PHOTOBIOMODULATION THERAPY IMPROVES PERFORMANCE AND ACCELERATES RECOVERY OF HIGH-LEVEL RUGBY PLAYERS IN FIELD TEST: A RANDOMIZED, CROSSOVER, DOUBLE-BLIND, PLACEBO-CONTROLLED CLINICAL STUDY

HENRIQUE D. PINTO,^{1,2} ADRIANE A. VANIN,^{1,2} EDUARDO F. MIRANDA,¹ SHAIANE S. TOMAZONI,³ DOUGLAS S. JOHNSON,⁴ GIANNA M. ALBUQUERQUE-PONTES,^{1,5} IVO DE O. ALEIXO JUNIOR,^{1,2} VANESSA DOS S. GRANDINETTI,⁵ HELIODORA L. CASALECHI,^{1,2} PAULO DE TARSO C. DE CARVALHO,^{1,2,5} AND ERNESTO CESAR P. LEAL JUNIOR,^{1,2,5}

¹Laboratory of Phototherapy in Sports and Exercise, University of Nove de Julho (UNINOVE), São Paulo, Brazil; ²Postgraduate Program in Rehabilitation Sciences, University of Nove de Julho (UNINOVE), São Paulo, Brazil; ³Department of Pharmacology, University of São Paulo, São Paulo, Brazil; ⁴Multi Radiance Medical, Solon, Ohio; and ⁵Postgraduate Program in Biophotonics Applied to Health Sciences, University of Nove de Julho (UNINOVE), São Paulo, Brazil

Abstract

Pinto, HD, Vanin, AA, Miranda, EF, Tomazoni, SS, Johnson, DS, Albuquerque-Pontes, GM, de Oliveira Aleixo Junior, I, Grandinetti, VdS, Casalechi, HL, de Tarso Camillo de Carvalho, P, and Pinto Leal Junior. Photobiomodulation therapy improves performance and accelerates recovery of high-level rugby players in field test: A randomized, crossover, double-blind, placebo-controlled clinical study. J Strength Cond Res 30(12): 3329-3338, 2016-Although growing evidence supports the use of photobiomodulation therapy (PBMT) for performance and recovery enhancement, there have only been laboratorycontrolled studies. Therefore, the aim of this study was to analyze the effects of PBMT in performance and recovery of highlevel 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. Photobiomodulation therapy irradiation was performed at 17 sites of each lower limb, employing a cluster with 12 diodes (4 laser diodes of 905 nm, 4 light emitting diodes [LEDs] of 875 nm, and 4 LEDs of 640 nm, 30 J per site, manufactured by Multi Radiance Medical). Average time of

Address correspondence to Ernesto Cesar P. Leal Junior, ernesto.leal. junior@gmail.com.

30(12)/3329-3338

Journal of Strength and Conditioning Research © 2016 National Strength and Conditioning Association 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. Photobiomodulation therapy significantly ($p \le 0.05$) improved the average time of sprints and fatigue index in BST. Photobiomodulation therapy significantly decreased percentage of change in blood lactate levels ($p \le 0.05$) and perceived fatigue ($p \le$ 0.05). Pre-exercise PBMT with the combination of superpulsed laser (low-level laser), red LEDs, and infrared LEDs can enhance performance and accelerate recovery of highlevel rugby players in field test. This opens a new avenue for wide use of PBMT in real clinical practice in sports settings.

KEY WORDS low-level laser therapy, light emitting diodes, sport, exercise, phototherapy

INTRODUCTION

he sport of rugby consists of intense physical activity with frequent bursts of high-intensity 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 with other noncollision sports. As

VOLUME 30 | NUMBER 12 | DECEMBER 2016 | 3329

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 risk of overuse injuries.

This is highly important in Rugby Sevens (7s), where the number of field players is limited to 7 rather than 15 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 after matches to best prepare the athlete for the next match.

Photobiomodulation therapy (PBMT), with lasers or light emitting diodes (LEDs), has been shown to prevent skeletal muscle fatigue and accelerate recovery (20). Previous studies have demonstrated that PBMT is able to reduce muscular fatigue, increase contraction strength, and muscle performance (16,19,20). Photobiomodulation therapy may prevent the onset of fatigue during activity, thereby improving athletic performance (20). Photobiomodulation therapy is a nonthermal (11), commercially available modality that can be used in a variety of clinical and athletic settings. The effects of PBMT are related to photochemical and photobiological effects within the tissue, and are not attributed to heat (11). Photobiomodulation therapy 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 a combination of different wavelengths synergistically (3,22), which suggests that the combined use of different wavelengths may optimize cytochrome c oxidase modulation, increasing the effects of PBMT (1).

Currently, all randomized clinical trials 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

3330 Journal of Strength and Conditioning Research

a combination of different wavelengths and light sources (lasers and LEDs), on performance and recovery of high-level rugby players in a noncontrolled 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 because 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, because 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 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 12 high-level male rugby players with a mean age of 23.50 (\pm 2.32) years (ranging 19–26-year-old), height of 178.00 (\pm 4.79) cm, and mean body mass of 86.00 (\pm 7.63) kg were recruited from São José Rugby Club (Brazil), and all experimental procedures for this study were completed 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, and oncologic), and had history of cardiac arrest that may limit performance of high-intensity exercises. A simple drawing of lots was used to determine which treatment each participant would receive at second and third exercise tests. Photobiomodulation therapy device was preset to either Program 1 or 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.

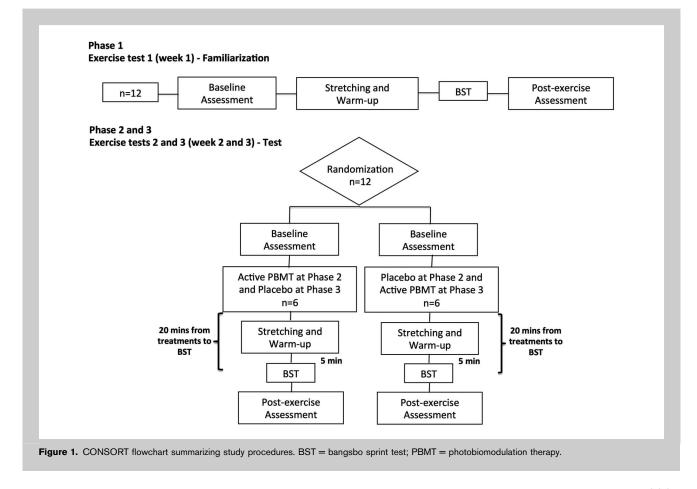
Randomization labels were created using a randomization table at a central office, where a series of sealed, opaque, and numbered envelopes were used to ensure confidentiality. The researcher who programmed the PBMT device based on the randomization results performed randomization. Thus, the researcher 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. Because 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 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. The study conforms to the Code of Ethics of the World Medical Association and required players to provide informed consent before participation.

Procedures

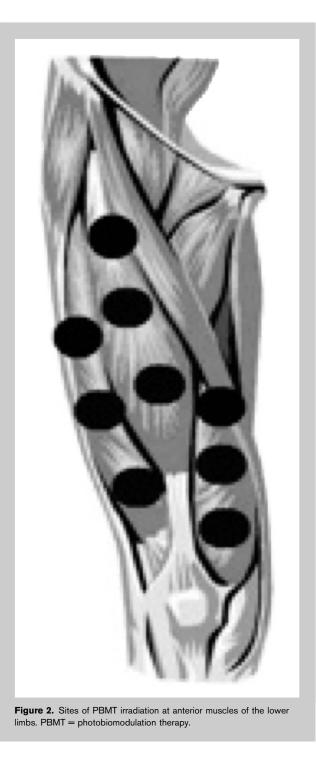
All exercise tests were conducted in an enclosed soccer or rugby field. The 3 test phases, administered 1 week apart, were performed on the same day of the week (Tuesday) and time (1–5 PM). The average temperature inside the building during the trials ranged from 26 to 28° C. At first phase (exercise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

Blood Samples

Blood samples were collected from the athlete's fingertips before stretching and warm-up (baseline), and at 3, 10, 30, and 60 minutes after BST at each of the 3 study stages or 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



VOLUME 30 | NUMBER 12 | DECEMBER 2016 | 3331



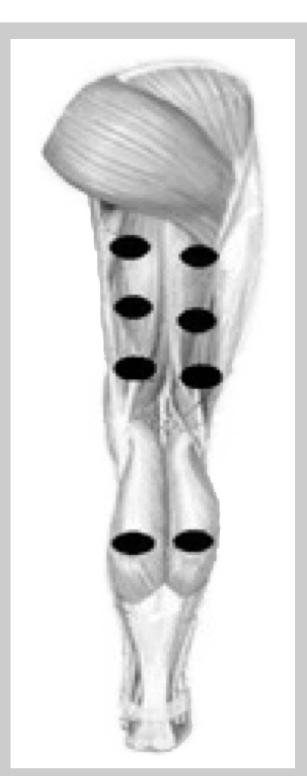


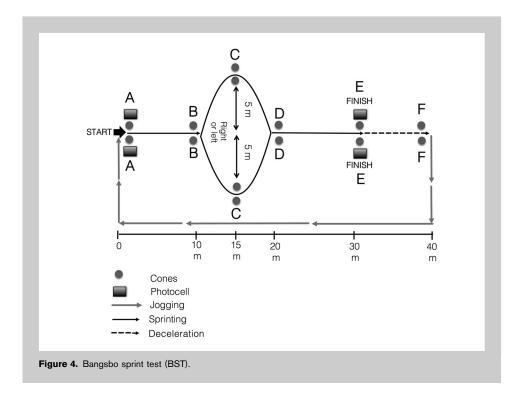
Figure 3. Sites of PBMT irradiation at posterior muscles of the lower limbs. PBMT = photobiomodulation therapy.

then 25 ul of blood was collected for biochemical analysis through electroenzymatic method, according to the instructions of the portable lactate analyzer manufacturer (Accutrend Lactate Plus Roche, Roche Diagnostics GmbH, Mannheim, Germany). The analyzer has a coefficient of variation between 1.8 and 3.3% (intraclass correlation [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

3332 Journal of Strength and Conditioning Research



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), and low-back and abdominal muscles. The stretching was followed by a warm-up comprised of low-intensity short running for 10 minutes. The stretching and warm-up lasted about 15 minutes, 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

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 7 maximum sprints 34.2 (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," to reach cone "C" (approximately 90 degrees), then runs 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 to athletes' preference in 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 a 25second 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 20–22 seconds. Verbal commands and cues were used to encourage the athletes and 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% confidence interval, 1.5–2.4).

Performance was evaluated by computing the average time of sprints performed during entire test (ST-mean) and for the fastest (best) time (ST-best) among the 7 sprints performed at each test. In addition, fatigue index was calculated using the following equation: FI (%) = (STmean/Stbest \times 100) –

100 to measure the percentage of decrease in performance between all sprints (6,26). This index is important because it shows the percentage of decrease in performance of athletes over the repeated sprints.

Questionnaire of Fatigue

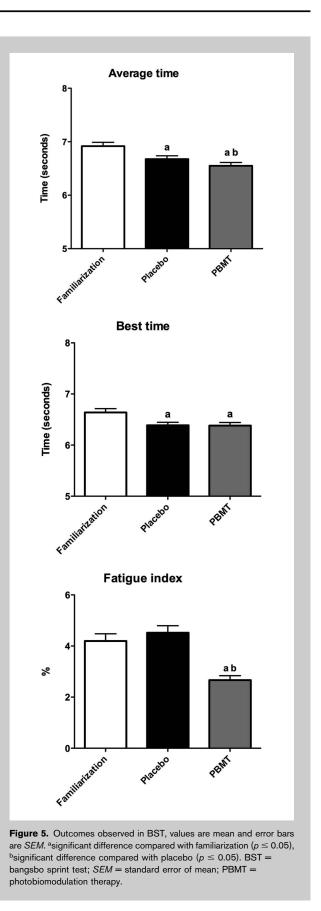
A quick perception of fatigue survey was administered 5 minutes after each exercise test to evaluate athletes' perceived fatigue for each experimental condition tested. The questionnaire consisted of 8 questions pertaining to perception of training, sleep, leg pain, concentration, effectiveness, anxiety, 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 that this questionnaire is a sensitive tool for monitoring fatigue.

Intervention

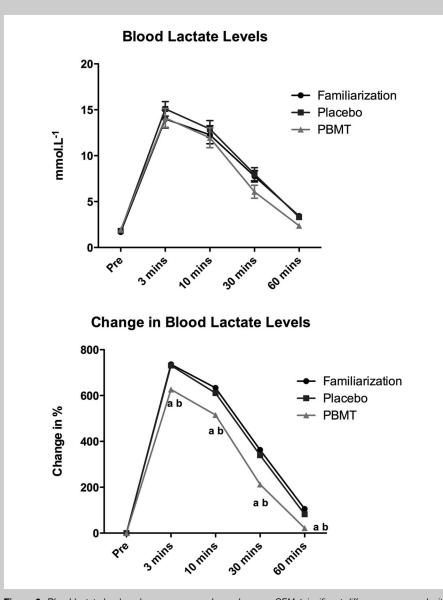
Photobiomodulation Therapy. Photobiomodulation therapy was applied employing MR4 Laser Therapy Systems outfitted with LaserShower 50 4D emitters (both manufactured by Multi Radiance Medical, Solon, OH, USA). The cluster style emitter contains 12 diodes comprising 4 super-pulsed laser diodes (905 nm, 0.3125 mW average power, and 12.5 W

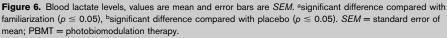
Number of lasers	4 Super-pulsed infrared
Wavelength (nm)	905 (±1)
Frequency (Hz)	250
Peak power (W)-each	12.5
Average mean optical	0.3125
output (mW)-each	0.71
Power density (mW/cm ²)-each	0.71
	0.162
Energy density (J/cm ²)-each	0.162
Dose (J)-each	0.07125
Spot size of laser	0.44
(cm ²)-each	0:44
Number of red LEDs	4 red
Wavelength of red	640 (±10)
LEDs (nm)	040 (=10)
Frequency (Hz)	2
Average optical output	15
(mW)-each	
Power density	16.66
(mW/cm ²)-each	
Energy density	3.8
(J/cm ²)-each	
Dose (J)-each	3.42
Spot size of red LED	0.9
(cm ²)–each	
Number of infrared LEDs	4 infrared
Wavelength of	875 (±10)
infrared LEDs (nm)	
Frequency (Hz)	16
Average optical output	17.5
(mW)-each	10.11
Power density	19.44
(mW/cm ²)-each	4.40
Energy density (J/cm ²)-each	4.43
Dose (J)-each	3.99
Spot size of LED	0.9
(cm ²)-each	0.9
Magnetic Field (mT)	35
Irradiation time per	228
site (s)	220
Total dose per site (J)	30
Total dose applied per	510
lower limb (J)	
Aperture of device (cm ²)	20
Application mode	Cluster probe held
	stationary in skin
	contact with a 90°
	angle and slight
	pressure

peak power for each diode), 4 red LED (640 nm, 15 mW average power for each diode), and 4 infrared LEDs (875 nm, 17.5 mW average power for each diode).



3334 Journal of Strength and Conditioning Research





The cluster probe was selected because of 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, and 2 sites on the calf (Figure 3) of both lower limbs (22). To ensure blinding, the device emitted the same sounds, regardless of the programmed mode (active or placebo). Furthermore, because the device produces a nonsignificant 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 irradi-

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 ($p \le 0.05$) between familiarization (6.91 \pm 0.24 seconds) and placebo (6.67 \pm 0.21 seconds), and familiarization and PBMT (6.55 \pm 0.21 seconds). Significant difference ($p \le 0.05$) was also demonstrated between PBMT and placebo groups.

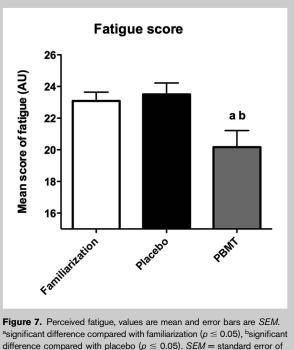
however, there were no dropouts in this study. The ICCs

ated 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 4

Photobiomodulation therapy 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.

Statistical Analyses

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 SD. The one-way analysis of variance test followed by the Bonferroni post hoc test was performed to verify statistical significance. The level of statistical significance was set at $p \leq 0.05$. In graphs, data are presented as mean and standard error of the mean. The intention-to-treat analysis would be followed a priori,



mean; PBMT = photobiomodulation therapy; AU = Arbitrary Unit.

For the best time among all 7 sprints (ST-best), there was significant difference ($p \le 0.05$) between familiarization (6.63 \pm 0.25 seconds) and placebo (6.38 \pm 0.20 seconds), and between familiarization and PBMT (6.38 \pm 0.21 seconds, $p \le 0.05$). However, no observed difference between PBMT and placebo (p > 0.05) was seen.

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

Although 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 ($p \le 0.05$) in favor of PBMT, when comparing both with familiarization and placebo at all postexercise time points tested (Figure 6).

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

DISCUSSION

This study details an important first 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 7 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.

Although no change was found between the active and placebo PBMT for ST-best, significant improvement was 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). Photobiomodulation therapy 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 byproducts 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 with the familiarization test. Higher levels of this biochemical marker should be and were expected in the active PBMT groups, because the athlete's performance was significantly better.

Therefore, it is reasonable to state that active PBMT prevented the expected increase of blood lactate levels, reduced muscular fatigue, and promoted faster recovery after 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 (17–19). There was a significant decrease in favor of the active PBMT compared with other 2 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 3–10 minutes after exercise test observed in this study was 15.10–12.91 mmol·L⁻¹ for placebo, 14.00–12.28 mmol·L⁻¹ for familiarization, and 14.11–11.95 mmol·L⁻¹ for PBMT condition, respectively. Our observations demonstrated that the experimental condition with the lowest lactate levels also correlated with lower fatigue index ratings, which suggests that improved performance can be accomplished by decreasing muscle acidosis in high-intense sports activities by applying PBMT before activity. However, additional research in this novel area is needed to provide insights into other sport-specific activities, and about mechanisms through which PBMT acts.

Our study employed the same short questionnaire by Elloumi et al. (8) previously used with rugby players 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 2 previously published studies using the same device where positive effects were noted (3,22). Antonialli et al. (3) tested 3 different doses against a placebo-control dose of 0 J, and found that 30 J was the best dose tested (compared with 10 J, 50 J, and placebo), that significantly increased performance, decreased the delay of onset muscle soreness, and modulated Creatine Kinase 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 used 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).

Photobiomodulation therapy demonstrates a modulatory effect on cytochrome c oxidase activity, and can explain how PBMT improves performance while protecting skeletal muscle from exercise-induced muscle damage. This has been considered the key mechanism for light-tissue interaction, promoting increase in cellular metabolism through increased mitochondrial function (1). Furthermore, Albuquerque-Pontes et al. (1) have 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 markers of skeletal muscle performance and 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.

PRACTICAL APPLICATIONS

The same outcomes previously observed in controlledlaboratory environment were confirmed in this study. Preexercise 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. Photobiomodulation therapy seems to have the potential to keep athletes at higher performance level, and consequently help to avoid injuries because of impaired recovery.

ACKNOWLEDGMENTS

The authors thank all rugby athletes that voluntarily participated this study, and to São José Rugby.

REFERENCES

- Albuquerque-Pontes, GM, Vieira Rde, P, Tomazoni, SS, et al. Effect of pre-irradiation with different doses, wavelengths, and application intervals of low-level laser therapy on cytochrome c oxidase activity in intact skeletal muscle of rats. *Lasers Med Sci* 30: 59–66, 2015.
- Allen, DG, Lamb, GD, and Westerblad, H. Skeletal muscle fatigue: Cellular mechanisms. *Physiol Rev* 88: 287–332, 2008.
- Antonialli, FC, De Marchi, T, Tomazoni, SS, et al. Phototherapy in skeletal muscle performance and recovery after exercise: Effect of combination of super-pulsed laser and light-emitting diodes. *Lasers Med Sci* 29: 1967–1976, 2014.
- Argus, CK, Gill, N, Keogh, J, Hopkins, WG, and Beaven, CM. Effects of a short-term pre-season training programme on the body composition and anaerobic performance of professional rugby union players. J Sports Sci 28: 679–686, 2010.
- Baldari, C, Bonavolonta, V, Emerenziani, GP, Gallotta, MC, Silva, AJ, and Guidetti, L. Accuracy, reliability, linearity of Accutrend and Lactate Pro versus EBIO plus analyzer. *Eur J Appl Physiol* 107: 105–111, 2009.
- Bangsbo, J. Fitness training in football A scientific approach. HO Storm: Bagsvaerd, 1994.
- Chaabène, H, Hellara, I, Ben Ghali, F, et al. Physiological stress and performance analysis to karate combat. J Sports Med Phys Fitness, 2015. Epub ahead of print.
- Elloumi, M, Makni, E, Moalla, W, et al. Monitoring training load and fatigue in rugby sevens players. *Asian J Sports Med* 3: 175–184, 2012.
- Gabbett, TJ. Physiological characteristics of junior and senior rugby league players. Br J Sports Med 36: 334–339, 2002.
- Girard, O, Mendez-Villanueva, A, and Bishop, D. Repeated-sprint ability - Part I: Factors contributing to fatigue. *Sports Med* 41: 673– 694, 2011.
- Grandinétti, VD, Miranda, EF, Johnson, DS, et al. The thermal impact of phototherapy with concurrent super-pulsed laser and red and infrared LEDs on human skin. *Lasers Med Sci* 30: 1575–1581, 2015.
- Halson, SL. Monitoring training load to understand fatigue in athletes. Sports Med 44: 139–147, 2014.

VOLUME 30 | NUMBER 12 | DECEMBER 2016 | 3337

- Huang, Y-Y, Chen, ACH, Carroll, JD, and Hamblin, MR. Biphasic dose response in low level light therapy. *Dase Response* 7: 358–383, 2009.
- Jastrzebski, Z, Zychowska, M, Konieczna, A, Ratkowski, W, and Radzimiński, L. Changes in the acid-base balance and lactate concentration in the blood in amateur ultramarathon runners during a 100-km run. *Biol Sport* 32: 261–265, 2015.
- Johnston, RD, Gabbett, TJ, and Jenkins, DG. Applied sport science of rugby league. *Sports Med* 44: 1087–1100, 2014.
- Leal Junior, EC, Lopes-Martins, RA, Dalan, F, et al. Effect of 655nm low-level laser therapy on exercise-induced skeletal muscle fatigue in humans. *Photomed Laser Surg* 26: 419–424, 2008.
- Leal Junior, EC, Lopes-Martins, RA, Frigo, L, et al. Effects of low-level laser therapy (LLLT) in the development of exercise-induced skeletal muscle fatigue and changes in biochemical markers related to postexercise recovery. J Orthop Sports Phys Ther 40: 524–532, 2010.
- Leal Junior, EC, Lopes-Martins, RA, Rossi, RP, et al. Effect of cluster multi-diode light emitting diode therapy (LEDT) on exerciseinduced skeletal muscle fatigue and skeletal muscle recovery in humans. *Lasers Surg Med* 41: 572–577, 2009.
- Leal Junior, EC, Lopes-Martins, RA, Vanin, AA, et al. Effect of 830nm low-level laser therapy on exercise-induced skeletal muscle fatigue in humans. *Lasers Med Sci* 24: 425–431, 2009.
- Leal-Junior, EC, Vanin, AA, Miranda, EF, et al. Effect of phototherapy (low-level laser therapy and light-emitting diode

therapy) on exercise performance and markers of exercise recovery: A systematic review with meta-analysis. *Lasers Med Sci* 30: 925–939, 2015.

- Menzies, P, Menzies, C, McIntyre, L, Paterson, P, Wilson, J, and Kemi, OJ. Blood lactate clearance during active recovery after an intense running bout depends on the intensity of the active recovery. *J Sports Sci* 28: 975–982, 2010.
- 22. Miranda, EF, Vanin, AA, Tomazoni, SS, et al. Pre-exercise photobiomodulation therapy combining super-pulsed lasers and light-emitting diodes improves performance in progressive cardiopulmonary exercise test. *J Athl Train* 51: 129–135, 2016.
- Mohr, M, Krustrup, P, and Bangsbo, J. Fatigue in soccer: A brief review. J Sports Sci 23: 593–599, 2005.
- 24. Santos, LA, Marcos, RL, Tomazoni, SS, et al. Effects of preirradiation of low-level laser therapy with different doses and wavelengths in skeletal muscle performance, fatigue, and skeletal muscle damage induced by tetanic contractions in rats. *Lasers Med Sci* 29: 1617–1626, 2014.
- Varley, MC, Gabbett, T, and Aughey, RJ. Activity profiles of professional soccer, rugby league and Australian football match play. *J Sports Sci* 32: 1858–1866, 2014.
- Wragg, CB, Maxwell, NS, and Doust, JH. Evaluation of the reliability and validity of a soccer-specific field test of repeated sprint ability. *Eur J Appl Physiol* 83: 77–83, 2000.