Lasers Med Sci (2016) 31:1555–1564
DOI 10.1007/s10103-016-2015-7

ORIGINAL ARTICLE

What is the best moment to apply phototherapy when associated to a strength training program? A randomized, double-blinded, placebo-controlled trial

Phototherapy in association to strength training

Adriane Aver Vanin[1,2] · Eduardo Foschini Miranda[1,3] · Caroline Santos Monteiro Machado[1] · Paulo Roberto Vicente de Paiva[1,3] · Gianna Móes Albuquerque-Pontes[1,3] · Heliodora Leão Casalechi[1] · Paulo de Tarso Camillo de Carvalho[1,2,3] · Ernesto Cesar Pinto Leal-Junior[1,2,3,4]

Received: 28 February 2016 / Accepted: 24 June 2016 / Published online: 1 July 2016

Abstract The effects of phototherapy (or photobiomodulation therapy) with low-level laser therapy (LLLT) and/or light-emitting diodes (LEDs) on human performance improvement have been widely studied. Few studies have examined its effect on muscular training and no studies have explored the necessary moment of phototherapy irradiations (i.e., before and/or after training sessions). The aim of this study was to determine the optimal moment to apply phototherapy irradiation when used in association with strength training. Forty-eight male volunteers (age between 18 to 35 years old) completed all procedures in this study. Volunteers performed the strength training protocol where either a phototherapy and/or placebo before and/or after each training session was performed using cluster probes with four laser diodes of 905 nm, four LEDs of 875 nm, and four LEDs of 640 nm—manufactured by Multi Radiance Medical™. The training protocol duration was 12 weeks with assessments of peak torque reached in maximum voluntary contraction test (MVC), load in 1-repetition maximum test (1-RM) and thigh circumference (perimetry) at larger cross-sectional area (CSA) at baseline, 4 weeks, 8 weeks, and 12 weeks. Volunteers from group treated with phototherapy before and placebo after training sessions showed significant ($p < 0.05$) changes in MVC and 1-RM tests for both exercises (leg extension and leg press) when compared to other groups. With an apparent lack of side effects and safety due to no thermal damage to the tissue, we conclude that the application of phototherapy yields enhanced strength gains when it is applied before exercise. The application may have additional beneficial value in post-injury rehabilitation where strength improvements are needed.

Keywords Photobiomodulation therapy · Low-level laser therapy · Light-emitting diode · Muscle adaptation · Muscle fatigue · Phototherapy

Introduction

The benefits of strength training have been studied across a variety of health conditions and age populations with the aim of improving physical fitness and quality of life [1–5]. Considered to be an essential component of rehabilitation, strength training has now been incorporated into preventive programs to reduce financial costs related to absence of employees in work, prevent injuries, and improve athletic performance in sports settings [6].

Strength training with heavy loads develops neural adaptations followed by muscular hypertrophy responsible for increasing strength in the exercised muscle [7–9]. The mechanical stimulation produced by the exercise load is crucial and

✉ Ernesto Cesar Pinto Leal-Junior
ernesto.leal.junior@gmail.com

1 Laboratory of Phototherapy in Sports and Exercise, Universidade Nove de Julho (UNINOVE), São Paulo, SP, Brazil

2 Postgraduate Program in Rehabilitation Sciences, Universidade Nove de Julho (UNINOVE), São Paulo, Brazil

3 Postgraduate Program in Biophotonics Applied to Health Sciences, Universidade Nove de Julho (UNINOVE), São Paulo, Brazil

4 Laboratory of Phototherapy in Sports and Exercise, Rua Vergueiro 235/249, CEP: 01504-001 São Paulo, SP, Brazil

without it other stimuli are irrelevant [10]. Exercise-induced skeletal muscle adaptation may differ according to the type of exercise performed, previous experience to the same exercise activity, age, and gender [11, 12]. Factors such as muscle actions, intensity, volume, exercise order, rest time, and frequency are also directly related to increases in muscle strength [13]. Currently, research projects have been designed to identify the different combinations of exercise sets and number of repetitions to explain the gains reached with strength training [9, 14, 15].

First developed during the 1960s, lasers (acronym of light amplification by stimulated emission of radiation) are characterized as having light that is monochromatic and of low divergence. Light-emitting diodes (or LEDs) were developed much later and share similarities to laser but the emitted light has far less coherence and a wider bandwidth. Phototherapy (or photobiomodulation therapy) using low-level laser therapy (LLLT) and/or light-emitting diode therapy (LEDT) has been used to promote tissue regeneration, reduce inflammation, accelerate wound healing, and relieve pain [16, 17].

Recently, phototherapy (with LLLT and/or LEDT) has demonstrated novel ergogenic effects on exercise human performance and post-exercise recovery [18–24]. Two systematic reviews [25, 26], one of them with meta-analysis [26], about the effects and the use of phototherapy in exercise performance and recovery, found positive results in improvement of performance and in biochemical markers related to recovery with the use of phototherapy. Positive outcomes have been demonstrated by a variety of wavelengths and different light sources (lasers and LEDs).

The effects of combination of super-pulsed-laser, red and infrared LEDs on muscle recovery, and performance in healthy volunteers have been analyzed [18]. Three doses were tested against placebo (10, 30, and 50 J), and 30-J dose showed better results in improvement of performance and decreased the delayed onset muscle soreness (DOMS) and creatine kinase activity (CK). It was suggested that a combination of previously successful phototherapy parameters may further optimize the effects on exercise performance and recovery.

It is suggested that pre-exercise irradiation with phototherapy may beneficially improve the overall progress and enhance strength gains by reducing fatigue and catabolic effect and could result in cumulative gains being realized over time. However, there are concerns that pre-exercise phototherapy could negatively affect muscle remodeling since exercise-induced muscle damage is important to increase muscle mass [27, 28], and phototherapy has shown protective effects on muscle tissue if applied before exercises [26, 29].

Post-exercise phototherapy may prevent an exaggerated inflammatory response caused by exercise-induced muscle damage [30]. On the other hand, post-exercise phototherapy application could disrupt the signaling of the inflammatory response for muscular remodeling through activation of satellite cells [27, 28, 31, 32]. Therefore, the moment of phototherapy irradiation, either before and/or after exercise, is of crucial importance to determine if an effect is either beneficial or detrimental to a strength training program.

While a large volume of work exists in this area, there is a lack of data on the use of phototherapy for improvement of strength training. With this perspective in mind, the aim of this study is to analyze the effects of phototherapy with combination of different light sources (super-pulsed laser, red and infrared LEDs) applied in different time points (before and/or after) of each training session to evaluate the potential outcomes on a muscle strengthening program.

Materials and methods

The study performed was a randomized, double-blinded placebo-controlled trial.

Subjects

The project received approval from the institutional research ethics committee (protocol number XXXX). Subjects were informed about the study design and the possible risks and discomfort related to procedures. All volunteers agreed to participate and signed a written informed consent. The CONSORT flowchart summarizing experimental procedures and number of volunteers at each study phase are shown in Fig. 1.

In a previous study with the same device, there was no evidence of thermal damage to the skin in various shades of skin pigmentation [33]; therefore, volunteers were not excluded based upon their skin color. The number of participants per group was calculated based on a previous study with the same phototherapy device [18]. For sample size calculation, we considered the β value of 20 % and α of 5 %. In a reference study [18], phototherapy improved maximum voluntary contraction—MVC (our primary outcome) to 336.88 N.m (±27.92), compared to baseline (286.63 ± 38.86). The calculation resulted in a sample of 12 volunteers per group, 48 volunteers in total. Predicting a sample loss of 20 %, 14 healthy volunteers were recruited per group (56 volunteers in total). Volunteers that completed all procedures in study ($n = 48$) had a mean age of 26 years old (±5.24), height of 174.5 cm (±7.59), and body mass 76.5 kg (±10.8).

Inclusion and exclusion criteria

Males between the age of 18 and 35 years old who performed less than one exercise activity per week with light, intermediate, or dark skin pigmentation [33] met the inclusion criterion.

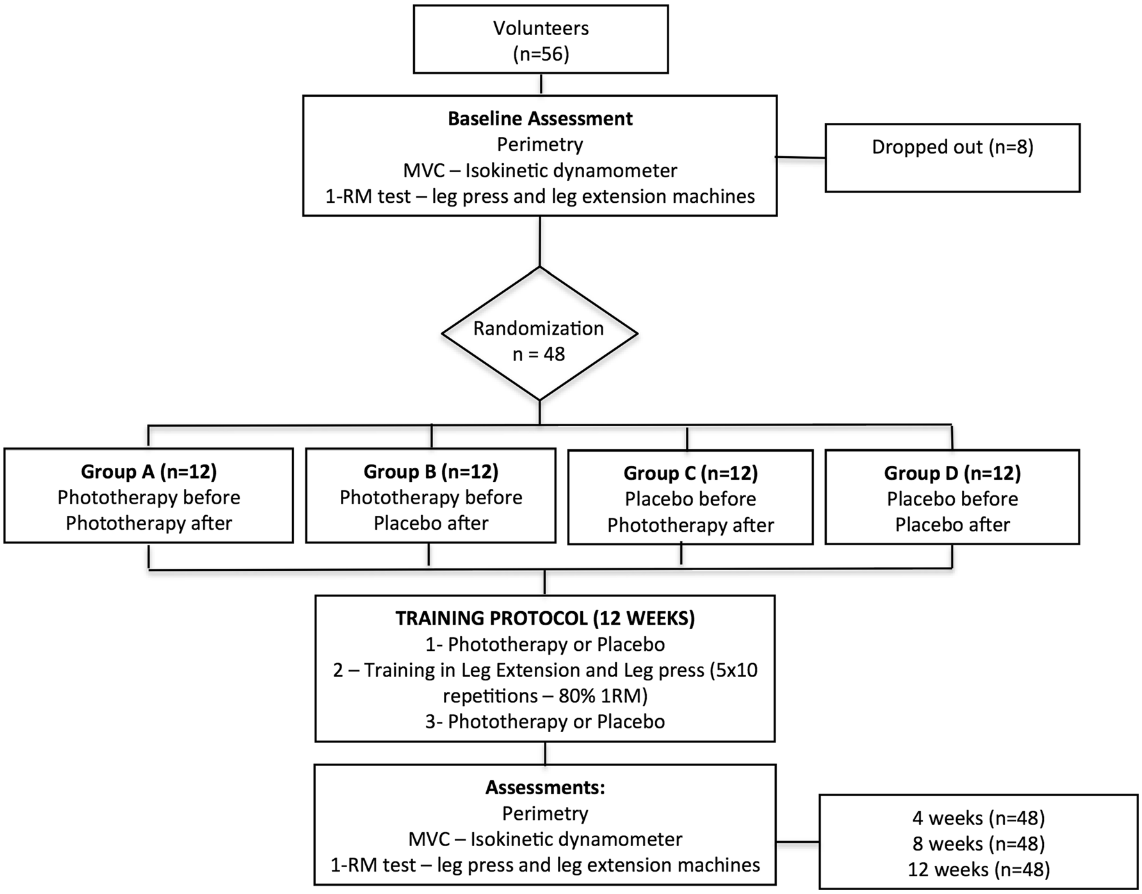

Fig. 1 CONSORT flowchart

Volunteers were excluded if they presented with a musculoskeletal injury to hips or knees in the previous 2 months or during 3 months of execution training, regular use of pharmacological agents, or nutritional supplementation. Volunteers that were unable to attend a minimum rate of 80 % of the strength training sessions and volunteers with immune diseases that require continuous use of anti-inflammatory drugs were also excluded. No statistical difference ($p > 0.05$) exists among groups regarding number of included volunteers that could not perform 100 % of training sessions and average number of missed training sessions (Table 1).

Composition of sample and randomization procedures

Of the initial 56 recruited volunteers, 8 dropped out during baseline assessments prior to the randomization process. The remaining 48 volunteers were distributed in four experimental groups (12 volunteers in each group) through a simple drawing of lots (A, B, C, or D) that determined the moment they would receive active and/or placebo phototherapy treatment:

- Photo + photo: volunteers were treated with active phototherapy before and after each training session;
- Photo + placebo: volunteers were treated with active phototherapy before and placebo phototherapy after each training session;
- Placebo + photo: volunteers were treated with placebo phototherapy before and active phototherapy after each training session;
- Placebo + placebo: volunteers were treated with placebo phototherapy before and after each training session;

The phototherapy device was pre-programmed with different programs to ensure blinding of volunteers and researchers. None of the researchers involved with strength

Table 1 Number of volunteers that did not perform 100 % of training sessions and average number of missed training sessions (± SD)

	Number of volunteers that did not perform 100 % of training sessions	Average number of missed training sessions
Photo + photo	3	2.33 (±0.47)
Photo + placebo	3	2.00 (±0.82)
Placebo + photo	3	2.33 (±0.47)
Placebo + placebo	2	2.00 (±1.00)

training, assessments and data collection had knowledge about which program corresponded to active or placebo phototherapy.

Blinding procedures

Identical phototherapy devices were programmed with two programs (one active, one placebo) by a researcher who was not involved in any phase of the projected data collection to ensure study blinding. All displays and sounds emitted were identical regardless of the selected program. The active phototherapy treatment did not demonstrate discernable amounts of heat [33], therefore, volunteers were unable to differentiate between active or placebo treatments. All volunteers were required to wear opaque goggles during treatments to maintain the double-blind design.

Procedures

Assessments

Baseline assessments recording the subjects' thigh perimeter and a maximum voluntary contraction (MVC) test were performed in the morning. In the afternoon, the 1-repetition maximum test (1-RM) was performed and recorded. Assessments of thigh perimeter, MVC, or 1-RM tests were performed by the same researcher, blinded to the device programming (active or placebo) and volunteers' allocation in the different experimental groups. The assessments were repeated at 4th, 8th, and 12th week of strength training. Participants were instructed to sleep well, continue their usual physical and nutritional behavior, and avoid alcoholic drinks and nutritional supplements.

1-RM test All volunteers performed a brief warm-up on a cycle ergometer (Inbramed®, Brazil), with 100 rpm with no load for 5 min. The range of motion for both leg press and knee extension exercises was from 90° of knee flexion to 0° (full knee extension) and the anatomical references were the lateral condyle of the knee and lateral malleolus of ankle. A familiarization exercise set with an estimated load less than 60 % of 1-RM using the strength training machines was performed prior to the test. This subjective load was identified in accordance with the OMNI scale (0 equal extremely easy and 10 equal extremely hard) [34].

One repetition maximum was determined by progressively increasing the load until the subject was unable to perform the activity with full range of motion [35] and identified on the OMNI scale [34]. To avoid metabolic disorders and interferences in test quality, load selections were limited to five attempts with 5-min intervals and were performed both for leg extension and for the leg press exercises on both legs (unilaterally). The volunteers were verbally encouraged to achieve maximum effort. The 1-RM test was recorded at baseline and at 4th, 8th, and 12th week of strength training, and training load was adjusted at 4th, 8th week of training protocol according to 1-RM re-assessments.

Maximal voluntary contraction (MVC) Volunteers were seated and attached to an isokinetic dynamometer (System 4, Biodex®—EUA) by a seat strap and two straps crossing the trunk with an angle of 100° between the trunk and hip and the leg positioned with 60° of knee flexion (0° corresponds to complete knee extension) and the axis of the dynamometer was positioned parallel to the center of the knee joint.

Volunteers were instructed to cross their arms over the trunk as they performed the MVC test that consisted of three 5-s isometric contractions of the knee extensors of the leg. The highest torque value of the three contractions (peak torque) was used for statistical analysis and reflects the maximum generation of force by the muscle in this condition [36]. Volunteers were given instructions on how to execute the test and given verbal encouragement during the test. MVC tests were recorded at baseline and at 4th, 8th, and 12th week of strength training for both legs (unilaterally).

Perimetry This measurement was performed in orthostatic position, feet slightly apart, the body weight equally distributed between legs with the thigh muscle relaxed. We used the measure relating to one third of distance between the gluteal fold and femoral-tibial joint space (popliteal line) and considered the point with the larger cross-sectional area of the anterior muscle. This assessment was performed at baseline and at 4th, 8th, and 12th week of strength training for both legs (unilaterally).

Intervention

Strength training protocol Volunteers initiated the strength training program based on Ferraresi et al. [19] study, 2 days following baseline assessments. The training protocol utilized a load of 80 % of 1-RM, five sets of ten repetitions for leg press and leg extension exercises, unilaterally (for both legs) and the rest between sets was 2 min. If a volunteer could not complete a full set of the exercise, he was instructed to continue until concentric muscle failure. The training protocol consisted of two sessions a week on non-consecutive days (72 h of rest) for 12 consecutive weeks (total of 24 training sessions) and the workload was adjusted by retesting the 1-RM test at 4th and 8th week. The room temperature was maintained between 23 and 26 °C.

Phototherapy Active or placebo phototherapy treatments were performed before and/or after each training session depending of each volunteer allocation to different experimental groups. Phototherapy was applied in direct contact with skin with slight pressure to six different sites of the anterior muscle of the thigh (two centrally—rectus femoris and vastus intermedius, two laterally—vastus lateralis, and two medially—vastus medialis) for both legs, as shown in Fig. 2.

Phototherapy was applied using the MR4 Laser Therapy System with LaserShower 50 4D cluster emitters (both manufactured by Multi Radiance Medical, Solon—OH, USA). The cluster emitters have 12 diodes, and each cluster emitter combines four super-pulsed laser diodes (905 nm), four red LEDs (640 nm), and four infrared LEDs (875 nm). The protocol for irradiation (active or placebo) required the use of four MR4 control units with three cluster emitters connected to each one, totalizing 12 cluster emitters to allow all sites of the anterior muscles of the thigh to be irradiated simultaneously and bilaterally for a total treatment time of 228 s (3 min and 48 s). The dose of 30 J (0.285 J of 905 nm, 13.68 J of 640 nm, 15.96 J of 875 nm) was selected based on a previous study using this same device performed by Antonialli et al. [18], and it was applied to six spots on each leg for both for pre- and post-exercise treatments. The time length between irradiation and the exercise protocol both to pre- and post-treatments was 5 to 10 min.

All phototherapy parameters are shown in Table 2.

Statistical analysis

The Kolmogorov–Smirnov test was used to verify the normal distribution of data. The data exhibited normal distribution and is expressed as mean values with standard deviations (SD). Data were analyzed both in absolute values and in percentage of change from baseline assessments. Two-way ANOVA test was performed to test between-group differences (followed by Bonferroni post hoc test). The significance level was set at $p < 0.05$. In graphs, data are presented as mean and standard error of the mean (SEM).

Results

Forty-eight male volunteers completed all experimental procedures for this study. The data were analyzed and no significant differences ($p > 0.05$) were observed at baseline for all experimental groups for MVC, 1-RM test, or perimetry and summarized in Table 3. No significant differences ($p > 0.05$) were observed between groups for any experimental time regarding perimetry.

MVC values, both in absolute and percentages, were significantly improved ($p < 0.05$) by pre-exercise phototherapy as recorded in Fig. 3. The same positive effect was observed for 1-RM test with the leg press and leg extension exercises (Figs. 4 and 5).

Fig. 2 Sites of phototherapy irradiation on anterior muscle of the thigh

Discussion

To our knowledge, this is the first study to identify the optimal moment to provide phototherapy irradiation when used in conjunction with strength training programs. To validate the

Table 2 Parameters for phototherapy

Number of lasers	4 super-pulsed infrared
Wavelength (nm)	905 (±1)
Frequency (Hz)	250
Peak power (W)—each	12.5
Average mean optical output (mW)—each	0.3125
Power density (mW/cm^2)—each	0.71
Energy density (J/cm^2)—each	0.162
Dose (J)—each	0.07125
Spot size of laser (cm^2)—each	0.44
Number of red LEDs	4 red
Wavelength of red LEDs (nm)	640 (±10)
Frequency (Hz)	2
Average optical output (mW)—each	15
Power density (mW/cm^2)—each	16.66
Energy density (J/cm^2)—each	3.8
Dose (J)—each	3.42
Spot size of red LED (cm^2)—each	0.9
Number of infrared LEDs	4 infrared
Wavelength of infrared LEDs (nm)	875 (±10)
Frequency (Hz)	16
Average optical output (mW)—each	17.5
Power density (mW/cm^2)—each	19.44
Energy density (J/cm^2)—each	4.43
Dose (J)—each	3.99
Spot size of LED (cm^2)—each	0.9
Magnetic field (mT)	35
Irradiation time per site (s)	228
Total dose per site (J)	30
Total dose applied in muscular group (J)	180
Aperture of device (cm^2)	20
Application mode	Cluster probe held stationary in skin contact with a 90° angle and slight pressure

result, we decided to assess peak torque reached in maximum voluntary contraction test (MVC), load in 1-repetition maximum test (1-RM), and thigh circumference (perimetry) at larger cross-sectional area (CSA).

While strength gains were observed, perimetry data did not demonstrate statistical significance and no significant changes in enhancement of muscle mass were observed until the end of training protocol (12 weeks). It is well established that in first weeks of muscular training, strength enhancement is attributed to the sum of neural and morphological adaptations. With no occurrence of significant changes in muscle thickness, the neural aspect is more evident [7, 19, 37]. This neural adaptation usually occurs until 6 to 8 weeks of training [27] and may partially explain our findings.

In agreement with Baroni et al. [38], we suggest that strength gains noted in both MVC and 1-RM tests for the two exercises in the present study may be attributed to intrinsic adaptation of muscle cells and changes in the muscle fibers, which are not detectable in whole muscle. Moreover, we believe that intramuscular fat content may have been replaced by contractile tissue leading to absence of changes in muscle circumference until the end of the 12-week training period as suggested by Baroni et al. [38].

The same researcher did perform all perimetry measurements and the assessments were done at the same time of day. However, the method is known to have intra-evaluator errors despite its use in clinical practice. A more accurate CSA assessment method is the use of magnetic resonance imaging multiscan (MRI) [7, 35, 39] and tomography [35, 40]. Baroni et al. [38] assessed muscle thickness using ultrasonography imaging correlated with electric activity from EMG analysis and observed increased muscle mass up to 8 weeks of training and stabilized at the end of 12 weeks. However, despite a higher accuracy, these methods are expensive and not commonly available in daily clinical settings.

The experimental group that received active phototherapy only before strength training sessions (and placebo after) increased MVC from 39 to 46 % (right and left leg, respectively); the experimental group that received active phototherapy after each strength training session (and placebo before) increased MVC from 20 to 21 % (right and left leg, respectively). Interestingly, the experimental group that received active phototherapy before and after strength training sessions training increased MVC from 12 % to 19 % (right and left leg, respectively). The group receiving only placebo phototherapy irradiation before and after strength training sessions increased MVC from 14 to 15 % (right and left leg, respectively). Significant statistical difference ($p < 0.05$) in MVC (for both legs) was observed at the 4th week of strength training in favor of group that received phototherapy before training sessions (and placebo after) compared to the group that received placebo irradiations before and after training sessions. Furthermore, pre-exercise phototherapy was statistically significantly better ($p < 0.05$) than all experimental groups at 8th and 12th week of strength training (for both legs).

A similar pattern was observed for the 1-RM test for the leg press and leg extension exercises. Our results show for 1-RM test in leg press with an increase in 169 to 176 % (right and left leg, respectively) and for leg extension an increase in 95 to 100 % (right and left leg, respectively) for group treated with pre-exercise phototherapy (and placebo after). The placebo group (before and after training sessions) demonstrated an increase of 86 to 120 % (right and left leg, respectively) for leg press exercise and an increase in 49 and 48 % (right and

Table 3 Data for functional assessments (mean ± SD)

		Baseline	4 weeks	8 weeks	12 weeks
MVC (N.m)	Photo + photo	193.20 (±23.27)	200.54 (±19.98)	215.43 (±21.89)	216.72 (±25.18)
Right leg	Photo + placebo	202.13 (±24.55)	227.07 (±33.75)	251.45 (±35.76)[a]	280.90 (±38.68)[a,b,c]
	Placebo + photo	196.24 (±21.38)	203.23 (±25.15)	224.48 (±28.04)	235.64 (±31.84)
	Placebo + placebo	204.97 (±17.86)	213.33 (±23.74)	226.0 (±30.0)	233.16 (±27.99)
MVC (N.m)	Photo + photo	204.73 (±11.02)	215.66 (±23.71)	229.23 (±23.86)	243.78 (±24.16)
Left leg	Photo + placebo	213.22 (±14.14)	239.04 (±24.96)[b]	281.98 (±28.10)[a,b,c]	311.27 (±31.36)[a,b,c]
	Placebo + photo	197.42 (±18.57)	207.62 (±24.68)	227.53 (±27.08)	239.13 (±23.86)
	Placebo + placebo	209.44 (±17.21)	215.46 (±19.92)	225.47 (±21.11)	240.70 (±26.15)
1-RM test	Photo + photo	48.00 (±7.46)	65.83 (±14.49)	78.75 (±15.56)	90.08 (±15.59)
Leg press	Photo + placebo	53.92 (±8.04)	83.83 (±8.79)[a,b]	109.67 (±13.14)[a,b,c]	144.83 (±22.53)[a,b,c]
Right leg (kg)	Placebo + photo	48.67 (±4.81)	69.58 (±7.89)	82.42 (±9.66)	95.83 (±9.03)
	Placebo + placebo	56.08 (±5.96)	72.25 (±12.05)	88.42 (±17.05)	104.42 (±19.46)
1-RM test	Photo + photo	47.92 (±6.86)	74.25 (±14.40)	89.00 (±16.45)	104.33 (±15.76)
Leg press	Photo + placebo	52.67 (±7.48)	88.25 (±11.52)[a]	114.00 (±17.04)[a,b]	145.33 (±18.23)[a,b,c]
Left leg (kg)	Placebo + photo	49.58 (±4.38)	76.17 (±8.99)	90.50 (±5.89)	103.42 (±7.94)
	Placebo + placebo	55.83 (±5.84)	83.42 (±9.63)	106.92 (±12.94)[a,b]	123.08 (±16.98)[a,b]
1-RM test	Photo + photo	58.42 (±7.20)	67.67 (±9.85)	76.67 (±9.93)	89.17 (±11.04)
Leg extension	Photo + placebo	65.50 (±8.88)	95.83 (±14.80)[a,b,c]	114.75 (±20.33)[a,b,c]	127.83 (±22.93)[a,b,c]
Right leg (kg)	Placebo + photo	60.67 (±8.94)	70.67 (±9.25)	83.00 (±12.20)	92.33 (±13.28)
	Placebo + placebo	63.17 (±6.67)	76.67 (±11.52)	83.25 (±14.37)	94.17 (±13.58)
1-RM test	Photo + photo	60.00 (±8.73)	69.17 (±10.51)	80.50 (±11.00)	92.83 (±11.56)
Leg extension	Photo + placebo	66.42 (±8.73)	96.67 (±14.67)[a,b,c]	117.33 (±15.88)[a,b,c]	132.92 (±16.14)[a,b,c]
Left leg (kg)	Placebo + photo	59.83 (±8.44)	69.92 (±10.78)	81.33 (±12.63)	93.67 (±13.91)
	Placebo + placebo	64.50 (±6.13)	74.67 (±8.27)	85.33 (±11.80)	95.75 (±11.76)
Perimetry	Photo + photo	55.43 (±7.05)	56.43 (±6.98)	56.70 (±6.72)	57.05 (±6.59)
Right leg (cm)	Photo + placebo	58.33 (±8.17)	59.33 (±8.33)	59.35 (±7.79)	59.69 (±7.79)
	Placebo + photo	56.34 (±2.08)	57.31 (±1.88)	57.28 (±2.00)	57.89 (±1.52)
	Placebo + placebo	59.11 (±2.28)	59.90 (±1.90)	59.70 (±2.02)	60.30 (±2.25)
Perimetry	Photo + photo	55.56 (±7.21)	55.81 (±6.58)	56.27 (±6.64)	57.12 (±6.74)
Left leg (cm)	Photo + placebo	57.63 (±7.84)	58.50 (±8.00)	58.66 (±7.53)	59.00 (±7.46)
	Placebo + photo	56.25 (±1.97)	56.78 (±1.73)	57.23 (±1.98)	57.55 (±1.80)
	Placebo + placebo	58.95 (±2.56)	59.27 (±1.51)	59.38 (±1.98)	59.78 (±1.75)

[a] Indicates significant difference compared to photo + photo group ($p < 0.05$)

[b] Indicates significant difference compared to placebo + photo group ($p < 0.05$)

[c] Indicates significant difference compared to placebo + placebo group ($p < 0.05$)

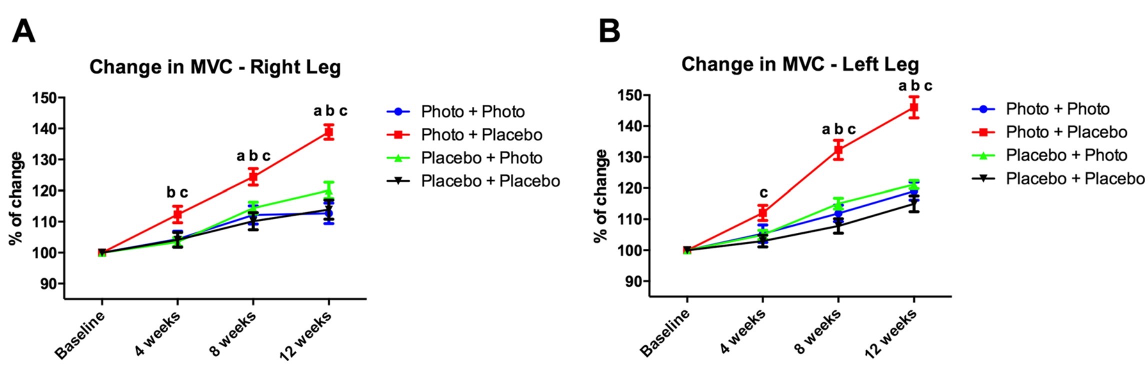

Fig. 3 Change in MVC (percentage values). *Values* are mean and *error bars* are SEM. *a* indicates significant difference compared to photo + photo group ($p < 0.05$), *b* indicates significant difference compared to placebo + photo group ($p < 0.05$), and *c* indicates significant difference compared to placebo + placebo group ($p < 0.05$)

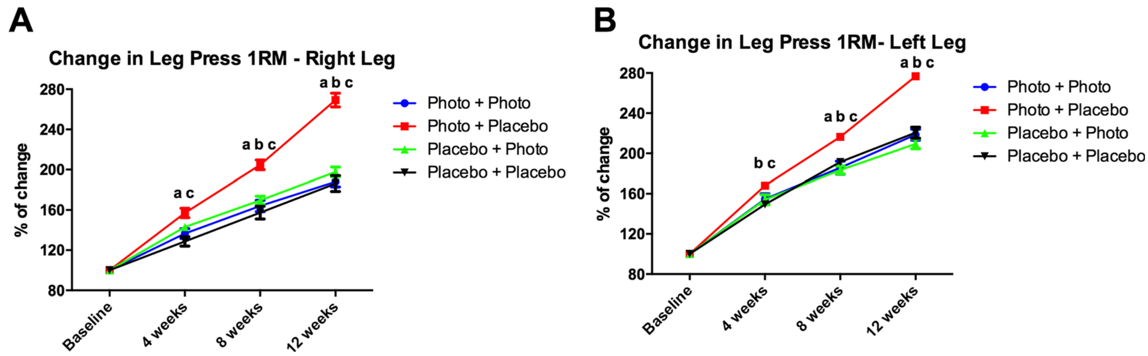

Fig. 4 Change in 1-RM test for leg press exercise (percentage values). *Values* are mean and *error bars* are SEM. *a* indicates significant difference compared to photo + photo group ($p < 0.05$), *b* indicates significant difference compared to placebo + photo group ($p < 0.05$), and *c* indicates significant difference compared to placebo + placebo group ($p < 0.05$)

left leg, respectively) for leg extension exercise. Of note, the 1-RM outcomes for the group treated with phototherapy before exercise (and placebo after) for the leg press exercise at 4th week reached the same improvement as all other groups at the 12th week time point. It suggests that pre-exercise phototherapy can potentiate the effects of muscular training by improving muscular strength three times faster than the placebo-control group (or exercise alone).

Baroni et al. [41] associated the application of pre-exercise phototherapy (with LLLT) before each eccentric exercise training session performed on an isokinetic dynamometer for an 8-week training duration and noted a significant increase ($p < 0.05$) in muscle mass (15.4 %) and in eccentric (32.3 %) and isometric peak torques (20.5 %) in volunteers. Authors concluded that addition of pre-exercise phototherapy can be beneficial to training programs where increase in muscular strength is desired.

Our outcomes partially agree with the findings observed by Baroni et al. [41]. Despite lack of significant results observed in muscle mass assessment, our outcomes demonstrated greater improvement in isometric muscular strength (39 to 46 %) than found previously (20.5 %). The irradiation of healthy muscles by phototherapy leads to increased cytochrome c-oxidase activity and may explain the effects of this therapy in stimulating intact (non-injured) skeletal muscles [42, 43]. Recently, Albuquerque-Pontes et al. [42] identified that a unique dose and time profile of activation of cytochrome c-oxidase exist for each wavelength (660, 830, and 905 nm). We believe that increase of 90 to 124 % observed in isometric torque observed in our study compared to Baroni et al. [41] is due to the synergistic use of concurrent multiple wavelengths and light sources. The increase in strength without gross muscle mass gain may suggest that pre-muscle training phototherapy may improve the overall muscle quality and efficiency.

Phototherapy applied after strength training sessions (and placebo before) and phototherapy performed before and after training sessions did not increase strength statistically compared to the placebo group. The application of phototherapy may disrupt signaling of the inflammatory response for muscular remodeling through activation of satellite cells [27, 28, 31, 32], which could explain the lack of positive results for post-exercise phototherapy. The lack of positive results for phototherapy before and after strength training sessions may be related to the overall dose delivered to the muscle tissue. While optimal doses have been identified as 180 J for the quadriceps [18], this dose was applied twice (before and after exercise sessions). The cumulative dose applied was effectively doubled for this group, which may have downregulated or eliminated

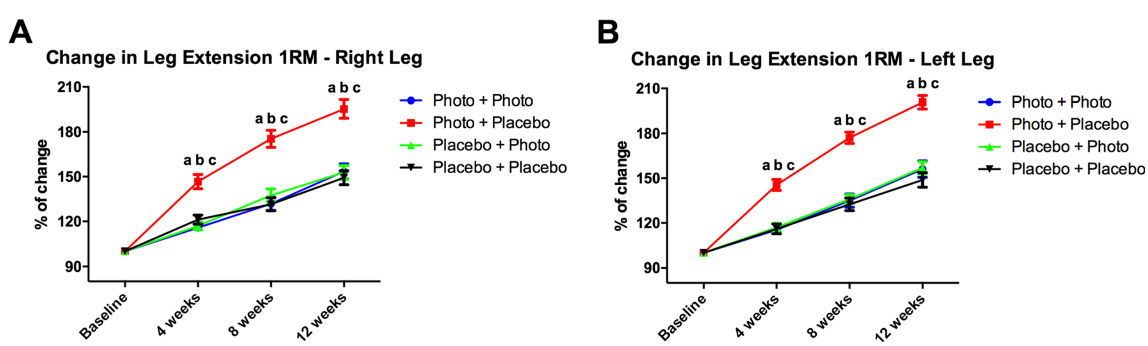

Fig. 5 Change in 1-RM test for leg extension exercise (percentage values). *Values* are mean and *error bars* are SEM. *a* indicates significant difference compared to photo + photo group ($p < 0.05$), *b* indicates significant difference compared to placebo + photo group ($p < 0.05$), and *c* indicates significant difference compared to placebo + placebo group ($p < 0.05$)

the beneficial cytoprotective effect. Further studies are needed to clarify these points.

The time window of 5 to 10 min between irradiation and the exercise protocol was selected based upon previously observed positive outcomes with this phototherapy device in clinical studies [18, 44, 45] when used in a time window of 3 to 20 min. However, this may not be the only appropriate time window that will elicit positive outcomes. Therefore, we suggest that further clinical studies with additional time windows should be tested to further optimize treatment parameters.

A limitation of this study is the variability of perimetry measurement. Assessment methods such as X-ray absorptiometry (DEXA) [39, 46] or ultrasonography imaging [41] may provide more accurate assessment and deserve to be considered in future studies. However, it is important to highlight that although perimetry assessment lacks sensitivity, this method is accepted and often utilized in clinical practice. Our study demonstrates consistency in data regarding strength gain. MVC tests performed with isokinetic dynamometers are currently considered gold standard for strength assessment, and outcomes found in MVC corroborate with 1-RM test. Finally, we conclude that application of phototherapy leads to better results in order to enhance strength gain when it is applied before exercise.

The data presented in this study confirm the positive interaction of pre-exercise phototherapy in muscle strength gain in muscular training when performed twice a week over 12 weeks. The application of phototherapy before exercise increased isometric strength in 39 to 46 %, whereas the placebo group strength increased from 14 to 15 %. In summary, our data show that pre-exercise phototherapy with parameters and device used in this study can lead to enhancement in the effects of strength training.

Compliance with ethical standards All procedures were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in study.

Conflict of interests Professor Ernesto Cesar Pinto Leal-Junior receives research support from Multi Radiance Medical (Solon, OH, USA), a laser device manufacturer. The remaining authors declare that they have no conflict of interests.

Disclosure of funding received for this work Adriane Aver Vanin received PhD scholarship from São Paulo Research Foundation (FAPESP) (grant number 2013/19355-3). Caroline Santos Monteiro Machado received undergraduate scholarship from São Paulo Research Foundation (FAPESP) (grant number 2013/25814-0). Professor Ernesto Cesar Pinto Leal-Junior would like to thank São Paulo Research Foundation—FAPESP (grant number 2010/52404-0) and Brazilian Council of Science and Technology Development—CNPq (grant numbers 472062/2013-1 and 307717/2014-3).

References

1. Benson AC, Torode ME, Fiatarone Singh MA (2008) Effects of resistance training on metabolic fitness in children and adolescents: a systematic review. Obes Rev 9:43–66
2. Hovanec N, Sawant A, Overend TJ, Petrella RJ, Vandervoort AA (2012) Resistance training and older adults with type 2 diabetes mellitus: strength of the evidence. J Aging Res 2012:284635
3. Mota MR, Oliveira RJ, Terra DF, Pardono E, Dutra MT, de Almeida JA, Silva FM (2013) Acute and chronic effects of resistance exercise on blood pressure in elderly women and the possible influence of ACE I/D polymorphism. Int J Gen Med 6:581–587
4. Nagamatsu LS, Handy TC, Hsu CL, Voss M, Liu-Ambrose T (2012) Resistance training promotes cognitive and functional brain plasticity in seniors with probable mild cognitive impairment. Arch Intern Med 172:666–668
5. Rosendahl E, Gustafson Y, Nordin E, Lundin-Olsson L, Nyberg L (2008) A randomized controlled trial of fall prevention by a high-intensity functional exercise program for older people living in residential care facilities. Aging Clin Exp Res 20:67–75
6. Murton AJ, Greenhaff PL (2013) Resistance exercise and the mechanisms of muscle mass regulation in humans: acute effects on muscle protein turnover and the gaps in our understanding of chronic resistance exercise training adaptation. Int J Biochem Cell Biol 45: 2209–2214
7. Hakkinen K, Alen M, Kraemer WJ, Gorostiaga E, Izquierdo M, Rusko H, Mikkola J, Hakkinen A, Valkeinen H, Kaarakainen E, Romu S, Erola V, Ahtiainen J, Paavolainen L (2003) Neuromuscular adaptations during concurrent strength and endurance training versus strength training. Eur J Appl Physiol 89:42–52
8. Moritani T, deVries HA (1979) Neural factors versus hypertrophy in the time course of muscle strength gain. Am J Phys Med 58:115–130
9. Seynnes OR, de Boer M, Narici MV (2007) Early skeletal muscle hypertrophy and architectural changes in response to high-intensity resistance training. J Appl Physiol 102:368–373
10. Baird MF, Graham SM, Baker JS, Bickerstaff GF (2012) Creatine-kinase- and exercise-related muscle damage implications for muscle performance and recovery. J Nutr Metab 2012:960363
11. Peake J, Nosaka K, Suzuki K (2005) Characterization of inflammatory responses to eccentric exercise in humans. Exerc Immunol Rev 11:64–85
12. Yamin C, Duarte JA, Oliveira JM, Amir O, Sagiv M, Eynon N, Sagiv M, Amir RE (2008) IL6 (-174) and TNFA (-308) promoter polymorphisms are associated with systemic creatine kinase response to eccentric exercise. Eur J Appl Physiol 104:579–586
13. American College of Sports M (2009) American College of Sports Medicine position stand. Progression models in resistance training for healthy adults. Med Sci Sports Exerc 41:687–708
14. Campos GE, Luecke TJ, Wendeln HK, Toma K, Hagerman FC, Murray TF, Ragg KE, Ratamess NA, Kraemer WJ, Staron RS (2002) Muscular adaptations in response to three different resistance-training regimens: specificity of repetition maximum training zones. Eur J Appl Physiol 88:50–60
15. McBride JM, Blaak JB, Triplett-McBride T (2003) Effect of resistance exercise volume and complexity on EMG, strength, and regional body composition. Eur J Appl Physiol 90:626–632
16. Chung H, Dai T, Sharma SK, Huang YY, Carroll JD, Hamblin MR (2012) The nuts and bolts of low-level laser (light) therapy. Ann Biomed Eng 40:516–533
17. Huang YY, Chen AC, Carroll JD, Hamblin MR (2009) Biphasic dose response in low level light therapy. Dose Response 7:358–383
18. Antonialli FC, de Marchi T, Tomazoni SS, Vanin AA, dos Santos Grandinetti V, de Paiva PR, Pinto HD, Miranda EF, de Tarso C, de

Carvalho P, Leal-Junior EC (2014) Phototherapy in skeletal muscle performance and recovery after exercise: effect of combination of super-pulsed laser and light-emitting diodes. Lasers Med Sci 29: 1967–1976
19. Ferraresi C, de Brito OT, de Oliveira ZL, de Menezes Reiff RB, Baldissera V, de Andrade Perez SE, Matheucci Junior E, Parizotto NA (2011) Effects of low level laser therapy (808 nm) on physical strength training in humans. Lasers Med Sci 26:349–358
20. Leal Junior EC, Lopes-Martins RA, Dalan F, Ferrari M, Sbabo FM, Generosi RA, Baroni BM, Penna SC, Iversen VV, Bjordal JM (2008) Effect of 655-nm low-level laser therapy on exercise-induced skeletal muscle fatigue in humans. Photomed Laser Surg 26:419–424
21. Leal Junior EC, Lopes-Martins RA, Vanin AA, Baroni BM, Grosselli D, De Marchi T, Iversen VV, Bjordal JM (2009) Effect of 830 nm low-level laser therapy in exercise-induced skeletal muscle fatigue in humans. Lasers Med Sci 24:425–431
22. Paolillo FR, Corazza AV, Borghi-Silva A, Parizotto NA, Kurachi C, Bagnato VS (2013) Infrared LED irradiation applied during high-intensity treadmill training improves maximal exercise tolerance in postmenopausal women: a 6-month longitudinal study. Lasers Med Sci 28:415–422
23. Toma RL, Tucci HT, Antunes HK, Pedroni CR, de Oliveira AS, Buck I, Ferreira PD, Vassao PG, Renno AC (2013) Effect of 808 nm low-level laser therapy in exercise-induced skeletal muscle fatigue in elderly women. Lasers Med Sci 28:1375–1382
24. Vieira WH, Ferraresi C, Perez SE, Baldissera V, Parizotto NA (2012) Effects of low-level laser therapy (808 nm) on isokinetic muscle performance of young women submitted to endurance training: a randomized controlled clinical trial. Lasers Med Sci 27:497–504
25. Borsa PA, Larkin KA, True JM (2013) Does phototherapy enhance skeletal muscle contractile function and postexercise recovery? A systematic review. J Athl Train 48:57–67
26. Leal-Junior EC, Vanin AA, Miranda EF, de Carvalho PT, Dal Corso S, Bjordal JM (2015) Effect of phototherapy (low-level laser therapy and light-emitting diode therapy) on exercise performance and markers of exercise recovery: a systematic review with meta-analysis. Lasers Med Sci 30:925–939
27. Folland JP, Williams AG (2007) The adaptations to strength training: morphological and neurological contributions to increased strength. Sports Med 37:145–168
28. Kadi F, Charifi N, Denis C, Lexell J, Andersen JL, Schjerling P, Olsen S, Kjaer M (2005) The behaviour of satellite cells in response to exercise: what have we learned from human studies? Pflugers Arch 451:319–327
29. Leal-Junior EC (2015) Photobiomodulation therapy in skeletal muscle: from exercise performance to muscular dystrophies. Photomed Laser Surg 33:53–54
30. Ziemann E, Zembron-Lacny A, Kasperska A, Antosiewicz J, Grzywacz T, Garsztka T, Laskowski R (2013) Exercise training-induced changes in inflammatory mediators and heat shock proteins in young tennis players. J Sports Sci Med 12:282–289
31. Mackey AL (2013) Does an NSAID a day keep satellite cells at bay? J Appl Physiol (1985) 115:900–908
32. Takagi R, Fujita N, Arakawa T, Kawada S, Ishii N, Miki A (2011) Influence of icing on muscle regeneration after crush injury to skeletal muscles in rats. J Appl Physiol 110:382–388
33. Grandinetti Vdos S, Miranda EF, Johnson DS, de Paiva PR, Tomazoni SS, Vanin AA, Albuquerque-Pontes GM, Frigo L, Marcos RL, de Carvalho PT, Leal-Junior EC (2015) The thermal impact of phototherapy with concurrent super-pulsed lasers and red and infrared LEDs on human skin. Lasers Med Sci 30:1575–1581
34. Irving BA, Rutkowski J, Brock DW, Davis CK, Barrett EJ, Gaesser GA, Weltman A (2006) Comparison of Borg- and OMNI-RPE as markers of the blood lactate response to exercise. Med Sci Sports Exerc 38:1348–1352
35. Abe T, Kojima K, Kearns CF, Yohena H, Fukuda J (2003) Whole body muscle hypertrophy from resistance training: distribution and total mass. Br J Sports Med 37:543–545
36. Ahtiainen JP, Pakarinen A, Alen M, Kraemer WJ, Hakkinen K (2003) Muscle hypertrophy, hormonal adaptations and strength development during strength training in strength-trained and untrained men. Eur J Appl Physiol 89:555–563
37. Wernbom M, Augustsson J, Thomee R (2007) The influence of frequency, intensity, volume and mode of strength training on whole muscle cross-sectional area in humans. Sports Med 37: 225–264
38. Baroni BM, Rodrigues R, Franke RA, Geremia JM, Rassier DE, Vaz MA (2013) Time course of neuromuscular adaptations to knee extensor eccentric training. Int J Sports Med 34:904–911
39. Bellamy LM, Joanisse S, Grubb A, Mitchell CJ, McKay BR, Phillips SM, Baker S, Parise G (2014) The acute satellite cell response and skeletal muscle hypertrophy following resistance training. PLoS One 9:e109739
40. Shepstone TN, Tang JE, Dallaire S, Schuenke MD, Staron RS, Phillips SM (2005) Short-term high- vs. low-velocity isokinetic lengthening training results in greater hypertrophy of the elbow flexors in young men. J Appl Physiol 98:1768–1776
41. Baroni BM, Rodrigues R, Freire BB, Franke Rde A, Geremia JM, Vaz MA (2015) Effect of low-level laser therapy on muscle adaptation to knee extensor eccentric training. Eur J Appl Physiol 115: 639–647
42. Albuquerque-Pontes GM, Vieira Rde P, Tomazoni SS, Caires CO, Nemeth V, Vanin AA, Santos LA, Pinto HD, Marcos RL, Bjordal JM, de Carvalho PT, Leal-Junior EC (2015) Effect of pre-irradiation with different doses, wavelengths, and application intervals of low-level laser therapy on cytochrome c oxidase activity in intact skeletal muscle of rats. Lasers Med Sci 30:59–66
43. Hayworth CR, Rojas JC, Padilla E, Holmes GM, Sheridan EC, Gonzalez-Lima F (2010) In vivo low-level light therapy increases cytochrome oxidase in skeletal muscle. Photochem Photobiol 86: 673–680
44. Miranda EF, Vanin AA, Tomazoni SS, Grandinetti Vdos S, de Paiva PR, Machado Cdos S, Monteiro KK, Casalechi HL, de Tarso P, de Carvalho C, Leal-Junior EC (2016) Using pre-exercise photobiomodulation therapy combining super-pulsed lasers and light-emitting diodes to improve performance in progressive cardiopulmonary exercise tests. J Athl Train 51:129–135
45. Pinto HD, Vanin AA, Miranda EF, Tomazoni SS, Johnson DS, Albuquerque-Pontes GM, Aleixo Junior IO, Grandinetti VD, Casalechi HL, de Carvalho PT, Leal-Junior EC (2016) Photobiomodulation therapy (PBMT) improves performance and accelerates recovery of high-level Rugby players in field test: a randomized, crossover, double-blind, placebo-controlled clinical study. J Strength Cond Res. [Epub ahead of print]
46. Kwon HJ, Ha YC, Park HM (2015) The reference value of skeletal muscle mass index for defining the sarcopenia of women in Korea. J Bone Metab 22:71–75