## ORIGINAL ARTICLE



# Does photobiomodulation therapy is better than cryotherapy in muscle recovery after a high-intensity exercise? A randomized, double-blind, placebo-controlled clinical trial

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Received: 9 September 2016 / Accepted: 26 December 2016 / Published online: 5 January 2017 © Springer-Verlag London 2017

**Abstract** This study aimed to determine the effectiveness of photobiomodulation therapy (PBMT) and cryotherapy, in isolated and combined forms, as muscle recovery techniques after muscle fatigue-inducing protocol. Forty volunteers were randomly divided into five groups: a placebo group (PG); a PBMT group (PBMT); a cryotherapy group (CG); a cryotherapy-PBMT group (CPG); and a PBMT-cryotherapy group (PCG). All subjects performed four sessions at 24-h intervals, during which they submitted to isometric assessment (MVC) and blood collection in the pre-exercise period, and 5 and 60 min post-exercise, while the muscle fatigue induction protocol occurred after the pre-exercise collections. In the remaining sessions performed 24, 48, and 72 h later, only blood collections and MVCs were performed. A single treatment with PBMT and/or cryotherapy was applied after

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only 2 min of completing the post-5-min MVC test at the first session. In the intragroup comparison, it was found that exercise led to a significant decrease (p < 0.05) in the production of MVC in all groups. Comparing the results of MVCs between groups, we observed significant increases in the MVC capacity of the PBMT, CPG, and PCG volunteers in comparison with both PG and CG (p < 0.05). We observed a significant decrease in the concentrations of the biochemical markers of oxidative damage (TBARS and PC) in all groups and muscle damage (creatine kinase—CK) in the PBMT, PCG, and CPG compared with the PG (p < 0.01). The clinical impact of these findings is clear because they demonstrate that the use of phototherapy is more effective than the use of cryotherapy for muscle recovery, additionally cryotherapy decreases PBMT efficacy.

**Keywords** Phototherapy · Cryotherapy · High-intensity exercise · Oxidative stress · Muscle damage

## Introduction

The practice of physical activity promotes health and quality of life, but there is a wide range of inherent risks associated with each type of sport as well as the physical demands that each sport imposes on its practitioners. Every athlete, at either the professional or the amateur level, is subject to injuries; thus, a rapid recovery is always desired to accelerate the return to sports activities. Therefore, therapies such as cryotherapy and phototherapy are used to rehabilitate and prevent injuries. Both therapies aim to decrease the duration of the muscle recovery period in between the game and/or the training sessions.

The use of cold therapy (cryotherapy) is one of the cheapest, most commonly recommended and used forms in the rehabilitation and treatment of acute injuries, pain of musculoskeletal origin, traumatic sports and other types of injuries, postoperative pain and edema, inflammatory processes, and muscle contractures [1]. According to Knight [2], the term cryotherapy means cold therapy, so any use of cold or ice for therapeutic purposes is defined as cryotherapy. Cryotherapy is widely used for muscle recovery after high-intensity exercise, but it can also be used to decrease the pain of musculoskeletal injuries, by reducing local tissue temperature, promoting local vasoconstriction, and decreasing both inflammation and edema [3]. In acute injuries, cold is indicated to minimize the inflammatory process, decrease metabolism and hypoxia secondary to injury, pain, and edema; during rehabilitation, cold decreases pain and muscle spasm enabling early mobilization [2].

Photobiomodulation therapy (PBMT) low-level laser therapy (LLLT) is the application of laser light (1-500 mW) to a pathologic condition and is applied by means of a light (usually low-powered laser and/or light emitting diodes-LEDs, with power between 1 and 500 mW) to a pathological or preventive clinical condition [4-7] and, unlike other procedures with a medical (surgical) laser, PBMT does not have ablative or thermal effects, but rather has photochemical effects in which light is absorbed and induces a chemical change in the tissues [8, 9]. PBMT is generally used to promote tissue regeneration, reduce swelling and inflammation, and relieve pain [5, 10]. The first randomized clinical trial to investigate its effects on disorders of the musculoskeletal system was carried out in the 1980s, in patients with rheumatoid arthritis [9]. Since the publication of this first clinical trial, additional positive effects of phototherapy have been identified in several other pathologies, such as osteoarthritis [11], tendinopathies [12, 13], back pain [14, 15], and neck pain [16, 17]. Thus, phototherapy presented a new form of therapy that has been used to treat muscular pain; however, the mechanisms responsible for the effects observed in clinical trials remain partially unclear [18].

Metabolism during contractile activity produces reactive oxygen species, which can cause the muscle to develop oxidative stress. This may be a factor associated with a reduction in contractile function and the development of muscle fatigue [7]. Skeletal muscle fatigue is characterized by a deficiency of the muscle's ability to both generate and maintain force produced during muscle activity. In submaximal activities, skeletal muscle fatigue is denoted as a failure to continue the activity at its initial intensity. The development of muscle fatigue is a complex and multifaceted process involving many physiological and biomechanical elements, including the muscle fiber type, oxidative stress, and both the intensity and duration of the activity [19].

The use of PBMT before and after exercise has shown positive results in slowing skeletal muscle fatigue and improving skeletal muscle recovery in both athletes and non-athletes [4]. Some studies have compared the effects of cryotherapy (ice immersion and application) and PBMT in both rats [20, 21] and humans [22]. However, to our knowledge, no studies have compared the ability of these therapies to act in combination or the effectiveness of such a joint therapeutic intervention for upper limbs.

To address these issues, this study aims to determine the effectiveness of PBMT and cryotherapy, when used in both isolated and combined forms following muscle fatigue, induced by performing high-intensity exercise protocols having a predominance of eccentric contraction.

# Methods

## Ethical aspects

The study was approved by the Ethics Committee of the University of Caxias do Sul. In accordance with the Declaration of Helsinki, all subjects were advised about the procedure and they signed an informed consent prior to participation in the study (CAEE 31344214.3.3001.5341).

## Subjects

Forty volunteers were selected for this study. The number of the participants was calculated using a statistical power of 80% and a significance level of p < 0.05 (or 5%). The individuals were recruited from among healthy physically active male volunteers aged between 19 and 29 years, from the University of Caxias do Sul. Exclusion criteria were any previous musculoskeletal injury in the previous 3 months and the use of any kind of nutritional supplements or pharmacological agents.

## **Randomization and blinding procedures**

Prior to the study, volunteers were randomly divided into five groups: (1) placebo group (PG); (2) PBMT group (PBMT); (3) cryotherapy group (CG); (4) cryotherapy-PBMT group (CPG); and (5) PBMT-cryotherapy group (PCG). Individuals of all groups attended the Institute of Sports Medicine for four sessions, with 24-h intervals. On the first day, they were subjected to a muscle fatigue-inducing protocol (MFIP) and blood collection during the pre-exercise period, 5 min post-exercise, and 60 min post-exercise. In the remaining sessions performed 24, 48, and 72 h later, blood collection and isometric evaluation in the isokinetic dynamometer were repeated. To ensure the blind nature of the study, the researchers responsible for verbal stimulation during the performance of the isokinetic dynamometry protocol had no knowledge about the allocation of the volunteers in the groups, thereby ensuring impartiality during the evaluations. A single

#### Table 1 Parameters for PBMT

Number of LEDs	69 (34 red LEDs and 35 infrared LEDs)
Wavelength	660 nm (red) and 850 nm (infrared)
Frequency	Continuous output
Optical output	10 mW (red) and 30 mW (infrared)
LED spot size	$0.2 \text{ cm}^2$ (for both—red and infrared), total spot sizes $13.8 \text{ cm}^2$
Power density	$0.05 \text{ W cm}^{-2}$ (for red) and $0.15 \text{ W cm}^{-2}$ (for infrared)
Energy	41.7 J (0.3 J from each red LED, 0.9 J from each infrared LED)
Energy density	1.5 J cm <sup><math>-2</math></sup> (for red) and 4.5 J cm <sup><math>-2</math></sup> (for infrared)
Treatment time	30 s
Number of irradiation points per muscle	1
Total energy delivered per muscle	41.7 J
Total area irradiated	$13.8 \text{ cm}^2$
Application mode	Cluster held stationary in skin contact with a 908 angle and slight pressure

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researcher, responsible for randomization and programming the PBMT equipment, was aware of the correct allocation of the volunteers in the groups.

### **Exercise protocol**

Initially, the volunteers were subjected to kinanthropometric measurements (body mass and height) and information about DOMS through the 100-mm visual analog scale (VAS). Next, the volunteers were properly positioned with their non-dominant upper limb in the position of evaluation by the isokinetic dynamometer *Biodex System 4 Pro* (Biodex Medical Systems, USA) according to the resolutions provided by the manufacturer for evaluation points of muscle recovery), the measurements and positioning of the equipment remained the same.

The first part of the protocol on the isokinetic dynamometer consisted of determining the maximum isometric torque of the elbow flexors (biceps brachii). To this end, three maximal voluntary contractions (MVCs) were performed in the isometric mode, in the position of 45° of elbow flexion, having a 5-s duration and a 5-s interval between contractions. During the MVCs, a constant and standardized verbal stimulus was

presented by the researchers. The maximum isometric torque value reached in the MVCs was considered the maximum capacity of power generation of the volunteer prior to exercise (PRE-MVC).

After determining the PRE-MVC, a period of 180 s of rest before the MFIP by eccentric exercise was allowed. After the rest period, the MFIP was initiated, consisting of five sets of 10 eccentric/concentric contractions of the elbow flexors separated by 30 s. The contractions were performed with an amplitude of 90° and speed of 90°.seg<sup>-1</sup> for the eccentric contractions and 180°.seg<sup>-1</sup> for concentric contractions. The guidance provided to the volunteers was to employ the highest strength possible to execute the elbow flexion movement and resist the elbow extension movement imposed by the dynamometer from the first to the last repetition.

Exactly 30 s after the MFIP, volunteers were subjected to a new MVC following the parameters of the MVCs prior to MFIP in relation to the limb's position, the duration, and the verbal stimulation provided by the researchers. The value found in this isolated MVC will be considered the maximum capacity of power generation of the volunteer after the exercise (POST-MVC). The MVC will be evaluated 24 (MVC24), 48 (MVC48), and 72 (MVC72) hours after the execution of MFIP.

**Fig. 1** a Application of PBMT. b Application of cryotherapy



**Table 2** Performance in maximalvoluntary contraction  $(N \times m)$ 

	Placebo group	Cryotherapy	PBMT	Cryotherapy + PBMT	PBMT + cryotherapy
Pre	67.11 ± 10.39	$64.02 \pm 17.02$	$71.66 \pm 16.03$	73.31 ± 8.27	$72.75 \pm 16.54$
Post	$41.63\pm9.13$	$43.44 \pm 15.51$	$49.04 \pm 10.94$	$55.49 \pm 21.35$	$45.65 \pm 13.68$
60′	$47.06\pm5.43$	$46.52\pm11.47$	$64.14 \pm 9.83*$	$62.68 \pm 13.56^{*}$	$60.93 \pm 14.14*$
24 h	$56.86 \pm 7.22$	$57.01 \pm 10.11$	$70.73 \pm 10.04 *$	$69.67 \pm 5.49 *$	$71.21 \pm 15.54*$
48 h	$58.08 \pm 5.67$	$52.91 \pm 9.28$	$72.09 \pm 10.71 *$	$70.76 \pm 11.97 *$	$72.30 \pm 13.80^{*}$
72 h	$58.14 \pm 9.44$	$59.66 \pm 13.13$	$76.66 \pm 6.45^{*}$	$75.98 \pm 9.75 \ast$	$75.94 \pm 11.63 ^{\ast}$

\*Statistical difference between the treated groups compared with the placebo group and cryotherapy (p < 0.05)

### **PBMT and cryotherapy**

A single treatment with PBMT and/or cryotherapy was applied 2 min after the completion of the post-exercise MVC test. For the application of PBMT (Table 1), we used a cluster of 69 LEDs (34 red LEDs and 35 infrared LEDs), with 660 and 850 nm, 10 mW (red) and 30 mW (infrared) output power (each diode), manufactured by THOR® Photomedicine (London, UK). The application of PBMT was held with the cluster in direct contact with the skin, on the muscle belly of the biceps, as illustrated in Fig. 1. Thus, volunteers received phototherapy with the 41.7-J dose (30 s of irradiation) or 0 J-placebo (3 seconds of irradiation, but without effective irradiation). The choice of these parameters was based in a previous study that used this same PBMT device and observed positive outcomes in performance enhancement and in biochemical markers of recovery [23]. Cryotherapy was performed on the muscle belly of the biceps, with the patient lying down, using thermal bags containing ice cubes, and fixed on the segment with compression. As for duration of cryotherapy, there is good [24, 25] and fair evidence [26] that support cryotherapy should not exceed 20 min. Therefore, the application of ice was limited to 20 min total. The PBMT device was calibrated before and after data acquisition and the equipment showed the same power output in both calibrations. The optical power was measured using a Newport multifunction optical meter model 1835 C. The stability of the



Fig. 2 Pre and post-exercise MVC. *Values* are mean and *error bars* are SEM. \*Different of placebo (p < 0.05); \*different of cryotherapy (p < 0.05)

laser during the laser irradiation was measured collecting light with a partial reflect (4%).

#### Blood samples and biochemical assays

Blood samples were collected by a qualified nurse blinded to group allocation and were obtained from an antecubital vein before exercise and exactly 5 min, 60 min, 24 h, 48 h, and 72 h after the end of the exercise protocol. Blood was centrifuged at 2700×g for 10 min at 4 °C. Serum was immediately pipetted into Eppendorf tubes and stored at -80 °C until analysis. Lipid damages were measured spectrophotometrically (Shimadzu spectrophotometer Model UV-1700, Shimadzu®, Japan) by determining thiobarbituric acid reactive substances (TBARS), as previously described by Wills [27]. Results were expressed as nanomole per milliliter. The oxidative damage to proteins was assessed by determining carbonyl groups based on the reaction with 2,4-dinitrophenylhydrazine (DNPH), as previously described by Levine et al. [28]. Results were expressed as DNPH nanomole per milligram of protein. Total protein levels were evaluated using the Total Protein kit from Labtest® (Protein Kit, Labtest Diagnostica S.A., Brazil). Creatine kinase (CK) activity was measured by using a commercial kit (CK-Labtest®, Brazil). CK catalyzes the dephosphorylation of creatine phosphate to produce adenosine if thiotriphosphate, which reacts with glucose in the presence of hexokinase forming glucose-6-phosphate. Glucose-6phosphate is acted on by glucose-6-phosphate dehydrogenase,



Fig. 3 Pre and post-exercise DOMS. *Values* are mean and *error bars* are SEM. <sup>#</sup>Different of placebo (p < 0.05); \*different of cryotherapy (p < 0.05)

Table 3Pre and post-exerciselevels of the oxidative damage tolipids (TBARS nmol/ml)

	Placebo group	Cryotherapy	PBMT	Cryotherapy + PBMT	PBMT + cryotherapy
	2.5( + 0.1(	2.50 + 0.10	2.55 + 0.10	2.57 + 0.15	2.51 + 0.19
Pre	$3.56 \pm 0.16$	$3.59 \pm 0.16$	$3.55 \pm 0.19$	$3.5/\pm0.15$	3.51±0.18
Post	$4.14 \pm 0.16$	$4.01 \pm 0.27$	$4.15 \pm 0.17$	$4.14 \pm 0.16$	$4.16 \pm 0.12$
60'	$4.08 \pm 0.18$	$3.20 \pm 0.19^*$	3.45 ± 0.40*	$3.22 \pm 0.36^*$	$3.19 \pm 0.14^*$
24 h	$4.10 \pm 0.21$	$4.06 \pm 0.71$	$3.50 \pm 0.27$ *	$3.30 \pm 0.19^*$	$3.40 \pm 0.26^{*}$
48 h	$4.42 \pm 0.32$	$3.93 \pm 0.63 **$	$3.72 \pm 0.32*$	$3.44 \pm 0.27*$	$3.54 \pm 0.24^{*}$
72 h	$4.27\pm0.35$	$3.70 \pm 0.44*$	$3.55 \pm 0.15*$	$3.37 \pm 0.25*$	$3.54 \pm 0.13*$

\*Statistical difference between the treated groups compared with the placebo group (p < 0.01); \*\*statistical difference between the treated groups compared with the placebo group (p < 0.05)

is oxidized to phosphogluconate, and reduces NADP+ to NADPH. The rate of increase in absorbance at 340 nm is proportional to CK activity in the sample. Results were expressed as units per liter.

## Statistical analysis

Data from the exercise protocol, oxidative stress, and muscle damage markers were expressed as mean and standard deviation ( $\pm$ SD) and tested statistically by an ANOVA and post hoc Tukey-Kramer and the significance level was set a *p* < 0.05. The software used was SPSS 18.0 for Windows.

## Results

Volunteers in this study were 25.30 years old ( $\pm 3.32$ ), weighed 77.98 kg ( $\pm 11.43$ ), with a height of 176.55 cm ( $\pm 5.55$ ). The results of the MVCs (mean  $\pm$  SD) containing the muscle recovery protocols are presented in Table 2 and Fig. 2. Initially, we observed that there were no significant differences between the groups in the pre-exercise evaluations in all the variables analyzed (MVC, TBARS, PC, CK, and DOMS). In an intragroup statistical test (pre and post comparison), it was found that exercise led to a significant decrease (p < 0.05) in the production of MVC after the fatigue protocol in all groups. Comparing the results of MVCs and DOMS between the groups, we observed that after treatment (from 1 to 72 h

after), we obtained significant increases in the MVC capacity and decrease in DOMS (Fig. 3) of the volunteers who received treatment with PBMT, CPG, and PCG, compared with the PG and CG (p < 0.05). The CG showed no differences compared to the PG.

The concentrations of the biochemical marker of oxidative damage to lipids, as shown in Table 3, indicate that after treatment (from 1 to 72 h after), we obtained a significant decrease in TBARS concentrations in PBMT, CPG, and PCG, compared with the PG (p < 0.01). In the CG, we observed a significant decrease in TBARS concentrations at 1 h (p < 0.01), 48 h (p < 0.05), and 72 h (p < 0.01) after treatment. In addition, our results of the concentrations of the biochemical marker of oxidative damage to proteins indicate that after treatment (from 1 to 72 h after), we obtained a significant decrease in PC concentrations in the PBMT, CG and PCG, compared with the PG (p < 0.01) as shown in Table 4. In the CPG, we observed a significant decrease in PC concentrations the treatment.

From the results found in the concentrations of the biochemical marker of muscle damage (CK) presented in Table 5 and Fig. 4, we can see that after treatment (from 1 to 72 h after), we obtained a significant decrease in CK concentrations in the PBMT, compared with the PG (p < 0.01). The PCG and CPG groups presented a significant decrease in CK concentrations in 48 and 72 h after treatment (p < 0.05 and p < 0.01, respectively).

Table 4Pre and post-exerciselevels of the oxidative damage toproteins (carbonylated proteinsnanomole of DNPH/gram/deciliter of proteins)

	Placebo group	Cryotherapy	PBMT	Cryotherapy + PBMT	PBMT + cryotherapy
Pre	$3.18 \pm 0.42$	$3.01\pm0.49$	$3.15\pm0.28$	$3.08\pm0.55$	$3.11 \pm 0.44$
Post	$3.82\pm0.90$	$3.48 \pm 0.80$	$3.50\pm0.56$	$3.44\pm0.60$	$3.49\pm0.35$
60′	$4.51 \pm 1.00$	$3.06 \pm 0.71 *$	$3.33 \pm 0.45*$	$3.65\pm0.57$	$2.81\pm0.48*$
24 h	$4.77\pm0.80$	$3.43\pm0.56*$	$3.30 \pm 0.39*$	$3.26 \pm 0.48*$	$3.17\pm0.39*$
48 h	$5.48 \pm 1.09$	$3.48\pm0.44*$	$3.61\pm0.30^*$	$3.47 \pm 0.46*$	$3.22\pm0.91*$
72 h	$5.09 \pm 0.89$	$3.61 \pm 0.47 *$	$3.63 \pm 0.31*$	$2.95 \pm 0.36*$	$2.93\pm0.33^*$

\*Statistical difference between the treated groups compared with the placebo group (p < 0.01)

	Placebo group	Cryotherapy	PBMT	Cryotherapy + PBMT	PBMT + cryotherapy
Pre	$63.95 \pm 5.44$	$76.64 \pm 14.12$	$66.91 \pm 8.70$	$72.08 \pm 6.43$	$73.66 \pm 13.75$
Post	$132.37 \pm 45.34$	$154.02\pm50.45$	$109.61 \pm 34.48$	$103.16 \pm 45.46$	$128.37\pm58.43$
60′	$131.57\pm84.45$	$202.95 \pm 28.32$	$82.67 \pm 38.02*$	$143.00 \pm 45.66$	$130.01 \pm 49.84$
24 h	$294.53 \pm 120.60$	$212.91 \pm 33.09$	$111.00 \pm 69.00 *$	$185.40 \pm 68.17 **$	$147.61 \pm 47.91^*$
48 h	$291.82 \pm 182.05$	$299.83 \pm 44.74$	$101.49 \pm 69.01 *$	$128.44 \pm 45.08*$	$163.28 \pm 45.35^*$
72 h	$226.02 \pm 101.12$	$145.72 \pm 43.52$	$73.48 \pm 27.00*$	$227.80\pm90.33$	$184.31 \pm 80.82$

**Table 5** Pre and post-exercise levels of the muscle damage (creatine kinase $-U.l^{-1}$ )

\*Statistical difference between the treated groups compared with the placebo group (p < 0.01); \*\*statistical difference between the treated groups compared with the placebo group (p < 0.05)

## Discussion

In the rehabilitation process, many therapeutic options are used in an associated manner, one after another, with virtually no recovery interval between their uses. The verification of its actual effects is rare and we found only one study when we searched the effects of PBMT and cryotherapy association in humans. To our knowledge, this is the first time that the synergistic effects of cryotherapy and PBMT have been tested in order to improve the performance of exercise and postexercise muscle recovery for upper limbs. Some authors [7, 23] have shown the efficacy of both the PBMT dose and the duration of cryotherapy used in this study.

It is known that high-intensity exercises are associated with hyperthermia, energy depletion, muscle injury, oxidative stress, inflammation, and fatigue that lead to decreased performance due to both fatigue and the start of delayed-onset muscle soreness [29]. Prevention and treatment of such afflictions are important tools for the maintenance of exercise programs. The use of non-steroidal anti-inflammatory drugs, stretching, compression therapy, ultrasound, acupuncture, deep massage, nutritional supplements, antioxidants, and electrical stimulation have all been tested, with varying degrees of success, to reduce the symptoms of muscle injury, fatigue, and delayedonset muscle soreness [29–34]. However, there is no consensus regarding the most appropriate method to prevent delayed-



**Fig. 4** Pre and post-exercise CK activity. *Values* are mean and *error bars* are SEM. <sup>#</sup>Different of placebo (p < 0.05); \*different of cryotherapy (p < 0.05). &Different of the all other groups (p < 0.05)

onset muscle pain and muscle injury effectively. Many studies [4, 7] have demonstrated the protective effects of PBMT when applied prior to exercise; thus, we used phototherapy and cryotherapy with resources to assist the muscle recovery process, and applied the modalities subsequent to performing MFIP.

We noted that PBMT has considerable potential not only for the prevention of muscle fatigue and damage caused by high-intensity exercises, but also can also improve performance conditions when applied post-exercise, in order to attain the goal of muscle recovery. Skeletal muscle is designed to withstand mechanical and metabolic overloads up to a certain limit. When stimulated, it rapidly reaches its maximum contraction load and increases oxygen flow up to 100%, which may lead to increased oxidative stress [35]. It is known that this phenomenon accompanies skeletal contractile activity [36] and may cause a decrease in the contractile function of the muscle groups involved and produce fatigue [37].

Cryotherapy has been widely used in sports to both prevent muscle injury and improve recovery [30, 38]. Therefore, it is not surprising that cryotherapy, despite not having demonstrated any effect on maintaining or increasing MVC after use in isolation, has demonstrated some effect on the reduction of markers of oxidative damage to lipids and proteins, probably through their known effects on vasoconstriction, reduction in muscle temperature, and inflammatory activity [39, 40]. This implies that the oxidative damage to lipids and proteins generated by the ischemia-reperfusion process may have been reduced by cryotherapy.

Associated with these findings, it is important to highlight that cryotherapy had no influence on maintaining the MVC capacity, having a behavior similar to the placebo group from the time of its application. Previous studies report that cryotherapy can reduce nerve conduction velocity by not only changing the perception of pain but also interfering with the recruitment of motor units [41]. In contrast, the groups that received the PBMT application exhibited a significant improvement in MVC after 60 min after the application of the muscle recovery protocol. The results obtained by cryotherapy in reducing markers of oxidative damage to lipids and proteins have also been reached in the group that received only the application of PBMT; moreover, this group had a significant decrease in the marker of muscle damage (CK), which was not observed in the cryotherapy group. Similar results have been reported in studies conducted in animals [20, 21, 42], where the use of cold water immersion and cryotherapy proved ineffective in providing effective muscle recovery, while the use of PBMT was capable of improving muscle condition 24 h after exercise.

Another interesting factor is the fact that joint application of therapeutic interventions has not shown much relevance, as indicated by the results achieved with joint application. For example, regardless of the application order, combined application of therapeutic interventions is not seen to be more effective than the individual application of PBMT and it corroborates with recent literature [43].

Recently, Albuquerque-Pontes et al. [44] have demonstrated that one single irradiation with PBMT is capable of increasing the activity of cytochrome c-oxidase in intact skeletal muscle tissue up to 24 h after irradiation, and this upregulation is dependent of dose and wavelength. Furthermore, the combined use of three wavelengths is beneficial for this aim [45–50]. This study [44] shows that PBMT plays a leading role in the self-regulation of mitochondrial activity by increasing the mitochondrial respiratory chain. This, in turn, consequently increases the production of ATP in muscle cells and leads to the reduction of oxidative stress and subsequently to the production of reactive oxygen species (ROS). It is important to emphasize that, in this study, uninjured animal muscles were irradiated.

The results observed lead to the discussion of using cryotherapy as a tool to speed recovery; therefore, more studies need to be conducted to confirm this hypothesis. However, the clinical impact of these findings is obvious because they demonstrate that the use of PBMT is more effective than the use of cryotherapy for muscle recovery. It is worth remembering that only one session of each mode was performed, that is, the potential shown by PBMT may be even higher if treatment is continued throughout the same week.

# Conclusion

Based on the above results and discussion, this study demonstrates that the application of cryotherapy associated with PBMT does not improve the effects of the application of PBMT, so the isolated application of PBMT seems to be the best option to improve muscle recovery in both the short and long term. In contrast, the use of cryotherapy in isolation was unable to provide muscle recovery. Additional field studies should be performed to optimize dose parameters for differences in elite and recreational athletic recovery and to examine the long-term effects of PBMT.

#### Compliance with ethical standards

**Competing interests** Professor Ernesto Cesar Pinto Leal-Junior receives research support from Multi Radiance Medical (Solon, OH -USA), a laser device manufacturer. Multi Radiance Medical had no role in the planning of this study, and the laser device used was not theirs. They had no influence on study design, data collection and analysis, decision to publish, or preparation of the manuscript. The remaining authors declare that they have no conflict of interests.

**Ethical aspects** The study was approved by the Ethics Committee of the University of Caxias do Sul. In accordance with the Declaration of Helsinki, all subjects were advised about the procedure and they signed an informed consent prior to participation in the study (CAEE 31344214.3.3001.5341).

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