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Study Title:

Whole-body Photobiomodulation Use in Professional Soccer Players During a State Championship: Randomized Controlled Trial

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1. Introduction

Photobiomodulation (PBM) is a non-invasive therapy that uses red and near-infrared light to stimulate mitochondrial activity, reduce muscle fatigue, and improve recovery. This protocol aims to evaluate the effects of whole-body PBM in professional soccer players during a state championship.

2. Methods

This study is a randomized, placebo-controlled clinical trial conducted in accordance with the SPIRIT 2013 guidelines (Chan et al., 2013).

2.1 Sample Description

The study sample will consist of a convenience sample comprising athletes from a professional soccer team, Desportivo Brasil. This club, located in Porto Feliz, São Paulo, is widely recognized for its excellence in player development and high-standard facilities, encompassing athletes from multiple youth categories as well as the main professional team competing in the São Paulo State Championship – Série A3 Division.

For this study, only athletes from the professional team will be considered, totaling 35 active players in the main roster. The predominant age range of these athletes is between 18 and 35 years, with anthropometric and physical performance characteristics consistent with the high-performance standards required in competitive soccer.

It is important to emphasize that participation in the study will be voluntary, and each athlete will be individually invited to take part in the research. No advantage or disadvantage will result from their decision, ensuring ethical conduct and respect for all participants.

2.1.1 Eligibility Criteria

Inclusion Criteria:

• Male individuals aged between 18 and 35 years;

• Professional soccer players who engage in regular training sessions at least five times per week;

• Availability to participate in all study sessions and assessments;

• Voluntary participation with signed informed consent.

Exclusion Criteria:

• Athletes currently undergoing treatment for injuries or temporarily removed from training for medical reasons;

• Presence of neuromuscular or orthopedic dysfunctions that may impair full physical activity or prevent execution of the study protocol;

• Any condition or medical limitation that may interfere with muscle performance or safety during the procedures.

Study Location and Sample Selection

Data collection will be conducted at Desportivo Brasil Football Club, located in Porto Feliz, São Paulo, Brazil. From a total of 35 professional athletes in the main roster, 24 participants will be selected based on the sample size calculation described in Section 3.7. All experimental procedures and assessments will take place within the club facilities, ensuring practicality and standardization throughout the process.

2.1.2 Training Routine Description

The athletes perform daily training sessions throughout the week, following the routine described below:

Table 1 – Weekly Training Composition

Period	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Morning	Rest day	Strength training at the gym (30 min) + Field session (aerobic activity – 60 min)	Strength training at the gym (30 min) + Field session (aerobic activity – 90 min)	Strength training at the gym (30 min)	Field session (aerobic activity – 45–60 min)	Training match or official match

Afternoon	Strength training at the gym (30 min) + Field session (aerobic activity – 45 min)	Field session (aerobic activity – 60 min)	Rest	Field session (aerobic activity – 60 min)	Rest	Day off

This training schedule reflects the standard weekly routine of the professional team during the competitive season, ensuring consistency in workload and recovery management.

2.1.3 Randomization and Allocation Concealment

Participants will be randomly allocated according to the type of intervention (PBM or Placebo). The randomization process will be performed using the website **randomization.com**, with participants distributed into two blocks of 12 individuals each.

To ensure allocation concealment, sealed opaque envelopes will be used, containing the randomized assignments: **PBM** or **Placebo**.

Only one researcher (Therapist 1) will be involved in this process and will be responsible for administering PBM to all participants. This researcher will be blinded to all other assessments, which will be conducted by another investigator (Evaluator 2).

2.2 Study Design

The selected participants will be randomly allocated into two balanced groups, as follows:

1) PBM Group: Participants will follow the club's standard physical training program (Table 1), combined with whole-body photobiomodulation (PBM) twice per week, after training sessions, on non-consecutive days.

2) Placebo Group: Participants will follow the club's standard physical training program (Table 1), combined with whole-body placebo photobiomodulation twice per week, after training sessions, on non-consecutive days.

On the **first day (T1)**, participants will undergo the following baseline assessments: medical history (anamnesis), jump performance tests (Squat Jump – SJ and Countermovement Jump – CMJ), dynamometry, complete blood count, Numeric Rating

Scale (NRS) for pain, pain map, and the **Pittsburgh Sleep Quality Index – Portuguese version (PSQI-PT)**.

Subsequently, participants will continue their daily training routines for **eight weeks**, coinciding with the professional soccer championship period. Out of the five weekly training sessions, athletes will receive whole-body PBM (or placebo) **twice a week**, always on non-consecutive days and immediately after training.

The assessments performed at T1—except for anamnesis, sleep quality index, and complete blood count—will be repeated **weekly (T2)**. All assessments will be repeated again **after eight weeks (T3)**.

The study design is summarized in **Figure 1 below**:

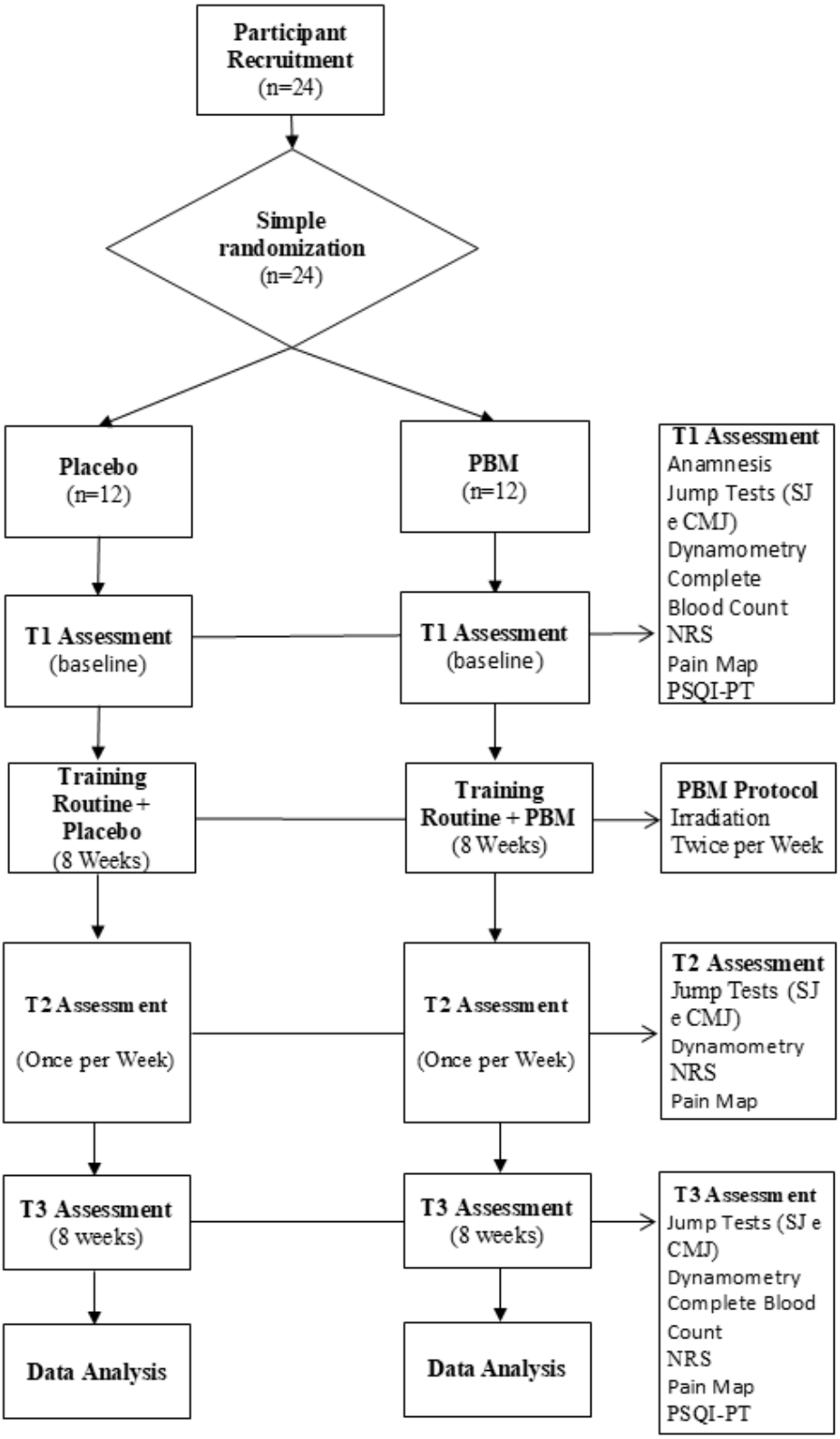

Figure 1. Study flow diagram

2.3 Assessments / Measurement Instruments

a) Anamnesis:

During this initial assessment, participants' personal information will be collected, including name, age, medical history, duration of sports practice, body mass, height, and body mass index (BMI) calculation.

b) Vertical Jump Tests (SJ - Squat Jump; CMJ - Countermovement Jump):

Vertical jumps will be performed in two formats: **Squat Jump (SJ)** and **Countermovement Jump (CMJ)**. Both tests will be performed twice, and the mean jump height (in centimeters) will be used for analysis.

Jump height will be measured using a contact platform that records flight time and calculates height based on the equations of vertical kinematics:

$$h = t^2 \times g \times 8^{-1}$$

where h = jump height, t = flight time (in seconds), and g = gravitational acceleration (9.81 m/s²) (Moreira, Lizana, Martins & Oliveira, 2008). The system automatically converts the measured flight time into height values, expressed in centimeters.

To perform the vertical jumps, participants will keep their hands on their hips and receive verbal cues before initiating the movement.

For the **Squat Jump (SJ)** test, the athlete will start from a semi-squat position, maintaining it for approximately 3 seconds before takeoff.

For the **Countermovement Jump (CMJ)** test, the athlete will start from a standing position and execute a quick downward movement (squat) immediately followed by an upward movement leading to takeoff (Van Hooren & Zolotarjova, 2017).

c) Dynamometry of the Knee Extensor Muscles:

The manual dynamometry test will be performed on the participant's **dominant leg**, according to a previous study (Lesnak, Anderson, Farmer, Katsavelis et al., 2019), using a **portable hand-held dynamometer** (Manual Muscle Test – Lafayette Instrument).

The athlete will be seated on a wooden examination table with the legs hanging freely over the edge, and the knee positioned at approximately **90° of flexion** (Figure 2).

All participants will perform **two maximal voluntary contractions (MVCs)**, each lasting **five seconds**, with **verbal encouragement** provided during the test. A **one-**

minute rest interval will be allowed between contractions, and the **mean value** of the two attempts will be used for analysis.

Figure 2. Positioning for the test adapted from Lesnak et al., 2019. (A) The test will be performed with the participant seated, legs hanging freely over the edge of an examination table, with the knee positioned at approximately 90° of flexion. A small wedge-shaped cushion will be placed under the distal posterior thigh to minimize posterior thigh discomfort, and a standard gait belt will be used to stabilize the thighs on the table. Participants will be instructed to keep their arms crossed during the test to isolate quadriceps activation. A foam pad will be positioned on the anterior aspect of the leg, where the dynamometer will be placed (5 cm proximal to the lateral malleolus), with the strap wrapped around the pad and the device anchored to the leg of the table (B). A small elastic wrap will be positioned between the table leg and the triceps surae muscle to minimize slack in the strap (A).

d) Complete Blood Count (CBC):

Blood collection will be performed by qualified professionals from a partner clinical analysis laboratory. To ensure convenience and practicality for participants, all collections will take place directly in the medical department room of the Desportivo Brasil Football Club, eliminating the need for external travel.

The laboratory professionals will be responsible for bringing all necessary materials and equipment to the club and for conducting the tests safely and efficiently, in full compliance with biosafety protocols. This approach avoids the need for athletes to travel to an external laboratory, thereby minimizing potential interference with their training routine and the study protocol.

The analysis will include the identification and quantification of the following blood markers:

- Red Blood Cells (Erythrocytes): Assess the number of red blood cells responsible for oxygen transport.

- Hemoglobin: Measures the concentration of hemoglobin, the protein responsible for oxygen carriage within red blood cells.
- Hematocrit: Indicates the proportion of red blood cells in the total blood volume.
- Iron, Ferritin, and Transferrin: The combined evaluation of these parameters provides insight into hematologic health and iron homeostasis. Understanding the levels of iron, ferritin, and transferrin is crucial for diagnosing and managing conditions such as anemia, hemochromatosis (iron overload), and other disorders related to iron metabolism.

e) Numeric Rating Scale (NRS) and Pain Map:

The perception of **delayed onset muscle soreness (DOMS)** will be assessed using a **Numeric Rating Scale (NRS)**. The scale ranges from **0 to 10**, where **0** indicates *no muscle soreness* and **10** represents *extreme muscle soreness* during a maximal voluntary knee extension performed in the seated position (see Appendices). The NRS was selected due to its **reliability and responsiveness** in assessing pain intensity (Ferreira-Valente, Pais-Ribeiro & Jensen, 2011; Thong, Jensen, Miró & Tan, 2018).

The **location of DOMS** will be evaluated using a **pain mapping tool** (see Appendices). Participants will be instructed to color a **body chart** representing the human body from **frontal, lateral, and dorsal views**. Two variables will be analyzed from the drawings: **pain extent** and **pain location** (Barbero, Moresi, Leoni, Gatti et al., 2015).

f) Pittsburgh Sleep Quality Index – Portuguese Version (PSQI-PT):

Sleep quality perception will be assessed using the Pittsburgh Sleep Quality Index – Portuguese Version (PSQI-PT).

This instrument consists of 19 self-rated questions covering seven components related to sleep, including duration, efficiency, and disturbances.

The total score ranges from 0 to 21, with higher scores indicating poorer sleep quality.

The PSQI-PT is widely used due to its reliability and validity across different populations.

2.4 Risks and Benefits

The risks and discomforts involved in this study are **minimal** and are described below according to each stage of the research:

Complete Blood Count (CBC): This procedure consists of drawing blood from a vein in the antecubital fossa using a 25x8 mm multiple-sample collection needle. A 5 mL vacuum collection tube with gel will be attached to the needle. The procedure will be performed by a qualified professional from the partner laboratory, using sterile gloves and proper aseptic techniques to ensure biosafety.

Blood collection may cause mild pain or slight bleeding, which typically resolves spontaneously within 5 minutes. An adhesive bandage will be applied, and participants will be advised to use ice if minor bruising occurs.

Muscle Soreness Assessment: This assessment involves rating muscle soreness using a Numeric Rating Scale (NRS) ranging from 0 to 10, where 0 indicates no pain and 10 indicates severe pain. Participants will be instructed to sit and perform a maximal voluntary isometric knee extension, which may cause temporary muscle soreness or discomfort. However, such sensations are normal responses to physical activity and do not require medication or intervention, only rest if necessary.

Jump Tests: This evaluation measures jump height (in centimeters) through four jumps—two Squat Jumps (SJ) and two Countermovement Jumps (CMJ). The test is quick and painless, though participants may experience mild discomfort upon landing. The evaluator will provide guidance on proper posture before each trial to minimize potential discomfort.

Muscle Strength Test: Muscle strength of the quadriceps of the dominant leg will be assessed using a portable hand-held dynamometer. Two consecutive measurements will be taken. The test poses no harm to participants, though it may cause temporary fatigue or mild discomfort due to pressure from the stabilizing strap used during the test.

To minimize discomfort, foam pads will be placed between the strap and the participant's skin to prevent localized pressure.

Whole-Body Photobiomodulation (PBM): The therapy is non-invasive, painless, and free of adverse effects. Participants may feel a mild warmth on the skin during light exposure. All participants and the operator will wear protective goggles, and instructions will be provided to avoid looking directly at the LED panels. If accidental exposure occurs, participants may experience mild transient visual discomfort, but this will not cause ocular damage. The device used in this study is FDA-approved and regulated by the U.S. Food and Drug Administration, ensuring safety and compliance with international health standards.

Potential Benefits: The potential benefits of this study include the identification of new therapeutic and ergogenic strategies that may effectively enhance muscle performance, accelerate post-training recovery, and reduce delayed-onset muscle soreness (DOMS) in athletes.

2.5 Photobiomodulation Protocol (PBM)

Whole-body photobiomodulation (PBM) will be administered using a **Joovv Elite System** (Figure 3), composed of **six panels** containing **76 red LEDs (660 ± 10 nm)** and **74 near-infrared LEDs (850 ± 10 nm)**, totaling **900 LEDs** covering an **irradiated area of 12,193 cm²**.

Participants will stand **20 cm in front of the device**, wearing only swimming trunks to ensure optimal light exposure to the thigh muscles and other body areas. The **total irradiation time** will be **15 minutes** (450 seconds for the anterior body and 450 seconds for the posterior body).

The **effective PBM dose** applied to each body region (anterior or posterior) will be **25.71 J/cm²**, with an **irradiance of 81.62 mW/cm²**.

The **placebo condition** will consist of **sham irradiation**, in which the device remains turned off. Additional **non-therapeutic accessory lights** will remain active to minimize visual differences between the placebo and the active PBM sessions. All PBM (active and placebo) sessions will be performed by a **researcher not involved in data collection or analysis**.

The **order of treatments** will follow a **simple balanced randomization**, as illustrated in Figure 1.

PBM (active or placebo) applications will occur **twice per week on non-consecutive days**, always **after the standard physical training sessions** prescribed by the professional soccer club, over a total period of **8 weeks**, corresponding to the duration of the professional soccer championship.

In all sessions, participants will wear **blindfolds** and **headphones** (listening to neutral background music) to prevent visual or auditory bias during treatment. All PBM parameters will be **verified beforehand using a power and energy meter** (PM100D, Thorlabs Inc.) equipped with a **S130C light sensor** (sensitive area = 0.70 cm²).

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Figure 3. Whole-body photobiomodulation using the Joovv Elite System, containing light-emitting diodes (LEDs) in the near-infrared (850 nm) and red (660 nm) wavelength ranges.

Photobiomodulation Parameters

Table 1. Irradiation Parameters (Units)		
Manufacturer	Joovv	
Wavelength	Red Light	Near-Infrared Light
Central Wavelength (nm)	660 – 10	850 – 10
Number of LEDs (6 panels)	456	444
Beam Area per LED (cm²)	2,54	2,54
Operational Mode	Continuous	Continuous
Distance from Body (cm)	20	20
Radiant Power (mW)	31,85	25,29
Power Density (mW/cm²)	45,50	36,13
Irradiation Time – Front (s)	450	450
Irradiation Time – Back (s)	450	450
Energy Density – Front (J/cm²)	14,33	11,38
Energy Density – Back (J/cm²)	14,33	11,38
Total Average Radiant Power (mW)	(31,85 + 25,29) = 57,14	
Total Average Power Density (mW/cm²)	(45,50 + 36,13) = 81,62	
Total Average Energy Density (J/cm²)	(14,33 + 11,38) = 25,71	
Total Irradiated Area (6 panels, cm²)	12.193	
Energy Meter Distance (cm)	20	20
Sensor Detection Area (cm²)	0,70	0,70

2.6 Sample Size Calculation

The sample size of **24 participants** was calculated *a priori* using **G*Power 3.1** software, considering **two groups** and **three time points** (T1, T2, and T3). A **two-way repeated-measures analysis of variance (ANOVA)** was selected, with a **minimum effect size of 0.25**, **statistical power of 80% (1-β = 0.80)**, and a **significance**

level (α) of 5%. To account for potential participant dropout, a total of **24 participants** will be recruited.

2.7 Data Analysis

Data analysis will be performed by a researcher blinded to group allocation, meaning that the evaluator will not have access to participants' group assignments. The **primary outcome** of the study will be **Delayed-Onset Muscle Soreness (DOMS)** assessed using the **Numeric Rating Scale (NRS)**.

Descriptive statistics will be used to summarize the data, including **absolute and relative frequency**, **mean**, and **standard deviation**. The **independent variables** will be the **experimental conditions** (i.e., PBM and Placebo) and the **assessment time points** (T1, T2, and T3).

Assumptions of **normality** and **homogeneity of variances** will be tested in advance using the **Shapiro–Wilk** and **Levene tests**, respectively.

The **dependent variables** will include:

- DOMS (measured by NRS)
- Jump height in the **Squat Jump (SJ)** and **Countermovement Jump (CMJ)** tests
- **Quadriceps muscle strength** assessed by manual dynamometry

A **two-way mixed analysis of variance (ANOVA)** will be conducted to examine the **interaction between factors (condition × time)** and the **main effects** of condition and time on the dependent variables. When significant interactions are detected, **simple effects analyses** will be performed to identify differences between groups and across time points.

The **level of significance** will be set at **5% ($p < 0.05$)**. All statistical analyses will be performed using **SPSS Statistics for Windows, version 20.0 (IBM Corp., Chicago, IL, USA)**.