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Effect of whey protein supplementation combined with resistance training on cellular health in pre-conditioned older women: A randomized, double-blind, placebo-controlled trial

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ABSTRACT

Aim: The purpose of study was to analyze the effects of a combined whey protein (WP) and RT intervention on cellular health in pre-conditioned older women.
Methods: The protocol is a randomized controlled clinical trial with a sample of seventy older women, divided into 3 groups: WP-placebo (WP-PLA, n = 24), placebo-WP (PLA-WP, n = 23), and placebo-placebo (PLA-PLA, n = 23). Each group drank 35 g of product (placebo or WP) pre- and post- training. The RT program was carried out over 12 weeks (3x/week; 3 × 8–12 repetitions maximum). Total body water (TBW), intra (ICW) and extracellular (ECW) water, resistance (R), reactance (Xc), and phase angle (PhA) assessed by bioimpedance spectroscopy. Lean soft tissue (LST) was measured using dual energy X-ray absorptiometry; and food consumption was assessed by means of the average of two 24-hour recalls. ANCOVA for repeated measures was applied for comparisons, with baseline scores used as covariates.
Results: A group by time interaction ($P < 0.05$) was observed for LST, ICW and the ECW/ICW ratio. There was a time effect ($P < 0.05$) for TBW, Xc, and PhA. A reduction ($P < 0.05$) in R was found only in the WP-PLA and PLA-WP groups.
Conclusion: Whey protein supplementation (pre- or post-) combined with RT promoted an increase in ICW and LST, and also a reduction in ECW/ICW ratio in pre-conditioned older women. Regardless of the supplementation intake, the RT regimen improved PhA in older adult women. This trial was registered at ClinicalTrials.gov: NCT03247192.

1. Introduction

The aging process predisposes individuals to a reduction in total body water (TBW) and its intracellular fraction (ICW), and a concomitant increase in extracellular water (ECW) (Saragat et al., 2014). Bioimpedance spectroscopy (BIS) has been shown to be a very useful alternative to evaluate cellular fluids as well as the phase angle (PhA)2. Phase angle has been considered an important indicator of nutritional status, sarcopenia, functional capacity, and mortality risk (Fukuda et al., 2016; Kyle, Soundar, Genton, & Pichard, 2012; Norman, Stobaus, Pirlich, & Bosy-Westphal, 2012), besides which, a decline in the phase angle has been associated with a decline in muscle mass in the elderly (Norman et al., 2012).

Resistance, reactance, and PhA are dependent on age, sex, height, fat-free mass, nutritional status, body mass index (BMI), and level of physical activity (Norman et al., 2012, Fukuda et al., 2016). In this context, the combination of resistance training (RT) and protein supplementation plays an important role, since these represent the most

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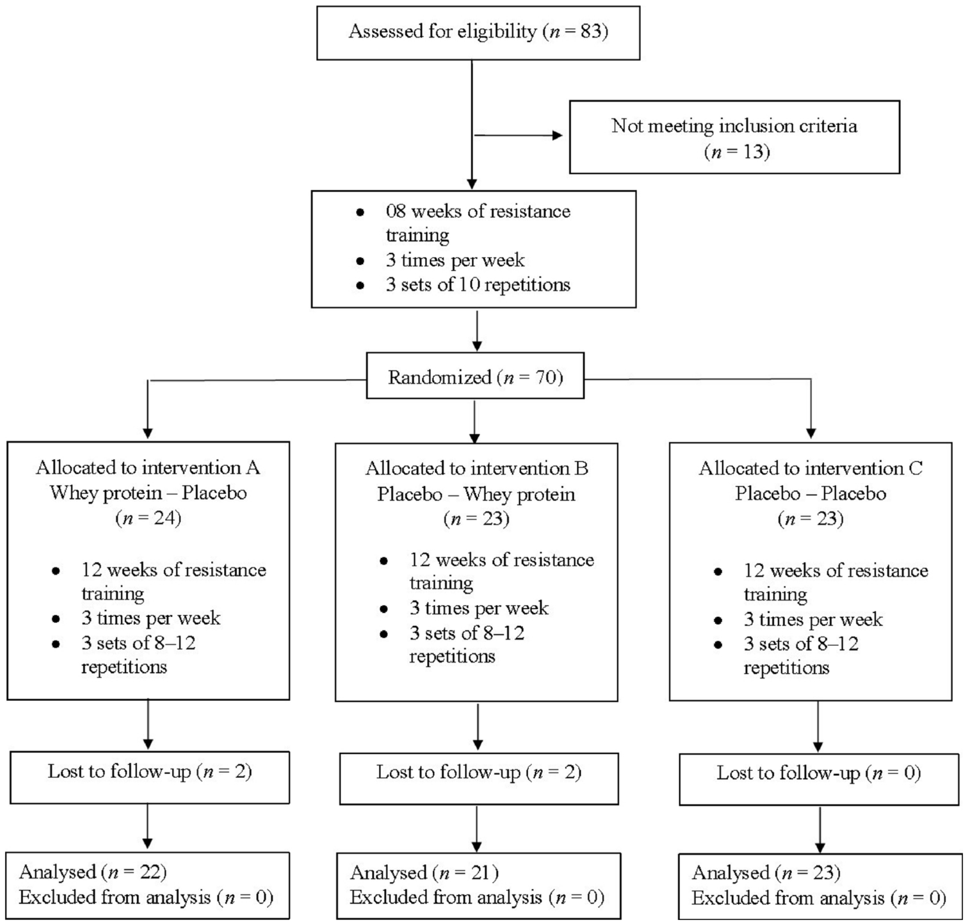

Fig. 1. Flowchart of the study.

common non-drug strategies with important implications for the health of older adults (Breen & Phillips, 2013; Morton et al., 2017). Resistance training has been widely recommended for the older population, since this type of exercise has been shown to be effective for increasing muscle mass (Ribeiro et al., 2017, 2019; Souza et al., 2016; Tomeleri et al., 2016), ICW (Ribeiro et al., 2017, 2019; Souza et al., 2016), and PhA [Souza et al., 2016; Ribeiro et al., 2017, 2019), as well as, for decreasing ECW (Souza et al., 2016). Moreover, increased protein intake has been associated with an increase in fat-free mass in older adults (Morton et al., 2017; Sugihara Junior et al., 2019; Nabuco et al., 2018), which may influence ICW and ECW, as well as favor increases in PhA.

To optimize the beneficial results of this combination strategy on the health of older adults, it is imperative to consider the training program, quality of the protein offered, and time of ingestion (Breen & Phillips, 2013). Regarding the RT program to increase muscle mass, training regimes that induce the greatest metabolic stress, with sets of 6–12 repetitions, rest intervals of 60–90 seconds, and multiplane and multiangular exercises to stimulate as many muscle fibers as possible, seem to be the most highly indicated (Schoenfeld, 2010).

The quality of the protein consumed also has great influence on the adaptive responses to RT. In this sense, whey protein (WP) is a protein that has been associated with increased muscle mass in older people due to its composition rich in leucine, a protein synthesis activator (Devries & Phillips, 2015; Nabuco et al., 2018; Sugihara Junior et al., 2019). Moreover, WP has antioxidative effects, because of its ability to increase the availability of reduced glutathione and the activity of the endogenous antioxidative enzyme system, which can promote an improvement in cellular health (Draganidis et al., 2016).

Whey protein and creatine plus RT was found to decrease ECW in men aged 48 to 72 years, without significant changes in lean soft tissue (Eliot et al., 2008). However, this investigation was conducted in both middle-aged and older adults, in an untrained condition. This is an important issue as the adaptive response to RT is dependent on training experience, with untrained individuals being more responsive (Morton et al., 2017; Ribeiro et al., 2015; Schoenfeld & Contreras, 2013). Moreover, there is a correlation between baseline scores of PhA and the percentage change from pre- to post-training in PhA, indicating that the greatest responsiveness in PhA occurs in those older women with a lower PhA at baseline (Ribeiro et al., 2019).

Another relevant aspect is the time of protein intake that can be used as a tool for maximizing RT-induced adaptations and optimizing recovery from tissue damage (Aragon & Schoenfeld, 2013). Resistance training in the fed state has the potentiated effect of antioxidant amino acids attenuating the formation of free radicals, protecting the cell membrane.

To our knowledge, no research has been conducted to assess changes in body water and cellular health after a combined intervention of WP supplementation and RT in pre-conditioned older women. Therefore, the purpose of this randomized, double-blind, placebo-controlled design was to analyze the effects of a combined WP and RT intervention on cellular health and body water in pre-conditioned older women. We hypothesized that WP plus RT would result in improvements in body water (increased ICW and reduced ECW) and cellular health (PhA) over a placebo and that the order of supplementation intake (pre or post RT session) would not influence the results.

2. Material and methods

2.1. Experimental design

This three-arm randomized, double-blind, placebo-controlled design was carried out over a period of 26 weeks divided in two phases. The first phase consisted of an eight-week period where participants underwent a pre-conditioning RT program (weeks 3–10), which participants were familiarized with RT. This period had the objective of standardizing training status, including the neural adaptations that occur within the first few weeks of training (Folland & Williams, 2007). In the second phase (supplementation phase) the participants were randomized into three groups and then started the WP plus RT for 12 weeks (weeks 13–24).

At the beginning and end of each phase of the experiment, two weeks were allocated for evaluations (weeks 1–2, 11–12, and 25–26) consisting of anthropometric, body composition and dietary intake measurements, performed at the university in a temperature-controlled room (22–24 °C), whereas the RT sessions were conducted at the university facilities. Supplementation was also conducted in a temperature-controlled room at the university. Fig. 1 presents a schematic representation of the participant recruitment and allocation adopted in this investigation.

2.2. Participants

Recruitment was carried out through newspaper and radio advertising. All participants completed health history and physical activity questionnaires and met the following inclusion criteria: 60 years old or more, physically independent, free from cardiac or orthopedic dysfunction, not receiving hormonal replacement and/or thyroid therapy, not using equipment that would prevent the accomplishment of protocols and tests, and not performing any regular physical exercise for 6 months preceding the beginning of the investigation. Participants underwent a diagnostic graded exercise stress test with a 12-lead electrocardiogram reviewed by a cardiologist and were released with no restrictions for participation in this investigation. Eighty-three Brazilian older women ($\geq$ 60 years old) volunteered to participate in this investigation. After individual interviews, thirteen volunteers were excluded as they did not meet the inclusion criteria. Seventy participants were submitted to a standardized RT program, for eight weeks. After the assessments, the participants were randomly divided into three groups according to their relative strength [ratio of total strength obtained in one repetition maximal tests (1-RM) by body mass]: (1) WP pre- and placebo post- RT (WP-PLA, n = 24), (2) placebo pre- and WP post- RT (PLA-WP, n = 23), and (3) placebo pre- and post- RT (PLA-PLA, n = 23). A blinded researcher was responsible for generating random numbers for participant allocation. All groups were submitted to the same RT program and 66 participants completed the experiment. The reasons for withdrawal from the investigation were reported as personal reasons and transportation issues.

Written informed consent was obtained from all participants after provision of a detailed description of investigation procedures. This investigation was conducted according to the Declaration of Helsinki and was approved by the local University Ethics Committee (no. 1.700.756).

2.3. Anthropometry

Body mass was measured to the nearest 0.1 kg using a calibrated electronic scale (Balmak, Laboratory Equipment Labstore, Curitiba, PR, Brazil) and height was measured using a stadiometer to the nearest 0.1 cm. The participants wore light clothing without shoes. Body mass index was calculated as the body mass in kilograms divided by the square of the height in meters.

2.4. Body composition

Whole-body dual-energy X-ray absorptiometry (DXA) (Lunar Prodigy, model NRL 41990, GE Lunar) was used to assess lean soft tissue (LST) according to previously described procedures (Tomeleri et al., 2016). A BIS device (Xitron Hydra, model 4200, Xitron Technologies, USA) was used to estimate resistance (R) and reactance (Xc) at a single frequency (50 kHz), and subsequently the PhA was calculated as the arc-tangent (Xc/R) x 180°/π. The TBW, ECW, and ICW were obtained from the BIS measurements.

All BIS measurements were performed during the morning period. Before measurement the participants were instructed to remove all objects containing metal. Participants were instructed to lie in a supine position, legs abducted at an angle of 45° relative to the body midline, and hands pronated. After cleaning the skin with alcohol, two electrodes were placed on the surface of the right hand and two on the right foot in accordance with procedures described by Sardinha et al (Sardinha, Lohman, Teixeira, Guedes, & Going, 1998). Participants were instructed to urinate about 30 min before the measures, refrain from ingesting food or drink in the previous 4 h, avoid strenuous physical exercise for at least 24 h, and refrain from consumption of alcoholic and caffeinated beverages for at least 48 h. To ensure that participants were in a neutral hydration state, combined information on the first-morning body weight prior to the body composition visit and observation of urine color were used (Casa, Clarkson, & Roberts, 2005).

Previous test-retest scans of 12 older women measured 24–48 h apart resulted in a standard error of measurement (SEM) of 0.38 kg for LST, 0.42 L for ECW, 0.48 L for ICW, 0.71 L for TBW, 17.86 ohms for resistance, 2.2 ohms for reactance, and 0.13 degrees for PhA, with an intraclass correlation coefficient (ICC) > 0.94 for all these variables.

2.5. Dietary intake

Food intake was assessed by the 24-hour dietary recall method applied on two non-consecutive days of the week, with the aid of a photographic record taken during an interview (Monego et al., 2013). The homemade measurements of the nutritional values of food and supplementation were converted into grams and milliliters using the online software program Virtual Nutri Plus (Keeple®, Rio de Janeiro, Brazil). Some foods were not found in the program database and were therefore added from food tables (Pinheiro, Lacerda, Benzecry, MCdS, & da Costa, 2009).

2.6. Supplementation protocol

Participants received a dose of 35 g of hydrolyzed WP (Lacprodan®, Arla Foods, Denmark) and/or placebo pre and post-RT. Maltodextrin (New Millen®, São Paulo, SP, Brazil) was used as a placebo. The hydrolyzed WP drink contained 27.1 g of protein, 5.2 g of carbohydrates, and 0.2 g of fat per portion (200 mL, 131 kcal), whereas the carbohydrate drink contained 0.3 g of protein and 33.3 g of carbohydrates per portion (200 mL, 134 kcal). The supplements were mixed with non-caloric sugar-free drinks to mask the contents (grape or passion fruit flavor). Participants ingested the drinks under the supervision of the research staff and were instructed to drink the solution. Supplementation was consumed only on training days. Both the subjects and the researchers responsible for RT were blinded as to which supplement was given until the end of the trial. Participants were asked to provide any information if they felt any side effects related to supplement administration, but they report no side effects

2.7. Resistance training program

Supervised RT was performed during the morning hours. The protocol was based on recommendations for RT in an older population to improve muscular strength and hypertrophy (American College of

Sports, 2009). In both phases, the sessions were performed 3 times per week on Mondays, Wednesdays, and Fridays. The RT program was a whole-body program with eight exercises, including: chest press, horizontal leg press, seated row, knee extension, preacher curl (free weights), leg curl, triceps pushdown, and seated calf raise. During the first phase, the participants trained following a protocol of three series of 10 RM, alternated by segment. During the supplementation plus RT period (second phase), the participants were submitted to conventional RT, alternated by segment, which consisted of the execution of three series of 8–12 repetitions, with fixed loads. Instructors adjusted the loads of each exercise according to the subject's ability and improvement in exercise capacity throughout the investigation in order ensure that the subjects were exercising with as much resistance as possible while maintaining proper exercise technique. The load was adjusted weekly using procedures described elsewhere (American College of Sports, 2009).

2.8. Statistical analyses

The Shapiro Wilk test was used to assess data distribution. Descriptive statistics are presented as means and standard deviation. One-way analysis of variance (ANOVA) and the chi-square test were used to compare groups regarding the general characteristics, dietary intake, and clinical history (categorical variables) at baseline. The paired *t*-test was used to analyze the effects (pre- vs. post-) of the first phase. For the second phase, two-way analysis of covariance (ANCOVA) for repeated measures was applied for comparisons, with baseline scores used as covariates. When the F-ratio was significant, the Bonferroni post hoc test was employed to identify the mean differences. The effect size (ES) was calculated to verify the magnitude of the differences, with an ES of 0.20–0.49 considered as small, 0.50–0.79 as moderate, and ≥ 0.80 as large (Cohen, 1992). For all statistical analyses, significance was accepted at $P < 0.05$. The data were analyzed using SPSS software version 20.0 (SPSS, Inc., Chicago, IL, USA).

The sample size estimation was conducted using G*Power (version 3.0.10, Universitat Kiel, Germany). Data from a previous investigation (Børsheim et al., 2008) were utilized to perform the sample size estimation. We based the calculation on an ES of 0.33, α level of 0.05, and power (1 − β) of 80%, giving a total of 72 volunteers required. Considering a drop-out of ˜15%, 83 older women were recruited.

Table 2
Participant scores pre and post the 8-week intervention (first phase) (n = 70).

	Pre	Post	Δ%	ES	*P*
LST (kg)	34.7 ± 5.5	35.1 ± 5.0	2.6	0.17	< 0.001
ECW (L)	13.4 ± 2.1	13.2 ± 2.1	−1.4	0.08	< 0.001
ICW (L)	13.9 ± 2.7	14.2 ± 2.5	1.9	0.09	< 0.001
TBW (L)	27.4 ± 4.5	27.5 ± 4.5	0.3	0.02	< 0.001
ECW/ICW ratio	0.97 ± 0.11	0.93 ± 0.09	−3.6	0.33	< 0.001
Resistance (ohms)	603.9 ± 76.1	591.8 ± 73.0	−2.0	0.16	< 0.001
Reactance (ohms)	53.5 ± 9.2	55.1 ± 8.4	2.9	0.17	< 0.001
PhA (degree)	5.1 ± 0.8	5.3 ± 0.6	4.6	0.34	< 0.001

Note: Paired *t*-test. Data are expressed as mean and standard deviation. ES = effect size; LST = lean soft tissue; ECW = extracellular water; ICW = intracellular water; TBW = total body water; PhA = phase angle.

3. Results

The general characteristics of the supplementation groups are described in Table 1.

The changes from pre to post first 8 weeks of RT (first phase) are displayed in Table 2. Changes (P < 0.001) in all outcomes were observed after this first phase of 8 weeks of RT.

Table 3 displays the changes from pre- to post-intervention after the second intervention phase. A group by time interaction was observed ($P < 0.05$) for LST, ICW and the ECW/ICW ratio, in which the WP groups presented higher increases when compared with the placebo-placebo. There was a time effect interaction ($P < 0.05$) for TBW, reactance, and PhA.

4. Discussion

The main finding of this investigation was that supplementation with WP combined with RT resulted in increases in ICW and LST and reductions in ECW/ICW ratio in the WP groups, compared to the placebo group. Thus, our hypothesis that the effects of combined supplementation plus RT would be superior to the placebo was partially confirmed.

To our knowledge, only one investigation has evaluated the effects of WP plus RT on cellular hydration (Eliot et al., 2008), in which protein supplementation was found to reduce ECW without effects on LST. However, the sample consisted of middle-aged men and the results cannot be extrapolated to other populations.

Our investigation showed that WP resulted in a significant increase

Table 1
General characteristics, dietary intake, and clinical history of the participants at baseline by supplementation group (n = 66).

	Whey protein-placebo (n = 22)	Placebo-whey protein (n = 21)	Placebo-placebo (n = 23)	*P*
General characteristics[a]				
Age (years)	67.5 ± 5.2	66.2 ± 9.4	66.5 ± 7.1	0.825
Body mass (kg)	69.0 ± 14.8	65.4 ± 16.6	62.2 ± 10.4	0.271
Height (cm)	156.8 ± 4.6	155.5 ± 6.5	155.7 ± 5.4	0.698
Body mass index (kg/m^{-2})	26.3 ± 5.2	25.3 ± 5.4	23.8 ± 3.7	0.256
Dietary intake[a]				
Protein (g/kg/day)	0.92 ± 0.20	0.94 ± 0.36	0.95 ± 0.27	0.949
Carbohydrates (g/day)	203.3 ± 48.8	202.7 ± 45.5	187.1 ± 35.9	0.374
Lipids (g/day)	51.9 ± 18.1	47.4 ± 13.1	41.2 ± 10.0	0.060
Total energy (kcal/day)	1532 ± 347	1467 ± 283	1348 ± 239	0.111
Sodium (mg/day)	1820.7 ± 753.7	1764.0 ± 666.3	1509.6 ± 306.1	0.190
Potassium (mg/day)	2219.0 ± 663.1	2343.8 ± 658.3	2097.3 ± 494.4	0.410
Clinical history[b]				
Hypertension (n)	10	12	11	0.721
Dyslipidemia (n)	10	14	15	0.280
Type 2 diabetes (n)	03	03	03	0.933

Note. Data are presented as mean and standard deviation. Clinical history is presented as absolute number.
[a] ANOVA one-way.
[b] chi-square test.

Table 3
Participant scores at baseline (pre) and after (post) the 12-week intervention (second phase) (n = 66).

	Whey protein-placebo (n = 22)				Placebo- whey protein (n = 21)				Placebo-placebo (n = 23)				Interaction *P*-value
	Pre	Post	Δ%	ES	Pre	Post	Δ%	ES	Pre	Post	Δ%	ES	
LST (kg)	36.5 ± 5.2	37.6 ± 5.1*§	2.7	0.19	35.1 ± 6.0	36.5 ± 6.1*§	3.7	0.22	35.6 ± 3.5	36.2 ± 3.7*	1.5	0.17	< 0.001
ECW (L)	13.7 ± 2.0	13.4 ± 1.9	−2.4	0.16	13.4 ± 2.6	13.1 ± 2.5	−2.6	0.13	12.6 ± 1.6	12.3 ± 1.4	−2.1	0.17	0.937
ICW (L)	15.0 ± 2.4	15.9 ± 2.1*§	5.5	0.36	14.1 ± 2.9	15.0 ± 2.9*§	6.6	0.30	13.5 ± 2.0	13.9 ± 2.1*	3.5	0.21	< 0.001
TBW (L)	28.8 ± 4.1	29.3 ± 3.9*	1.8	0.13	27.5 ± 5.4	28.1 ± 5.4*	2.1	0.11	26.1 ± 3.5	26.3 ± 3.4*	0.7	0.05	0.473
ECW/ICW ratio	0.92 ± 0.12	0.85 ± 0.08*§	−8.0	0.71	0.96 ± 0.09	0.87 ± 0.07*§	−9.1	1.05	0.93 ± 0.06	0.89 ± 0.07*	−4.9	0.71	0.014
R (ohms)	573.7 ± 68.5	563.2 ± 61.2	−1.8	0.16	579.4 ± 85.1	567.3 ± 82.2	−2.1	0.15	620.2 ± 58.2	607.9 ± 61.1	−2.0	0.21	0.885
Xc(ohms)	53.5 ± 8.6	56.6 ± 8.8*	5.9	0.36	54.5 ± 9.4	58.2 ± 9.8*	6.7	0.38	57.6 ± 6.2	60.5 ± 5.6*	5.0	0.48	0.701
PhA (degree)	5.3 ± 0.7	5.7 ± 0.7*	7.6	0.58	5.4 ± 0.6	5.8 ± 0.5*	8.8	0.85	5.3 ± 0.5	5.7 ± 0.5*	7.3	0.78	0.545

Note: ANCOVA two-way. Data are expressed as mean and standard deviation. ES = effect size; ECW = extracellular water; ICW = intracellular water; TBW = total body water; R = resistance; Xc = reactance; PhA = phase angle. *$P < 0.05$ vs. pre training; §$P < 0.05$ vs. placebo-placebo.

in ICW and decrease in the ECW/ICW ratio. A possible mechanism that may explain the effect of WP on ICW may be related to the increase in LST. Hydrolyzed WP is rapidly digested and absorbed and also contains high leucine content, a key anabolic amino acid for muscle protein synthesis (Devries & Phillips, 2015). Thus, an increase in LST will result in an increase in ICW, since it is a well-hydrated tissue, contributing to the decrease in the ECW/ICW ratio. Moreover, we found a positive correlation between changes in LST and ICW ($r = 0.28$, $P < 0.05$), which is in agreement with previous work from our laboratory with RT in a different cohort of older adult women (Souza et al., 2016).

No differences between groups were found in changes in PhA. However, a main effect of time was observed, i.e., RT improved PhA, which is in agreement with previous investigations that concluded that RT enhances PhA in older women (Ribeiro et al., 2017; Souza et al., 2016). The mechanisms that explain the influence of RT on PhA are still not well elucidated. However, some facts may be explained this phenomenon. Phase angle is derived from resistance and reactance, both influenced by the electrical conductivity of the tissues, thus an alteration in cellular membrane integrity (reactance) or body fluid (resistance) consequently results in PhA changes (Kyle et al., 2004; Xu et al., 2016). It is worth noteworthy that the reduction observed in reactance in this study occurred without significant changes in resistance values probably led to an increase in PhA. Gonzalez et al (Gonzalez, Barbosa-Silva, Bielemann, Gallagher, & Heymsfield, 2016) documented that LST is the body composition compartment that most influences PhA, both in men and women. When an electrical current passes through a biological system at a certain frequency, the LST act as conductors, mainly due to the large amount of water and electrolytes these tissues contain. Moreover, the increase in ICW, due to an increase in LST, also results in better conductivity, contributing to improve PhA.

This investigation is not without limitations. The results are specific to healthy pre-conditioned older women and should not be extrapolated to other populations. Physical activity was not assessed during the free-living condition, although performance of RT beyond that provided by the university was not expected. A major strength of our investigation was the inclusion in the design of a first phase of standardization of participants' training status as well as the randomized, double-blind, placebo-controlled design.

Whey protein supplementation (pre- or post-) combined with RT promoted an increase in ICW and LST and a reduction in ECW/ICW ratio in pre-conditioned older women. Regardless of the supplementation intake, the RT regimen improved PhA in older adult women. These findings suggest that WP supplementation is a good strategy to maximize the response to RT in pre-conditioned older adult woman, in particular cellular hydration.

Disclosure statement

The authors declare no conflict of interest.

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