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Effects of pre- or post-exercise whey protein supplementation on body fat and metabolic and inflammatory profile in pre-conditioned older women: A randomized, double-blind, placebo-controlled trial

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Abstract *Background and aim:* Protein supplementation and resistance training (RT) are interventions that may counteract decline in muscle mass and increase in fat mass, thus reducing the risk of developing chronic diseases during the aging process. The objective of this study was to investigate the effect of whey protein (WP) pre- or post-RT on metabolic and inflammatory profile in pre-conditioned older women.
Methods and results: Seventy older women participated in this investigation and were randomly assigned to one of three groups: WP pre-RT and placebo post-RT (WP-PLA, n = 24), placebo pre-RT and WP post-RT (PLA-WP, n = 23) and placebo pre and post-RT (PLA-PLA, n = 23). Each group ingested 35 g of PLA or WP pre- and post-RT. RT was carried out over 12 weeks (three times/week; 3 x 8–12 repetition maximum). Body composition, blood pressure, blood samples and dietary intake were assessed pre- and post-intervention. After the intervention, WP groups showed greater improvements in appendicular lean soft tissue (ALST: WP-PLA, 3.1%; PLA-WP, 3.9%; PLA-PLA, 1.8%) and total cholesterol/high density lipoprotein cholesterol ratio (TC/HDL-C: WP-PLA, −12.11%; PLA-WP, −13.2%; PLA-PLA, −0.7) when compared with PLA-PLA. WP post-RT also showed improvements ($P < 0.05$) in ALST/appendicular fat mass ratio (PLA-WP, 5.8%; PLA-PLA, 1.3%), total body fat (PLA-WP, −3.8%; PLA-PLA: −0.1) and trunk fat mass (PLA-WP, −3.1%; PLA-PLA, −0.3%) when compared with PLA-PLA.
Conclusion: WP pre- or post- RT promotes improvements in ALST and TC/HDL-C ratio in pre-conditioned older women. WP administered after RT was more effective in improving metabolic health Z-score and in reducing body fat compared to placebo group.

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Introduction

Aging is a process associated with different physiological alterations, such as a decline in muscle mass (sarcopenia) and increase in fat mass, mainly visceral fat and intramuscular fat [1,2]. As a consequence, older individuals present an increased risk of developing dyslipidemia, type 2 diabetes, metabolic syndrome, and cardiovascular diseases favoring a higher risk of death [3,4]. It is worth noting that advancing age is a major drive to deregulate the cardio-metabolic profile, as sarcopenia affects the metabolic system [5], contributing to chronic and systemic inflammation [4]. Higher plasmatic levels of interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) are found in older adults [6]. Indeed, these inflammatory cytokines are relevant markers of frailty during the aging process in humans [7].

Lifestyle interventions are non-pharmacological therapeutic approaches that may attenuate or counteract the adverse effects of age on metabolic and body composition parameters. Resistance training (RT) has been considered an effective strategy for increasing muscle mass [8,9] and reducing fat mass [9,10], inflammatory markers [8,9] and blood pressure [11], along with improving lipid and glycemic profiles in older people [8,9].

Previous studies have reported that increased dietary protein intake can reduce body fat, glucose, the lipid profile and abdominal fat, as well as increase lean soft tissue (LST) and energy expenditure, in overweight/obese adults, postmenopausal women, adults with hypertension and sarcopenic older adults [12–15]. Among several dietary approaches to increase protein intake, whey protein has been widely used to improve the metabolic profile and LST [13–15], and reduce fat mass [16,17]. However, these studies did not combine whey protein supplementation with RT.

Our laboratory recently showed, in another cohort of women [18,19], that 12 weeks of WP supplementation post-RT performed 3 times per week improved muscle mass, muscular strength and total cholesterol/high density lipoprotein (TC/HDL) ratio in pre-conditioned older women. However, in these studies, we were unable to analyze the impact of timing of protein intake.

In this sense, in a recent study, we have shown that WP pre- or post- RT is effective in promoting increases in skeletal muscle mass and muscular strength, and improves functional capacity in pre-conditioned older women [20]. Nevertheless, we did not analyze the metabolic and inflammatory profiles, which are variables that we believe, can be influenced by the timing of protein supplementation [14]. Moreover, a previous study from our research group showed that low protein consumption is related to altered metabolic syndrome components in older women [21]. In addition, trained individuals respond differently to training stimuli when compared to untrained, both in hypertrophic responses [22] and in metabolic responses [8]. In this sense, we decided to evaluate the effect of protein supplementation combined with RT on inflammatory and metabolic markers, considering an optimal dose for the elderly population, the best ingestion time and the level of training.

Based on the previous findings, our hypothesis was that regardless of the time of whey protein administration, before or after RT, improvements in the metabolic and inflammatory profile would be similar. Therefore, the aim of this investigation was to analyze the effects of whey protein supplementation, pre- or post- RT, on metabolic and inflammatory profile in pre-conditioned older women.

Methods

Experimental design

The design of this three-arm randomized, double-blind, placebo-controlled trial was previously described [20]. Briefly, the investigation 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 [23]. In the second phase (supplementation phase) the participants were randomized into three groups and then started the whey protein supplementation 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 body composition, blood sample and dietary intake measurements. Figure 1 presents the experimental design adopted for the present study.

Participants

The present study is part of the Active Aging Project, a longitudinal cohort study designed to examine the role of RT on older women's health. And this population has also been investigated in a previous study about the effect of whey protein associated with RT on muscle mass, muscle strength and functional capacity [20]. Recruitment was carried as previously described [20]. Briefly, 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 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 study. Participants passed 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

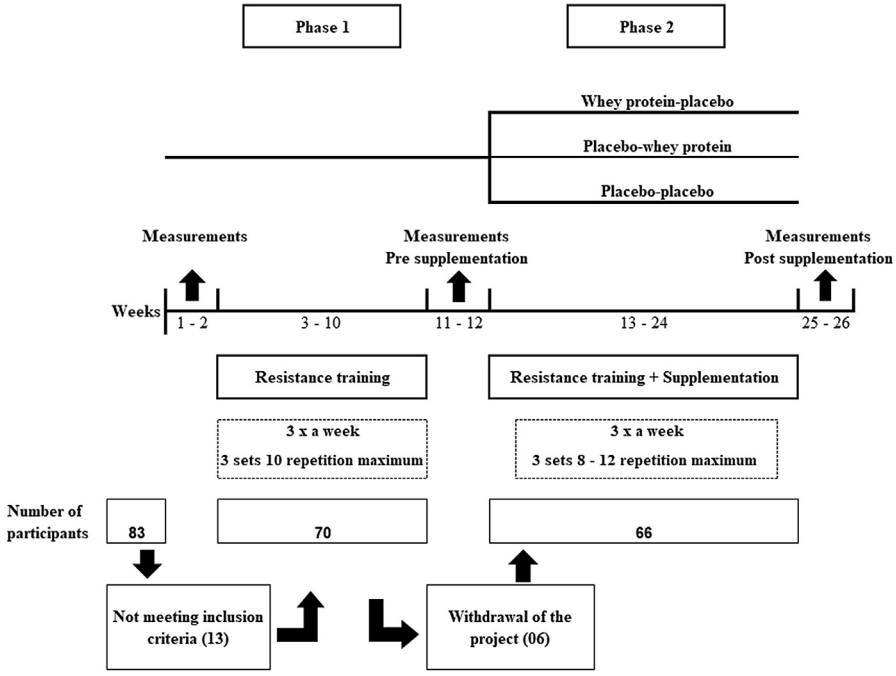

Figure 1 Experimental study design.

groups according to their relative strength (ratio of total strength obtained in 1-repetition maximum tests by body mass): (1) whey protein pre- and placebo post- RT (WP-PLA, n = 24), (2) placebo pre- and whey protein 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 study 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 approved by the local University Ethics Committee (nº 1.700.756).

Anthropometry

Body mass, height, waist circumference (WC) and hip circumference (HP) were measured according to previously described procedures [21]. 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. 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 an intraclass correlation coefficient (ICC) > 0.99 for both variables.

Body composition

Whole-body dual-energy X-ray absorptiometry (DXA) (Lunar Prodigy, model NRL 41990, GE Lunar, Madison, WI) was used to assess appendicular lean soft tissue (ALST), total body fat (BF), trunk fat mass and appendicular fat mass (AFM) according to previously described procedures [9,20]. A SEM of 0.19 for ALST, 0.10 kg for BF, 0.67 kg for trunk fat mass and 0.23 kg for AFM were found, with an ICC > 0.99 for all these variables.

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 performed, according to previously described procedures [9,24,25] to determine glucose, total cholesterol (TC), high-density lipoprotein (HDL-C), triglycerides (TG), high-sensitivity C-reactive protein (CRP), Tumor necrosis factor alpha (TNF-α) and Interleukin-6 (IL-6). For determination of low-density protein (LDL-c) the Friedewald equation was used: LDL-C = TC − (HDL-C + TG/5). Insulin was determined by the chemiluminescence method using LIASON equipment and the Homeostasis Assessment Model (HOMA-IR), calculated by the formula: Fasting insulin (μUI/mL) x Fasting glucose (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.

Blood pressure

The resting blood pressure (BP) assessment was 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 [11,21]. The SEMs for systolic BP and diastolic BP were 1.33 mmHg and 1.11 mmHg, with ICCs of 0.99 and 0.98, respectively.

Dietary intake

Food consumption was assessed by the 24-h dietary recall method applied on two non-consecutive days of the week during a personal interview. A photographic manual of food portion size was used to improve the precision of dietary intake reporting [26]. The homemade measurements of the nutritional values of food and supplementation were converted into grams and milliliters using the online software Virtual Nutri Plus (Keeple®, Rio de Janeiro, RJ, Brazil) for diet analysis. Some foods were not found in the program database and were therefore added from food tables [27].

Supplementation protocol

Participants received a dose of 35 g of hydrolyzed whey protein (Lacprodan®, Arla Foods, Viby J, Aarhus, Denmark) and/or placebo pre and post-RT. Maltodextrin (New Millen®, São Paulo, SP, Brazil) was used as a placebo. The hydrolyzed whey protein 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 study staff and were instructed to drink the solution as quickly as possible. 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.

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 [28]. 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 pre-supplementation training period (first phase), the participants performed training of three series of 10 repetition maximum (RM), in a protocol alternated by segment. During the supplementation plus RT period, the participants were submitted to conventional RT, alternated by segment, which consisted of the execution of three series of 8–12 RM, with fixed loads. Instructors adjusted the loads of each exercise according to subject's ability and improvements in exercise capacity throughout the investigation 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 the procedures described [28]. All groups were asked to maintain similar physical activity as they did before the study. All groups were also assessed by the International Physical Activity Questionnaire (IPAQ) [29] to assess physical activity performed external to the intervention.

Z-score of cardio-metabolic risk factors

A Z-score was calculated for each variable using individual data, and standard deviations of data for the entire group at baseline and post intervention. Metabolic health and inflammatory markers Z-scores were calculated as per the following formulas:

Metabolic health Z-score pre intervention = [(50 − HDL)/14.6+ (TG − 150)/48.4)] + [(fasting blood glucose − 100)/19.8)] + [(total cholesterol − 240)/33.1)] + [(low density lipoprotein − 130)/30.3].

Metabolic health Z-score post intervention = [(50 − HDL)/14.4+ (TG − 150)/40.1)] + [(fasting blood glucose − 100)/19.0)] + [(total cholesterol − 240)/34.4)] + [(low density lipoprotein − 130)/33.9].

Inflammatory markers pre intervention: [(TNF-α − 8.1)/3.6 + (IL-6 − 5.9)/1.6) + (CRP − 0.8)/2.0)].

Inflammatory marker Z-score post intervention = [(TNF-α − 8.1)/4.1 + (IL-6 − 5.9)/1.5) + (CRP − 0.8)/1.6)].

Statistical analyses

The Shapiro Wilk test was used to assess data distribution. Data are presented as mean, standard deviations, Z-scores, and percentage changes. One-way analysis of variance (ANOVA) and the chi-square test were used to compare groups regarding the general characteristics and clinical/medical history (categorical variables). Two-way ANOVA for repeated measures was applied for comparisons pre and post 12 weeks of intervention. The differences between pre-to post-training were calculated, transformed into z-scores, and means for each variable compared by two-way ANOVA. 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 by Cohen's d, with an ES of 0.20–0.49 considered as small, 0.50–0.79 as moderate, and ≥0.80 as large [30]. 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).

Results

Table 1 displays the general characteristics and clinical conditions of the participants at baseline. No differences ($P > 0.05$) were observed among groups for any characteristics at baseline. Results of the IPAQ showed no

Table 1 General characteristics, clinical history and dietary intake of the participants randomly assigned to the three intervention groups, at baseline (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
Total physical activity (minutes/week)	2472 ± 926	2353 ± 858	2372 ± 787	0.935
Clinical history[b]				
Hypertension (n)	10	12	11	0.721
Dyslipidemia (n)	10	14	15	0.280
Type 2 diabetes (n)	03	03	03	0.993
Medical treatment[b]				
Statins (n)	04	04	06	0.776
Calcium blockers (n)	02	01	01	0.765
ACE − inhibitors/angiotensin II-antagonists (n)	09	06	08	0.698
Diuretic (n)	01	02	02	0.801
Antidiabetic agents (n)	03	03	03	0.476
Dietary intake[a]				
Protein (g/kg/d)	0.92 ± 0.21	0.94 ± 0.34	0.95 ± 0.27	0.902
CHO (g/kg/d)	3.1 ± 0.96	3.3 ± 1.1	3.1 ± 0.94	0.703
Lipids (g/kg/d)	0.77 ± 0.29	0.77 ± 0.28	0.70 ± 0.17	0.221
Energy (kcal/kg/d)	22.9 ± 6.3	23.8 ± 7.4	22.4 ± 5.9	0.728

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

significant differences between groups at any point (from pre-to post-training) during the study.

The first eight weeks of the RT period were used to standardize the training level of the participants (Table 2). During first training phase, no supplementation was provided, data are presented separately to show the groups' behavior during the pre-supplementation phase in the same training phase. All groups improved ($P > 0.05$) their scores for ALST, total fat mass, HOMA-IR, TNF-α and IL-6, without significant differences between conditions. In placebo-whey protein group, a main effect of time ($P > 0.05$) was observed for AFM and HDL. In placebo-placebo group, a main effect of time ($P > 0.05$) was observed for HDL, SBP and HC. There were no significant ($P > 0.05$) changes in trunk fat, total cholesterol, triglycerides, LDL, TC/HDL, LDL/HDL, glucose, insulin, diastolic blood pressure, WC, waist-hip ratio and CRP.

Table 3 presents body composition of the participants at baseline and after 12 weeks of intervention. An interaction of time vs. group was observed ($P < 0.001$) for ALST with the whey protein supplementation groups showing greater increases compared with the PLA-PLA group (PLA-WP = 3.9%; PLA-PLA = 1.8%), without differences between the timing of protein intake. Participants supplemented with whey protein post-RT presented a decrease ($P < 0.05$) in total fat mass (−3.1% vs. −0.3%), trunk fat mass (−3.8% vs. −0.1%), and an increase in ALST/AFM ratio (5.8% vs. 1.3%) compared with the PLA-PLA group. In all the groups, a main effect of time ($P < 0.05$) was observed for ALST and ALST/AFM ratio.

Metabolic and inflammatory profile assessed before and after the intervention is presented in Table 4. A time vs. group interaction was observed ($P < 0.001$) for the TC/HDL-C ratio, with both WP-PLA and PLA-WP presenting greater decreases compared with the PLA-PLA, without differences ($P > 0.05$) between the timing of protein intake. In all the groups, a main effect of time ($P < 0.05$) was observed for HDL-c, TC, glucose, the LDL-c/HDL-c ratio, WC, and inflammatory biomarkers.

Figure 2 showed that a significant interaction was observed ($P < 0.05$) for metabolic health Z-score, with placebo-whey protein group showing great improvements when compared with placebo-placebo group. No difference was found in inflammatory marker Z-score.

Discussion

The findings from this investigation showed that combined WP supplementation with RT led a reduction in the TC/HDL-C ratio and an increased in ALST in pre-conditioned older women. In addition, whey protein supplementation after RT significantly improved fat mass and the metabolic Z-score compared with the PLA-PLA. Our hypothesis was partially confirmed, as no significant differences between the WP-PLA and PLA-WP groups were observed.

This is the first study to investigate the effects of whey protein supplementation, administered before and after RT, on fat mass, and the metabolic and inflammatory profile of pre-conditioned older women. Previous studies with older men showed no differences between the time protein intake was administered to evaluated body composition, muscular strength, glucose, 24-h urine collection, myofibrillar degradation and bone resorption [31,32]. However, these studies did not investigate

Table 2 Participants' scores at baseline (pre) and after (post) 8-week intervention (Phase 1) period (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	
Body composition													
ALST (kg)	15.8 ± 1.9	16.3 ± 2.1*	3.1	0.25	15.6 ± 2.7	16.1 ± 2.7*	2.6	0.15	14.6 ± 1.8	15.1 ± 1.9*	2.7	0.21	0.600
AFM (kg)	11.6 ± 3.7	11.2 ± 3.8	−3.5	0.11	11.3 ± 4.5	10.8 ± 4.5*	−5.2	0.13	10.2 ± 3.8	9.8 ± 3.0	−3.1	0.09	0.721
Trunk fat (kg)	15.4 ± 5.7	15.2 ± 6.0	−1.3	0.03	14.1 ± 6.4	14.1 ± 6.7	−0.1	0	13.2 ± 4.7	12.9 ± 4.8	−1.6	0.04	0.476
Total fat mass (kg)	27.1 ± 8.9	25.2 ± 7.8*	−2.2	0.07	25.4 ± 10.8	23.2 ± 8.4*	−2.4	0.06	23.9 ± 7.6	22.9 ± 7.5*	−3.6	0.11	0.605
Lipid profile													
TC (mg/dL)	190.8 ± 40.0	200.2 ± 34.7	4.9	0.25	205.6 ± 32.8	214.4 ± 30.8	4.3	0.28	196.8 ± 28.4	200.8 ± 31.3	2.0	0.13	0.357
TG (mg/dL)	130.8 ± 41.7	132.0 ± 43.1	0.9	0.03	115.6 ± 46.9	111.5 ± 55.4	−3.5	0.08	133.0 ± 55.7	124.6 ± 40.7	−6.3	0.17	0.348
LDL-C (mg/dL)	128.4 ± 51.1	124.1 ± 32.9	−3.3	0.10	129.9 ± 37.6	131.2 ± 28.7	1.0	0.04	127.6 ± 31.1	128.0 ± 27.2	0.3	0.01	0.683
HDL-C (mg/dL)	46.7 ± 11.5	46.5 ± 11.5	−0.5	0.02	55.7 ± 11.7	55.6 ± 12.3*	3.3	0.14	50.4 ± 12.8	52.3 ± 12.9 *	3.7	0.15	0.129
TC/HDL-C	4.3 ± 1.2	4.5 ± 1.2	5.2	0.18	3.7 ± 0.9	3.7 ± 1.1	0	0	4.1 ± 1.2	3.9 ± 1.1	−3.7	0.13	0.081
LDL-C/HDL-C	2.9 ± 1.3	2.4 ± 1.0	−1.9	0.05	2.4 ± 0.9	2.3 ± 0.8	−4.5	0.12	2.6 ± 1.1	2.5 ± 0.9	−2.9	0.08	0.925
Insulin resistance index													
Glucose (mg/dL)	112.1 ± 25.0	106.5 ± 8.8	−5.0	0.33	103.0 ± 9.8	102.8 ± 9.0	−0.2	0.02	107.5 ± 11.4	107.4 ± 11.4	−0.2	0.01	0.319
Insulin (μU/mL)	12.1 ± 4.5	11.6 ± 4.1	−4.1	0.12	11.2 ± 6.9	12.0 ± 6.7	7.3	0.12	10.7 ± 6.2	9.5 ± 5.1	0.5	0.01	0.125
HOMA-IR	3.6 ± 2.0	3.2 ± 1.2*	−11.6	0.26	3.4 ± 3.4	2.8 ± 1.6*	−18.8	0.26	2.9 ± 1.8	2.7 ± 1.6*	−8.1	0.14	0.372
Blood pressure													
SBP (mmHg)	122.4 ± 14.8	119.8 ± 11.3	−2.1	0.20	117.7 ± 12.3	117.6 ± 11.0	−0.1	0.01	122.6 ± 15.1	117.9 ± 12.6*	−3.8	0.34	0.304
DBP (mmHg)	67.8 ± 8.6	67.0 ± 6.8	−1.1	0.09	64.2 ± 7.0	64.6 ± 7.0	0.5	0.05	68.8 ± 6.3	66.3 ± 7.2	−3.7	0.38	0.178
Anthropometric measurements													
WC (cm)	89.4 ± 12.9	88.9 ± 13.7	−0.5	0.03	85.7 ± 12.2	83.7 ± 12.5	−2.3	0.16	83.0 ± 10.2	83.1 ± 10.7	0.10	0.01	0.841
HC (cm)	103.3 ± 9.9	101.9 ± 8.4*	−1.3	0.15	100.7 ± 12.4	100.1 ± 11.2	−0.6	0.06	99.7 ± 8.3	98.4 ± 7.2*	−1.3	0.17	0.644
Waist-hip ratio	0.86 ± 0.05	0.87 ± 0.07	1.0	0.12	0.85 ± 0.04	0.85 ± 0.07	0.3	0.05	0.83 ± 0.05	0.84 ± 0.07	0.4	0.06	0.883
Inflammatory biomarkers													
TNF-α (pg/mL)	4.7 ± 3.8	4.2 ± 3.4*	−10.8	0.14	5.5 ± 4.2	4.7 ± 3.28	−15.2	0.23	5.2 ± 6.7	4.3 ± 3.2*	−17.2	0.18	0.895
IL-6 (pg/mL)	4.4 ± 2.9	3.2 ± 1.8*	−27.4	0.51	3.9 ± 1.1*	3.2 ± 1.4	−17.9	0.56	4.3 ± 4.6	2.9 ± 1.4*	−33.3	0.48	0.199
CRP (mg/L)	2.9 ± 2.8	2.8 ± 1.9	−5.5	0.07	3.4 ± 2.8	3.2 ± 2.6	−6.3	0.08	2.7 ± 2.0	2.6 ± 1.5	−7.4	0.12	0.994

Note: Two-way ANOVA. 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; WC = Waist circumference; HC = Hip circumference; TNF-α = Tumor necrosis factor-α; IL-6 = interleukin-6; CRP = C-reactive protein. *$P < 0.05$ vs. pre intervention. §$P < 0.05$ vs. placebo-placebo. During the Phase 1 no supplementation was provided, data are presented separately to show that both groups demonstrated the same behavior during the pre-supplementation phase in the same training phase.

Table 3 Body composition of the participants before and after Phase 2 intervention, according with supplementation intake (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	
ALST (kg)	16.3 ± 2.1	16.8 ± 2.0*§	3.1	0.25	16.1 ± 2.7	16.7 ± 2.7*§	3.9	0.23	15.1 ± 1.9	15.3 ± 1.8*	1.8	0.14	<0.001
AFM (kg)	11.2 ± 3.8	11.3 ± 3.7	0.9	0.03	10.8 ± 4.5	9.8 ± 3.0	−0.9	0.02	9.8 ± 3.0	9.9 ± 3.1	0.8	0.03	0.581
ALST/AFM ratio	1.62 ± 0.61	1.65 ± 0.64*	2.1	0.06	1.67 ± 0.57	1.76 ± 0.64*§	5.8	0.16	1.64 ± 0.43	1.66 ± 0.44*	1.3	0.05	<0.05
Trunk fat (kg)	15.2 ± 6.0	14.8 ± 5.6*	−2.5	0.06	14.1 ± 6.7	13.5 ± 6.6*§	−3.8	0.08	12.9 ± 4.8	12.9 ± 4.8	−0.1	0	<0.05
Total fat mass (kg)	25.2 ± 7.8	24.9 ± 7.6	−1.2	0.04	23.2 ± 8.4	22.6 ± 8.3*§	−3.1	0.09	22.9 ± 7.5	22.8 ± 7.6	−0.3	0.01	<0.05

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

whether there were differences in the metabolic or in the inflammatory profile of previously resistance-trained individuals. A previous investigation reported no differences between protein intake before or after 2 h of RT on body composition and cardio-metabolic risk factors in middle-aged diabetics [33]. However, in this investigation, participants followed an energy-restricted diet and post-RT supplementation was delayed by 2 h.

Our investigation suggests that whey protein supplementation, administered either before or after RT, was effective for increasing ALST. These data were previously shown and discussed in our previous study which showed the effect of whey protein on muscle mass, functional capacity and muscle strength [20].

Whey protein post-RT reduced total fat mass and trunk fat mass and promoted further increases in the ALST/AFM ratio when compared to the PLA-PLA. It is expected that the improvement in the ALST/AFM ratio was due to the significant increase in ALST and body fat mass reduction. Similarly, a previous study conducted with sarcopenic overweight older men [34] after a 12-week RT protocol, found fat mass reductions and ALST gains without caloric restriction, after dairy supplementation associated with RT. On average, the PLA-WP group lost 0.7 kg of total body fat, partially extending the findings of a previous review showing an average loss of 1.7 kg in 24 weeks in energy-restricted individuals [35].

We observed that regardless of the timing of whey protein administration, a significant reduction occurred in the TC/HDL ratio, an important index of cardiovascular risk compared to TC alone [36]. Previous investigations have demonstrated improvements in lipid metabolism after whey protein supplementation [12–14]. In pre-conditioned older women, high protein intake post-RT also reduces this ratio [19]. Mechanisms that could explain the effects of whey protein on lipid metabolism have not been fully determined. It seems that whey protein inhibit the activity of HMG-CoA reductase (rate-limiting enzyme for cholesterol synthesis) reducing de novo cholesterol synthesis in the liver [16]. Furthermore, reduction in fat mass and especially trunk fat mass, also contributed to improvements in blood markers, since abdominal fat mass has a greater association with metabolic alterations [37]. Improvements in CT and HDL were greater in the groups supplemented with whey protein, however, there was no statistical significance. We speculate that it might be due to the participants being healthy and also the wide variation of the standard deviation. The CT/HDL ratio is a more sensitive measure than the isolated variables, so perhaps only in this variable has statistical significance been reached. However, we must consider that there may have been a type I statistical error, that is, a limitation of our study, to find the result statistically significant, when in fact it may have been at random. Thus, more studies are needed to confirm these data and identify possible mechanisms. Another point to be considered is that the lipid profile even though it was within the normal range was observed important reductions, which should also be considered, since in the elderly population is expected an increase of these parameters [3,4]. Thus, although not reaching the statistical significance between the groups, there was a clinical improvement in all the parameters of the lipid profile.

Previous investigations conducted by our research group found that changes in fat mass (promoted by RT) have more influenced on metabolic and inflammatory profile than lean mass [9,38]. Moreover, RT alone is effective in improving the metabolic and inflammatory profile [9,10], although these improvements may be influenced by the training status [8].

Considering the general improvement in metabolic profile, the PLA-WP presented a superior benefit (Fig. 2a). Although the mechanisms are not well elucidated, we may speculate that digestion processes associated with mechanical stress during RT may have attenuated the effects of whey protein administered before RT. It is possible a longer intervention period (whey protein + RT) may be required for additional benefits of whey protein supplementation in such a healthy and physically active population.

There are some limitations in this study. First, we used maltodextrin to blind the study. Maltodextrin has been used as a control in several studies, since it is a good source of energy and promotes an isocaloric condition. However, maltodextrin may have influenced some outcomes such as glucose, TG, and total fat mass. The whey protein groups also consumed this product and demonstrated improvements. Second, our participants are healthy older individuals, and this may explain in part why we did not observe many changes in metabolic and inflammatory profiles. Finally, it was not possible to objectively monitor (free-living) physical activity levels outside the intervention protocol. However, subjects were asked to

Table 4 Cardio-metabolic risk factors of older women, before and after Phase 2 intervention, according with supplementation intake (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	
Lipid profile													
TC (mg/dL)	200.2 ± 34.7	190.6 ± 33.7*	−4.8	0.28	214.4 ± 30.8	203.2 ± 28.3*	−5.2	0.38	200.8 ± 31.3	201.2 ± 40.2	0.2	0.01	0.157
TG (mg/dL)	132.0 ± 43.1	123.5 ± 41.0	−6.5	0.20	111.5 ± 55.4	105.8 ± 42.0	−5.2	0.12	124.6 ± 40.7	118.4 ± 39.8	−5.0	0.15	0.953
LDL-C (mg/dL)	124.1 ± 32.9	119.4 ± 33.7	−3.8	0.14	131.2 ± 28.7	123.8 ± 32.6	−5.7	0.24	128.0 ± 27.2	127.5 ± 38.5	−0.4	0.02	0.479
HDL-C (mg/dL)	46.5 ± 11.5	50.2 ± 11.2*	7.9	0.32	55.6 ± 12.3	60.3 ± 11.1*	8.3	0.39	52.3 ± 12.9	54.5 ± 13.1*	4.2	0.17	0.382
TC/HDL-C	4.5 ± 1.2	3.9 ± 0.9*§	−12.1	0.51	3.7 ± 1.1	3.3 ± 0.7*§	−13.2	0.57	3.9 ± 1.1	3.9 ± 1.2	−0.7	0.02	0.010
LDL-C/HDL-C	2.4 ± 1.0	2.5 ± 0.8*	−13.4	0.41	2.3 ± 0.8	2.1 ± 0.7*	−11.5	0.34	2.5 ± 0.9	2.5 ± 1.0	−2.0	0.05	0.077
Insulin resistance index													
Glucose (mg/dL)	106.5 ± 8.8	102.0 ± 9.6*	−4.2	0.49	102.8 ± 9.0	97.9 ± 8.0*	−4.8	0.57	107.4 ± 11.4	105.5 ± 11.2	−1.7	0.16	0.266
Insulin (μU/mL)	11.6 ± 4.1	11.6 ± 4.2	0.1	0	12.0 ± 6.7	11.3 ± 6.3	−6.0	0.11	9.5 ± 5.1	10.2 ± 5.0	7.2	0.14	0.401
HOMA-IR	3.2 ± 1.2	3.1 ± 1.3	−3.8	0.09	2.8 ± 1.6	2.6 ± 1.6	−7.5	0.13	2.7 ± 1.6	2.8 ± 1.6	3.6	0.06	0.596
Blood pressure													
SBP (mmHg)	119.8 ± 11.3	122.4 ± 12.9	2.2	0.21	117.6 ± 11.0	118.5 ± 10.4	+0.8	0.09	117.9 ± 12.6	120.6 ± 14.3	2.5	0.22	0.651
DBP (mmHg)	67.0 ± 6.8	67.2 ± 6.9	0.3	0.03	64.6 ± 7.0	63.2 ± 8.6	−2.0	0.16	66.3 ± 7.2	65.7 ± 8.9	−0.9	0.08	0.747
Anthropometric measurements													
WC (cm)	88.9 ± 13.7	87.5 ± 12.5*	−1.7	0.11	83.7 ± 12.5	83.6 ± 11.7*	−2.1	0.15	83.1 ± 10.7	82.4 ± 10.1*	−0.8	0.07	0.276
HC (cm)	101.9 ± 8.4	102.0 ± 8.1	0.1	0.02	100.1 ± 11.2	100.2 ± 10.9	0.1	0.01	98.4 ± 7.2	99.4 ± 7.6	1.1	0.15	0.143
Waist-hip ratio	0.87 ± 0.07	0.85 ± 0.07	−1.8	0.21	0.85 ± 0.07	0.83 ± 0.05	−2.2	0.31	0.84 ± 0.07	0.83 ± 0.06	−1.9	0.24	0.878
Inflammatory biomarkers													
TNF-α (pg/mL)	4.2 ± 3.4	3.3 ± 4.2*	−21.2	0.23	4.7 ± 3.2	3.9 ± 2.5*	−18.3	0.31	4.3 ± 3.2	3.6 ± 3.3*	−15.9	0.21	0.961
IL-6 (pg/mL)	3.2 ± 1.8	2.9 ± 1.8*	−10.9	0.19	3.2 ± 1.4	2.6 ± 1.2*	−18.8	0.45	2.9 ± 1.4	2.7 ± 1.3*	−5.3	0.11	0.545
CRP (mg/L)	2.8 ± 1.9	2.3 ± 1.3*	−18.2	0.32	3.2 ± 2.6	2.3 ± 1.7*	−27.7	0.41	2.6 ± 1.5	2.1 ± 1.7*	−17.9	0.29	0.582

Note: ANOVA two-way. 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; WC = Waist circumference; HC = Hip circumference; TNF-α = Tumor necrosis factor-α; IL-6 = interleukin-6; CRP = C-reactive protein. *$P < 0.05$ vs. pre intervention. §$P < 0.05$ vs. placebo-placebo.

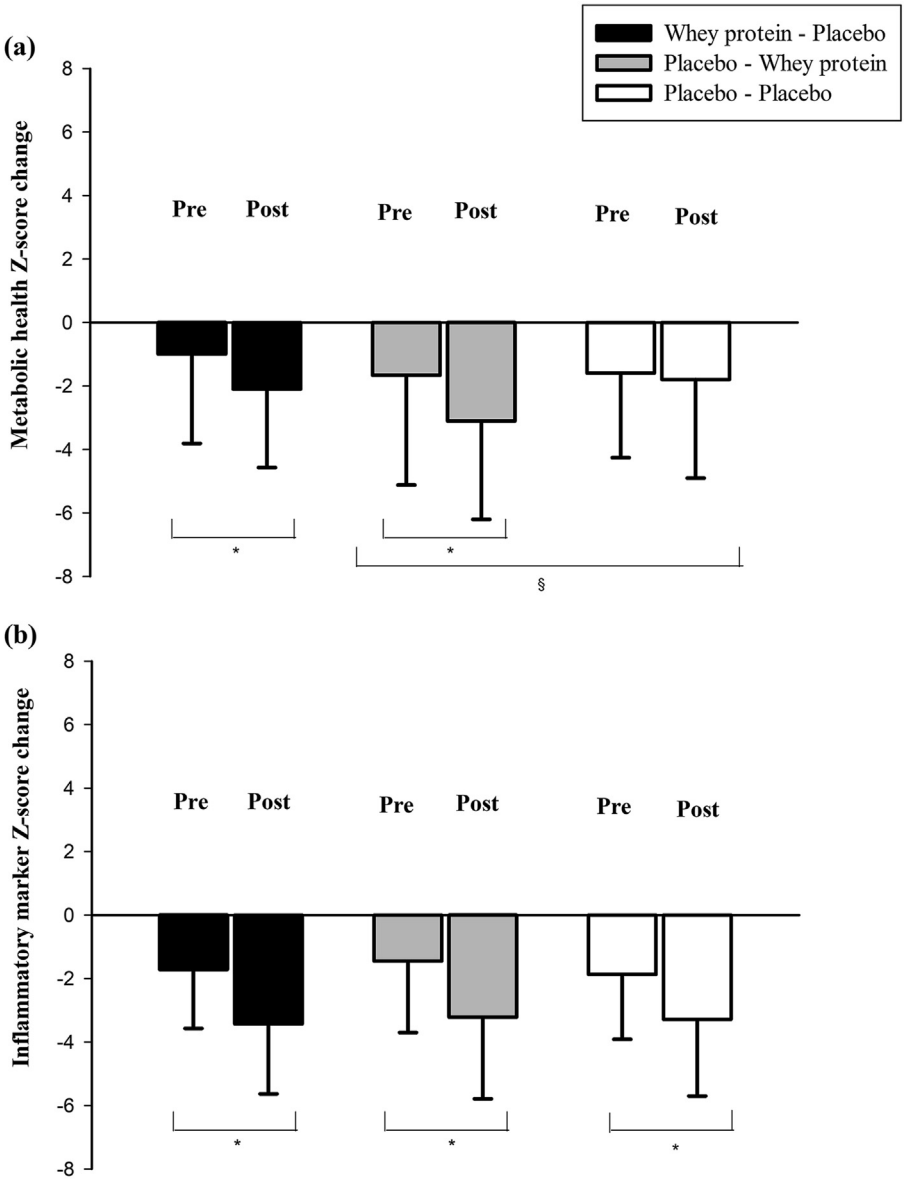

Figure 2 Z-score of the change in metabolic health (a) and inflammatory (b) parameters after 12 weeks of a combined whey protein supplementation with resistance training. §$P < 0.05$ vs. placebo-placebo.

maintain their usual daily living activities throughout the investigation period to minimize any interference from lifestyle factors. Conversely, the present study is a randomized clinical trial with a control group, which strengthens the results presented. This is the first study to investigate the effects of whey protein supplementation, administered before and after RT on the cardio-metabolic profile in older women. Dietary intake pre- and post-intervention was monitored to assure that no changes in habitual food intake occurred during the study.

Conclusion

Whey protein supplementation pre- or post- RT promotes an increase in ALST and a reduction in TC/HDL-C ratio in pre-conditioned older women. Whey protein administered after RT was more effective in improving Z-score of metabolic profile and reduces body fat compared to the placebo group, but without differences from the pre-RT supplementation group. This trial was registered at ClinicalTrials.gov: NCT03247192.

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, 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; ASR, CMT, AMS, LBS, and ESC: provided critical revision and important intellectual content. All authors have read and approved the final version of the manuscript.

Conflicts of interest

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

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