastus lateralis* and *rectus femoris* (27.66±46.90 vs. 16.13±19.66 %, respectively), with no difference between groups. The same pattern was found in the submaximal neuromuscular activity of *vastus lateralis* and *rectus femoris*, with significant increases in both RA and AR (*vastus lateralis* −5.26±16.44 vs. −6.04±10.74 %, respectively; *rectus femoris* −16.73±12.74 vs. −12.39±19.56 %, respectively) and no difference between groups.

Discussion

The main finding of the present study was that water-based concurrent training performed twice weekly during 12-week induced relevant improvements in dynamic and isometric maximal strength, muscle thickness, and muscle activity in sedentary postmenopausal women. In

Table 3 Maximal strength and muscle thickness before and after training: aerobic–resistance and resistance–aerobic; mean±SD

	Resistance–aerobic, $n=10$				Aerobic–resistance, $n=11$						
	Pre		Post		Pre		Post		Group	Time	Group * time
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*p*	*p*	*p*
EFLE 1RM (kg)	15.89	±2.37	17.67	±2.24	17.82	±3.12	19.09	±3.78	0.212	0.001[a]	0.516
KEX 1RM (kg)	45.00	±9.05	60.22	±11.31	54.00	±9.90	60.91	±8.45	0.258	<0.001[a]	0.006[b]
VL MT (mm)	19.50	±3.89	20.31	±3.97	20.03	±2.07	20.85	±2.22	0.698	<0.001[a]	0.926
BB MT (mm)	17.77	±2.33	18.67	±2.58	18.02	±2.48	19.24	±2.90	0.717	<0.001[a]	0.316

EFLE and *KEX 1RM* elbow flexors and knee extensors one maximal repetition, *VL MT* and *BB MT vastus lateralis* and *biceps brachii* muscle thickness

[a] Significant difference between pre and post-training in both groups (RA and AR)

[b] Significant time vs. group interaction

Table 4 Isometric torque and electromyography recordings before and after training: aerobic–resistance and resistance–aerobic; mean±SD

	Resistance–aerobic, *n*=10				Aerobic–resistance, *n*=11						
	Pre		Post		Pre		Post		Group	Time	Group * time
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*p*	*p*	*p*
KEX PT (N m)	153.38	±17.02	169.12	±20.10	165.78	±26.27	175.94	±27.39	0.535	<0.001[a]	0.587
EMG VL (v)	0.08	±0.04	0.09	±0.05	0.09	±0.04	0.10	±0.04	0.658	0.019[a]	0.644
EMG RF (v)	0.07	±0.03	0.09	±0.05	0.06	±0.02	0.08	±0.03	0.573	0.001[a]	0.911
EMG VL 40 % (%)	39.47	±11.54	36.69	±9.50	35.00	±6.08	32.83	±5.97	0.256	0.054[a]	0.806
EMG RF 40 % (%)	34.84	±9.41	28.94	±7.94	33.60	±8.52	28.41	±5.67	0.785	0.003[a]	0.824

KEX PT knee extensors maximal isometric peak torque, *EMG VL* and *EMG RF* maximal neuromuscular activity of *vastus lateralis* and *rectus femoris*, *EMG VL 40%* and *EMG RF 40%* neuromuscular economy (normalized EMG at 40 % of pre-training MVC) of *vastus lateralis* and *rectus femoris*

[a] Significant difference between pre and post-training in both groups (RA and AR)

addition, greater strength gains in knee extensors were found when resistance exercises were performed prior to aerobic exercises during water-based concurrent training. Furthermore, no differences were observed in the neural and morphological adaptations between groups suggesting that the intrasession exercise sequence influenced strength performance but not the magnitude of neuromuscular activity and hypertrophy in postmenopausal women. These results suggest that the high-intensity water-based concurrent training protocol used in the present study was an effective intervention to improve muscle strength and size in postmenopausal women, a population at greater risk of neuromuscular and functional decline.

Both RA and AR groups presented maximal dynamic strength gains in upper (EFLE 1RM 12 vs. 7 %, respectively) and lower limbs (KEX 1RM 35 vs. 14 %, respectively). Such improvements are similar to those found in studies with water-based resistance training in postmenopausal women. Tsourlou et al. (2006) evaluated the effects of water-based aerobic and resistance training during 24 weeks (three sessions a week) and observed an increase corresponding to 29 % in the knee extensors dynamic strength measured by three maximal repetitions (3RM) in elderly women. This percent increment for the knee extensors dynamic strength observed by Tsourlou et al. (2006) was greater than those found in the aerobic–resistance intrasession sequence order group in the present study (i.e., 14 %). However, we found in the opposite order (i.e., resistance–aerobic) greater gains in this muscle group (i.e., 35 %) in a program with a shorter duration (12 vs. 24 weeks) and lower weekly frequency (2 vs. 3). Regarding the elbow flexors maximal dynamic strength, the percent gains found in the present study (RA 12 %, AR 7 %) were similar to the values observed (12 %) in the study developed by Kruel et al. (2005) that evaluated the effects of 11 weeks of water-based aerobic and resistance training (two sessions a week) on strength of postmenopausal women. It is important to highlight that both studies employed similar periodization during the water-based resistance training.

Regarding the water-based concurrent training order, the present study demonstrated greater knee extensors maximal dynamic strength in the RA compared to AR group (35 vs. 14 %). This finding is in accordance to the results showed by Cadore et al. (2013) and Pinto et al. (2014), who analyzed the intrasession exercise sequence during 12 weeks of concurrent training in dry land and aquatic environments in elderly men and young women, respectively. Cadore et al. (2013) observed greater gain in the maximal dynamic strength in this muscle group in the RA compared to AR exercise sequence (35 vs. 22 %, respectively). The control of the intensity of aerobic training in this study should be highlighted, since it was based on percents of the second ventilatory threshold (80–100 %) using a cycle ergometer. Pinto et al. (2014) also found such greater gain in RA compared to AR during water-based concurrent training (43 vs. 27 %, respectively). These authors used specific water-based exercises in the aerobic training with the intensity corresponding to 100 % of the second ventilatory threshold. It may be speculated that the residual fatigue from the water-based aerobic exercises (i.e., stationary running, cross-country skiing, and frontal kick) influenced the subsequent water-based resistance exercises, since both of them involved knee extension movements.

In the present study, the knee extensors maximal isometric peak torque presented a similar significant increase in both RA and AR groups (7 vs. 6 %, respectively). These results are in accordance to study developed by Tsourlou et al. (2006) which analyzed this variable after water-based program in elderly (10 %). Regarding water-based concurrent training order, the present results corroborate to Cadore et al. (2013) and Pinto et al. (2014) which showed no difference between the intrasession sequence order resistance–aerobic (8 and 7 %, respectively) and aerobic–resistance (6 and 11 %, respectively). The lower percent gains in the maximal isometric peak torque compared to the maximal dynamic strength of the knee extensors could be explained by the fact that the water-based concurrent training is performed with dynamic contractions (i.e., training specificity).

Regarding the neuromuscular activity, both groups (i.e., RA and AR) presented similar stimulus to improve the EMG variables analyzed (maximal and submaximal EMG amplitude). These results are in accordance with the study of Pöyhönen et al. (2002) that demonstrated 10 weeks of water-based resistance training significant improvements in the maximal neuromuscular activity (EMG *quadriceps* 26 %). In the present study, increases in the maximal neuromuscular activity from vastus lateralis and rectus femoris were 28 and 34 % for the RA and 16 and 31 % for the AR group, respectively. In addition, both groups showed improvements in the neuromuscular economy (i.e., submaximal neuromuscular activity), since a reduction ranging from 5 to 6 % and 12 to 16 % for *vastus lateralis* and *rectus femoris*, respectively, was found to reach 40 % MVC. The present results agree with those from Cadore et al. (2013) who demonstrated that 12 weeks of concurrent training in dry land environment in elderly men resulted in a neuromuscular economy from *vastus lateralis* of −17 and −12 % to reach 50 % MVC in RA and AR groups.

The muscle thickness of upper and lower limbs presented increases between 4 and 7 %, independent from the intrasession exercise sequence. These values are similar to those found by Pöyhönen et al. (2002), who observed that 10 weeks of water-based resistance training was effective to improve the muscle mass of lower limbs in young women (*quadriceps* 4 %). Regarding the order of the concurrent training, Cadore et al. (2013) also demonstrated that 12 weeks of concurrent training in dry land environment in elderly men resulted in similar gains in upper and lower limbs muscle thickness (3–7 %) between RA and AR groups. It is important to highlight that 12 weeks of water-based concurrent training are enough to promote significant increases in muscle thickness of upper and lower limbs in untrained postmenopausal women. These results are important because this population has a greater risk of sarcopenia, which contributes to decline in the functional capacity.

In summary, water-based concurrent training was quite effective to improve neuromuscular function in postmenopausal women, especially when resistance exercises were performed prior to aerobic exercises (i.e., RA group). From a practical standpoint, the resistance–aerobic intrasession exercise sequence in water-based programs should be prioritized in order to optimize the strength gains in lower limbs in postmenopausal women. Nevertheless, both intrasession exercise sequences in water-based programs performed twice weekly (i.e., resistance–aerobic and aerobic–resistance) presented relevant improvements to promote health and physical fitness in postmenopausal women.

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