

# Photobiomodulation therapy (PBMT) and/or cryotherapy in skeletal muscle restitution, what is better? A randomized, double-blinded, placebo-controlled clinical trial

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**Abstract** Cryotherapy for post-exercise recovery remains widely used despite the lack of quality evidence. Photobiomodulation therapy (PBMT) studies (with both low-level laser therapy and light-emitting diode therapy) have demonstrated positive scientific evidence to suggest its use. The study aims to evaluate PBMT and cryotherapy as a single or combined treatment on skeletal muscle recovery after eccentric contractions of knee extensors. Fifty healthy male volunteers were recruited and randomized into five groups (PBMT, cryotherapy, cryotherapy + PBMT, PBMT + cryotherapy, or placebo) for a randomized, double-blinded, placebo-controlled trial that evaluated exercise performance (maximum voluntary contraction (MVC)), delayed onset muscle soreness (DOMS), and muscle damage (creatine kinase (CK)). Assessments were performed at baseline; immediately after; and at 1, 24, 48, 72, and 96 h. Comparator treatments was performed 3 min after exercise and repeated at 24, 48, and 72 h. PBMT was applied employing a cordless, portable GameDay™ device (combination of 905 nm super-

pulsed laser and 875- and 640-nm light-emitting diodes (LEDs); manufactured by Multi Radiance Medical™, Solon - OH, USA), and cryotherapy by flexible rubber ice packs. PBMT alone was optimal for post-exercise recovery with improved MVC, decreased DOMS, and CK activity ( $p < 0.05$ ) from 24 to 96 h compared to placebo, cryotherapy, and cryotherapy + PBMT. In the PBMT + cryotherapy group, the effect of PBMT was decreased ( $p > 0.05$ ) but demonstrated significant improvement in MVC, decreased DOMS, and CK activity ( $p < 0.05$ ). Cryotherapy as single treatment and cryotherapy + PBMT were similar to placebo ( $p > 0.05$ ). We conclude that PBMT used as single treatment is the best modality for enhancement of post-exercise restitution, leading to complete recovery to baseline levels from 24 h after high-intensity eccentric contractions.

**Keywords** Phototherapy · Low-level laser therapy · Light-emitting diodes · Performance · Exercise recovery

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## Introduction

From professional to recreational, athletic performance whether training or competing requires imposes a large physiological demands on the body. Fatigue is linked to a decrease in athletic performance and is experienced by all athletes in every sport. Enoka and Duchateau [1] describe the development of muscle fatigue as a quantified decline in the maximal force or power capacity of muscle. Physiological recovery from fatigue is necessary to prevent overtraining and minimizing injuries and is often overlooked aspect of athletic performance.

A wide array of traditional and non-conventional modalities has been suggested to accelerate recovery following exercise or sport. While supportive evidence may be scarce or limited, massage [2], water immersion [3], contrast baths [4], compressive garments, stretching [5], and cryotherapy [6] have been employed in clinical practice and generally accepted as beneficial. In clinical practice, it is not uncommon for suggested modalities to be performed as monotherapies or as adjuncts to other method, modalities, or treatments.

Cryotherapy remains one of most popular and more often used modalities in the management of acute musculoskeletal injuries. Recently published systematic reviews [7, 8] have challenged the effectiveness of cryotherapy for aiding in post-exercise recovery. Despite the lack of scientific evidence for its use related to recovery [7, 8], various cooling procedures are widely utilized by physicians, clinicians, and laymen as a first means of reducing the delayed onset muscle soreness (DOMS) and minimizing muscular damage.

On the other hand, two recent systematic reviews [9, 10] support the positive ergogenic effects of pre-activity exposure to photobiomodulation therapy (PBMT) by both low-level lasers and light-emitting diode (LED) light sources. Furthermore, recent studies [11–15] suggest that combining the use of multiple wavelengths and light sources before exercise can improve performance. The presence of strong positive evidence suggests a paradigm shift from using PBMT only following an injury for rehabilitation but now also as a means for prophylactic protection of skeletal muscles. With minimal contraindications, PBMT offers a unique, non-invasive, non-pharmacological means to accelerate recovery from muscular fatigue and exercise-induced damage.

In clinical practice, it is often necessary to decide between the application of two or more different therapeutic agents. In some instances, it is common to combine two or more therapeutic agents in an effort to combine them synergistically and maximize the limited available time with the patient. Therefore, a greater understanding of the most effective therapeutic agent or combination of modalities for a given condition is necessary to optimize the clinical decision-making process.

A few direct comparisons between PBMT and other physical agents [16, 17] have been published, but to our knowledge, only two small animal trials [18, 19] and one preliminary clinical trial [20] have directly compared cryotherapy to PBMT. Despite scientific evidence indicates that pre-exercise PBMT has ergogenic and protective effects on skeletal muscle tissue, it is important to highlight that cryotherapy is usually applied after exercise sessions when it is used to accelerate post-exercise restitution. Thus, to perform a reliable and fair head-to-head comparison, both modalities need to be applied at same conditions.

With this perspective in mind, the aim of this study was to evaluate the effects of cryotherapy and PBMT on post-

exercise recovery in untrained male volunteers to determine which of the comparators or sequence of combined comparators can accelerate recovery following eccentric exercise-induced muscle fatigue. The result of this study will assist clinicians in determining where ice, PBMT, or a combination of both modalities influences recovery rates and provides evidence for best clinical practice.

## Materials and methods

### Study design and ethics statement

A randomized, double-blind, placebo-controlled clinical trial was performed. The project has received approval from the institutional research ethics committee.

### Characterization of sample

Sample size calculations were based on a previous study [12] that utilized the same PBMT device that observed positive significant improvement in maximum voluntary contraction (MVC), DOMS, and creatine kinase (CK) activity. Fifty healthy untrained male subjects were recruited from university staff and students to participate in the study. All participants voluntarily agreed to participate and signed the informed consent statement. Despite no dropouts occurred in study, the intention-to-treat analysis was followed. CONSORT flowchart summarizing experimental procedures and subjects are shown in Fig. 1.

### Inclusion criteria and exclusion criteria

Males between the ages of 18 and 25 were included in the study if they performed up to one exercise session per week. In some instances, the outcomes of PBMT may be impacted by skin pigmentation due to rapidly increasing surface temperature. However, the device used in this study has demonstrated previously a lack of damaging thermal effects that is independent of amount of skin pigmentation [21]. Therefore, volunteers were not excluded due to skin color and none adjustment in PBMT parameters was needed. Volunteers were excluded if they presented with any musculoskeletal injury to hips or knees within the previous 2 months, were currently using pharmacological agents or nutritional supplements regularly, if a musculoskeletal injury during the study occurred, or if they reported use of either alcohol or tobacco.

### Composition of groups and randomization process

The volunteers were randomly allocated to five experimental groups ( $n = 10$  per group) according to the applied comparator. Randomization was carried out by a simple drawing of lots (A,

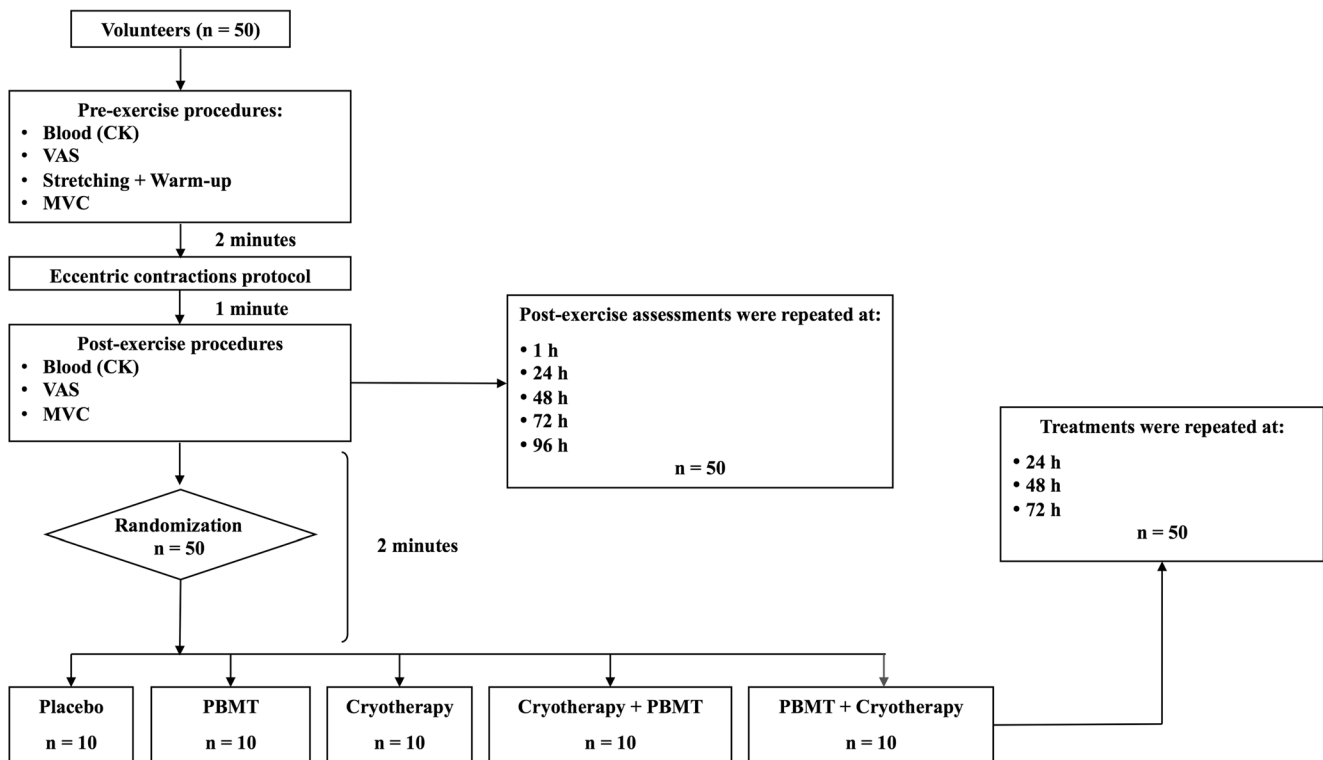


Fig. 1 CONSORT flowchart

B, C, D, or E). The placebo PBMT devices were identical to the active devices and displayed the same settings and emitted the same sound regardless of the comparator. Randomization labels were created using a randomization table at a central office where a series of sealed, opaque, and numbered envelopes were used to ensure confidentiality. The researcher who programmed the PBMT devices according to the randomization results was instructed to not disclose the identity of the devices to anyone involved in the study. A different researcher applied the various comparators and was blinded to which devices provided active or placebo treatments to the volunteers.

## Experimental protocol

### Blood samples and biochemical analyses

Following the informative process and randomization, blood samples (10 ml) were taken by a qualified nurse blinded to the allocation of the volunteers to the five experimental groups from the antecubital vein to establish baseline values. Additional samples were collected at 1 min, 1, 24, 48, 72, and 96 h after the eccentric contraction protocol. One hour after collection, each sample was centrifuged at 3000 rpm for 20 min. Pipettes were used to transfer the serum to Eppendorf® tubes, which were stored at  $-80^{\circ}\text{C}$  until analysis.

Spectrophotometry and specific reagent kits (Labtest®, São Paulo, SP, Brazil) were utilized to analyze CK activity as an indirect marker of muscle damage. The researcher performing

the blood analysis was blinded to randomization and allocation of volunteers in experimental groups.

### Evaluation of DOMS

A visual analogue scale (VAS) of 100 mm was used as a self-rating of volunteer DOMS intensity, with assistance of a blinded researcher. We decided to use VAS since this is a valid and reliable method to assess DOMS [22–24]. DOMS assessments were performed at baseline, immediately after eccentric exercise protocol (1 min), and were repeated at 1, 24, 48, 72, and 96 h after the exercise protocol.

### Stretching and warm-up

Prior to the isokinetic protocol, each volunteer performed three 60-s sets of active stretching of the knee extensors of the non-dominant lower limb followed by a light warm up consisting of pedaling a stationary bike (Inbramed®, Porto Alegre, RS, Brazil) at 100 rpm for 5 min without resistance.

### MVC

Immediately following the stretching and warm-up exercises, an eccentric exercise protocol was performed on an isokinetic dynamometer to measure MVC and evaluate muscle function. Volunteers were seated upright on the isokinetic dynamometer (System 4, Biodex®, USA) with an angle of  $100^{\circ}$  between the

trunk and hip and fixated by two straps crossing the trunk. The non-dominant leg was positioned with the knee at 60° of flexion (0° corresponds to complete knee extension) and fixated to the seat of the dynamometer by straps. The dominant leg was positioned at 100° of hip flexion and fixated as well. The volunteers were instructed to cross their arms over the trunk, and the axis of the dynamometer was positioned parallel to the center of the knee. The MVC test consisted of three 5-s isometric contractions of the knee extensors of the non-dominant leg. The highest torque value of the three contractions (peak torque) was used for the statistical analysis. Instructions on how to execute the test were given first, and the volunteers received verbal encouragement during the execution of the test. Despite the diversity of protocols proposed for the execution of eccentric exercises on isokinetic dynamometers, previous studies [12, 15, 25] employing PBMT have utilized the same protocol and demonstrated reliability and reproducibility for exercise-induced muscle damage. MVC measurements were repeated at the same time intervals as the CK measures. The researcher that performed assessment of MVC was blinded to randomization and allocation of volunteers in experimental groups.

#### *Eccentric exercise protocol*

Precisely 2 min after baseline MVC, volunteers performed the eccentric contraction protocol, which consisted of 75 eccentric isokinetic contractions of the knee extensor musculature in the non-dominant leg (5 sets of 15 repetitions, 30-s rest interval between sets) at a velocity of 60°.seg<sup>-1</sup> in both the flexion and extension of the knee movements with a 60° range of motion (between 90° and 30° of knee flexion). At each contraction, the dynamometer automatically (passively) positioned the knee at 30°; the dynamometer then flexed the knee until reaching 90° [12, 25]. The volunteers were instructed to resist against knee flexion movement imposed by the dynamometer with maximum force. Instructions on how to execute the maneuver were given first, and the volunteers received verbal encouragement throughout the protocol. The researcher in charge was blinded to randomization and allocation of volunteers in experimental groups.

#### **Treatments**

All comparators tested were applied 3 min after eccentric exercise protocol. Comparators were repeated at 24, 48, and 72 h following the collection of additional blood samples for CK analyses and MVC and VAS (DOMS) assessments.

#### *PBMT*

PBMT was applied employing a cordless, portable GameDay™ device (manufactured by Multi Radiance Medical, Solon, OH, USA) to six sites of quadriceps femoris in direct contact with the skin (two centrally—rectus femoris

and vastus intermedius, two laterally—vastus lateralis, and two medially—vastus medialis) (Fig. 2). PBMT parameters were chosen based on parameters tested in a previous study [12]. The description of parameters is provided in Table 1.

The optical power was checked before irradiation in each volunteer using a Thorlabs thermal power meter (model S322C, Thorlabs®, Newton, NJ, USA). The researcher that performed PBMT was blinded to randomization and allocation of volunteers in experimental groups.

#### *Cryotherapy*

For cryotherapy treatment, the PRICE protocol was employed using two flexible rubber ice packs filled with ice cubes and water (with a volume of 1.15 l each), in order to cover the entire quadriceps. Rubber belts were used to apply compression (40 mmHg ± 6.2) and to affix the packs tightly to the volunteers' quadriceps. As for duration of cryotherapy, there is good [26, 27] and fair evidence [28] that support cryotherapy should not exceed 20 min. Therefore, the application of ice was limited to 20 min total.



**Fig. 2** Sites of PBMT irradiation on quadriceps

**Table 1** Parameters for GameDay™ device

Number of lasers	One super-pulsed infrared
Wavelength (nm)	905 ( $\pm 1$ )
Frequency (Hz)	1000
Peak power (W)—each	12.5
Average mean optical output (mW)—each	1.25
Power density (mW/cm <sup>2</sup> )—each	2.84
Energy density (J/cm <sup>2</sup> )—each	0.85
Dose (J)—each	0.375
Spot size of laser (cm <sup>2</sup> )—each	0.44
Number of red LEDs	4 red
Wavelength of red LEDs (nm)	640 ( $\pm 10$ )
Frequency (Hz)	2
Average optical output (mW)—each	15
Power density (mW/cm <sup>2</sup> )—each	16.67
Energy density (J/cm <sup>2</sup> )—each	5
Dose (J)—each	4.5
Spot size of red LED (cm <sup>2</sup> )—each	0.9
Number of infrared LEDs	4 infrared
Wavelength of infrared LEDs (nm)	875 ( $\pm 10$ )
Frequency (Hz)	16
Average optical output (mW)—each	17.5
Power density (mW/cm <sup>2</sup> )—each	19.44
Energy density (J/cm <sup>2</sup> )—each	5.83
Dose (J)—each	5.25
Spot size of LED (cm <sup>2</sup> )—each	0.9
Magnetic field (mT)	35
Irradiation time per site (s)	300
Total dose per site (J)	39.37
Aperture of device (cm <sup>2</sup> )	4
Application mode	Cluster probe held stationary in skin contact with a 90° angle and slight pressure

#### Combined comparators (cryotherapy + PBMT and PBMT + cryotherapy groups)

Two of the five groups had combined comparators; however, they were delivered sequentially and not concurrently. Each modality was applied as described in earlier section; however, the sequence of comparators was changed to verify if the order of the application did have an impact on the outcomes.

Therefore, one group had PBMT applied before cryotherapy and the other had the order reversed (PBMT + cryotherapy or cryotherapy + PBMT).

#### Placebo PBMT treatment

The placebo PBMT device was identical to the active devices and displayed the same settings and emitted the same sound regardless of the comparator. The treatment was performed by a blinded researcher.

#### Statistical analysis

The intention-to-treat analysis was followed a priori. The primary outcome was the peak torque obtained from MVC at different time points. Secondary outcomes were VAS (DOMS) and CK. The researcher that performed statistical analysis was blinded to randomization and allocation of volunteers in experimental groups. Data were firstly tested regarding normal distribution using Shapiro-Wilk test and were expressed as mean and standard deviation. Two-way ANOVA test were 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

Fifty healthy, untrained male subjects were recruited in our study and completed all procedures; there were no dropouts. They had average age of 24.98 years old ( $\pm 5.90$ ), height of 176.00 cm ( $\pm 7.00$ ), and body weight of 76.77 kg ( $\pm 12.26$ ). Table 2 reports all outcomes in absolute values regarding functional and biochemical aspects of performance and recovery that we observed in our study.

Figure 3 demonstrates that PBMT as single therapy significantly increased ( $p < 0.05$ ) MVC compared to placebo-control group from 24 to 96 h. PBMT + cryotherapy presented similar outcomes to PBMT as single therapy. However, cryotherapy used as single treatment and cryotherapy + PBMT were similar to the placebo-control group ( $p > 0.05$ ).

Significant differences ( $p < 0.05$ ) were observed between PBMT as single treatment and placebo-control group for DOMS from 1 to 96 h after eccentric exercise protocol ( $p < 0.05$ ). PBMT + cryotherapy just showed positive results in decreased pain ( $p < 0.05$ ) compared to placebo-control group from 1 to 48 h after eccentric exercise contractions. Cryotherapy used as single treatment and cryotherapy + PBMT were similar to placebo-control group ( $p > 0.05$ ) at all time points. Results are summarized in Fig. 4.

Volunteers treated with PBMT as single treatment did not experience the expected increase in CK activity following the

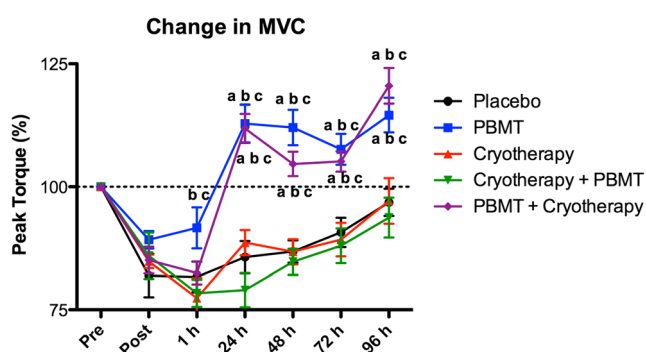
**Table 2** Functional and biochemical markers of performance and recovery (mean  $\pm$  SD)

		Pre	Post	1 h	24 h	48 h	72 h	96 h
MVC (N.m)	Placebo	258.24 ( $\pm 30.81$ )	211.59 ( $\pm 29.50$ )	210.84 ( $\pm 20.76$ )	221.24 ( $\pm 22.93$ )	224.18 ( $\pm 16.16$ )	234.25 ( $\pm 22.12$ )	250.05 ( $\pm 21.91$ )
	PBMT	256.31 ( $\pm 12.51$ )	228.64 ( $\pm 12.91$ )	234.88 ( $\pm 31.08$ ) <sup>b,c</sup>	289.34 ( $\pm 34.88$ ) <sup>a,b,c</sup>	287.24 ( $\pm 32.71$ ) <sup>a,b,c</sup>	275.91 ( $\pm 27.56$ ) <sup>a,b,c</sup>	293.71 ( $\pm 32.32$ ) <sup>a,b,c</sup>
	Cryotherapy	241.34 ( $\pm 20.82$ )	204.65 ( $\pm 8.87$ )	186.69 ( $\pm 24.09$ )	213.89 ( $\pm 17.37$ )	209.41 ( $\pm 16.96$ )	215.39 ( $\pm 23.26$ )	234.38 ( $\pm 34.40$ )
	Cryotherapy + PBMT	232.13 ( $\pm 30.90$ )	199.53 ( $\pm 29.71$ )	181.88 ( $\pm 15.85$ )	183.36 ( $\pm 20.05$ ) <sup>a,b</sup>	196.69 ( $\pm 16.52$ )	204.25 ( $\pm 22.88$ ) <sup>a</sup>	217.54 ( $\pm 28.01$ ) <sup>a</sup>
	PBMT + Cryotherapy	253.46 ( $\pm 30.17$ )	215.65 ( $\pm 18.47$ )	208.96 ( $\pm 15.12$ )	283.59 ( $\pm 26.61$ ) <sup>a,b,c</sup>	265.26 ( $\pm 20.90$ ) <sup>a,b,c</sup>	266.55 ( $\pm 17.49$ ) <sup>a,b,c</sup>	305.46 ( $\pm 35.08$ ) <sup>a,b,c</sup>
VAS (mm)	Placebo	0.00 ( $\pm 0.00$ )	3.84 ( $\pm 1.73$ )	3.68 ( $\pm 1.84$ )	4.10 ( $\pm 1.94$ )	4.68 ( $\pm 2.09$ )	2.36 ( $\pm 1.94$ )	1.66 ( $\pm 1.80$ )
	PBMT	0.00 ( $\pm 0.00$ )	3.76 ( $\pm 1.10$ )	1.36 ( $\pm 1.03$ ) <sup>a,b,c</sup>	0.91 ( $\pm 0.87$ ) <sup>a,b,c,d</sup>	0.45 ( $\pm 0.71$ ) <sup>a,b,c,d</sup>	0.19 ( $\pm 0.37$ ) <sup>a,b,c,d</sup>	0.06 ( $\pm 0.14$ ) <sup>a</sup>
	Cryotherapy	0.00 ( $\pm 0.00$ )	4.30 ( $\pm 1.90$ )	4.18 ( $\pm 1.81$ )	4.79 ( $\pm 1.57$ )	5.89 ( $\pm 1.30$ )	2.93 ( $\pm 2.42$ )	1.41 ( $\pm 1.26$ )
	Cryotherapy + PBMT	0.00 ( $\pm 0.00$ )	3.19 ( $\pm 0.94$ )	3.03 ( $\pm 0.98$ )	3.83 ( $\pm 1.49$ )	4.59 ( $\pm 0.96$ )	1.93 ( $\pm 0.85$ )	0.75 ( $\pm 0.64$ )
	PBMT + Cryotherapy	0.00 ( $\pm 0.00$ )	3.16 ( $\pm 0.86$ )	1.89 ( $\pm 1.35$ ) <sup>a,b</sup>	2.53 ( $\pm 0.44$ ) <sup>a,b</sup>	2.86 ( $\pm 0.69$ ) <sup>a,b,c</sup>	1.81 ( $\pm 1.30$ )	0.45 ( $\pm 0.44$ )
CK (U.L <sup>-1</sup> )	Placebo	44.11 ( $\pm 7.77$ )	51.30 ( $\pm 6.79$ )	56.92 ( $\pm 16.86$ )	100.84 ( $\pm 13.66$ )	118.91 ( $\pm 12.45$ )	99.55 ( $\pm 10.38$ )	99.47 ( $\pm 11.01$ )
	PBMT	51.01 ( $\pm 12.35$ )	55.53 ( $\pm 15.58$ )	56.69 ( $\pm 16.03$ )	54.63 ( $\pm 16.65$ ) <sup>a,b,c,d</sup>	56.55 ( $\pm 17.63$ ) <sup>a,b,c,d</sup>	52.35 ( $\pm 16.26$ ) <sup>a,b,c</sup>	43.66 ( $\pm 16.30$ ) <sup>a,b,c</sup>
	Cryotherapy	43.96 ( $\pm 7.43$ )	42.85 ( $\pm 7.42$ )	46.37 ( $\pm 6.03$ )	118.62 ( $\pm 13.85$ )	118.61 ( $\pm 18.77$ )	100.72 ( $\pm 16.78$ )	89.90 ( $\pm 13.17$ )
	Cryotherapy + PBMT	46.02 ( $\pm 11.46$ )	43.97 ( $\pm 15.04$ )	58.19 ( $\pm 20.90$ )	105.56 ( $\pm 18.60$ )	106.87 ( $\pm 17.70$ )	97.60 ( $\pm 23.04$ )	91.93 ( $\pm 17.40$ )
	PBMT + Cryotherapy	44.54 ( $\pm 19.72$ )	49.49 ( $\pm 17.70$ )	46.45 ( $\pm 19.23$ )	78.99 ( $\pm 22.97$ ) <sup>a,b,c</sup>	78.97 ( $\pm 21.43$ ) <sup>a,b,c</sup>	67.94 ( $\pm 19.06$ ) <sup>a,b,c</sup>	58.53 ( $\pm 15.61$ ) <sup>a,b,c</sup>

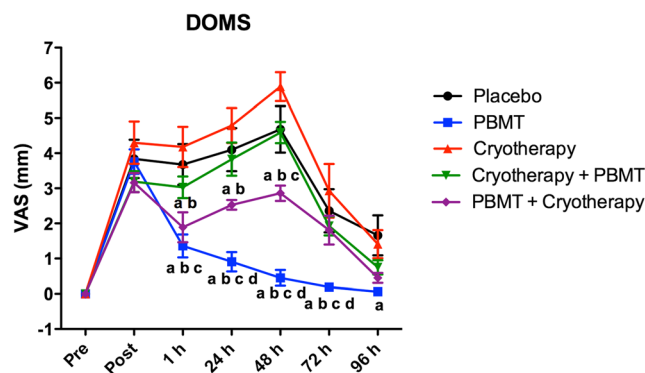
<sup>a</sup> Difference of placebo ( $p < 0.05$ )<sup>b</sup> Difference of cryotherapy ( $p < 0.05$ )<sup>c</sup> Difference of cryotherapy + PBMT ( $p < 0.05$ )<sup>d</sup> Difference of PBMT + cryotherapy ( $p < 0.05$ )

eccentric exercise protocol with significant difference ( $p < 0.05$ ) compared to placebo-control group from 24 to 96 h. PBMT + cryotherapy showed a noted decrease in efficacy compared to PBMT as single treatment; however, it was still significantly better than placebo-control group ( $p < 0.05$ ).

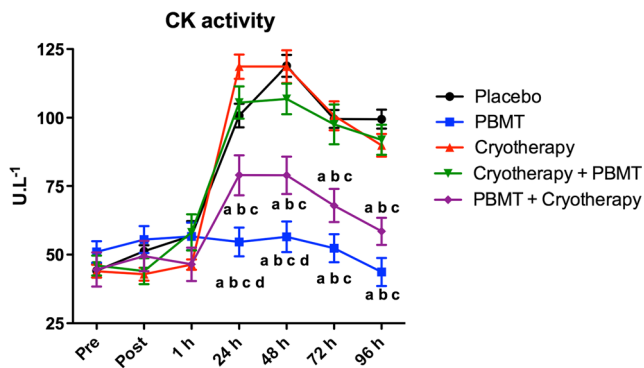
Cryotherapy used as single treatment and cryotherapy + PBMT were similar to the effects seen by the placebo-control group ( $p > 0.05$ ). The results of the CK analysis are summarized in Fig. 5.



**Fig. 3** MVC in percentage values. Values are mean and error bars are standard error of the mean (SEM). Letter *a* indicates the significant difference compared to placebo ( $p < 0.05$ ), letter *b* indicates the significant difference compared to cryotherapy ( $p < 0.05$ ), and letter *c* indicates the significant difference compared to cryotherapy + PBMT ( $p < 0.05$ )



**Fig. 4** DOMS assessment using 100 mm VAS. Values are mean and error bars are standard error of the mean (SEM). Letter *a* indicates the significant difference compared to placebo ( $p < 0.05$ ), letter *b* indicates the significant difference compared to cryotherapy ( $p < 0.05$ ), letter *c* indicates the significant difference compared to cryotherapy + PBMT ( $p < 0.05$ ), and letter *d* indicates the significant difference compared to PBMT + cryotherapy ( $p < 0.05$ )



**Fig. 5** CK activity. Values are mean and error bars are standard error of the mean (SEM). Letter *a* indicates the significant difference compared to placebo ( $p < 0.05$ ), letter *b* indicates the significant difference compared to cryotherapy ( $p < 0.05$ ), letter *c* indicates the significant difference compared to cryotherapy + PBMT ( $p < 0.05$ ), and letter *d* indicates the significant difference compared to PBMT + cryotherapy ( $p < 0.05$ )

## Discussion

To our understanding, this is the first study designed to evaluate and analyze the singular and combined effects of PBMT and cryotherapy for recovery. As mentioned before, it is common in clinical practice to combine two or more therapeutic agents. To aide in better clinical decisions making, a greater understanding of the most efficient interaction between therapeutic agents is required. Knowing when or if modalities should be combined and the order in which two or more modalities are applied is crucial for best clinical practice.

In previous experiments, the scientific evidence recommends the use of PBMT prior to exercise to provide both ergogenic and protective effects to skeletal muscle tissue [9, 10]. Since cryotherapy is widely used in after-exercise sessions to promote the recovery of skeletal muscles after physical activity [29, 30], our study looked to evaluate the use of PBMT post-exercises to better understanding the post-exercise PBMT effect and provide an “equal” comparison between the two investigated modalities.

The eccentric exercise protocol employed in our study was effective in reducing muscle strength (MVC), increasing both muscle damage (CK), and muscle soreness (VAS) as observed in outcomes from placebo-control group. Regarding the MVC, groups treated with cryotherapy as single treatment and cryotherapy + PBMT showed similar effects as the placebo-control group. These outcomes are consistent with Yamane et al. [31] that observed that cryotherapy might have negative effect on muscular strength. Compared to the other experimental groups (placebo-control, cryotherapy, and cryotherapy + PBMT), both the PBMT as monotherapy and PBMT + cryotherapy demonstrated statistically significant difference and complete recovery of MVC at 24 h after eccentric exercise protocol. Furthermore, in the PBMT and

PBMT + cryotherapy groups, an overcompensation in MVC surpassed the baseline values by 14 to 20 %.

Compared to prior studies that have utilized the same eccentric exercise protocol for fatigue where PBMT was performed only once and previously to exercise [12], our study performed all interventions after the exercise protocol and were repeated after 24, 48, and 72 h. The differences among outcomes, we believe, can be attributed to the repeated treatment protocol every 24 h for four consecutive days and not necessarily related to only the initial post-exercise intervention. These aspects need to be clarified in further studies.

The eccentric exercise protocol was also effective in promoting DOMS as demonstrated by the significant increase of pain, especially in the period of 24 to 48 h after exercise protocol. As before, the groups treated with cryotherapy as a monotherapy and cryotherapy + PBMT showed results similar to that of the placebo-control group. The group treated with PBMT as monotherapy demonstrated the best results with a significant decrease in pain from 1 to 96 h after exercise when compared to placebo-control group, from 1 to 72 h compared to cryotherapy and cryotherapy + PBMT groups, and from 24 to 72 h when compared to PBMT + cryotherapy group. However, at 72 h post-exercise, the pain experienced by the PBMT group had returned to baseline. Of note, the combined application of cryotherapy after PBMT (PBMT + cryotherapy) did reduce the overall PBMT effects compared to the group treated only with PBMT.

The exercise protocol was effective also in inducing muscle damage and consequently increased CK activity as can be observed in placebo-control group. Similar to the outcomes noted with MVC, the groups treated with cryotherapy as monotherapy and cryotherapy + PBMT exhibited similar outcomes as the placebo-control group. The group treated with PBMT + cryotherapy showed significant decrease of CK activity when compared to the placebo-control group, cryotherapy group, and cryotherapy + PBMT group from 24 to 96 h after exercise protocol. A statistical significant decrease in the PBMT effect was noted when the ice application followed the treatment from 24 to 48 h after eccentric exercise protocol (comparison between PBMT vs PBMT + cryotherapy). The PBMT as single treatment was able to prevent the expected increase of CK activity, which corresponds to consider the protective effects of PBMT on skeletal muscle tissue previously observed in other studies [19, 20, 32, 33]. Previously, this protective effect of PBMT on skeletal muscle damage was observed using this same device, settings, and dose range, when the active therapy was performed prior to the exercise [12].

Our outcomes clearly demonstrate that cryotherapy decreases or nullifies the effects of PBMT; however, the exact reason is unclear. We believe that the down-regulation of cellular metabolism promoted by

cryotherapy may partially explain the observed phenomenon. In cryotherapy + PBMT group, possibly the decrease in cell and mitochondrial metabolism was decreased too greatly by the application of cryotherapy to an extent the PBMT was not able to reverse. In the PBMT + cryotherapy group, only a decrease in recovery was observed, minimizing the PBMT effects but not negating them. Obviously, these hypotheses need to be properly tested in further mechanistic studies.

The absence of positive effects with cryotherapy treatment was expected, and it corroborates with literature [7, 8]. While cryotherapy exhibits some effects on inflammatory events at a cellular and physiological level after acute tissue damage, overall, the evidence-supporting cryotherapy as a recovery modality is insufficient [7, 8]. A clinical trial showed that cryotherapy is ineffective to minimize or prevent symptoms of muscle damage after eccentric exercise with young individuals [34]. Therefore, the use and effectiveness of cryotherapy as recovery strategy must be questioned.

In an animal trial comparing the use of PBMT and cryotherapy post-exercise, da Costa et al. [18] observed that PBMT showed better results in decrease of muscle damage and inflammation. These findings agree with reported by Camargo et al. [19] in a similar study. A preliminary clinical trial has compared cryotherapy and PBMT after exercise and demonstrated that treatment with PBMT 5 min post-exercise inhibits the increase of CK activity and suggested that PBMT is better than cryotherapy in post-exercise restitution [20]. Although outcomes of several previous studies have suggested that ice may not be effective, our findings provide a clearer understanding in regards to clinical impact by testing both therapies as mono and/or combined therapies, and analyzed their effects over seven different time points, from immediately after to 96 h after exercise.

Of note, our outcomes showed that treatment with PBMT or PBMT + cryotherapy recovers 100 % of muscle strength at 24 h after exercise, three times faster than other groups (or treatments). However, only group treated with PBMT as monotherapy led to prevention of expected increase in CK activity and faster recovery of pain. We believe that superiority of our outcomes compared to previously observed by Leal-Junior et al. [20] has been achieved due to optimized parameters, such as use of three wavelengths simultaneously rather than only one or two wavelengths. It is known that PBMT improves cytochrome c oxidase activity [35]; however, only the simultaneous use of three wavelengths is able to promote the modulation of cytochrome c oxidase activity in an optimized way, in a time window from 5 min to 24 h after irradiation, as demonstrated by Albuquerque-Pontes et al. [36]. Furthermore, the synergistic use of three wavelengths can partially explain how PBMT can promote ergogenic effects and at same time protects muscles against damage [37].

## Conclusion

In this study, the use of PBMT as single treatment provided superior post-exercise recovery and provided the greatest reduction in DOMS post-exercise. PBMT treatment is safe and with minimal contraindications. This may serve to improve athletic performance by minimizing recovery time by reducing the muscular fatigue response, delay the onset of fatigue, and protect cells from exercise-induced damage while accelerating post-exercise recovery. Utilizing this new data could assist with proper recommendations for athletes looking to maximize training and recovery to improve short-term performance. Additional field studies should be performed to optimize dose parameters for differences in elite and recreational athletic recovery and examine the long-term effects of PBMT recovery on adaptive strength, endurance, and hypertrophy training.

## Compliance with ethical standards

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