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ORIGINAL ARTICLE

Quadriceps femoris performance after resistance training with and without photobiomodulation in elderly women: a randomized clinical trial

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Abstract

The study assessed if quadriceps femoris muscle performance of older women can be improved by applying photobiomodulation therapy after a resistance training program. This study is a randomized, controlled trial with concealed allocation, intention-to-treat analysis, and blinded outcome evaluators. Forty-five healthy sedentary older women classified as active or insufficiently active were randomized to groups receiving 8 weeks of quadriceps femoris resistance training plus active group or placebo group, or a control group (no training or photobiomodulation). Surface electromyographic fatigue indexes of vastus medialis, rectus femoris, and vastus lateralis; one-maximum repetition (1-MR); and analysis of inflammatory biomarkers (IL-6, IL-8, and TNF-α cytokines, plus CK and LDH enzymes) were measured at baseline and twice in a 24 h-period after 8 weeks. No differences among the three groups were found in fatigue indexes for all three muscles, although in general, the active group presented improved fatigue indexes from baseline to 8-week outcome, while the other groups did not. Both training groups improved in 1-MR over the 8-week period. Inflammatory biomarkers were not different at long- or short-term among the three groups, except differences in groups for long-term IL-8 changes, differences in time for long-term LDH and short-term TNF-α changes, and interactions of time by group for short-term LDH changes. Quadriceps femoris performance of older women was not improved when photobiomodulation was associated to the proposed quadriceps femoris resistance training, when compared to training without photobiomodulation and a sedentary group.

Keywords Fatigue · Quadriceps femoris · Electromyography · Photobiomodulation · Resistance training

Introduction

Aging is a process marked by significant alterations in the functional capacity of tissues and organs, including the cardiopulmonary, endocrine, immunological, and neurological systems [1]. Impairments in the musculoskeletal tissue are also common and are closely related to functional disability [1, 2]. Decreases in the size of muscle cells and in the amount of muscle fibers are commonly observed and may lead to both decline in muscle strength and dysfunction in motor control [3]. Muscle mass is reduced by approximately 3 to 8% per decade after the age of 30, with even higher rates after the age of 60 [4]. As a consequence, older adults are more susceptible to falls and physical disabilities, with decreasing quality of life and increasing levels of morbidity [5, 6].

Aerobic and resistive exercises improve muscle performance by increasing muscle hypertrophy and contractile muscle properties [7]. Thus, physical exercise programs are one of the most efficient interventions to attenuate age-related musculoskeletal tissue modifications [8]. However, the prescription of physical exercise to the population of older adults requires special considerations due to the high incidence of injuries and susceptibility to fatigue [9, 10].

Current literature shows that photobiomodulation has been used in aged rats and in adult humans with the aim of increasing

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the already beneficial effects of physical exercise training by improving muscle performance [5, 11–13]. Photobiomodulation is a recent terminology used to describe the non-thermal light therapy that utilizes lasers, light-emitting diodes, and/or broad-band light, in the visible (400–700 nm) and near-infrared (700–1100 nm) electromagnetic spectrum [11, 12].

Photobiomodulation interacts with cells and tissues and is absorbed by chromophores in the mitochondria, causing an increase in the adenosine triphosphate (ATP) production and in the synthesis of proteins [14]. Some studies suggest that the biological stimulation produced by photobiomodulation delays muscle fatigue, decreasing lactate levels and inflammatory markers [15–17]. The prevailing theory is that muscle fatigue [18] might be attenuated when photobiomodulation is used in association with physical activity [5, 11].

There are several types of photobiomodulation devices that vary from a single diode or light source emitter that irradiates a small tissue area to clusters that irradiates larger tissues area. Consequently, an important consideration in the field of photobiomodulation and muscle performance is the technique used for delivery [17]. Recent studies have used multi-diode cluster, with several visible red and infrared diodes [17, 19]. One of the advantages of using clusters is the possibility of simultaneously irradiating a large portion of the muscle belly, which seems to improve the biological effects in the tissue [17, 19]. We used a cluster device in our study.

Guaraldo et al. [11] demonstrated that photobiomodulation associated to swimming aerobic training reduced oxidative stress and increased VO_2 max, which improved performance in aged animals. Amadio et al. [5] observed a decrease in the expression of inflammatory markers such as interleukins 6 (IL-6) and tumor necrosis factor-α (TNF-α) in aged rats submitted to photobiomodulation and swimming exercises. Toma et al. [13] and Vassão et al. [20] demonstrated a beneficial effect of photobiomodulation in reducing quadriceps femoris fatigue in older women submitted to a strength training and a fatigue protocol, respectively. However, research on the physiologic effects, such as changes in inflammatory markers, of strength and resistance training in elderly population with and without addition of photobiomodulation is still lacking [12]. We found only one publication that analyzed the effect of photobiomodulation in addition to physical exercises in the rectus femoris muscles of older women [13].

The present study assessed fatigue and inflammation biomarkers in healthy older women receiving resistance training with and without photobiomodulation applied after each training session and compared them to a group of healthy sedentary older women.

The research hypothesis was that women who received resistance training plus active photobiomodulation would have a greater muscle performance improvement compared to those who received resistance training plus placebo photobiomodulation or who did not receive any training.

Material and methods

Experimental procedure

This study was a three-arm, parallel, randomized controlled trial. The study was approved by the University Ethics Committee and registered at the Brazilian Registry of Clinical Trials, RBR-82BCMZ.

Participants

Inclusion criteria were female 60 years or older, considered activity or insufficiently active by The International Physical Activity Questionnaire-short version (IPAQ, described later in the manuscript), and not participating in exercise programs in the past 3 months. Exclusion criteria were previous spine or lower extremities surgery; no full range of motion in knees or hips; history of luxation of hip joint; and presence of uncontrolled hypertension or *diabetes mellitus*; rheumatoid, neurological, or degenerative disease; and dietary supplements that might lead to muscle gain.

Forty-two untrained women were enrolled in this study. Participants were recruited by flyers and word-of-mouth. They were randomized into one of three groups of 14 participants each: quadriceps femoris resistance training plus photobiomodulation (denominated "active group"), quadriceps femoris resistance training plus placebo photobiomodulation ("placebo group"), and a group without exercise program and without photobiomodulation ("control group"). Participants who were eligible and agreed to participate received information about experimental procedures, signed a written informed consent before any evaluation, and were informed that they could withdraw from the study at any time if they decided to do so. A randomization list was computer-generated and individual assignments were inserted in numbered opaque envelopes and sealed. The researcher who prepared the envelopes was not involved in the participant's evaluation or in applying the intervention. Participants of the active and placebo groups were blinded to the type of photobiomodulation (active or placebo) they were receiving. The intervention and evaluation were performed by two different physical therapists, and the evaluator was blinded to the intervention group to which the person belonged.

Procedures

Resistance training and photobiomodulation intervention

The control group did not receive any resistance training during the study. The timeline of surface electromyography (SEMG), force, and blood sample measurements for control group follow the timeline for collecting SEMG, force, and blood sample of active and placebo groups. After the baseline session, the examiner explained to the participants of the control group that they would be invited to come back to the laboratory after 8 weeks for two more sessions of fatigue protocol (described below in detail) and for one more session of one-maximum repetition (MR) test (described below).

For training groups, the quadriceps femoris resistance program consisted of 8 weeks of individual training in two weekly sessions, with intervals of 48 h between sessions. Training was performed in a XR-5 leg-extension machine (Righetto Fitness Equipment, Campinas, Brazil) and consisted of two sets of 15 repetitions in the first 2 weeks and three sets of 15 repetitions in subsequent weeks, with 2 min of rest between sets. Parameters of exercise training were based on previous studies reported in the literature [13, 21]. During training sessions, participants performed training with both lower extremities at the same time with a range of motion of 90° (starting position = 90° of knee flexion; final position = 0° of flexion or total knee extension). Participants were removed from the study if any of the following happened: they had blood pressure higher than 160/120 mmHg at the beginning of three consecutive training sessions, missed four training sessions in total or more than two consecutive sessions, had lower extremity or trunk injuries, or did not agree to have a blood draw.

We assessed 1-MR for all groups at baseline session, 3 to 5 days before starting the 8-week training program for active and placebo group and at enrollment for control group. For the control group, we assessed 1-MR only at baseline and after a period of 8 weeks. For active and placebo group, 1-MR was also assessed at the end of weeks 2, 4, 6, and 8 to monitor and adjust the loads for each participant in the training groups. Load for the resistance training was 60% of baseline 1-MR for the first 2 weeks and 80% of the 1-MR values obtained in the subsequent 2 weeks. Parameters of load increasing during exercise training were based on loads previously reported in the literature [13, 21].

At the end of each training session, participants in the active group received the photobiomodulation (device "on"), while the placebo group had the device positioned in the same body parts but with the device turned off. The cluster used was a device with 14 outputs for photobiomodulation (7 light-emitting diodes [LED] with visible wavelengths of 660 nm and 7 diodes [LLLT] with infrared wavelengths of 808 nm) (DMC Equipment Ltda, São Carlos, Brazil). In this study, the photobiomodulation was delivered only by the 7 diodes of 808 nm (red LEDs were turned off during the active therapy), with continuous laser frequency (Hz), optimal output of 100 mW/diode, spot size of 0.05 cm^2, power density of 2 W/cm^2, dose of 4 J with energy density of 91 J/cm^2, time per application 40 s, and application mode perpendicular contact to the skin. Due to the cluster's size, four applications were necessary to cover the quadriceps femoris muscle, totalizing 112 J in 120 s 100 mW/diode. The chosen photobiomodulation parameters were within the effective range for reducing fatigue and improving performance of quadriceps femoris that were found in a systematic review and meta-analysis by Vanin et al. [22]. That systematic review included primarily studies that used photobiomodulation plus physical exercises but also included studies that used combined therapies (photobiomodulation plus magnetic field). Although the inclusion of other therapies in the meta-analysis makes it difficult to isolate the effect of photobiomodulation, we used this reference as a starting point for the choice of the parameters for this study.

The distribution of diodes in the cluster was alternated (Fig. 1a, middle panel: outputs used are indicated with a square). In one row, there were four diodes of 808 nm and three diodes of 660 nm and in the other row, there were four diodes of 660 nm and three diodes of 808 nm. The cluster dimension is 150 mm (L) × 100 mm (W) × 55 mm (H), with 1 cm of distance between infrared diodes. The placement of the cluster in all sessions for all participants is shown in Fig. 1b.

Electromyography and force measurements during quadriceps femoris fatigue protocol

Surface electromyography (SEMG) and force measurements were sampled from quadriceps femoris during the fatigue protocol performed at baseline and at 24 and 48 h after the last quadriceps femoris resistance-training session for training groups or after 8 weeks for control group. To perform the fatigue protocol, participants sat on a leg-extension machine and kept their knee at 90° of flexion and their hip at 80° of flexion. The fatigue protocol consisted of performing 30 s of quadriceps femoris submaximal isometric voluntary contraction. The leg-extension machine was locked to maintain knee angle in 90° during the entire fatigue protocol. SEMG and force measurements were collected with participants in this position. Before placing electrodes, the participant's skin was shaved and cleaned with alcohol. SEMG of dominant lower extremity was sampled from vastus medialis, rectus femoris, and vastus lateralis according to SENIAM recommendations [23]. Myoelectric signals were sampled by a disposable bipolar electrode (Ag/AgCl, 20 mm inter-electrode distance) connected to a pre-amplifier with input impedance of 10 GΩ, common mode rejection ratio of 130 dB, and gain of 20 times (Miotec, Rio Grande do Sul, Brazil). A circular disposable electrode was fixed in the tibial plateau of dominant lower extremity to reduce acquisition noise.

The fatigue protocol was performed for 30 s, since a muscular voluntary isometric contraction (MVIC) should be maintained for a minimum of 30 s to produce changes in the spectral analyses of the electromyographic signal [24]. In addition, we wanted to ensure that our fatigue protocol would produce mechanical muscular failure, that is, that participants had enough muscle fatigue to cause muscle microdamage. Mathur et al. [25] showed that when the level of muscular force decreases 20% from the initial peak force value, there is fatigue of quadriceps femoris. We also collected data on

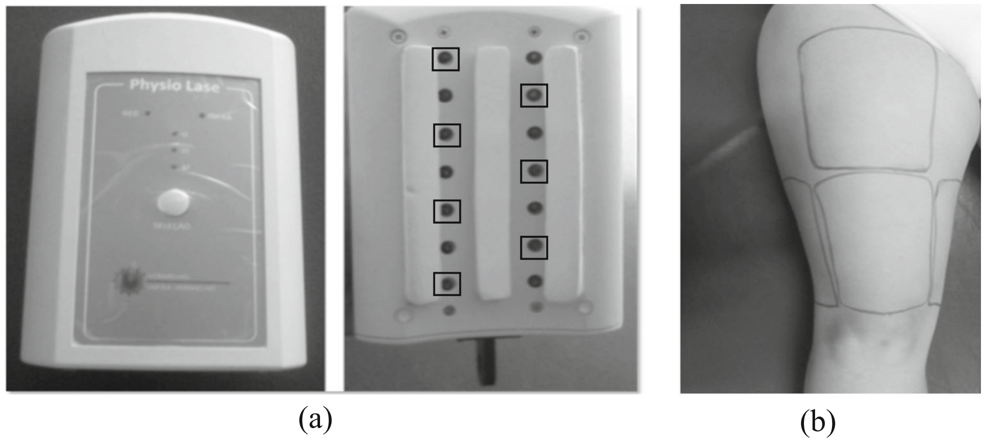

Fig. 1 Front and back views of the cluster with squares indicating the 7 diodes of 850 nm used (**a**). Quadriceps femoris area of phototherapy application (**b**)

peak force and time for the force to decrease 20% from the peak.

Force signal was collect simultaneously to the SEMG signal. The force signal was obtained using a load cell (Miotec Ltda., Rio Grande do Sul, Brazil) connected to the same 16-bit A/D converter board. Both signals were sampled at 2 kHz of frequency (Miotec, Rio Grande do Sul, Brazil), so that the force and SEMG signals would be automatically synchronized. SEMG signals were filtered at a frequency bandwidth of 20-1000 Hz. Force data were analyzed in the Matlab program R2014a. The load cell force values were filtered with a fourth-order Butterworth filter, with cutoff frequency of 2 Hz. Subsequently, the force data were rectified and filtered again with low pass filter of fourth-order with a cutoff frequency of 0.2 Hz to obtain the linear envelope. The peak force value (maximum value = 100%) was found, and the signal was divided in windows of 500 frames. When the average signal of a window reached 80% of the peak force value (20% drop of peak force), the first frame of that window was determined as the fatigue moment. The 30-s SEMG raw signal was windowed in three parts of 10 s each, and fatigue indexes for each of muscle were determined by the ratio of two median frequencies: the third 10-s window by the first 10-s window [13, 16, 18, 26].

One-maximum repetition

The 1-MR was assessed according to The American College of Sports Medicine [27], using a leg-extension machine. The test consists of the maximum weight that the person can lift once and it has been applied to older adults [13, 20]. Participants performed 1-MR test with both lower extremities together in a knee range of motion of 90° (initial knee position = 90° of flexion; final knee position = 0° of flexion or total extension). The protocol to define the 1-MR was conducted in five series, with a rest of 3 min between them. The first series was performed with a minimum weight. Then, participants performed 10 repetitions of knee flexion-extension to warm-up and to be familiarized with the exercise. The second and third series consisted of five repetitions of knee flexion-extension, and the fourth and the fifth series consisted of only one repetition of knee flexion-extension. The weight of each series was determined subjectively by the examiner who was experienced in doing this test in the elderly population. If it was not possible to determine the 1-MR in five series, another session was supposed to be scheduled. However, all participants had their 1-MR found in the first session. To allow comparison among individuals, values of 1-MR of each participant were normalized by dividing the maximum weight lifted by the woman's body mass, thus avoiding potential confounding with measurements of strength.

Blood collection and measurement of inflammatory markers

All groups had blood draw at baseline. Active and placebo groups had blood draws at 24 and 48 h after 8 weeks of resistance training, while controls had blood draws at 24 and 48 h after an 8-week period from baseline. Peripheral blood was collected aseptically, and the prepared plasma was stored at − 80 °C until sent to a certified laboratory. Serum marker levels of IL-6, IL-8, and TNF-α were quantified using ELISA assays kits (Enzo Life Sciences, Farmingdale, NY, USA). Chemiluminescence methods were used to quantify creatine kinase (CK) and lactate dehydrogenase (LDH) (Beckman Coulter). These enzymes and cytokines are usually released into the blood stream after physical activity [28–31] and are indicators of inflammatory response from the muscle lesions and chemical stress caused by physical activity.

Data analysis

We aimed to recruit the largest sample size possible within the time frame and with the resources available for the study. Forty-two women were enrolled, and the enrollment was ended when the funding ended.

One-way analysis of variance (ANOVA) was performed to compare means for age, body mass, height, body mass index (BMI), and 1-MR among the three groups at baseline. This analysis was made to assure that no detectable differences existed among the three groups before the intervention period, which could bias the observed results. This analysis was chosen after we checked for possible departures from Gaussian distribution thorough Q-Q plots.

Repeated-measures ANOVA was used to compare fatigue indexes and inflammatory biomarkers in two models: from baseline fatigue protocol (denominated baseline) to 24 h post-fatigue protocol after 8 weeks of intervention (denominated 24 h) to assess the long-term effect of the intervention and from 24 to 48 h post-fatigue protocol after 8 weeks of intervention (denominated 24 and 48 h, respectively) to assess the short-term decline in inflammatory biomarkers as the tissue is healed. All models included group (control, placebo, and active) and time (baseline and 24 h for long term, or 24 h and 48 h for short term) as main factors, and a time by group interaction term.

Normalized 1-MR was analyzed by repeated-measures ANOVA with group (Active and Placebo), time (baseline, 2, 4, 6, and 8 weeks) and an interaction of time by group. The control group was not analyzed for 1-MR over time, since we did not collect that data for intermediate weeks and the purpose of the 1-MR was to assess that the training program had an effect over time.

For the repeated-measures ANOVA, we first checked the interaction between time and group for statistical significance. If the interaction is present, the results of the time effect must be interpreted separately for each group. If not present, then the time and group effects are interpreted as separate main effects.

All participants were analyzed in an intent-to-treat principle. Significance level was set at $\alpha = 0.05$ for all tests in the ANOVA procedures. Since this was not a confirmatory study, we did not adjust for multiple comparisons, to avoid stringent tests that would possibly reject null hypotheses that still need to be confirmed in future studies. The interpretation of the results takes this issue into account. Statistical analyses were performed using SPSS 24 for Mac (for ANOVAs) and R Studio Version 0.98.1083 for Mac (for the figures).

Results

A total of 163 women were assessed for eligibility, and 121 of them were not included for various reasons listed in the Fig. 2. In the active group, one woman dropped out of the study and another discontinued training due to a fall injury outside the study. In the placebo group, one woman was excluded due to uncontrolled high blood pressure. In the control group, one woman did not have the outcomes assessed at week 8. Thus, 38 women had data for the statistical analysis for the final outcome measures. All participants were classified as insufficiently active according to the IPAQ questionnaire.

Table 1 shows descriptive statistics for age, anthropometric characteristics, and 1-MR of the participants at baseline. There were no differences among groups at the baseline for age (means varied from 65.1 to 65.9 years, $p = 0.78$), height (means from 1.58 to 1.62 m, $p = 0.21$), body mass (means from 65.3 to 71.6 kg, $p = 0.31$), BMI (means from 26.1 to 28.3, $p = 0.30$), and normalized 1-MR (means from 0.27 to 0.32, $p = 0.55$).

Table 2 shows the time to have a decrease of 20% in the peak force at the baseline, and 24 and 48 h post-intervention. The mean and median times were always below 30 s.

Table 3 shows the results for fatigue indexes for all groups, times (baseline, and 24 and 48 h post-intervention), and muscles. When comparing fatigue indexes from baseline to 24 h (see second column of Table 3), neither time, nor group, nor their interaction was statistically significant for all three muscles, although the mean fatigue index was usually higher (better) for the active group after the training program. When comparing the groups for changes from 24 to 48 h (last column of Table 3), neither time, nor group, nor their interaction was statistically significant for all three muscles, though the active group maintained the fatigue index and the placebo group decreased it (worse) for all muscles.

Plots of individual values and group means (Fig. 3) show that the fatigue index was more often larger from baseline to 24 h (change over 8 weeks) and stable from 24 to 48 h (change in 24 h) for the active group in all three muscles, while in the other groups, the fatigue index showed more variation. While there were few women for whom the values seemed very different from the others, it would be premature to consider them as outliers, since a larger study might show that the variance in the population is actually large.

Table 4 shows the results for the normalized 1-MR. The control group is presented for reference only, and it shows that the women in that group had a slight decrease in normalized 1-MR from baseline to 8 weeks. Both, placebo and active groups had increasing mean 1-MR over time. The active group started with slightly lower 1-MR when compared to the placebo group, although the difference was not statistically significant. Repeated-measures ANOVA showed no interaction ($p = 0.37$) and no effect of group ($p = 0.18$). Time on training was statistically significant ($p < 0.001$) and 1-MR increased with time, as it would be expected when individuals are in a training program.

Table 5 shows the results for the inflammatory biomarkers. Results of TNF-α at 48 h are not reported, because the observed values were extremely low for all three groups and these were unlikely results for TNF-α. It was not possible to redo the laboratory tests for TNF-α due to lack of funding resources. When comparing baseline to the end of week 8 (second column of Table 5), only time for LDH ($p < 0.001$) was statistically significant at 0.05. When comparing the two

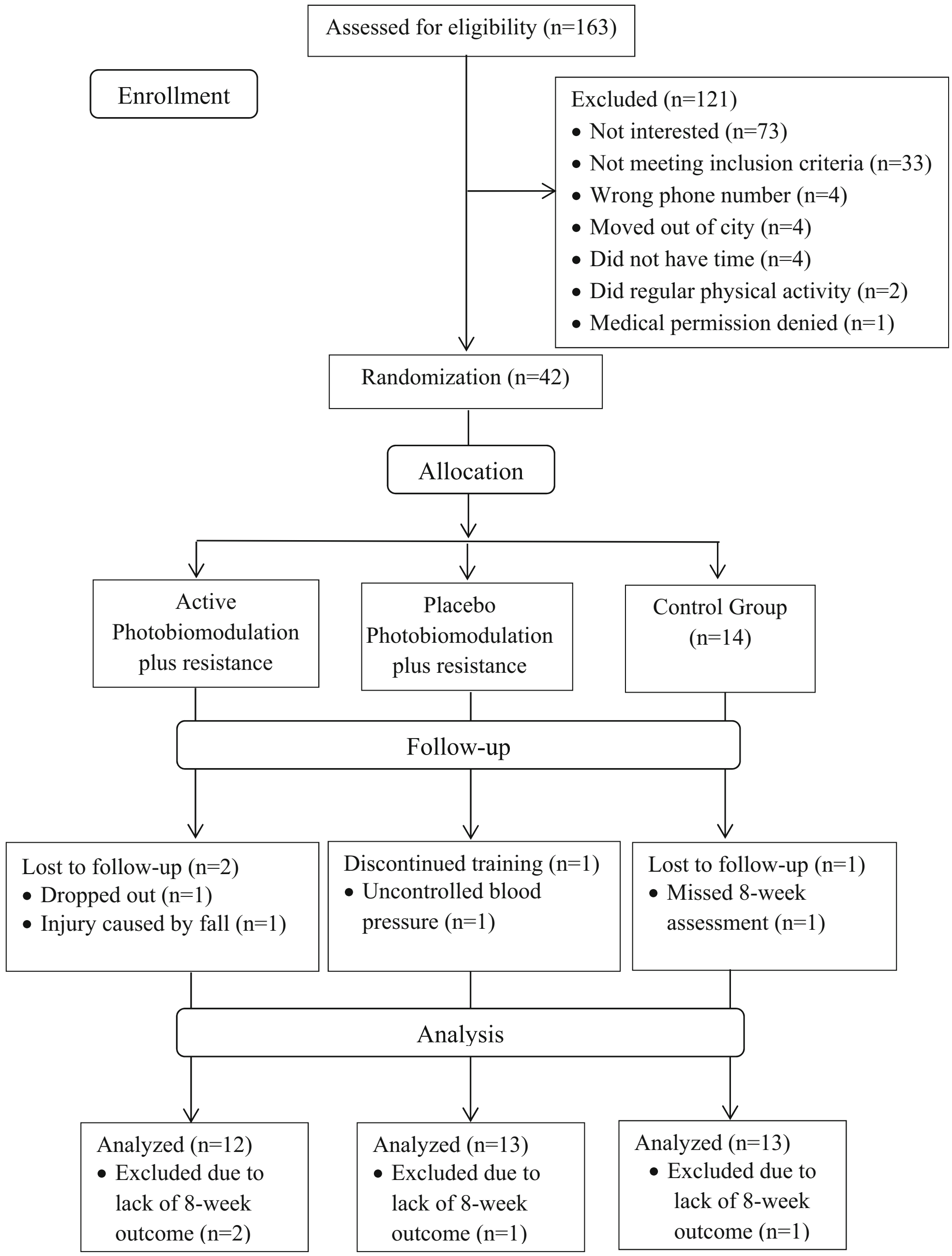

Fig. 2 Flowchart from recruitment to data analysis

training groups at 24 and 48 h (last column of Table 5), time was statistically significant for IL-8 ($p = 0.001$) and the interaction was statistically significant for LDH ($p = 0.04$).

Plots of individual values and group means for all cytokines and enzymes (Fig. 4) showed large variation for some groups and follow-up times.

Discussion

The purpose of this study was to assess the effect of applying photobiomodulation after resistance-training sessions for the quadriceps femoris on measures of fatigue, load, and inflammatory biomarkers in older sedentary women. Results showed that the resistance training increased load progression after 8 weeks and showed no effect of photobiomodulation on reducing muscle fatigue or on inflammatory biomarkers. The results were not expected, since studies had shown efficacy of photobiomodulation on reducing muscle fatigue and modulating inflammatory biomarkers in other populations [4, 16]. We discuss below some of factors that might explain the differences in results.

Our photobiomodulation parameters were in the therapeutic window shown to be effective to reduce muscle fatigue and

Table 1 Descriptive statistics for age, anthropometric, and outcome characteristics of participants at baseline. Presented values of 1-MR were normalized per body weight

Characteristic	Group		
	Control	Placebo Photobiomodulation	Active Photobiomodulation
Sample size	14	14	14
Age in years, mean (SD)	65.1 (3.5)	65.1 (3.5)	65.9 (3.6)
Median (min, max)	66.0 (60.0, 70.0)	66.0 (60.0, 70.0)	67.5 (60.0, 70,0)
Height (m)[a], mean (SD)	1.62 (0.05)	1.58 (0.07)	1.59 (0.06)
Median (min, max)	1.62 (1.52, 1.70)	1.60 (1.43, 1.68)	1.59 (1.52, 1.70)
Body mass[a] (kg), mean (SD)	68.7 (9.7)	65.3 (8.9)	71.6 (12.8)
Median (min, max)	67.4 (57.0, 92.0)	63.2 (48.0, 82.0)	71.0 (57.0, 107.0)
Body Mass Index[a], mean (SD)	26.1 (3.3)	26.1 (3.9)	28.3 (5.2)
Median (min, max)	25.7 (21.2, 32.0)	27.5 (18.5, 31.6)	26.5 (23.7, 42.3)
1-MR[b], mean (SD)	0.27 (0.16)	0.32 (0.12)	0.29 (0.12)
Median (min, max)	0.24 (0.11, 0.63)	0.30 (0.15, 0.56)	0.27 (0.14, 0.54)
IPAQ classification	Insufficient activity	Insufficient activity	Insufficient activity

Body mass index = weight/(height^2)

SD standard deviation, *kg* kilograms, *m* meters, 1-RM one maximum repetition, *IPAQ* International Physical Activity Questionnaire

[a] One missing value in the active photobiomodulation group

[b] One missing value in the control group

to modulate inflammatory biomarkers, but the data came from a systematic review that also included some studies of photobiomodulation in conjunction with other therapies [22]. Based on previous research, photobiomodulation can be efficacious in improving muscle performance if applied in combination with exercises [5, 20]. However, the time period for long-term benefits of using photobiomodulation combined with resistance exercises has not yet been established and needs more research.

Acute effects of photobiomodulation on electromyographic fatigue of older women were previously reported in the literature. Toma et al. [12] observed that photobiomodulation did not reduce rectus femoris electromyographic fatigue. In contrast, Vassão et al. [20] showed a reduction of rectus femoris electromyographic fatigue after photobiomodulation application. One recent clinical trial of older women who received quadriceps femoris resistance training associated with photobiomulation [13] showed a reduction of rectus femoris electromyographic fatigue. To date, we found no studies on the effects of long-term exposure to photobiomodulation that could be compared to some variables of the present study. Based on the current literature, results (effective or not effective) for muscle activity and muscle performance, for instance, have not been consistent when participants went to an exercise protocol associated with photobiomodulation.

One of the most common reasons cited as a possible cause of lack of effectiveness of photobiomodulation on improving

Table 2 Descriptive analysis of time between peak force and decrease to 80% of peak force, by group and time

Descriptive analysis	Control group			Placebo photobiomodulation			Active photobiomodulation		
	Baseline	Post-intervention		Baseline	Post-intervention		Baseline	Post-intervention	
		24 h	48 h		24 h	48 h		24 h	48 h
Sample Size	13	13	13	13	13	13	12	12	12
Mean	11.6	9.6	15.5	13.0	11.6	15.8	8.8	16.6	20.0
Standard Deviation	10.5	6.1	10.3	6.8	6.1	12.1	7.1	9.2	8.8
Median	5.0	7.5	14.8	10.8	10.5	14.5	7.6	16.9	20.2
Minimum	0.8	2.0	1.5	2.2	3.0	1.3	2.5	1.5	6.5
Maximum	29.0	20.0	32.0	24.5	21.5	46.0	26.8	29.0	32.2
% above 30 s (no. in total)	0	0	7.7 (1 in 13)	0	0	7.7 (1 in 13)	0	0	16.7 (2 in 12)

Table 3 Means (standard deviations) and repeated measures ANOVA for fatigue indexes of vastus medialis, rectus femoralis, and vastus lateralis muscles by group and follow-up time

Muscle and follow-up time	Models with baseline and 24 h[b]	Group			Models with 24 and 48 h[c]
		Control	Placebo Photobiomodulation	Active Photobiomodulation	
Vastus lateralis					
Baseline	Time: F = 0.75, df = 1, $p = 0.39$ Group: F = 0.10, df = 2, $p = 0.91$	0.95 (0.10)	0.95 (0.09)	0.92 (0.09)	
24 h[a]	Time by group: F = 1.51, df = 2, $p = 0.23$	0.93 (0.08)	0.95 (0.09)	0.98 (0.07)	Time: F = 0.28, df = 1, $p = 0.60$ Group: F = 1.14, df = 2, $p = 0.33$
48 h[a]		0.94 (0.12)	0.92 (0.09)	0.98 (0.07)	Time by group: F = 0.91, df = 2, $p = 0.41$
Rectus femoralis					
Baseline	Time: F = 0.06, df = 1, $p = 0.80$ Group: F = 0.36, df = 2, $p = 0.70$	0.93 (0.13)	0.97 (0.20)	0.91 (0.22)	
24 h[a]	Time by group: F = 0.40, df = 2, $p = 0.67$	0.92 (0.13)	0.96 (0.13)	0.96 (0.10)	Time: F = 2.86, df = 1, $p = 0.10$ Group: F = 0.66, df = 2, $p = 0.52$
48 h[a]		0.88 (0.14)	0.91 (0.14)	0.96 (0.10)	Time by group: F = 0.71, df = 2, $p = 0.50$
Vastus medialis					
Baseline	Time: F = 1.36, df = 1, $p = 0.25$ Group: F = 1.58, df=, $p = 0.22$	0.96 (0.14)	1.00 (0.24)	0.98 (0.20)	
24 h[a]	Time by group: 0.86, df = 1, $p = 0.43$	0.96 (0.14)	1.02 (0.22)	1.12 (0.20)	Time: F = 0.17, df = 1, $p = 0.68$ Group: F = 2.89, df = 2, $p = 0.07$
48 h[a]		0.98 (0.22)	0.96 (0.13)	1.12 (0.20)	Time by group: F = 0.81, df = 2, $p = 0.45$

[a] 24 h and 48 h after the last training session for Photobiomodulation groups and after 8 weeks for control group

[b] Results from repeated-measures ANOVA using baseline and 24 h data

[c] Results from repeated-measures ANOVA using 24 and 48 h data

muscle performance is the choice of photobiomodulation parameters [22]. Part of difficulty of establishing parameters is the lack of consensus in the literature about the best choice of parameters or the best therapeutic window for those parameters. Thus, as a starting point, our parameters were based on the systematic review and meta-analysis by Vanin et al. [22], although that review included some studies where photobiomodulation was combined with magnetic field. The type of exercise (resistance, strength, plyometric, etc.), number of repetitions, and the volume of exercise can also affect the results of training protocols [20], including in studies combining photobiomodulation and exercise training [22]. Different outcomes can be affected by different types of training, such as fatigue is likely to be more susceptible to changes after resistance training, or load increasing could be greater after strength training. Consequently, study designs associating different training protocols and photobiomodulation parameters are necessary and important in this field of research. One possibility is to have studies that keep the same photobiomodulation parameters in different types of exercises, for example, where we could test whether the same set of parameters is effective for various types of exercise.

The cytokines and enzymes assessed in this study are believed to increase when inflammation is present, and a strength-training program might induce some inflammation due to the breakdown of muscle fibers at microlevel [32]. To date, we have found no clinical trials that evaluated the effects of adding photobiomodulation to resistance training on inflammatory biomarkers in older adults. Studies of isolate resistance or strength training in this population showed no alterations in the levels of inflammatory biomarkers after training [28–30], even when comparing to a control group [2, 31]. Mikkelsen et al. [33] found lower IL-6 concentrations after strength exercises in older men who were long-time practitioners of physical activity when compared to a group of sedentary men of similar age, possibly because physical activity is able to regulate muscle physiological parameters.

Our study has several strengths. First, to our knowledge, there has been no study of photobiomodulation after resistance training in sedentary older women. Second, the study design is statistically sound and allowed the comparison of resistance exercise with and without photobiomodulation as well as the comparison with a control group. Third, we assessed fatigue in three different muscles of quadriceps instead of a single muscle of quadriceps femoris, as it is usual in the literature. Finally, we measured inflammatory biomarkers at three points during the study, which had not been done in past studies. To our knowledge, this is the

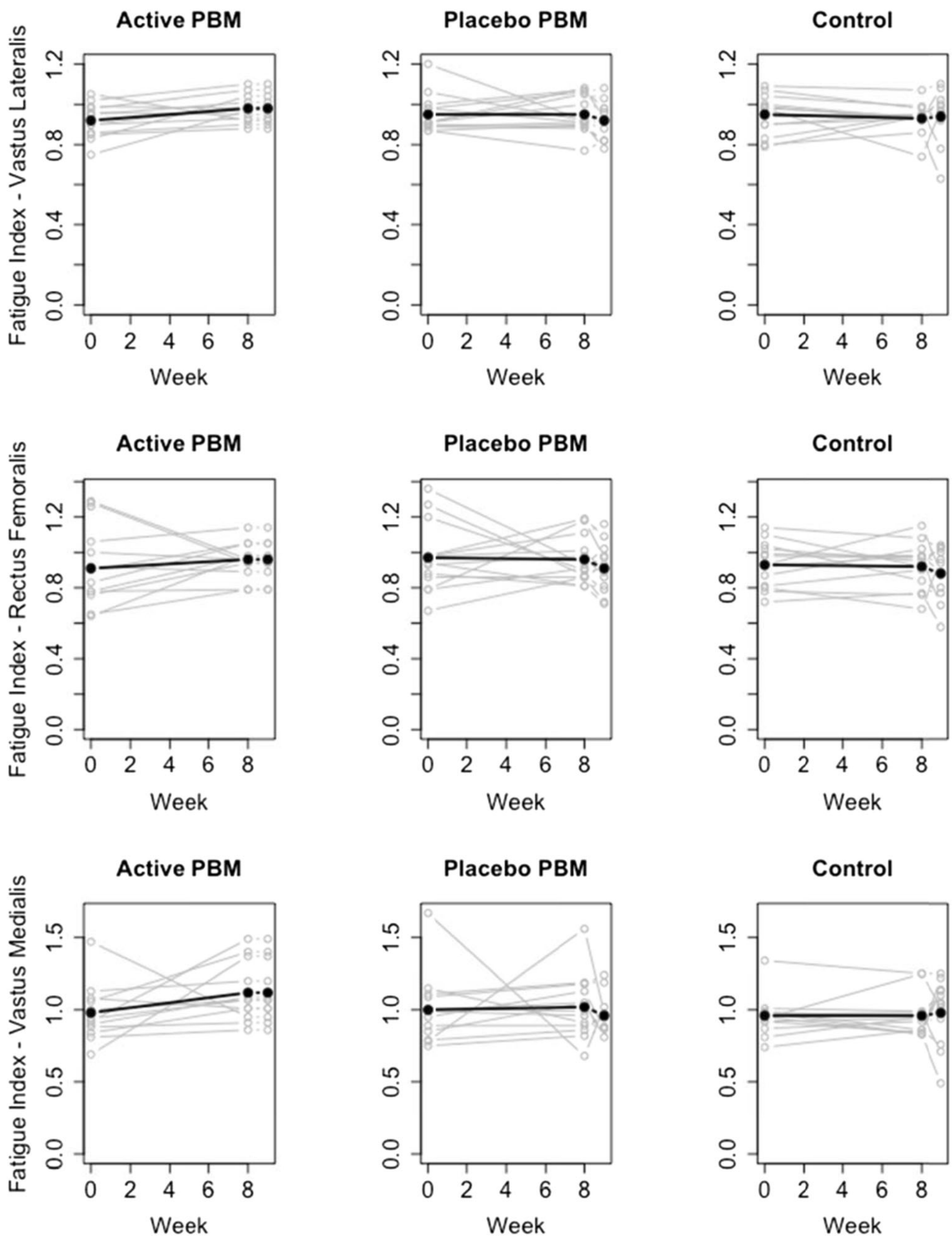

Fig. 3 Electromyographic fatigue indexes for three muscles, by group and time. Values for 24 h are plotted at 8 weeks, while values for 48 h are plotted at 9 weeks to allow better visualization of the follow-up time

first clinical trial on the effects of photobiomodulation after resistance training sessions on the levels of inflammatory biomarkers in older sedentary women. The control and placebo groups allowed us to test if fatigue indexes and inflammatory biomarkers were due to natural history vs. the training, or to the addition of active vs. placebo photobiomodulation. We applied photobiomodulation after each training session only, while other studies applied it before the sessions [17, 20]. This could be an explanation for the differences between previous and our results. Future studies could compare biomarkers in three groups: no photobiomodulation, photobiomodulation applied before the training session, and photobiomodulation applied after training session, to assess the effect of the timing of phototherapy.

Our study was designed based on what was known in the literature at the time. We can now identify some limitations, which should be taken into consideration in future studies. We analyzed only two blood samples for biomarkers after the end of the 8-week training, limiting our ability to study lasting effects of photobiomodulation. Blood samples were collected only after the fatigue protocol, but in the future, it might be informative to collect before and after the fatigue protocol. Unfortunately, currently there is no guidance in the literature regarding the number of times or time intervals for biomarker data collection. Another possibility for future studies is to add muscle biopsy, which would give more information about the effects of photobiomodulation on muscle tissue. To date, we

Table 4 Mean values (standard deviation) and ANOVA results of normalized 1-MR by group and follow-up time

Follow-up Time	Means (SD) for groups			Results of repeated-measures ANOVA[b]
	Control[a]	Placebo Photobiomodulation	Active Photobiomodulation	
Baseline	0.27 (0.16)	0.32 (0.12)	0.30 (0.13)	Time: F = 43.0, df = 1.6, $p < 0.001$
Week 2	–	0.35 (0.12)	0.34 (0.13)	Group: F = 0.41, df = 1, $p = 0.53$
Week 4	–	0.41 (0.12)	0.37 (0.13)	Time by group: F = 0.50, df = 1.6, $p = 0.57$
Week 6	–	0.46 (0.13)	0.41 (0.14)	
Week 8	0.25 (0.09)	0.51 (0.12)	0.48 (0.16)	

[a] Values for control group were collected at baseline and week 8 only, and are shown here for comparison only

[b] Repeated-measures ANOVA using five follow-up times for the placebo and active Photobiomodulation groups (using Greenhouse-Geisser adjustment for degrees of freedom due to lack of sphericity of covariance matrix)

have not found any clinical trials in exercise training and photobiomodulation that analyzed muscle biopsies or had several blood collections for analysis of biomarkers. An exception was a single study of muscle biopsy in monozygotic twins [34], which showed decreases in interleukin 1β and muscle atrophy when muscle was irradiated with active photobiomodulation as

Table 5 Means (standard deviations) and ANOVA for inflammatory markers by group and follow-up time

Biomarkers and follow-up time	Models with Baseline and 24 h[b]	Group			Models with 24 to 48 h[c]
		Control	Placebo Photobiomodulation	Active Photobiomodulation	
IL-6 (pg/ml)					
Baseline	Time: F = 1.48, df = 1, $p = 0.23$ Group: F = 1.04, df = 2, $p = 0.36$	28.6 (13.4)	22.7 (9.6)	29.8 (21.3)	
24 h[a]	Time by group: F = 0.06, df = 2, $p = 0.95$	31.3 (18.4)	28.2 (13.8)	33.7 (12.8)	Time: F = 0.46, df = 1, $p = 0.50$ Group: F = 1.79, df = 2, $p = 0.18$
48 h[a]		73.5 (132.4)	21.7 (21.6)	25.8 (26.6)	Time by group: F = 1.49, df = 2, $p = 0.24$
IL-8 (pg/ml)					
Baseline	Time: F = 0.67, df = 1, $p = 0.42$ Group: F = 2.31, df = 2, $p = 0.12$	12.8 (9.2)	49.4 (74.0)	12.0 (7.2)	
24 h[a]	Time by group: F = 3.14, df = 2, $p = 0.06$	31.3 (18.4)	28.2 (13.8)	33.7 (12.8)	Time: F = 14.0, df = 1, $p = 0.001$ Group: F = 0.09, df = 2, $p = 0.92$
48 h[a]		18.8 (13.4)	23.2 (16.5)	14.3 (6.5)	Time by group: 1.58, df = 2, $p = 0.22$
TNF-α(pg/ml)					
Baseline	Time: F = 1.30, df = 1, $p = 0.26$ Group: F = 0.83, df = 2, $p = 0.45$	22.6 (6.6)	26.4 (11.5)	18.0 (10.9)	
24 h[a]	Time by group: F = 3.09, df = 2, $p = 0.06$	24.1 (5.4)	23.2 (4.5)	25.7 (4.6)	
LDH (pg/ml)					
Baseline	Time: F = 18.5, df = 1, $p < 0.001$ Group: F = 2.80, df = 2, $p = 0.08$	320.0 (38.2)	389.3 (95.8)	367.0 (78.4)	
24 h[a]	Time by group: F = 0.69, df = 2, $p = 0.51$	304.3 (41.2)	357.6 (71.1)	339.9 (75.8)	Time: F = 0.54, df = 1, $p = 0.47$ Group: F = 2.85, df = 2, $p = 0.07$
48 h[a]		324.4 (42.1)	371.4 (50.8)	320.4 (61.5)	Time by group: F = 3.47, df = 2, $p = 0.04$
CK (pg/ml)					
Baseline	Time: F = 0.07, df = 1, $p = 0.80$ Group: F = 1.64, df = 2, $p = 0.21$	83.5 (29.1)	136.7 (142.0)	104.8 (42.3)	
24 h[a]	Time by group: F = 0.39, df = 2, $p = 0.68$	76.2 (28.2)	136.1 (113.0)	108.6 (49.1)	Time: F = 1.63, df = 1, $p = 0.21$ Group: F = 2.36, df = 2, $p = 0.11$
48 h[a]		79.0 (27.3)	145.0 (119.7)	108.0 (40.5)	Time by group: F = 0.90, df = 2, $p = 0.41$

IL-6 interleukin 6, *IL-8* interleukin 8, *TNF-α* tumor necrosis factor-alpha, *CK* creatine kinase, *LDH* lactate dehydrogenase

[a] 24 and 48 h after the last training session for photobiomodulation groups and after 8 weeks for control group

[b] Results from repeated-measures ANOVA using baseline and 24 h data

[c] Results from repeated-measures ANOVA using 24 and 48 h data

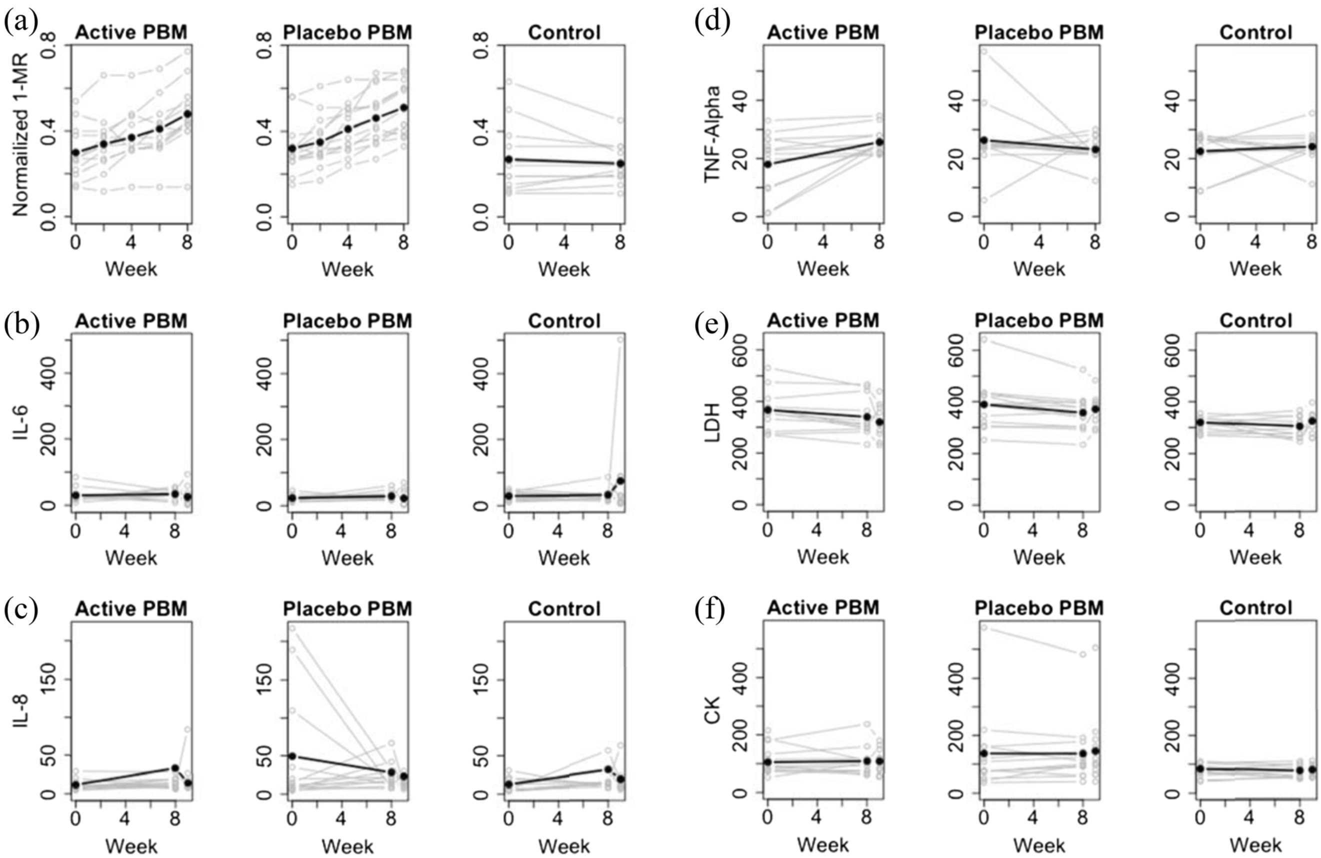

Fig. 4 Individual values (in gray) and group mean (in black) for variables measured at baseline (week 0), 24 and 48 h after last training session. Values for 24 h are plotted at 8 weeks, while values for 48 h are plotted at 9 weeks to allow better visualization of the follow-up time. Panel **a** Normalized one maximum repetition (1-RM). Panel **b** Interlukine-6 (IL-6). Panel **c** Interleukine-8 (IL-8). Panel **d** Tumor necrosis factor-alpha (TNF-α), which was plotted only for baseline and 24 h. Panel **e** Lactate dehydrogenase (LDH). Panel **f** Creatine kinase (CK)

compared to sham irradiation. The authors suggested that future researches with larger samples are needed to study the molecular effects of photobiomodulation on muscular tissue through biopsy. We agree with the importance of muscle biopsy as an objective to assess the effect of exercise training in conjunction with photobiomodulation. However, this approach is invasive and expensive and might be challenging to be implemented, especially in older adults. Finally, an 8-week training protocol is considered adequate to show positive effects of photobiomodulation on muscle performance in older women [13] but maybe not enough for changes in modulation of inflammatory biomarkers.

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Compliance with ethical standards

The study was approved by the University Ethics Committee and all participants signed the consent form before any type of assessment.

Conflict of interest The authors declare that they have no conflict of interest.

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