Original article

Effect of whey protein supplementation combined with resistance training on body composition, muscular strength, functional capacity, and plasma-metabolism biomarkers in older women with sarcopenic obesity: A randomized, double-blind, placebo-controlled trial

Hellen C.G. Nabuco [a, b, *], Crisieli M. Tomeleri [b, c], Rodrigo R. Fernandes [b],
Paulo Sugihara Junior [b], Edilaine F. Cavalcante [b], Paolo M. Cunha [b], Melissa Antunes [b],
João Pedro Nunes [b], Danielle Venturini [d], Décio S. Barbosa [d], Roberto Carlos Burini [e],
Analiza M. Silva [f], Luís B. Sardinha [f], Edilson S. Cyrino [b]

[a] Federal Institute of Science and Technology of Mato Grosso, Highway BR-364, Km 329, Cuiabá, MT 78106-970, Brazil
[b] Metabolism, Nutrition, and Exercise Laboratory, Physical Education and Sport Center, Londrina State University, Highway Celso Garcia Cid, Londrina, Paraná 86057-970, Brazil
[c] Faculty of Physical Education, University of Campinas, Érico Veríssimo Avenue, Campinas, São Paulo 13083-970, Brazil
[d] Clinical Analyses Laboratory, Londrina State University, Highway Celso Garcia Cid, Londrina, Paraná 86057-970, Brazil
[e] Exercise and Nutrition Metabolism Center, Department of Public Health, Botucatu School of Medicine, UNESP, Botucatu, São Paulo 18618-687, Brazil
[f] Exercise and Health Laboratory, CIPER, Faculdade de Motricidade Humana, Universidade de Lisboa, Estrada da Costa, Cruz Quebrada, Dafundo 1499-002, Portugal

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SUMMARY

Backgrounds & aims: Obesity and sarcopenia are independent illnesses associated with contemporary dietary and physical activity behaviors, aggravated by aging. Their coexistence is termed sarcopenic obesity (SO). Hence, increasing protein intake and resistance training (RT) are interventions that could counteract these illnesses. The objective of this investigation was to analyze the effects of whey protein (WP) supplementation associated with RT on body composition, muscular strength, functional capacity, and plasma-metabolism biomarkers in older women with SO.
Methods: Twenty six sarcopenic (appendicular lean soft tissue ALST < 15.02 kg) obese (body fat mass $\geq$ 35%) older women were randomly assigned to receive daily, either 35 g of WP (WP group) or placebo (PLA group), combined with supervised RT (8 exercises, 3×8–12 rep, 3 times a week), during a 12-week protocol. Blood samples, blood pressure, dietary intake, functional capacity tests, the one repetition maximum (1RM) test, and body composition were assessed before and after the intervention period. Two-way analysis of variance for repeated measures was applied for comparisons.
Results: The WP group presented greater ($P < 0.05$) increases in ALST (WP = 6.0% vs. PLA = 2.5%) and decreases in ($P < 0.05$) total (-3.3% vs. -0.3%) and trunk fat mass (WP = -5.1% vs. PLA = -1.1) and IL-6 (WP = -34.6% vs. PLA = 9.3%) compared with the PLA group. Both groups demonstrated improved ($P < 0.05$) scores for muscular strength, waist—hip ratio, functional capacity, and other plasma-metabolism biomarkers without significant differences between conditions.
Conclusion: Whey protein combined with RT increased ALST, and decreased total and trunk fat mass, improving sarcopenia and decreasing SO in older women, with a limited impact on inflammation. Registered under ClinicalTrials.gov Identifier n° NCT03752359.

* Corresponding author. Federal Institute of Science and Technology of Mato Grosso, Antônio Cesário de Figueiredo Street, 460, Cuiabá, Mato Grosso 78032-143, Brazil.
E-mail addresses: hellen.nabuco@svc.ifmt.edu.br (H.C.G. Nabuco), crisielitomeleri@gmail.com (C.M. Tomeleri), rodrigo.r.fernandes@gmail.com (R.R. Fernandes), juniornutricao@hotmail.com (P. Sugihara Junior), edilainefungari@gmail.com (E.F. Cavalcante), pcunha88@hotmail.com (P.M. Cunha), melissa.antunes@hotmail.com (M. Antunes), joaonunes.jpn@hotmail.com (J.P. Nunes), danielle.venturini@bol.com.br (D. Venturini), sabbatini2011@hotmail.com (D.S. Barbosa), burini@fmb.unesp.br (R.C. Burini), analiza@fmh.ulisboa.pt (A.M. Silva), lbsardinha55@gmail.com (L.B. Sardinha), edilsoncyrino@gmail.com (E.S. Cyrino).

1. Introduction

Aging is a natural process associated with modifications in biological systems such as a decline in muscle mass and muscle strength [1]. Another important alteration is an increase in fat mass, mainly in fat free mass (FFM) deposits, such as bones, liver, and muscles [1]. The possible causes for body modifications with aging are estrogen reduction and environmental causes such as muscle disuse through inactivity, malnutrition, and pharmacological interaction, among others [2,3]. The coexistence of very low age-related values of muscle mass (sarcopenia) and increased age-related values of fat mass (obesity), termed sarcopenic obesity (SO) [4,5]. SO is associated with metabolic syndrome, falls, frailty, hypertension, insulin resistance, inflammation, oxidative stress, alterations in the lipid profile, and reduction in muscular functional capacity, cardiovascular diseases, and mortality [4–7].

Although related to age, SO is a process that can be reversed [4]. Both resistance training (RT) and nutrition are interventions that have been shown to improve the quality of life of the elderly. RT increases skeletal muscle mass, muscular strength, and functional capacity and reduces inflammatory biomarkers and body fat [8–10]. Nutritional supplementation has been found to be effective for the management of sarcopenia [11–16].

Given this information, many studies have been evaluated the additive effect of protein supplementation on RT-induced adaptations [17–21]. However, there is considerably less information about obese sarcopenic older and in the current literature there is no consensus as to the effects of protein supplementation on muscular adaptations in older individuals. For example, meta-analyze from Morton et al. [21] concluded that protein supplementation augments strength and fat-free mass, but these effects were not observed in the elderly. However, Liao et al. [18] concluded that protein supplementation combined with RT effectively increases muscle mass in older people regardless of body weight. The discrepancies from these results may be related, at least in part, to some methodological issues, such as the amount of baseline protein consumption, training status of the participants, protein doses and protein sources [21,22].

Whey protein (WP) is rich in leucine, a key amino acid for activation of muscle protein synthesis [23], and WP has also been associated with fat mass reductions [24,25]. When associated with physical activity WP improves FFM, inflammation, functional capacity, muscular strength in sarcopenic older adults [26]. However, there are only two studies that investigated the effects of RT and nutritional supplementation on SO [27,28], and have shown no additional effects of nutritional supplementation on increased FFM in elderly individuals with SO. To our knowledge, no research has been conducted to evaluate the effects of WP combined with RT on older women with SO. Therefore, the purpose of this randomized controlled trial was to investigate the effects of WP following RT on body composition, muscular strength, functional capacity, and plasma-metabolism biomarkers in older women with SO.

2. Material and methods

2.1. Experimental design

This two-arm randomized, double-blind, placebo-controlled design was carried out over a period of 16 weeks. At the beginning and end of the experiment, two weeks were allocated for evaluations consisting of anthropometric (weeks 2 and 16), body composition (weeks 2 and 16), one repetition maximum tests (weeks 1 and 15), functional capacity tests (weeks 1 and 15), blood samples (weeks 2 and 16) and dietary intake measurements (weeks 1 and 15). The anthropometric, body composition, blood samples and dietary

intake measurements were carried out in a temperature-controlled room (22—24 °C), and the RT sessions were conducted at the university training facility. Fig. 1 presents a schematic representation of the women recruitment and allocation adopted in this investigation.

2.2. Participants

Twenty-six Brazilian older women (≥60 years old) volunteered to participate in this investigation. Recruitment was carried out through newspapers, radio advertising, and home delivery of leaflets in the central area and residential neighborhoods. All participants completed health history and physical activity questionnaires and were included in the study if they met the following inclusion criteria: 60 years old or more, physically independent, free from cardiac or orthopedic dysfunction that would prevent them from performing the prescribed exercise or exercise testing associated with the study, not receiving hormonal replacement therapy, and completed a previous 8-week RT program. In this study, only women with SO were included. SO was defined as a body fat mass ≥35% [29] combined with appendicular lean soft tissue (ALST) less than <15.02 kg [30], assessed by dual X-ray energy absorptiometry (DXA). 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.

Exclusion criteria were as follows: individuals with any metallic implant or artificial pacemaker, who had undergone surgery, who were unable to perform the tests and training sessions, volunteers who failed the medical examination for any reason, and refused or were unable to give consent. After the assessments, the participants were randomly divided into two groups according to relative strength (ratio of total strength obtained in 1-RM tests by body mass): whey protein (WP, $n = 13$) or placebo (PLA, $n = 13$). A blinded researcher was responsible for generating random numbers for participant allocation. Both groups were submitted to the same RT program and all participants completed the experiment. Written informed consent was obtained from all participants after a detailed description of investigation procedures was provided. This investigation was conducted according to the Declaration of Helsinki and was approved by the local University Ethics Committee (n° 1.700.756).

2.3. Anthropometry

Body mass, height, waist circumference (WC), and hip circumference (HP) were obtained according to previously described procedures [31]. The body mass index was calculated as the body mass in kilograms divided by the square of the height in meters. The waist—hip ratio was calculated by dividing WC by HP. A previous test—retest of 12 older women measured 24—48 h apart resulted in a standard error of measurement (SEM) of 0.51 and 0.38 for WC and HP, respectively, with intraclass correlation coefficient (ICC) > 0.99 for both variables.

2.4. Body composition

Whole-body dual-energy X-ray absorptiometry (Lunar Prodigy, model NRL 41990, GE Lunar, Madison, WI) was used to assess appendicular lean soft tissue (ALST), lower LST, total LST, total body fat mass, percentage of body fat mass (%BF), and trunk fat mass according to previously described procedures [9]. DXA-scan was performed in a non-fasted state. Previous test—retest scans of 12 older women measured 24—48 h apart resulted in an SEM of 0.19 kg for ALST, 0.10 kg for lower LST, 0.25 kg for total LST, 0.10 kg for total body fat mass, 0.25% for %BF, and 0.67 kg for trunk fat mass, with an ICC > 0.99 for all these variables.
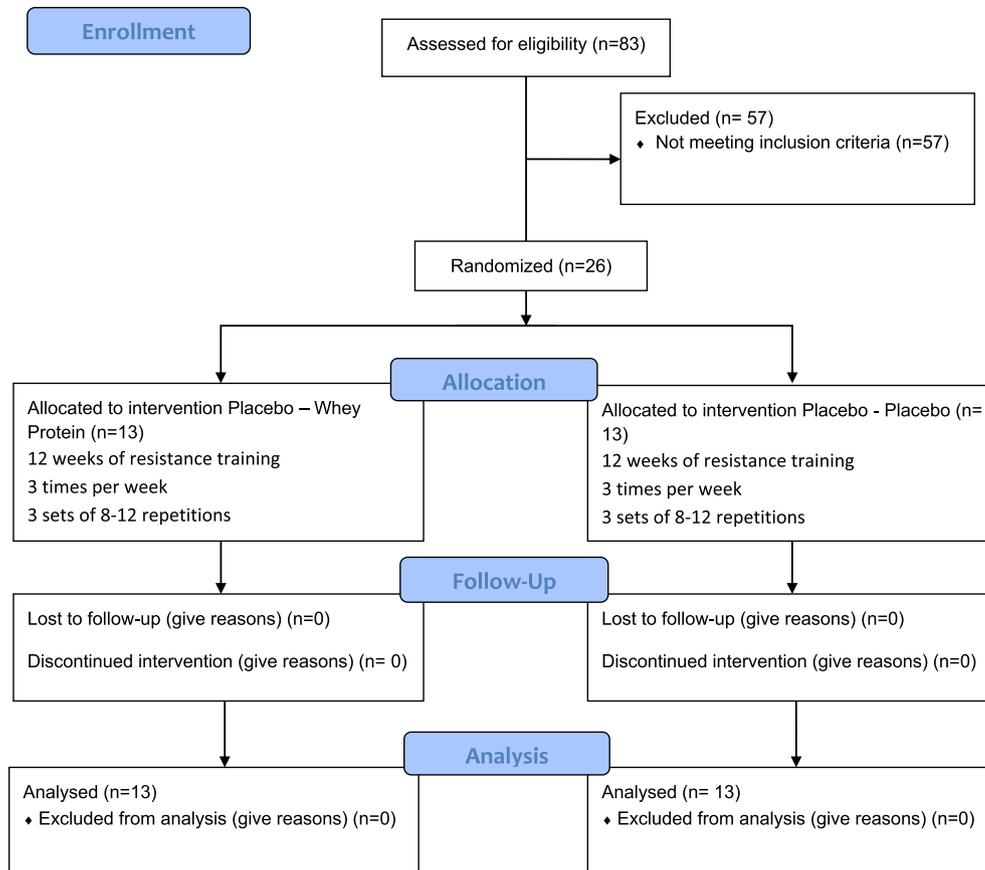
Fig. 1. Flow chart of the study.

2.5. Muscular strength

Maximal dynamic strength was evaluated using the 1RM tests assessed in the chest press, knee extension, and preacher curl exercises, performed in this exact order. Testing for each exercise was preceded by a warm-up set (6—10 repetitions), with approximately 50% of the estimated load used in the first attempt of the 1RM. The subjects were instructed to try to accomplish two repetitions with the imposed load and were given three attempts in both exercises. The rest period was 3—5 min between each attempt, and 5 min between exercises. The 1RM was recorded as the final load lifted in which the subject was able to complete only one single maximal execution. Three 1RM sessions were performed separated by 48 h. The highest load achieved in the 3 sessions was used for analysis in each exercise. Total strength was determined by the sum of the three. The SEMs for chest press, knee extension, and preacher curl were 0.46 kg, 1.67 kg, and 0.93 kg, respectively and the ICCs for chest press, knee extension, and preacher curl were 0.97, 0.91, and 0.93, respectively.

2.6. Functional capacity

Functional capacity was evaluated using two tests from the Latin American Development Group for Maturity protocol [32]: the 10-m walk test (10 MW) and rising from the sitting position test (RSP). For the 10 MW, participants were required to walk a distance of 10 m to evaluate gait speed. Participants initiated the walk two meters before the start line and stopped two meters after the end line. This procedure avoided the influence of initial acceleration and final deceleration. For the RSP, they were required to stand up and

sit down five times consecutively. The rest interval between tests was 3 min. A digital timer was used to record the time of the tests. The SEMs for the 10 MW and RSP were 0.12 s and 0.15 s, with ICCs of 0.94 and 0.98, respectively.

2.7. Biochemical analysis

Venous blood samples were collected between 7:00 am and 8:20 am after a 12 h fast and at least 72 h after the last physical exercise session, according to previously described procedures [9,33,34], to determine glucose, total cholesterol (TC), high-density lipoprotein (HDL-c), triglycerides (TG), albumin, high-sensitivity C-reactive protein (CRP), Tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), Advanced Oxidation Protein Products (AOPP), and Total Radical-trapping Antioxidant Parameter (TRAP). For determination of the LDL-c, the Friedewald equation was used: LDL-c $=$ TC $-$ (HDL-c $+$ TG/5). Insulin was determined by the chemiluminescence method using LIAISON equipment and the Homeostasis Assessment Model (HOMA-IR) was calculated by the formula: Insulin fasting (μUI/mL) $\times$ Glucose fasting (mmol/L)/22.5. All samples were determined in duplicate to guarantee the precision of the results. Inter- and intra-assay coefficients of variation were <10% as determined in human plasma.

2.8. Blood pressure

Resting blood pressure (BP) measurements were performed using automatic, oscillometric equipment (Omron HEM-7113). Participants attended the laboratory on three different days and, during each visit, remained seated at rest for 10 min with the cuff of

the equipment in place on the right arm, according to previously described procedures [8]. The procedures followed the recommendations of the VI Brazilian Guidelines on Hypertension [35]. The SEMs for SBP and DBP were 1.33 mmHg and 1.11 mmHg, with ICCs of 0.99 and 0.98, respectively.

2.9. Dietary intake

Food consumption was assessed by the 24-h dietary recall method applied on two non-consecutive days of the week, with the aid of a photographic record taken during an interview [36]. The homemade measurements of the nutritional values of food and supplementation were converted into grams and milliliters by the online software Virtual Nutri Plus (Keeple®, Rio de Janeiro, Rio de Janeiro, Brazil) for diet analysis. Some foods were not found in the program database and these items were added from food tables [37].

2.10. Supplementation protocol

Participants received a dose of 35 g of hydrolyzed whey protein (Lacprodan®, Arla Foods, Viby J, Aarhus, Jutland, Denmark) and/or placebo post every training session. Maltodextrin (New Millen®, São Paulo, SP, Brazil) was used as a placebo. 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 study staff and were instructed to drink the solution. Supplementation was consumed only on training days. Both the subjects and the researchers responsible for the RT were blinded as to which supplement was given until the end of the trial.

2.11. 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 [38,39]. Physical education professionals with experience in supervising RT programs directed the intervention, ensuring consistent and safe exercise procedures. 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. The participants were submitted to a conventional RT alternated by segment, which consisted of the execution of three series of 8—12 repetitions, with fixed loads. Throughout the investigation, instructors adjusted the loads of each exercise according to the subject's abilities and improvements in exercise capacity in order to 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 [38]. The loads were adjusted individually for each exercise during the 12 weeks where the upper limit of programmed repetitions (12 RM) was reached in 2 consecutive sessions in the 3 sets. Initial load for each exercise increases in load range from 2 to 5% for upper-limb exercises and 5—10% for lower-limb exercises, as recommended in the literature [38,40].

2.12. Statistical analyses

The Shapiro Wilk test was used to test data distribution. Data are presented as means, standard deviation, and Z-score. The student's independent t-test and chi-square test were used to compare groups regarding the general characteristics and clinical/medical history (categorical variables). Two-way analysis of variance (ANOVA) for repeated measures was used to assess between group comparisons. The effect size (ES) was calculated to verify the magnitude of the differences by Cohen's d, where an ES of 0.20—0.49 was considered as small, 0.50—0.79 as moderate, and ≥0.80 as large [41]. The Z-score of the percentage changes (from pre-to post-training) of the raw data for each parameter was calculated, as well as a total Z-score, derived from all the components. To verify the differences between groups in total Z-scores, an independent T test was also applied. 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).

3. Results

The sample characteristics are described in Table 1. Habitual daily energy and macronutrient intake were not different ($P > 0.05$) between groups. Overall, at baseline, there were no significant differences ($P > 0.05$) between groups for general characteristics and medical history.

Daily intake of total energy and macronutrients at pre- and post-12 week RT are shown in Table 2. There were no significant ($P > 0.05$) main effects, indicating that the habitual daily energy and macronutrients intake were not different between groups and did not change over time.

Table 3 presents body composition and anthropometric measures of the participants at pre and after 12 weeks of intervention. A significant interaction was observed ($P < 0.05$) for total LST, lower LST, ALST, total fat mass (absolute and relative), and trunk fat mass, with WP group presenting greater increases in ALST (6.0% vs. 2.5%) and decreases in total (−3.3% vs. −0.3%), relative (−2.8% vs. −0.3) and trunk (−5.1% vs. −1.1%) fat mass compared with the PLA group. Both groups improved ($P < 0.05$) their scores for WC and waist—hip ratio, without significant differences between conditions ($P > 0.05$).

After the intervention, the reduction in body fat mass was not large enough to remove either group from the obesity zone (≥35% body fat mass) (Table 3). However, Fig. 2 shows that WP group significantly reduced the frequency of sarcopenia (13 to 6). Overall, after the intervention, the SO decreased in both groups, with WP group presenting a significant reduction (61.5%, $P < 0.05$).

Table 1
General characteristics of the sample at baseline according to supplementation group in older women ($n = 26$).

	Whey protein ($n = 13$)	Placebo ($n = 13$)	P
General characteristics[a]			
Age (year)	68.0 ± 4.2	70.1 ± 3.9	0.785
Body mass (kg)	61.6 ± 6.8	62.9 ± 8.1	0.960
Height (m)	1.53 ± 0.04	1.52 ± 0.05	0.337
Body mass index (kg/m^2)	26.4 ± 3.0	27.4 ± 3.0	0.395
Medical history[b]			
Hypertension (n)	10	09	0.705
Dyslipidemia (n)	07	07	1.00
Type 2 diabetes (n)	02	03	0.619
Medical treatment[b]			
Antidiabetic agents (n)	02	03	0.619
ACE − inhibitors/angiotensin II-antagonists (n)	04	05	0.680
Diuretics (n)	01	01	1.0
Calcium blockers (n)	01	0	0.308

Note. The general characteristic values are displayed as means and standard deviation. Medical history is presented as an absolute number.
[a] Student's independent t-test.
[b] Chi-square test.

Table 2
Habitual dietary intake (without supplementation) at pre and post 12 weeks of intervention ($n = 26$).

	Whey protein ($n = 13$)				Placebo ($n = 13$)				Interaction P-value	Between ES
	Pre	Post	Δ%	ES	Pre	Post	Δ%	ES		
Protein (g/kg/d)	0.93 ± 0.36	1.0 ± 0.23	7.5	0.24	0.97 ± 0.28	1.0 ± 0.19	3.1	0.13	0.436	0.11
Carbohydrates (g/d)	212.6 ± 29.3	209.6 ± 38.9	−1.4	0.09	224.4 ± 28.7	220.9 ± 27.9	−1.6	0.12	0.945	0.03
Lipids (g/d)	51.8 ± 7.1	54.3 ± 11.9	4.7	0.25	50.1 ± 7.3	53.7 ± 11.7	7.2	0.38	0.755	0.13
Energy (kcal/d)	1539 ± 174	1585 ± 224	3.0	0.23	1589 ± 154	1618 ± 162	+1.8	0.18	0.761	0.05

Note: Data are expressed as mean and standard deviation. ES = effect size. *$P < 0.05$ vs. pre-intervention; §$P < 0.05$ vs. placebo.

Table 3
Body composition and anthropometric measures at pre and post 12 weeks of intervention ($n = 26$).

	Whey protein ($n = 13$)				Placebo ($n = 13$)				Interaction P-value	Between ES
	Pre	Post	Δ%	ES	Pre	Post	Δ%	ES		
Total LST	31.8 ± 2.8	33.0 ± 2.9*§	3.8	0.42	32.0 ± 2.9	33.5 ± 2.8*	1.0	0.11	<0.001	1.37
Lower LST	10.7 ± 0.9	11.2 ± 0.9*§	4.8	0.57	10.9 ± 0.9	11.0 ± 0.8*	1.3	0.17	<0.001	1.23
ALST (kg)	13.9 ± 0.9	14.7 ± 1.1*§	6.1	0.81	13.9 ± 0.8	14.2 ± 0.8*	2.4	0.41	<0.001	1.51
Total fat mass (kg)	23.8 ± 5.4	23.0 ± 5.3*§	−3.3	0.15	23.8 ± 5.9	23.7 ± 6.4	−0.3	0.01	<0.05	0.85
Total fat mass (%)	40.9 ± 4.1	39.7 ± 4.1*§	−2.8	0.22	39.6 ± 4.4	39.5 ± 4.9*	−0.3	0.02	<0.05	0.71
Trunk fat mass (kg)	13.6 ± 3.4	13.0 ± 3.3*	−4.1	0.16	13.9 ± 4.2	13.8 ± 4.6	−1.1	0.04	0.125	0.62
WC (cm)	83.4 ± 8.6	82.4 ± 7.9*	−1.2	0.12	84.0 ± 10.3	83.2 ± 9.7*	−1.0	0.08	0.765	0.12
HP (cm)	97.7 ± 6.2	98.0 ± 5.9	0.3	0.05	100.2 ± 5.0	100.8 ± 6.4	0.7	0.12	0.618	0.20
Waist−hip ratio	0.85 ± 0.06	0.84 ± 0.05*	−1.4	0.19	0.84 ± 0.05	0.82 ± 0.04*	−1.7	0.19	0.767	0.11

Note: Data are expressed as mean and standard deviation. ES = effect size; ALST = appendicular lean soft tissue; WC = waist circumference; HP = hip circumference. *$P < 0.05$ vs. pre-intervention; §$P < 0.05$ vs. placebo.
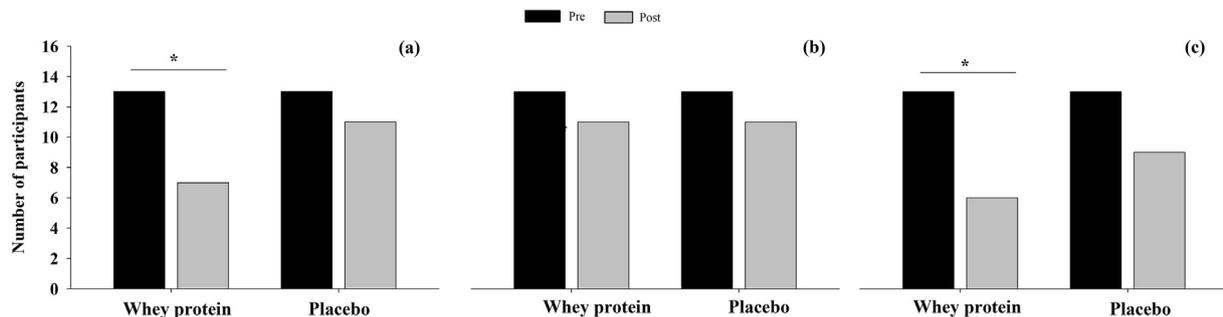

Fig. 2. Changes in prevalence of (a) sarcopenia, (b) obesity, and (c) sarcopenic obesity after the intervention by group. *$P < 0.05$ vs. pre intervention.

Muscular strength and functional capacity outcomes (12-weeks) are presented in Table 4. Both groups improved ($P < 0.05$) their scores for knee extension, chest press, preacher curl, total strength, 10 MW and RSP without significant differences between conditions ($P > 0.05$).

Both groups presented comparable biochemistry profiles except for a significant interaction observed ($P < 0.05$) for IL-6, with a greater decrease in the WP group compared with the PLA (Table 5). Resistance training increased HDL-c and TRAP, and also reduced glucose, TG, AOPP, CRP, and TNF-alpha, without affecting LDL-c, insulin or HOMA-IR.

The composite Z-scores of percent change from pre-to post-training, according to groups. In the WP group a greater change was found in the composite Z-scores compared to the PLA ($P < 0.05$) (total Z-score: WP = 0.15 ± 0.2; PLA = −0.14 ± 0.3).

4. Discussion

In our randomized controlled trial, we found that WP combined with RT promotes greater increases in ALST and greater decreases in total, relative and trunk fat mass. Both groups improved their scores for muscular strength, waist−hip ratio, functional capacity, and other plasma-metabolism biomarkers, without significant differences between conditions. Regarding the metabolic profile we only observed a decrease in IL-6.

Maltais et al. [27] and Kim et al. [28] did not find additional effects of nutritional supplementation on increased FFM in elderly individuals with SO. We speculate that differences between our results and those of previous studies may be related to the type and dose of the prescribed supplement. Maltais et al. [27] prescribed a dose of 12 g of protein (7 g of essential amino acids − EAA) and Kim et al. [28] offered a dose of 3 g of EAA (1.2 g of leucine). It has been found that older adults are not responsive to ~7 g of EAA ingestion containing ~1.7 g of leucine, however when the leucine dose is increased to ~2.8 g, the muscle protein synthesis increases [42]. On the other hand, Rondanelli et al. have shown that whey protein associated with physical activity improves FFM in sarcopenic older adults [26]. Sugihara Junior et al. [43] showed that whey protein (35 g WP and 3.8 g leucine) post-RT increased skeletal mass and muscular strength in older women.

We provided a 35 g-dose of WP, containing 3.8 g of leucine, as this amount was effective for the stimulation of muscle protein synthesis (≥ 2 g leucine) [21,44]. It has been suggested that 30−35 g of WP is required to allow appropriate stimulation of postprandial muscle protein synthesis in older individual [45,46]. In addition, WP is a fast protein to digest, promoting rapid release of amino

Table 4
Functional capacity and muscular strength at pre and post 12 weeks of intervention (n = 26).

	Whey protein (n = 13)				Placebo (n = 13)				Interaction P-value	Between ES
	Pre	Post	Δ%	ES	Pre	Post	Δ%	ES		
10 MW (s)	7.4 ± 0.8	6.9 ± 0.8*	−6.7	0.60	7.4 ± 0.8	6.8 ± 0.6*	−7.6	0.77	0.792	0.10
RSP (s)	12.1 ± 1.0	10.7 ± 1.4*	−11.5	1.17	11.9 ± 2.1	10.7 ± 1.6*	−10.1	0.66	0.694	0.15
Knee extension (kg)	48.7 ± 10.8	51.8 ± 10.9*	6.3	0.28	50.9 ± 9.9	53.4 ± 9.4*	4.8	0.25	0.347	0.38
Chest press (kg)	42.8 ± 8.3	44.8 ± 8.6*	4.7	0.24	40.9 ± 7.2	42.8 ± 7.1*	4.5	0.26	0.696	0.16
Preacher curl (kg)	21.0 ± 4.0	23.7 ± 4.3*	12.4	0.63	19.5 ± 2.5	21.5 ± 2.9*	10.2	0.74	0.274	0.44
Total strength (kg)	112.6 ± 20.9	120.3 ± 21.7*	6.8	0.36	111.4 ± 17.4	117.7 ± 17.0*	5.7	0.37	0.248	0.46

Note: Data are expressed as mean and standard deviation. ES = effect size; 10 MW = 10 m walk test; RSP = rising from sitting position test. *$P < 0.05$ vs. pre-intervention; §$P < 0.05$ vs. Placebo.

Table 5
Participants' scores at baseline (pre) and post 12 weeks of intervention (n = 26).

	Whey protein (n = 13)				Placebo (n = 13)				Interaction P-value	Between ES
	Pre	Post	Δ%	ES	Pre	Post	Δ%	ES		
TC (mg/dL)	200.9 ± 32.1	191.4 ± 29.8	−4.7	0.31	194.1 ± 31.1	186.7 ± 40.7	−3.8	0.21	0.847	0.11
TG (mg/dL)	116.2 ± 41.9	102.9 ± 38.1*	−12.0	0.34	129.6 ± 38.1	118.4 ± 37.6*	−8.7	0.30	0.782	0.11
LDL-c (mg/dL)	121.2 ± 30.4	111.8 ± 25.4	−7.8	0.34	111.9 ± 27.3	108.6 ± 32.7	−3.0	0.11	0.542	0.24
HDL-c (mg/dL)	54.8 ± 13.7	58.6 ± 15.1*	6.9	0.26	55.4 ± 12.5	58.2 ± 12.7*	5.1	0.23	0.689	0.16
Glucose (mg/dL)	110.3 ± 20.9	105.8 ± 22.2*	−4.1	0.36	111.8 ± 18.3	110.7 ± 18.2*	−1.0	0.11	0.251	0.35
Insulin (μU/mL)	10.5 ± 3.8	10.0 ± 4.1	−4.9	0.13	11.0 ± 5.0	10.8 ± 4.9	−1.9	0.04	0.774	0.12
HOMA-IR	2.9 ± 1.4	2.7 ± 1.5	−7.5	0.15	3.5 ± 2.1	3.5 ± 2.3	−0.2	0	0.511	0.26
SBP (mmHg)	123.4 ± 11.0	122.8 ± 12.7	−0.5	0.05	120.3 ± 13.4	122.1 ± 15.8	1.5	0.12	0.451	0.30
DBP (mmHg)	64.7 ± 6.9	63.5 ± 7.7	−1.9	0.17	65.6 ± 6.6	63.6 ± 8.5	−3.0	0.26	0.702	0.15
TNF-α (pg/mL)	3.6 ± 1.8	2.3 ± 1.6*	−34.0	0.27	4.0 ± 2.5	3.4 ± 3.2*	−16.9	0.18	0.503	0.26
IL-6 (pg/mL)	3.3 ± 1.2	2.1 ± 0.6*§	−34.6	1.21	3.2 ± 1.4	2.9 ± 1.6*	−9.3	0.20	0.038	0.86
CRP (mg/L)	2.9 ± 1.3	2.2 ± 1.0*	−24.5	0.62	2.6 ± 1.7	2.2 ± 2.1*	−13.5	0.18	0.181	0.54
AOPP (μmol/L)	99.4 ± 24.3	68.8 ± 19.7*	−30.8	1.39	101.3 ± 32.1	85.4 ± 24.3*	−15.7	0.57	0.152	0.58
TRAP (μmol Trolox®)	883.7 ± 154.1	956.7 ± 161.6*	+8.3	0.46	855.3 ± 53.3	909.9 ± 86.2*	+6.4	0.78	0.553	0.23

Note: Data are expressed as mean and standard deviation. ES = effect size; TC = total cholesterol; TG = triglycerides; LDL-c = low density lipoprotein; HDL-c = high density lipoprotein; SBP = systolic blood pressure; DBP = diastolic blood pressure; TNF-α = tumor necrosis factor; IL-6 = interleukin-6; CRP = C-reactive protein; AOPP = advanced oxidation protein products; TRAP = total radical-trapping antioxidant potential. *$P < 0.05$ vs. pre intervention. §$P < 0.05$ vs. placebo.

acids into the bloodstream [23], leading to a more pronounced stimulation of muscle protein synthesis rate during the first few hours following ingestion [47] and this is an important feature since slower digestion processes are observed in older people [48].

Overall, the recent literature has shown conflicted effects of protein supplementation following RT on lean body mass. A meta-analysis by Cermak et al. [19] found that, protein supplementation following RT conferred additional benefit for strength and FFM in older individuals. Morton et al. [21] concluded that protein supplementation (different sources) augments strength and FFM, but these effects are less effective with increasing chronological age. Liao et al. [18] concluded that protein supplementation combined with RT effectively increases muscle mass in older people regardless of body weight. Miller et al. [17] investigated specifically the combined effects of WP and RT, and reported a significant increase in lean body mass with WP supplementation. On the other hand, Colonetti et al. [20] failed to demonstrate additional effect of WP in elderly submitted to RT. A recent meta-analysis by Martí-nez-Amat et al. [49] that included only studies with older people with sarcopenic obesity concluded that results do not allow drawing a clear conclusion. Conflicting results from these reviews may be related to methodological differences among the studies, such as concentration of protein per dose, dose of essential amino acids, initial protein intake, days of intake (only on training days or daily), timing of protein intake, length of the intervention and source of protein.

The reduction in total and trunk fat mass observed in the WP is in agreement with previous studies [27,28]. For example, Maltais et al. [27] showed a reduction in total fat mass in the group who consumed dairy shake. Kim et al. [28] found that the exercise combined with nutritional supplementation group lost more adiposity without differences between active interventions (exercise, nutrition, and exercise + nutrition).

We were not able to identify which mechanisms are involved in the mobilization of stored lipids; however, some studies have raised hypotheses. . First, the WP is rich in calcium, contributing to impair dietary fat absorption through the formation of insoluble calcium soaps, thus reducing fat absorption [25]. Second, the high calcium content may have reduced the concentrations of calcio-tropic hormones, which in turn could reduce lipogenesis and increase lipolysis, thereby increasing fatty acid availability for fat oxidation [24,25]. Nevertheless, we did not measure the calcium content of our whey protein and we also did not measure the cal-citropic hormones, but we believe that these mechanisms deserve further investigation. However, even with the reduction in body fat of 2.8% (WP) and 0.3% (PLA) the values remained above 39%; the 7.7% reduction in total-body fat mass was not large enough to remove either group from the obesity zone (≥35% body fat mass). It is well known that RT has limited effects on body-fat loss [50].

Resistance training promoted improvements in muscular strength, 10 MW, and RSP. Previous studies using older individuals highlighted the role of RT as an effective method to improve health-related parameters in older people [9,40,51,52]. Previous works from our laboratory in another cohort of older women have shown that RT increased muscular strength in trained older women [53] and that RT improved osteosarcopenic obesity syndrome parameters in older women [54].

With respect to inflammatory markers, we found an effect of the combined WP with RT on IL-6, without significant changes in TNF-α or CRP. The decrease in IL-6 observed in the WP does not agree with

the previous studies of Maltais et al. [27] and Kim et al. [28]. However, our findings are in accordance with Daly et al. [55], in which showed that beef protein plus RT reduced IL-6 without changes in TNF-α or CRP in healthy elderly women. Moreover, Rondanelli et al. showed that WP associated with physical activity reduces C-reactive protein in sarcopenic older adults [26]. These contradictory results may be related to the characteristics of the population (age, sex, inflammation levels, training status, body composition, presence of diseases, etc.), as well as methodological differences in study design (type of training, duration of intervention, type of supplementation). It has been suggested that a reduction in fat mass could promote a reduction in pro-inflammatory biomarkers [9]. However, our results failed to confirm this hypothesis ($r = 0.1$, $P = 0.68$). Additionally, we also hypothesize that skeletal muscle mass secretes anti-inflammatory myokines that antagonize pro-inflammatory cytokines [56], although this association was not observed in our study ($r = -0.3$, $P = 0.13$). We speculate that the effect of WP on IL-6 is via increased antioxidant capacity, as the inflammatory response is associated with oxidative stress [57]. Whey protein is rich in cysteine, an important precursor of glutathione, which is a potent antioxidant that may counteract the production of reactive oxygen and nitrogen species and also delay signaling pathways that mediate immune cell mobilization [58]. Indeed, although we did not find a significant effect of WP on oxidative stress biomarkers, the WP group presented the largest reduction. Other mechanisms may involve the stimulation of insulin-like growth factor I (IGF-1) production on skeletal muscle mass. Previous studies have shown a positive effect of dairy products on IGF-1 [26,59,60], which has an inverse effect on IL-6 [61,62]. However, these hypotheses need to be tested in other studies.

In the present experiment, RT reduced CRP, TNF-α, TG and glucose as well as the pro-oxidant marker AOPP, and increased TRAP and HDL, an anti-oxidant marker. Previous investigations have shown that RT alone is effective in improving the metabolic and inflammatory profile [8,9,40,54,63], There are some limitations in this study. We used maltodextrin to blind the study. However, it has been used as a control in several studies, since it is a good source of energy and promotes an isocaloric condition. Physical activity was not assessed in free living conditions, but RT beyond that practiced at the university during the intervention was not expected.

Whey protein combined with RT increased ALST, and decreased total, relative and trunk fat mass, improving sarcopenia and decreasing SO in older women, with a limited impact on inflammation. Indeed, in this randomized controlled trial only IL-6 was found to decrease.

Statement of authorship

The authors' responsibilities were as follows: HCGN, PSJ, RRF and ESC: conceived and designed the study; HCGN, PSJ, RR, EFC, and MA recruited the participants; EFC, PMC, JPN, CMT, and MA supervised the resistance training; HCGN, PSJ, and RRF managed the supplements; HCGN analyzed and interpreted the data and wrote the first draft of the manuscript; DV, DSB, RCB, AMS, LBS, and ESC: provided critical revision and important intellectual content. All authors have read and approved the final version of the manuscript.

Conflict of interests

The authors declare that they have no conflict of interests regarding the publication of this paper.

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