

**Document Type:** Study Protocol and Statistical Analysis Plan

**NCT number:** NCT05989815

**Document Date:** 20-06-2025

**Prepared by:** Italo Amaral de Oliveira, PT, MSc.

**Institution:** Federal University of São Carlos (UFSCar), São Paulo, Brazil

## Title page

# **Whole-body photobiomodulation for muscle performance enhancement, attenuation of muscular damage and delayed onset muscle soreness in professional soccer athletes – randomized sham-controlled trial**

## Abstract

**Context:** Photobiomodulation (PBM) has recently been applied in sports to enhance physical performance and mitigate muscle damage and delayed-onset muscle soreness (DOMS). These effects may be particularly relevant when PBM is delivered to large body areas through whole-body irradiation. **Objective:** To evaluate the effects of whole-body PBM on muscle damage, DOMS, and muscle performance in professional soccer athletes undergoing a muscle damage induction protocol. **Methods:** A randomized, double-blind, sham-controlled trial was conducted with 30 professional soccer players from a Brazilian team. Participants were randomly allocated into three groups ( $n = 10$  each): (a) PBM-post, (b) PBM-pre, and (c) PBM-sham. Outcomes included serum creatine kinase (CK) levels, DOMS (numeric rating scale [NRS] and pain mapping [mapDOMS]), muscle performance (squat jump [SJ] and countermovement jump [CMJ]), and isometric dynamometry of the knee extensors. All groups received PBM irradiation (active or sham), followed by the muscle damage induction protocol, and subsequent PBM irradiation (active or sham). Assessments were performed at baseline, 24 h, 48 h, and 72 h post-induction. **Results:** CK levels significantly increased ( $p < 0.05$ ) at 24–72 h in the sham group and at 24–48 h in the PBM-post group. In contrast, the PBM-pre group showed no significant increase ( $p > 0.05$ ) across 24–72 h. For DOMS (NRS), the sham group

reported significant increases ( $p < 0.05$ ) from 24–72 h, the PBM-pre group only at 24 h, and the PBM-post group at no time point. DOMS assessed by mapDOMS showed no significant changes ( $p > 0.05$ ) across groups or time points. No significant differences ( $p > 0.05$ ) were observed in muscle performance outcomes among groups. **Conclusion:** Pre-exercise PBM provided protective effects against muscle damage (lower CK) and reduced DOMS at 48–72 h, while post-exercise PBM reduced DOMS consistently at all time points (24–72 h). Neither pre- nor post-exercise PBM improved or worsened muscle performance.

**Keywords:** CK; DOMS; light-emitting diode; low-level laser therapy; recovery

## Methods

The study was characterized as a randomized, double-blind, Sham-controlled trial and followed CONSORT guidelines. All procedures were approved by the Research Ethics Committee of the Federal University of São Carlos (CAAE 62842522.2.0000.5504) and registered at ClinicalTrials.gov (NCT05989815). All participants were informed about the study's objectives and signed an informed consent form.

## Sample Characterization

### Criteria for Eligibility

Male individuals between 18 and 35 years of age, practicing professional soccer and undergoing training at least 5 times per week, were selected. Individuals with any impediment to physical activity or dysfunctions affecting the neuromuscular system were excluded. Data collection took place at the Desportivo Brasil Football Club in Porto Feliz, SP. The sample consisted of team members, with a set number of 30 athletes established for the sample.

## Training Routine Description

The athletes underwent daily training sessions throughout the week, following the club's training routine as described on Table 1.

**Table 1** – Weekly Training Routine

| Period           | Monday                                                                       | Tuesday                                                                                    | Wednesday                                                                         | Thursday                                  | Friday                                     | Saturday                                |
|------------------|------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|-------------------------------------------|--------------------------------------------|-----------------------------------------|
| <b>Morning</b>   | Rest/Downtime                                                                | Gym<br>(strength<br>training<br>– 30<br>min) +<br>Field<br>(aerobic<br>activity<br>60 min) | Gym (strength<br>training – 30<br>min) + Field<br>(aerobic<br>activity 90<br>min) | Gym<br>(strength<br>training –<br>30 min) | Field (aerobic<br>activity 45 - 60<br>min) | Training<br>game or<br>Official<br>Game |
| <b>Afternoon</b> | Gym (strength<br>training 30<br>min) + Field<br>(aerobic<br>activity 60 min) | Field<br>(aerobic<br>activity 60 min)                                                      | Rest/Downtime                                                                     | Field<br>(aerobic<br>activity 60 min)     | Downtime/Rest                              | Rest                                    |

|  |                     |
|--|---------------------|
|  | activity 45<br>min) |
|--|---------------------|

## Randomization and Allocation Concealment

The randomization was conducted using the website randomization.com, and the participants were allocated into three groups of ten individuals each. To ensure allocation concealment, sealed opaque envelopes were used, containing the randomly assigned interventions: PBM-post, PBM-pre, and PBM-Sham. Only one researcher (therapist 1) was involved in this process, being responsible for applying PBM to all participants. This researcher was blinded to the other assessments in the study, which were conducted by therapist 2. Participants were unaware of which intervention was applied during the study.

## Study Design

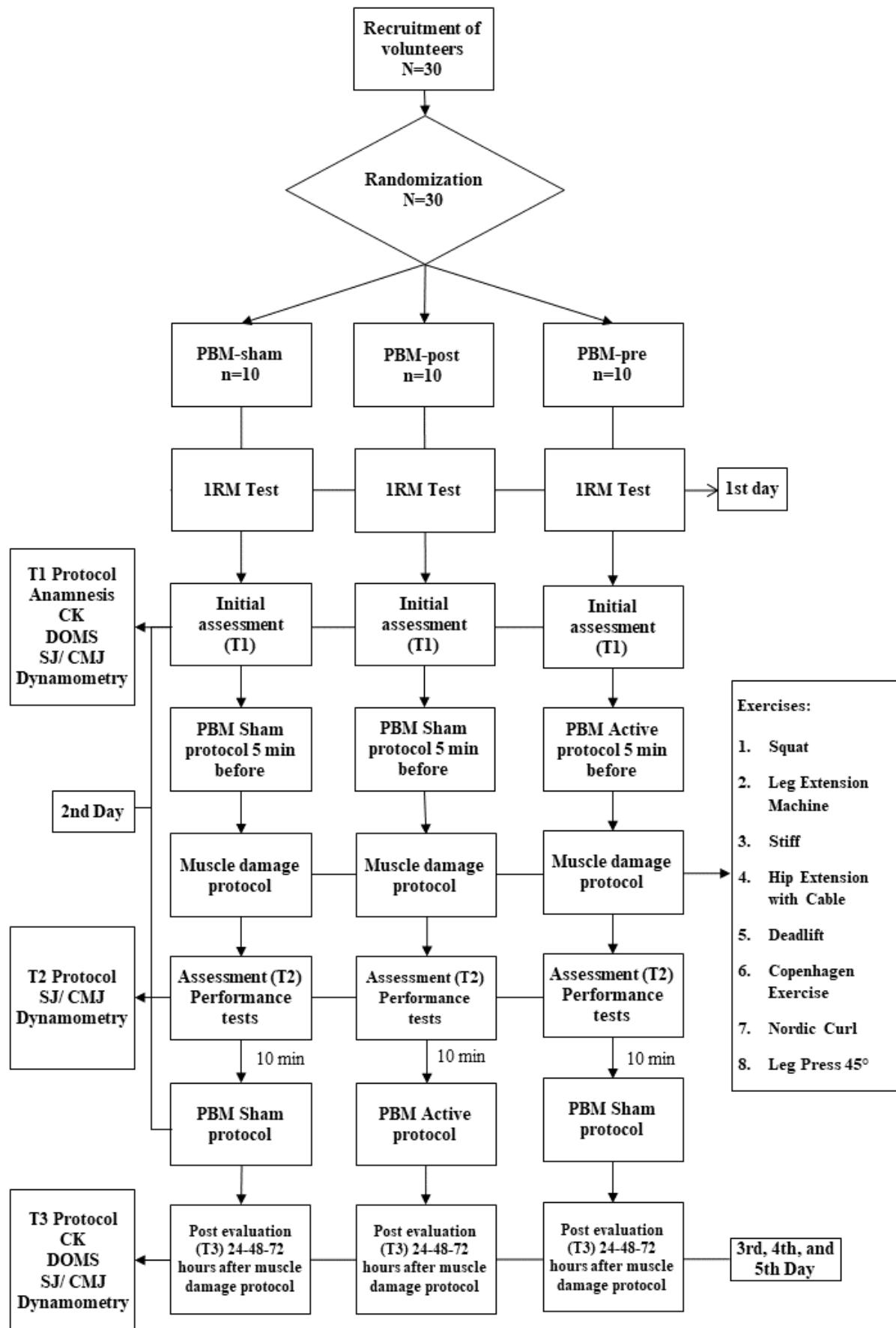
The selected individuals were allocated into 3 equal groups and randomized according to the type of intervention:

- **PBM Post-Exercise Group (PBM-Post):** Submitted to the protocol of muscle damage induction and Sham photobiomodulation pre-exercise, followed by active post-exercise photobiomodulation.
- **PBM Pre-Exercise Group (PBM-Pre):** Submitted to the protocol of muscle damage induction and active pre-exercise photobiomodulation, followed by Sham post-exercise photobiomodulation.
- **PBM Sham Group (PBM-Sham):** Submitted to the protocol of muscle damage induction and Sham photobiomodulation both pre and post-exercise.

On the 1<sup>st</sup> day, all individuals were assessed using the one-repetition maximum (1RM) test to measure the loads for the muscle damage induction protocol. On the 2<sup>nd</sup> day (24 hours after the 1RM test), all groups underwent initial assessment tests (T1), which included: a) Anamnesis through a questionnaire. b) Measurement of blood levels of creatine phosphokinase (CK) using the Roche Hitachi 917 biochemical analyzer. c) Delayed onset muscle soreness (DOMS) using the numeric rating scale (NRS) and the pain location area using the pain map (mapDOMS). d) Muscle performance tests on a jump platform (Squat Jump test – SJ and Counter Movement Jump - CMJ). e) Dynamometry of the dominant knee extensor muscles with the Manual Muscle Test equipment (Lafayette Instrument).

Also on the 2<sup>nd</sup> day, the groups were irradiated with whole-body PBM (Sham or active) followed by the muscle damage induction protocol. Sequentially, participants underwent vertical jump and dynamometry tests (T2). Ten minutes after T2, participants were irradiated with active PBM or Sham according to their allocation in the groups.

Finally, on the 3<sup>rd</sup>, 4<sup>th</sup>, and 5<sup>th</sup> days, participants were reassessed (T3) for CK, DOMS, SJ/CMJ, and Dynamometry at 24h, 48h, and 72h after the muscle damage induction protocol. The study design is summarized in Figure 1.



**Figure 1.** Study Flowchart

## **Assessments/Measurement Instruments**

### **a) Anamnesis:**

In the initial assessment, the volunteer's name, age, medical history, duration of sports practice, weight, height, and calculation of Body Mass Index (BMI) were collected. Additionally, the participants' skin phototype (Fitzpatrick scale) was determined.

### **b) Measurement of Blood Levels of Creatine Phosphokinase (CK):**

The analyses were carried out by a clinical analysis laboratory (LabCenter Diagnostics Integrates, Itu, SP, Brazil) in a blinded manner, meaning they were unaware of the therapies applied to each participant. Briefly, the athletes rested for about 10 minutes in a seated position before blood collection. The material was labeled, and the forearm was aseptically cleaned with 70% alcohol. Using a 25x8 multiple collection needle, a vein in the antecubital fossa was punctured, and a 5 ml vacuum collection gel tube was inserted for CK analysis. Subsequently, the measurement was performed on a Roche Hitachi 917 biochemical analyzer using enzymatic methodology. A biomedical professional from the laboratory, not involved in the study, assessed the results in U/L. This analysis was conducted at baseline (T1), i.e., before the muscle damage induction protocol, and at 24h, 48h, and 72h after (T3).

### **c) Delayed Onset Muscle Soreness (DOMS):**

The perception of Delayed Onset Muscle Soreness (DOMS) was assessed using the Numeric Rating Scale (NRS). The scale was numbered from 0 to 10, where 0 indicated the absence of muscle pain, and 10 indicated maximum muscle pain during a maximum voluntary contraction for knee extension in a seated position. The choice of NRS was based on its reliability and responsiveness, as reported by <sup>26,27</sup>.

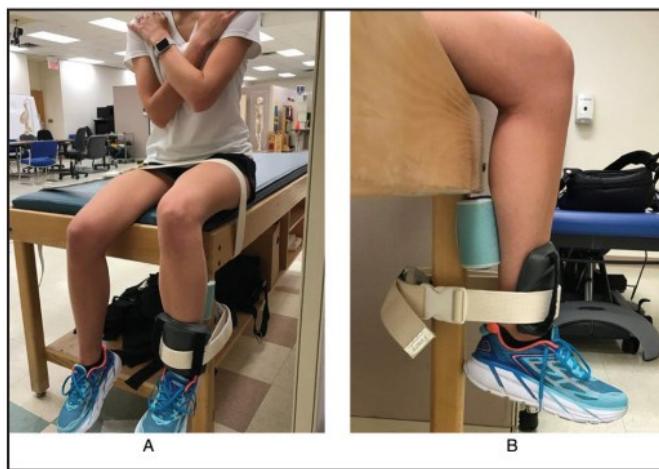
The location and extent (percentage area) of DOMS were assessed using a pain map (mapDOMS) using a body chart representing the human body from different views (frontal, lateral, and dorsal). Two variables, the extent and location of pain, were used following the approach proposed by <sup>28</sup>. Briefly, the areas where athletes reported pain were marked and later measured in cm. The painted area was then calculated using ImageJ software, an open-source software created by Wayne Rasband at the National Institutes of Health (NIH) in 1987 <sup>29</sup>. Finally, the painted area was calculated as a percentage (%) of the total area related to the lower limbs.

### **d) Muscle Performance Tests on Jump Platform:**

Vertical jumps were performed twice, and the mean value was used for the analyses. Initially, the athletes warmed up on a cycle ergometer for 5 minutes at over 90 rpm. To perform the vertical jumps on a jump platform (Cefise, SP, Brazil), participants kept their hands on their hips and received verbal commands to initiate the jumps. For the Squat Jump (SJ) test, the athlete started from a semi-squat position, maintaining this position for approximately 3 seconds before takeoff. For the Counter Movement Jump (CMJ) test, the athlete was instructed to initiate a downward movement (squatting) from a standing position, which was immediately followed by an upward movement leading to takeoff<sup>30</sup>. The mean value of two jumps was used for subsequent analyses.

**e) Muscle Performance Test of Knee Extensors by Dynamometry:**

The manual dynamometry test was performed on the individual's dominant leg, as indicated by a previous study<sup>31</sup>, using a portable dynamometer (Manual Muscle Test - Lafayette Instrument). The athlete was positioned in a seated position with legs hanging off the edge of a table, keeping the knee at approximately 90° of flexion, as illustrated in figure 2. All participants performed two maximum voluntary contractions, verbally encouraged during execution for five seconds, with a one-minute interval between contractions. The mean value, in kilogram-force (kgf), of the two contractions was used for analysis.



**Figure 2.** Positioning of the adapted test from<sup>31</sup>. A) The test was conducted with the participant in a seated position, legs hanging off the edge of a table, maintaining the knee at approximately 90° of flexion. To minimize posterior thigh discomfort, a small wedge-shaped cushion was placed on the posterior aspect of the distal thigh, and a standard gait belt was used to stabilize the thighs on the table. During the test, participants kept their arms crossed to isolate the quadriceps muscle. A foam pad was positioned on the anterior aspect of the leg, where the dynamometer was placed, situated 5 cm proximal to the lateral malleolus, with the strap wrapped around a pad and the dynamometer secured against the leg of the table (B). Additionally, a small elastic wrap was placed between the leg of the table and the triceps surae muscle to minimize belt slack (A).

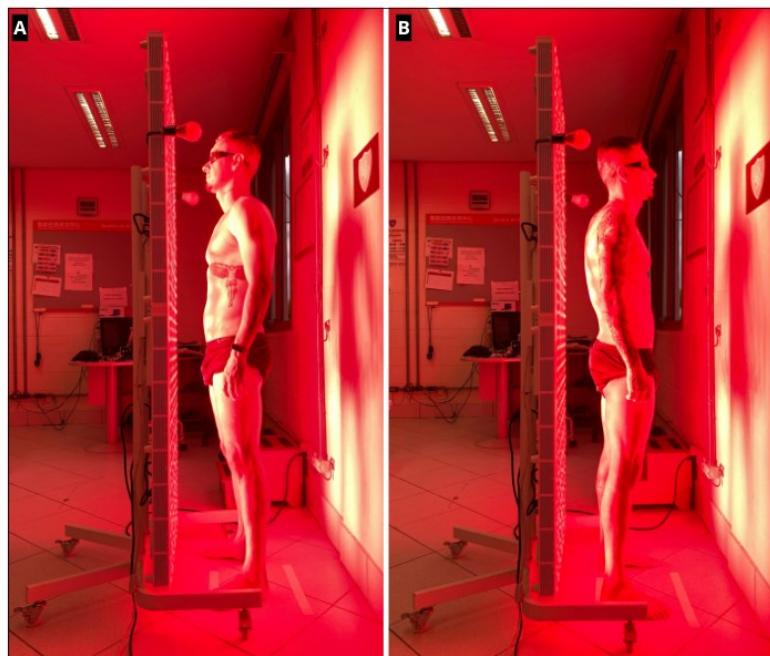
### f) 1RM Test

The 1RM (One Repetition Maximum) Test aimed to assess the maximum load an individual could use during the execution of a specific exercise. This test was conducted for the exercises included in the muscle damage induction protocol. Initially, a maximum load close to what was expected for the participant to endure was chosen. The test concluded when the individual completed only one full repetition of the movement with the determined load<sup>32</sup>. Five attempts were made, with a 3-minute interval between each attempt.

### **Photobiomodulation (PBM) Protocol**

Photobiomodulation (PBM) was performed using a whole-body irradiation system (Joovv Elite System), consisting of 6 panels with 76 red LEDs ( $660\pm10\text{nm}$ ) and 74 infrared LEDs ( $850\pm10\text{nm}$ ), totaling 900 LEDs in an area of  $12,193\text{ cm}^2$  (Figure 3). Athletes were positioned in front of the device (20 cm away from the thigh region), wearing only swim shorts to expose the muscle area of the lower limbs, trunk, and arms, with a total irradiation time of 20 minutes (600 seconds for the anterior region, 600 seconds for the posterior region). The effective PBM dose applied to each region (anterior and posterior) was  $48.97\text{ J/cm}^2$ , with an irradiance of  $81.62\text{ mW/cm}^2$ . The Sham condition consisted of irradiation with the device without effective light emission. An investigator not involved in data collection and analysis performed all irradiations (Sham and effective). The order of treatments was determined through simple balanced randomization, as shown in Figure 1.

The application of PBM (effective and/or Sham) was performed after the muscle performance tests (protocol T1), in the pre and post-protocol of muscle damage induction, as illustrated in Figure 1. In all conditions, participants were blindfolded during treatments. All PBM parameters were measured beforehand using a power and energy meter (PM100D, Thorlabs Inc.) equipped with the S130C light sensor (area of  $0.70\text{ cm}^2$ ) and are described in Table 2.



**Figure 3.** Whole-Body Photobiomodulation using the Joovv Elite System, containing LEDs (light-emitting diodes) in the infrared range ( $850\pm10\text{nm}$ ) and red range ( $660\pm10\text{nm}$ ). A. Frontal irradiation; B. Dorsal irradiation.

**Table 2** – Irradiation Parameters

**Table 2. Irradiation Parameters**

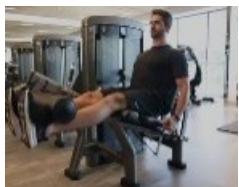
| Manufacturer                                                            | Joovv                     |            |
|-------------------------------------------------------------------------|---------------------------|------------|
| <b>Wavelength</b>                                                       | Red                       | Infrared   |
| <b>Central wavelength (nm)</b>                                          | $660\pm10$                | $850\pm10$ |
| <b>Number of LEDs (6 panels)</b>                                        | 456                       | 444        |
| <b>Beam area (<math>\text{cm}^2</math>) per LED</b>                     | 2.54                      | 2.54       |
| <b>Operational mode</b>                                                 | Continuous                | Continuous |
| <b>Body distance (cm)</b>                                               | 20                        | 20         |
| <b>Radiant power (mW):</b>                                              | 31.85                     | 25.29      |
| <b>Power density (<math>\text{mW}/\text{cm}^2</math>)</b>               | 45.50                     | 36.13      |
| <b>Irradiation time (front) (s)</b>                                     | 600                       | 600        |
| <b>Irradiation time (back) (s)</b>                                      | 600                       | 600        |
| <b>Energy density (front) (<math>\text{J}/\text{cm}^2</math>)</b>       | 27.30                     | 21.67      |
| <b>Energy density (back) (<math>\text{J}/\text{cm}^2</math>)</b>        | 27.30                     | 21.67      |
| <b>Total average radiant power (mW)</b>                                 | $(31.85 + 25.29) = 57.14$ |            |
| <b>Total average power density (<math>\text{mW}/\text{cm}^2</math>)</b> | $(45.50 + 36.13) = 81.62$ |            |
| <b>Total average energy density (<math>\text{J}/\text{cm}^2</math>)</b> | $(27.30 + 21.67) = 48.97$ |            |
| <b>Total area of six panels (<math>\text{cm}^2</math>)</b>              | 12,193                    |            |

|                                                 |      |      |
|-------------------------------------------------|------|------|
| <b>Distance from power meter (cm)</b>           | 20   | 20   |
| <b>Area sensor power meter (cm<sup>2</sup>)</b> | 0.70 | 0.70 |

### Muscle Damage Induction Protocol

The adopted muscle damage induction protocol followed the recommendations of ACSM <sup>33</sup> for trained individuals, which consists of 8-12 repetitions above 70% of the one-repetition maximum (1RM) with 1 minute of rest between sets and 2 minutes between exercises. The protocol consisted of 3 sets of 10 repetitions for each exercise, with emphasis on the eccentric phase of the exercise to induce microlesions in the muscle tissue, and it is described in Table 3.

**Table 3** – Muscle damage induction protocol

| <b>Exercise</b>              | <b>Illustration</b>                                                                 | <b>Sets</b> | <b>Repetitions</b> | <b>Total Repetitions</b> |
|------------------------------|-------------------------------------------------------------------------------------|-------------|--------------------|--------------------------|
| <b>Squat</b>                 |  | 3           | 10                 | 30                       |
| <b>Leg Extension</b>         |  | 3           | 10                 | 30                       |
| <b>Stiff</b>                 |  | 3           | 10                 | 30                       |
| <b>Hip Extension (Cable)</b> |  | 3           | 10                 | 30                       |

|                      |                                                                                    |   |    |    |
|----------------------|------------------------------------------------------------------------------------|---|----|----|
| <b>Deadlift</b>      |   | 3 | 10 | 30 |
| <b>Copenhagen</b>    |   | 3 | 10 | 30 |
| <b>Nordic Curl</b>   |   | 3 | 10 | 30 |
| <b>Leg Press 45°</b> |  | 3 | 10 | 30 |

---

### 3.7 Sample Size Calculation

The sample size ( $n=30$ ) was calculated a priori using GPower 3.1 software. It considered 3 groups, 4 assessment time points (immediately after the muscle damage induction protocol, and at 24h, 48h, and 72h after), a minimum effect size of 0.25, a test power of 80%, and a significance level of 5%, for comparisons through two-way repeated measures analysis of variance.

### STATISTICAL ANALYSIS PLAN (SUMMARY)

Primary endpoint: Serum CK levels (U/L) over time.

Secondary endpoints: DOMS (NRS and mapDOMS), SJ and CMJ jump heights (cm), and isometric knee extensor force (kgf).

Statistical tests:

- Normality (Shapiro–Wilk) and homogeneity (Levene).
- Two-way repeated measures ANOVA with Tukey post hoc (parametric).
- Friedman and Kruskal–Wallis (nonparametric when assumptions not met).
- Significance level:  $\alpha = 0.05$ .

Software: IBM SPSS Statistics v20.0.

## Data Analysis

Data analysis was conducted by a researcher blinded to participant group allocation. The primary outcome of the study was considered the muscle damage measured by CK levels.

The data were descriptively analyzed using mean and standard deviation. The independent variables were the experimental conditions (Sham, PBM-pre, and PBM-post) and assessment times pre, 24h, 48h, 72h after the muscle damage induction protocol. Normality and homoscedasticity assumptions were tested beforehand using the Shapiro-Wilk W Test and Levene test, respectively. The dependent variables included CK, DOMS, height of jumps in Squat Jump (SJ) and Counter Movement Jump (CMJ), and isometric manual dynamometry of the femoral quadriceps (kgf).

Two-way repeated measures analysis of variance (ANOVA) was conducted (considering pre, 24h, 48h, and 72h after the muscle damage induction protocol) followed by Tukey's post hoc test when the data showed normal distribution and variance homogeneity. In cases where the data did not present normal distribution, a logarithmic transformation (log10) was adopted, and normality and variance homogeneity were verified again for parametric tests. If the data did not meet the assumptions of normality and variance homogeneity, non-parametric tests such as Friedman's ANOVA (intragroup analyses) and Kruskal-Wallis (intergroup analyses) were employed. The significance level was set at 5%. Statistical analysis was performed using a statistical software (SPSS Statistics for Windows version 20.0, IBM, Chicago, IL, United States).

## RESULTS

Thirty male professional football players (high level) from the same team were recruited and completed all procedures of this study. Therefore, there were no dropouts, and intention-to-treat analysis was not necessary. Anthropometric data are described in Table 4. No adverse effects were reported during the study.

**Table 4.** Anthropometric data of the investigated athlete sample. Values in mean ( $\pm$  standard deviation):

| <b>Groups</b> | <b>Age (years)</b>  | <b>Body Mass (kg)</b> | <b>BMI<br/>(kg/m<sup>2</sup>)</b> | <b>Years of Practice</b> | <b>Fitzpatrick<br/>Phototype</b>          |
|---------------|---------------------|-----------------------|-----------------------------------|--------------------------|-------------------------------------------|
| <b>Sham</b>   | 18.70 ( $\pm$ 0.82) | 76.03 ( $\pm$ 13.27)  | 22.62 ( $\pm$ 2.10)               | 9.60 ( $\pm$ 1.77)       | Type I: 2,<br>Type II: 0,<br>Type III: 3, |

|                 |                      |                      |                      |                      |                                                                       |
|-----------------|----------------------|----------------------|----------------------|----------------------|-----------------------------------------------------------------------|
|                 |                      |                      |                      |                      | Type IV: 3,<br>Type V: 2                                              |
| <b>PBM-pre</b>  | 20.50 ( $\pm 4.30$ ) | 70.43 ( $\pm 4.90$ ) | 23.07 ( $\pm 0.94$ ) | 11.30 ( $\pm 3.74$ ) | Type I: 2,<br>Type II: 2,<br>Type III: 3,<br>Type IV: 2,<br>Type V: 1 |
| <b>PBM-post</b> | 21.22 ( $\pm 7.44$ ) | 76.13 ( $\pm 8.03$ ) | 23.48 ( $\pm 1.73$ ) | 11.44 ( $\pm 3.46$ ) | Type I: 3,<br>Type II: 5,<br>Type III: 1,<br>Type IV: 1,<br>Type V: 0 |

### Creatine Kinase (CK)

The CK data in U/L did not follow a normal distribution, and a logarithmic transformation ( $\log_{10}$ ) was applied. After the transformation, the data showed a normal distribution and homogeneity of variances for conducting parametric tests. Repeated measures ANOVA revealed a moment interaction ( $F_{2,27} < 0.001$ ) and a moment versus treatment interaction ( $F_{2,27} < 0.001$ ). Tukey's post hoc test identified significant differences as shown in the table below:

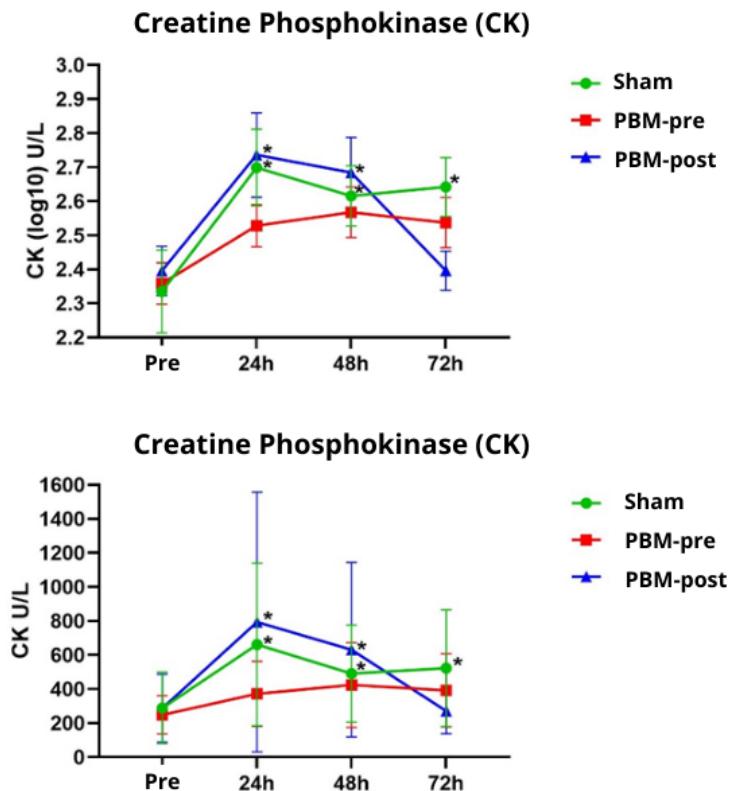
**Table 5.** Mean values ( $\pm$ standard deviation) of creatine phosphokinase (CK) in U/L for the Sham, PBM-pre, and PBM-post groups at pre, 24h, 48h, and 72h after the muscle damage induction protocol:

| Groups   | CK pre                 | CK 24h                  | CK 48h                  | CK 72h                  |
|----------|------------------------|-------------------------|-------------------------|-------------------------|
| Sham     | 289.2 ( $\pm 209.94$ ) | 661.5 ( $\pm 477.24$ )* | 491.6 ( $\pm 285.44$ )* | 523.2 ( $\pm 343.15$ )* |
| PBM-pre  | 249.2 ( $\pm 112.28$ ) | 372.7 ( $\pm 190.58$ )  | 424.2 ( $\pm 248.90$ )  | 392.6 ( $\pm 215.23$ )  |
| PBM-post | 287.8 ( $\pm 199.09$ ) | 794.5 ( $\pm 763.80$ )* | 631.2 ( $\pm 512.08$ )* | 271.44 ( $\pm 133.27$ ) |

**Abbreviations:** PBM (photobiomodulation); CK (creatine phosphokinase). \* p-value  $< 0.05$  in two-way repeated measures ANOVA followed by Tukey's post hoc analysis.

Compared to the pre-moment of the muscle damage induction protocol, there was a significant increase in CK levels in the Sham group ( $p = 0.001$ ) and PBM-post group ( $p = 0.001$ ) at the moment 24h after, while in the PBM-pre group the increase was not significant ( $p = 0.285$ ). When comparing the pre-moment of the muscle damage induction protocol to 48h after, the Sham group had a significant increase in CK ( $p = 0.028$ ), as well as the PBM-post group ( $p = 0.015$ ), while the PBM-pre group did not show a significant increase ( $p = 0.131$ ). In the

comparison between the pre-moment of the muscle damage induction protocol and 72h after, only the Sham group had a significant increase in CK ( $p = 0.010$ ), while the PBM-pre and PBM-post groups did not show significant increases ( $p = 0.506$ ;  $p = 1.000$ , respectively). In the comparison between groups for the respective CK assessment moments, there were no significant differences ( $p > 0.05$ ). The graphical representation of these results is in Figure 4.



**Figure 4.** Results for creatine phosphokinase (CK) at the evaluated time points (pre-moment of muscle damage induction protocol, 24h, 48h, and 72h after), and experimental groups (Sham, PBM-pre, PBM-post). Values presented in both log10-transformed and raw formats. Data expressed as mean ( $\pm$  standard error).

#### Delayed Onset Muscle Soreness (DOMS) measured by the Numeric Rating Scale (NRS).

Repeated measures ANOVA revealed a moment interaction ( $F_{2,27} < 0.001$ ) and a moment versus treatment interaction ( $F_{2,27} = 0.044$ ). Tukey's post hoc analysis identified significant differences as shown in Table 6.

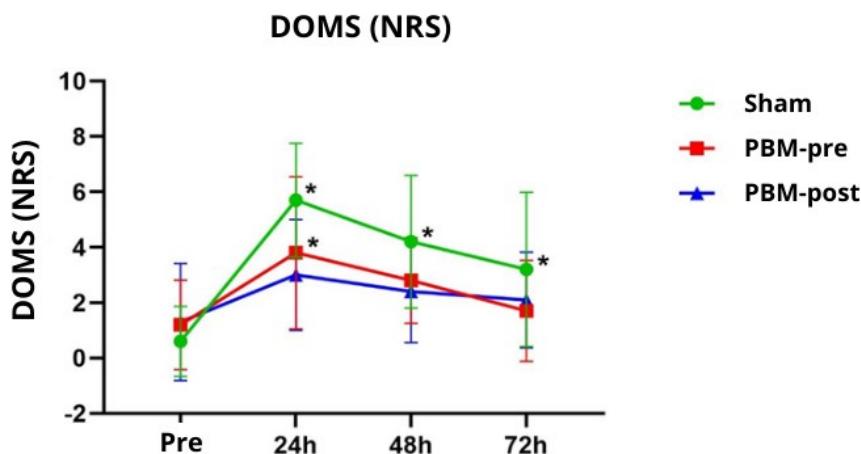
**Table 6.** Mean values ( $\pm$  standard deviation) of Delayed Onset Muscle Soreness (DOMS) measured by the Numeric Rating Scale (NRS) for the Sham, PBM-pre, and PBM-post groups at pre, 24h, 48h, and 72h after the muscle damage induction protocol:

| Groups | DOMS (NRS)          | DOMS (NRS)           | DOMS (NRS)           | DOMS (NRS)           |
|--------|---------------------|----------------------|----------------------|----------------------|
|        | pre                 | 24h                  | 48h                  | 72h                  |
| Sham   | 0.60 ( $\pm 1.26$ ) | 5.70 ( $\pm 2.05$ )* | 4.20 ( $\pm 2.39$ )* | 3.20 ( $\pm 2.78$ )* |

|                 |                     |                      |                     |                     |
|-----------------|---------------------|----------------------|---------------------|---------------------|
| <b>PBM-pre</b>  | 1.20 ( $\pm 1.61$ ) | 3.80 ( $\pm 2.74$ )* | 2.80 ( $\pm 1.54$ ) | 1.70 ( $\pm 1.82$ ) |
| <b>PBM-post</b> | 1.30 ( $\pm 2.11$ ) | 3.00 ( $\pm 2.00$ )  | 2.40 ( $\pm 1.83$ ) | 2.10 ( $\pm 1.72$ ) |

**Abbreviations:** PBM (photobiomodulation); DOMS (Delayed Onset Muscle Soreness); NRS (Numeric Rating Scale); \* p-value < 0.05 in two-way repeated measures ANOVA followed by Tukey's post hoc analysis.

Compared to the pre-moment of the muscle damage induction protocol, there was a significant increase in DOMS measured by the NRS in the Sham group ( $p < 0.001$ ) and PBM-pre group ( $p = 0.027$ ) at the moment 24h after, while the PBM-post group did not show a significant increase ( $p = 0.460$ ). In the comparison between the pre-moment of the muscle damage induction protocol and 48h after, the Sham group had a significant increase in DOMS (NRS) ( $p < 0.001$ ), while the PBM-pre and PBM-post groups did not show significant increases ( $p = 0.555$ ;  $p = 0.933$ , respectively). In the comparison between the pre-moment of the muscle damage induction protocol and 72h after, only the Sham group had a significant increase in DOMS ( $p = 0.027$ ), while the PBM-pre and PBM-post groups did not show significant increases ( $p = 0.999$ ;  $p = 0.993$ , respectively). In the comparison between groups for the respective moments of DOMS (NRS) assessment, there were no significant differences ( $p > 0.05$ ). The graphical representation of these results is in Figure 5.



**Figure 5.** Results for Delayed Onset Muscle Soreness (DOMS), measured by the Numeric Rating Scale (NRS) at the evaluated time points (pre-moment of muscle damage induction protocol, 24h, 48h, and 72h after), and experimental groups (Sham, PBM-pre, PBM-post). Data expressed as mean ( $\pm$  standard error).

### DOMS measured by Pain Map (mapDOMS)

The mapDOMS data did not follow a normal distribution, and a logarithmic transformation ( $\log_{10}$ ) was applied. After the transformation, the data exhibited a normal distribution and homogeneity of variances, allowing for the use of parametric tests. Repeated

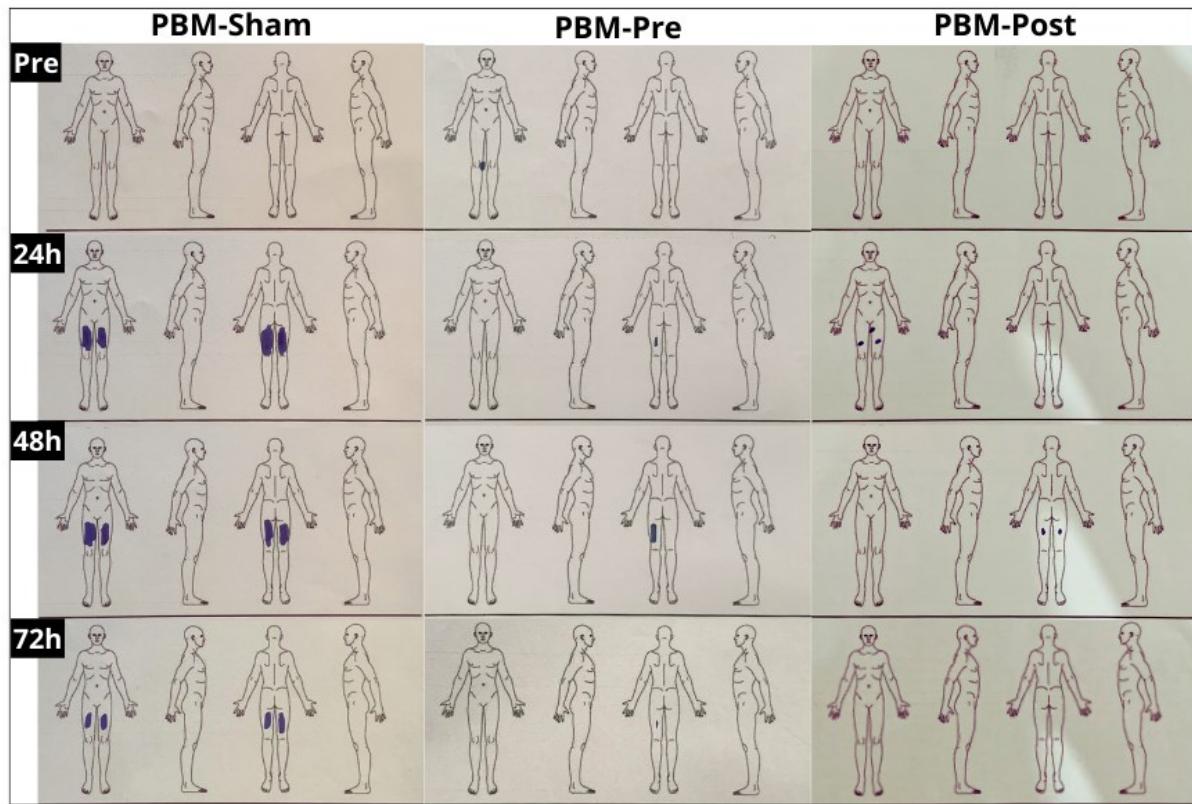
measures ANOVA did not reveal a moment interaction ( $F_{2,27} = 0.302$ ) and moment versus treatment interaction ( $F_{2,27} = 0.423$ ). As there was no significant interaction, Tukey's post hoc analysis was not conducted. The means ( $\pm$  percentage standard deviation) of mapDOMS are presented in Table 7.

**Table 7.** Mean values ( $\pm$  percentage standard deviation) of mapDOMS for the Sham, PBM-pre, and PBM-post groups at pre, 24h, 48h, and 72h after the muscle damage induction protocol:

| Groups   | mapDOMS pre            | mapDOMS 24h            | mapDOMS 48h            | mapDOMS 72h            |
|----------|------------------------|------------------------|------------------------|------------------------|
| Sham     | 2.99% ( $\pm 5.16\%$ ) | 9.55% ( $\pm 7.78\%$ ) | 8.51% ( $\pm 6.55\%$ ) | 5.97% ( $\pm 7.16\%$ ) |
| PBM-pre  | 0.96% ( $\pm 0.83\%$ ) | 5.42% ( $\pm 7.66\%$ ) | 3.73% ( $\pm 3.41\%$ ) | 3.85% ( $\pm 3.71\%$ ) |
| PBM-post | 1.53% ( $\pm 2.23\%$ ) | 4.88% ( $\pm 5.64\%$ ) | 3.54% ( $\pm 2.81\%$ ) | 3.08% ( $\pm 3.15\%$ ) |

**Abbreviations:** PBM (photobiomodulation); mapDOMS (Delayed Onset Muscle Soreness Map).

Figure 6 shows an example of the graphical representation of the map used during data collection for mapDOMS.



**Figure 6.** Pain Map (mapDOMS) of the experimental groups at pre, 24h, 48h, and 72h after.

### ***Squat Jump (SJ)***

A repeated measures ANOVA did not reveal a moment interaction ( $F_{2,27} = 0.177$ ) and moment versus treatment interaction ( $F_{2,27} = 0.834$ ). As there was no significant interaction,

Tukey's post hoc analysis was not conducted. The means and standard deviation for SJ are presented in Table 8.

**Table 8.** Mean values ( $\pm$  standard deviation) of SJ (cm) for the Sham, PBM-pre, and PBM-post groups at pre, immediately after, 24h, 48h, and 72h after the muscle damage induction protocol:

| Groups          | SJ pre              | SJ post             | SJ 24h              | SJ 48h              | SJ 72h              |
|-----------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| <b>Sham</b>     | 39.80 ( $\pm$ 4.60) | 39.65 ( $\pm$ 4.20) | 38.56 ( $\pm$ 4.09) | 40.10 ( $\pm$ 3.89) | 39.12 ( $\pm$ 4.13) |
| <b>PBM-pre</b>  | 39.96 ( $\pm$ 7.40) | 40.42 ( $\pm$ 7.54) | 38.91 ( $\pm$ 5.45) | 39.63 ( $\pm$ 6.94) | 40.32 ( $\pm$ 7.08) |
| <b>PBM-post</b> | 37.04 ( $\pm$ 3.74) | 38.39 ( $\pm$ 4.68) | 37.38 ( $\pm$ 5.22) | 38.18 ( $\pm$ 4.07) | 38.20 ( $\pm$ 3.77) |

**Abbreviations:** PBM (photobiomodulation); SJ (Squat Jump).

### **Counter Movement Jump (CMJ)**

The repeated measures ANOVA did not reveal a moment interaction ( $F_{2,27} = 0.166$ ) and moment versus treatment interaction ( $F_{2,27} = 0.127$ ). As there was no significant interaction, the Tukey post hoc analysis was not conducted. The means and standard deviations for the Counter Movement Jump (CMJ) are presented in Table 9.

**Table 9.** Mean values ( $\pm$  standard deviation) of Counter Movement Jump (CMJ) in centimeters for the Sham, PBM-pre, and PBM-post groups at pre, immediately after, 24h, 48h, and 72h after the muscle damage induction protocol:

| Groups          | CMJ pre             | CMJ post            | CMJ 24h             | CMJ 48h             | CMJ 72h             |
|-----------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| <b>Sham</b>     | 40.86 ( $\pm$ 4.68) | 40.49 ( $\pm$ 4.16) | 39.94 ( $\pm$ 3.64) | 41.11 ( $\pm$ 4.16) | 40.35 ( $\pm$ 4.76) |
| <b>PBM-pre</b>  | 41.17 ( $\pm$ 7.09) | 42.51 ( $\pm$ 7.66) | 40.93 ( $\pm$ 5.82) | 40.21 ( $\pm$ 6.22) | 42.98 ( $\pm$ 6.86) |
| <b>PBM-post</b> | 38.29 ( $\pm$ 3.55) | 39.57 ( $\pm$ 3.89) | 39.30 ( $\pm$ 4.59) | 39.64 ( $\pm$ 4.70) | 39.99 ( $\pm$ 4.60) |

**Abbreviations:** PBM (photobiomodulation); CMJ (Counter Movement Jump).

### **Dynamometry**

A repeated measures ANOVA revealed a significant moment interaction ( $F_{2,27} = 0.034$ ). However, there was no significant interaction between moment and treatment ( $F_{2,27} = 0.486$ ). Since there was a significant interaction for moment, Tukey's post hoc analysis was conducted. However, no significant differences were found ( $p > 0.05$ ). The means and standard deviations for dynamometry (kgf - kilogram-force) are presented in Table 10.

**Table 10.** Mean values ( $\pm$  standard deviation) of dynamometry (kgf) for the Sham, PBM-pre, and PBM-post groups at pre, immediately after, 24h, 48h, and 72h after the muscle damage induction protocol:

| Groups         | Dynamo pre           | Dynamo post          | Dynamo 24h           | Dynamo 48h           | Dynamo 72h          |
|----------------|----------------------|----------------------|----------------------|----------------------|---------------------|
| <b>Sham</b>    | 50.41 ( $\pm$ 14.98) | 53.83 ( $\pm$ 10.38) | 55.58 ( $\pm$ 10.45) | 56.46 ( $\pm$ 11.14) | 54.71 ( $\pm$ 9.61) |
| <b>PBM-pre</b> | 53.87 ( $\pm$ 9.81)  | 58.02 ( $\pm$ 7.64)  | 53.45 ( $\pm$ 8.63)  | 56.26 ( $\pm$ 10.95) | 57.75 ( $\pm$ 7.50) |

**PBM-post** 59.53 ( $\pm 8.87$ ) 58.67 ( $\pm 8.80$ ) 59.19 ( $\pm 8.34$ ) 64.38 ( $\pm 8.21$ ) 63.18 ( $\pm 11.32$ )

---

**Abbreviations:** PBM (photobiomodulation); Dynamo (dynamometry).

## FUNDING

This study did not receive external financial support. All study expenses were self-funded by the principal investigator.