Photobiomodulation therapy (PBMT) improves performance and accelerates recovery of high-level Rugby players in field test:

A randomized, crossover, double-blind, placebo-controlled clinical study

PBMT improves performance and accelerates recovery in Rugby players

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COMPETING INTERESTS

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

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ABSTRACT

While growing evidence supports the use of photobiomodulation therapy (PBMT) for performance and recovery enhancement, there have only been laboratory-controlled studies. Therefore, the aim of this study was to analyze the effects of PBMT in performance and recovery of high-level rugby players during an anaerobic field test. Twelve male high-level rugby athletes were recruited in this randomized, crossover, double-blinded, placebo-controlled trial. No interventions were performed before the Bangsbo Sprint Test (BST) at familiarization phase (week 1), at weeks 2 and 3 pre-exercise PBMT or placebo were randomly applied to each athlete. PBMT irradiation was performed at 17 sites of each lower limb, employing a cluster with 12 diodes (4 laser diodes of 905nm, 4 LED diodes of 875nm, and 4 LED diodes of 640nm, 30J per site - manufactured by Multi Radiance MedicalTM). Average time of sprints, best time of sprints, and fatigue index were obtained from BST. Blood lactate levels were assessed at baseline, and at 3, 10, 30 and 60 minutes after BST. Athletes' perceived fatigue was also assessed through a questionnaire. PBMT significantly (p<0.05) improved average time of sprints and fatigue index in BST. PBMT significantly decreased percentage of change in blood lactate levels (p<0.05) and perceived fatigue (p<0.05). Pre-exercise PBMT with the combination of super-pulsed laser (low-level laser), red and infrared LEDs can enhance performance and accelerate recovery of high-level rugby players in field test. This opens a new avenue for wide use of PBMT in real clinical practice in sports settings.

Keywords: Low-level laser therapy, Light emitting diodes, Sport, Exercise, Phototherapy.

INTRODUCTION

The sport of rugby consists of intense physical activity with frequent bursts of highintensity activities such as sprinting, tackling, and blocking intermingled with short intervals of low-intensity activities (between 4 and 8 seconds) like standing, walking or jogging. Preparation for play requires training to focus on a combination of muscular strength, power, agility, speed, aerobic and anaerobic endurance (4,8,9,15,25).

Varley et al. (25) compared activity profiles between rugby, American football and soccer players, and concluded that rugby players have less running load in matches. However, the frequent collisions increase the high-intensity efforts when compared to other non-collisions sports. As a high-intensity sport, rugby creates a higher physical demand and requires better conditioning from players. Identifying methods that not only promote recovery but also accelerate it are crucial to minimize accumulating fatigue and the risk of overuse injuries.

This is highly important in Rugby Sevens (7s), where the number of field players is limited to seven rather than fifteen on a full-sized field. This version of rugby increases the physical demands on the athletes and potentiates perceived fatigue. Moreover, according to tournament format, teams will play several matches per day with only a few hours between to recover from physical and physiological stress (8). Johnston et al. (15) state that symptoms of fatigue appear immediately after a match and usually persist for some days. Damage to large muscles, physiological stress and impairments in muscle function are commonly seen in players resulting in a decrease in field performance and skill (15). It is necessary to employ strategies that enhance and accelerate recovery following matches to best prepare the athlete for the next match. Photobiomodulation therapy (PBMT), with lasers and/or LEDs, has been shown to prevent skeletal muscle fatigue and accelerate recovery (20). Previous studies have demonstrated PBMT is able to reduce muscular fatigue, increase contraction strength and muscle performance (16,19,20). PBMT may prevent the onset of fatigue during activity thereby improving athletic performance (20). PBMT is non-thermal (11), commercially available modality that can be used in a variety of clinical and athletic settings. The effects of PBM are related to photochemical and photobiological effects within the tissue and are not attributed to heat (11). PBMT modulates biological processes of cells on mitochondrial level, increasing the oxygen consumption and production of adenosine triphosphate (ATP) (13).

Several studies have demonstrated the positive effects of PBMT on the improvement of biochemical markers related to muscle damage and recovery (20), including blood lactate levels. Furthermore, PBMT decreases the recovery time needed between exercise sessions (16,19). More recently, the literature showed beneficial effects in muscular recovery when PBMT is applied using the combination of different wavelengths synergistically (3,22). Which suggests that the combined use of different wavelengths may optimize cytochrome c oxidase modulation, increasing PBMT effects (1).

Currently all randomized clinical trials (RCTs) performed in this field demonstrating PBMT effectiveness in performance enhancement and accelerating recovery have been conducted in laboratory-controlled environment. To demonstrate real world application and translation to clinical practice, field tests are required to confirm the outcomes seen in the controlled laboratory trials. Therefore, the aim of this study is to analyze the effects of PBMT, with combination of different wavelengths and light sources (lasers and LEDs), on performance and recovery of high-level rugby players in a non-controlled field test environment.

METHODS

Experimental approach to the problem

A randomized, crossover, double-blind, placebo-controlled, clinical study was performed. To our knowledge, this novel study is the first to analyze the effects of PBMT on performance and recovery in professional athletes in an uncontrolled environment field test. Our hypothetical presumption was that PBMT can enhance athletes performance in field test, accelerate blood lactate clearance and lead athletes to decreased perceived fatigue. The Bangsbo Sprint Test (BST) (6) was chosen as field test since it mimics key actions performed during rugby matches such as sprints, change of direction, and active recovery between sprints (low-intensity running), and it is widely used by Rugby teams to testing athletes' anaerobic performance. We decided to assess blood lactate levels since it is a biochemical marker related to anaerobic metabolism and muscular acidosis (7,14,21), and it is often monitored in sports settings to evaluate athletes' recovery. Finally, the fatigue questionnaire (8) was used in order to evaluate athletes' perceived fatigue for each experimental condition tested. The dependent variables measured were: blood lactate levels; perceived fatigue score (from questionnaire); Mean Sprint Time (ST-Mean), Best Sprint Time (ST-Best) and Fatigue index from BST. The independent variables were: treatment with 3 levels (Familiarization, Placebo-Control and PBMT), and time for blood lactate (baseline, 3, 10, 30 and 60 minutes post).

Subjects

The study was approved by institutional ethics committee (process 665.347), and written informed consent was obtained from all volunteers. The number of participants per

group was determined based on a previous study conducted by Antonialli et al. (3) using the same PBMT device of the current study. A total of twelve high-level male rugby players with a mean age of 23.50 (\pm 2.32) years old (ranging 19 to 26 years old), height of 178.00 (\pm 4.79) cm and mean body mass of 86.00 (\pm 7.63) kg, from São José Rugby Club (Brazil) were recruited and completed all experimental procedures for this study with no dropouts. Each athlete played a minimum of once for the Brazilian national team with a mean time of sports practice of 9.33 (\pm 2.99) years.

Athletes were excluded if skeletal muscle injury was present, currently use any nutritional supplement or pharmacological agent, presented signs and symptoms of any disease (i.e., neurological, inflammatory, pulmonary, metabolic, oncologic), and had history of cardiac arrest that may limit performance of high-intensity exercises.

A simple drawing of lots that was used to determine which treatment each participant would receive at second and third exercise tests. PBMT device was preset to either Program 1 and Program 2 which corresponded to either active PBMT or the placebo treatment. The researcher that programmed the devices only knew the identity of the devices as either active or placebo and was instructed to not inform the participants or other researchers about the specific device programming.

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 device based on the randomization results performed randomization. Thus, the researcher who applied PBMT was blinded to which treatment was provided to the volunteers. Blinding was further maintained by the use of opaque goggles by the participants. Since this is a crossover study, participants who received program 1 at second exercise test, received program 2 at third exercise test, and vice-versa. Randomization was balanced in order to ensure that 50% of athletes would receive active

PBMT at second exercise test, and other 50% would receive active PBMT at third exercise test, avoiding further learning bias in our outcomes.

Procedures

All exercise tests were conducted in an enclosed soccer/rugby field. The three test phases, administered one week apart, were performed on the same day of the week (Tuesday) and time (1-5pm). The average temperature inside the building during the trials ranged from 26°C to 28°C. At first phase (exercise test 1) all athletes performed the Bangsbo Sprint Test (BST) (6) to familiarization with the procedure. No treatments were applied at this phase. However, at the second and third phases (exercise tests 2 and 3, respectively) either a placebo or active PBMT was applied immediately before athletes perform stretching and warm-up according to randomization. All procedures are summarized in figure 1.

<<Figure 1>>

Blood samples

Blood samples were collected from the athlete's fingertips prior to stretching and warming-up (baseline), and at 3, 10, 30 and 60 minutes after BST at each of the three study stages/phase (exercise tests). After finger asepsis with alcohol, puncture was performed with a disposable lancet. The first blood drop was discarded to avoid contamination with sweat, and then 25 microliters of blood were collected to biochemical analyses through electroenzimatic method, in accordance to portable lactate analyzer manufacturer (Accutrend Lactate Plus Roche[®]). The analyzer has a coefficient of variation ranged between 1.8 and 3.3% (ICC r=0.999), with good reliability for intra-, inter-analyzers, and between test strips (5).

Stretching and warm-up

After blood sample collection (to establish baseline), athletes performed a standardized warm-up and stretching, the same stretching and warm-up procedure was performed at each phase of study. The stretching lasted about 5 minutes and comprised dynamic stretches (30 seconds each) for knee extensors (bilaterally), knee flexors (bilaterally), calf muscles (bilaterally), low-back and abdominal muscles. The stretching was followed by a warm-up comprised by low-intensity short running for 10 minutes. The stretching and warming-up lasted about 15 minutes and followed by 5 minutes of rest. At phases 2 and 3, the stretching and warm-up procedure was performed immediately after PBMT or placebo treatments.

Bangsbo Sprint Test (BST)

The BST was performed immediately after stretching and warm-up procedure and 5 minutes of rest. Therefore, in exercise tests 2 and 3 BST started about 20 minutes after PBMT or placebo.

The BST protocol consists of seven maximum sprints 34.2 meters (m) in length (A-E). The time of each sprint was measured by infrared photocells positioned at the start (A) and finish line (E) of the track (34.2 m). The athlete changes direction after crossing cone "B" in order to reach cone "C" (approximately 90 degrees), to after run to cone "D " and finally cone "E". At each sprint the side to change direction is alternated (right and left, consecutively). The

first change of direction was performed according athletes' preference at exercise test 1, and the same order for change of direction was kept for exercise tests 2 and 3 (6).

Active recovery between sprints was performed with either a 25 seconds low-intensity run of 40 meters to return to the starting position on the track (F-A). The time of recovery and return to start was monitored with a manual chronometer to ensure a consistent return to starting position within 20 to 22 seconds. Verbal commands and cues were used to encourage the athletes as well as to provide feedback regarding the remaining time for recovery and to start the next sprint (6). According to Wragg et al. (26), the BST has high reliability and presents a subject mean coefficient of variation of 1.8% (95% CI, 1.5-2.4).

Performance was evaluated by computing the Average Time of sprints performed during entire test (ST mean) as well as for the fastest (best) time (ST best) among the seven sprints performed at each test. In addition, fatigue index was calculated by the following equation: FI(%)= (STmean / STbest x 100) \cdot 100 to measure the percentage of decrease in performance between all sprints (5,25). This index is important since it shows the percentage of decrease of decrease in performance of athletes over the repeated sprints.

<<Figure 4>>

Questionnaire of fatigue

A quick perception of fatigue survey was administered 5 minutes after each exercise test in order to evaluate athletes' perceived fatigue for each experimental condition tested. The questionnaire consisted of eight questions pertaining to perception of training, sleep, leg pain, concentration, effectiveness, anxiety, irritability and stress. Each question was evaluated according to a score scale where 1-2 points corresponded to "not at all", 3-4 points to "normal", and 5-7 to "very much". The scores were calculated according to the relative importance of each question and a lower score indicated better general well-being perception, and a higher score demonstrated greater fatigue perception (8). This questionnaire was used in a previous study (8), and demonstrated a high reliability and very good correlation (r=0.63-0.83) with objective measures of fatigue and performance. Which demonstrates this questionnaire is a sensitive tool for monitoring fatigue.

Intervention

Photobiomodulation therapy

PBMT was applied employing MR4 Laser Therapy Systems outfitted with LaserShower 50 4D emitters (both manufactured by Multi Radiance Medical, Solon - OH, USA). The cluster style emitter contains 12 diodes comprising of four super-pulsed laser diodes (905 nm, 0.3125 mW average power, and 12.5 W peak power for each diode), four red LED diodes (640 nm, 15 mW average power for each diode), and four infrared LEDs diodes (875 nm, 17.5 mW average power for each diode).

The cluster probe was selected due to the available coverage area and to reduce the number of sites needing treatment. Treatment was applied in direct contact with the skin with a slight applied overpressure to 9 sites on extensor muscles of the knee (Figure 2), 6 sites on knee flexors of the knee, and 2 sites on the calf (Figure 3) of both lower limbs (22). To ensure blinding, the device emitted the same sounds and regardless of the programmed mode (active or placebo). Furthermore, since the device produces a non-significant amount of heat (11), the volunteers were not able to know if active or placebo PBMT was administered. A total of 17 emitters were used to apply the treatments, all sites of left leg were irradiated simultaneously at first, followed by all sites of right leg. The treatments lasted about 10 minutes. The researcher,

who was blinded to randomization and the programming of PBMT device, performed the phototherapy.

<<Figure 2>>

<<Figure 3>>

PBMT parameters and irradiation sites were selected based upon previous positive outcomes demonstrated with the same device (3,22). Table 1 provides a full description of the PBMT parameters.

<<Table 1>>

Statistical analysis

The number of participants per group was determined based on a previous study conducted by Antonialli et al. (3) using the same PBMT device of the current study, for sample size calculation we considered the β value of 20% and α of 5%. The Kolmogorov-Smirnov test was used to verify the normal distribution of data. The collected data demonstrated normal distribution, therefore the data were expressed in mean values and standard deviation (SD). The one-way ANOVA test followed by the Bonferroni post-hoc test were performed to verify statistical significance. The level of statistical significance was set at p<0.05. In graphs data are presented as mean and standard error of the mean (SEM). The intention-to-treat analysis would be followed a priori, however, there weren't dropouts in this study. The intra-class correlations (ICCs) for dependent measures were: ST-Mean (0.82), ST-Best (0.62), Fatigue index (0.58), Blood lactate (0.99), and perceived fatigue score (0.40).

RESULTS

The average time of sprints (ST mean) was significantly different (p<0.05) between familiarization (6.91 \pm 0.24 sec) and placebo (6.67 \pm 0.21 sec), as well as familiarization and PBMT (6.55 \pm 0.21 sec). Significant difference (p<0.05) was also demonstrated between PBMT and placebo groups.

For the best time among all seven sprints (ST best), there was significant difference (p<0.05) between familiarization (6.63 ± 0.25 sec) and placebo (6.38 ± 0.20 sec) and between familiarization and PBMT (6.38 ± 0.21 sec - p<0.05). However, no observed difference between PBMT and placebo (p>0.05) was seen.

Regarding fatigue index during BST, a significant difference (p<0.05) was observed between PBMT (2.66% \pm 0.61) and familiarization (4.19% \pm 0.98) and between PBMT and placebo (4.51% \pm 0.95 - p<0.05). No differences were observed between familiarization and placebo (p>0.05). All outcomes observed during the BST are summarized in figure 5.

<<Figure 5>>

While no statistical differences (p>0.05) regarding blood lactate levels in absolute values among conditions tested was observed, the percentage of decrease in the levels was significant (p<0.05) in favor of PBMT when compared both to familiarization and placebo at all post-exercise time-points tested (Figure 6).

<<Figure 6>>

Lastly, the perceived fatigue was significantly lower (p<0.05) when athletes received pre-exercise PBMT (20.16 \pm 3.63) when compared both to familiarization (23.08 \pm 1.92) and placebo (23.50 \pm 2.50). Outcomes are summarized in figure 7.

<<Figure 7>>

DISCUSSION

This research details an important first important step for the adoption of PBMT by both professional sports teams and high-level athletes, and represents the bridge between laboratory controlled studies and real world clinical practice.

Bangsbo (6) proposes the use of the BST due the inclusion of directional change and the active recovery between the seven sprints. According to Wragg et al. (26), a reliable field test must be sport specific and reliably represent the activities of an individual sport. Only a few field tests contain these features (26) that mimic the key actions performed during rugby matches.

While no change was found between the active and placebo PBMT for ST best, there was significant improvements observed in the ST mean. It was noted that active PMBT maintained an optimal running performance over the entire 7 sprints that lead to a significant decrease in the fatigue index when there is typically a decrease in performance as the test progresses (10). PBMT was successfully able to maintain the athlete's running speed over the entire series of sprint tests.

Fatigue is comprised of multifactorial components and can be generally characterized as a decreasing generation of force (2,10,12). Girard et al. (10) explains that fatigue development is associated with the intramuscular accumulation of metabolic by-products, such as hydrogen ions and blood lactate, which changes cellular pH. Metabolite accumulation can impair contractile function through inhibition of ATP production, which affects muscular performance.

Blood lactate concentration is considered an important biochemical marker of muscular acidosis and it is often monitored in sports settings, mainly in high-intensity sports (7,14,21). No statistical difference was observed in the absolute values of blood lactate levels in between treatments or when compared to the familiarization test. Higher levels of this biochemical marker should be and were expected in the active PBMT groups since the athlete's performance was significantly better.

Therefore, it is reasonable to state that active PBMT prevented the expected increase of blood lactate levels and reduced muscular fatigue and promoted faster recovery following exercise bouts. These findings are consistent with previous reports that observed the same beneficial effects of PBMT on muscle recovery after a high-intensity exercise (17,18,19). There was a significant decrease in favor of the active PBMT compared to other two tested conditions (familiarization and placebo), when the percentage change in blood lactate levels were calculated.

As stated by Mohr et al. (23) in their review, increased lactate levels indicate muscular fatigue or acidosis that is associated with anaerobic metabolism during intense exercise activity. High levels of blood lactate are related to impaired performance during intense muscular contraction (21,23). The average blood lactate levels 3 to 10 minutes after exercise test observed in this study was 15.10 to 12.91 mmol/L⁻¹ for placebo, 14.00 to 12.28 mmol/L⁻¹ for familiarization, and 14.11 to 11.95 mmol/L⁻¹ for PBMT condition, respectively. Our observations demonstrated that the experimental condition with the lowest lactate levels also correlated to the lower fatigue index ratings. Which suggests that improved performance can be

accomplished by decreasing the muscle acidosis in high-intense sports activities by applying PBMT prior to activity. However, additional research in this novel area is needed to provide insights into other sport specific activities, as well as about mechanisms through PBMT acts.

Our study employed the same short questionnaire previously used with rugby players by Elloumi et al. (8) to evaluate perceived fatigue in rugby athletes among experimental conditions and was sensitive enough to assess physical effort and perception of fatigue (8). Our outcomes demonstrated that when irradiated with active PBMT athletes presented a lower index of fatigue perception. This finding corroborates with other assessments performed in this study such as fatigue index in BST and blood lactate levels. According to Halson (12) a reliable questionnaire must corroborate with collected physiological data, and our outcomes have demonstrated this.

It should be noted that the parameters chosen for PBMT treatment in our study were selected based upon two previously published studies utilizing the same device where positive effects were noted (3,22). Antonialli et al. (3) tested three different doses against a placebo-control dose of 0 J and found that 30 J was the best dose tested (compared to 10 J, 50 J and placebo) significantly increasing performance, decreased the delay of onset muscle soreness (DOMS) and modulated CK activity. A crossover study performed by Miranda et al. (22) found significant decreases in dyspnea sensation, improvement in time until exhaustion, pulmonary ventilation and distance covered when PBMT was applied before the progressive-intensity cardiopulmonary test. The main muscular groups of both lower limbs were irradiated. We utilized the same irradiation sites used by Miranda et al. (22) to provide reasonable coverage of the major muscles of the lower extremity needed to perform the exercise protocol (BST).

PBMT demonstrates a modulatory effect on cytochrome c oxidase activity and can explain how PBMT improves performance while protecting skeletal muscle from exerciseinduced muscle damage. This has been considered the key mechanism for light-tissue interaction, promoting increased in cellular metabolism through increased mitochondrial function (1). Furthermore, Albuquerque-Pontes et al. (1) has demonstrated that cytochrome c oxidase activity stimulation by different wavelengths and doses occurs along different time-profiles. This suggests that PBMT stimulation can be optimized when different wavelengths are used simultaneously (1). These optimized PBMT effects were supported by Santos et al. (24) when different wavelengths and doses were applied immediately before tetanic contractions. They reported positive effects on several makers of skeletal muscle performance as well as the protective effects on skeletal muscle tissue (24).

Finally, our outcomes demonstrate the potential use of PBMT as a prophylactic strategy for performance and recovery enhancement of high-level athletes, and it is an important step for wide clinical use of this therapeutic tool.

PRATICAL APPLICATIONS

The same outcomes previously observed in controlled-laboratory environment were confirmed in this study. Pre-exercise PBMT enhances performance and accelerates recovery of high-level rugby players, which may represent a shift in current clinical practice with wide use of PBMT in sports settings. PBMT seems to have the potential to keep athletes at higher performance level and consequently help to avoid injuries due impaired recovery.

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LEGENDS OF FIGURES

Figure 1: CONSORT flowchart summarizing study procedures.

Figure 2: Sites of PBMT irradiation at anterior muscles of the lower limbs.

Figure 3: Sites of PBMT irradiation at posterior muscles of the lower limbs.

Figure 4: Bangsbo Sprint Test (BST).

Figure 5: Outcomes observed in BST, values are mean and error bars are SEM. ^a indicates significant difference compared to Familiarization (p<0.05), ^b indicates significant difference compared to Placebo (p<0.05).

Figure 6: Blood lactate levels, values are mean and error bars are SEM. ^a indicates significant difference compared to Familiarization (p<0.05), ^b indicates significant difference compared to Placebo (p<0.05).

Figure 7: Perceived fatigue, values are mean and error bars are SEM. ^a indicates significant difference compared to Familiarization (p<0.05), ^b indicates significant difference compared to Placebo (p<0.05).

Table 1. Parameters for PBMT.

Number of lasers	4 Super-pulsed infrared
Wavelength (nm)	905 (±1)
Frequency (Hz)	250
Peak power (W) – each	12.5
Average mean optical output (mW) - each	n 0.3125
Power density (mW/cm^2) - each	0.71
Energy density (J/cm^2) - each	0.162
Dose (J) - each	0.07125
Spot size of laser (cm ²) - each	0.44
Number of red LEDs	4 Red
Wavelength of red LEDs (nm)	640 (±10)
Frequency (Hz)	2
Average optical output (mW) - each	15
Power density (mW/cm^2) - each	16.66
Energy density (J/cm^2) - each	3.8
Dose (J) - each	3.42
Spot size of red LED (cm ²) - each	0.9
Number of infrared LEDs	4 Infrared
Wavelength of infrared LEDs (nm)	875 (±10)
Frequency (Hz)	16
Average optical output (mW) - each	17.5
Power density (mW/cm^2) - each	19.44
Energy density (J/cm^2) - each	4.43
Dose (J) - each	3.99
Spot Size of LED (cm ²) - each	0.9
Magnetic Field (mT)	35
Irradiation time per site (sec)	228
Total dose per site (J)	30
Total dose applied per lower limb (J)	510
Aperture of device (cm ²)	20
Application mode	Cluster probe held stationary in
	skin contact with a 90-degree
	angle and slight pressure

Phase 1 Exercise test 1 (week 1) - Familiarization











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