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PHOTOBIOMODULATION ON HIGH-INTENSITY EXERCISE OF HEALTHY MEN: EFFECTS ON OXYGEN UPTAKE/HEART RATE KINETICS AND TIME TO EXHAUSTION

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ABSTRACT

Introduction and objective: Oxygen uptake (VO_2) kinetics is an important method for the evaluation of oxidative response during physical activity. Few studies have evaluated the acute effects of photobiomodulation therapy (PBMT) on oxygen uptake kinetics and exercise tolerance to fatigue (Tlim) of healthy men. As such, the present study aimed to evaluate the acute effects of PBMT on the metabolic and cardiorespiratory responses during high intensity physical exercise in health men. Materials and Methods: Thirteen participants (aged 26 ± 6 years) underwent two (PBMT x placebo-PBMT) constant power cardiopulmonary test (CPCT) using cycloergometer, using 75% of peak power reached in a primary maximal incremental power cardiopulmonary test. The PBMT (GaAlAs - 808 nm – 100mW – 250 J/cm2) was applied in eight points distributed in the anterior region of the femoral quadriceps of both limbs (7J of energy per point - 56J of energy on each limb), before the CPCT. The variables evaluated were VO_2 peak, VO2 kinetics (tau and a1), Tlim, recovery time (1st and 3rd minute) and the angulation coefficient of the slow component. Results: A significant increase in VO2peak (PBMT: 41.68 ± 8.8 x placebo-PBMT: 39.17 ± 9.25; p = 0.01) was found with no effects on Tlim. There was no effect on VO_2 or heart rate (HR) kinetics under exercise recovery variables. Conclusion: Despite the increase in VO_2 peak found, no effects on cardiorespiratory responses were observed. PBMT, as used in the present study, has no effect on the increase of aerobic responses to high intensity exercise.

Key words: Cardiopulmonary exercise test. Functional capacity. Aerobic capacity. Laser. Kinetics of oxygen consumption.

RESUMO

Fotobiomodulação no exercício de alta intensidade de homens saudáveis: efeitos na cinética da absorção de oxigênio/frequência cardíaca e no tempo de exaustão

Introdução e objetivo: A cinética do consumo de oxigênio (VO_2) é um método importante para avaliação da resposta oxidativa durante a atividade física. Poucos estudos avaliaram os efeitos agudos da terapia de fotobiomodulação (PBMT) na cinética de captação de oxigênio e na tolerância ao exercício à fadiga (Tlim) de homens saudáveis. Assim, o presente estudo teve como objetivo avaliar os efeitos agudos do PBMT nas respostas metabólicas e cardiorrespiratórias durante o exercício físico de alta intensidade em homens saudáveis. Materiais e Métodos: Treze participantes (idade 26 ± 6 anos) foram submetidos a dois testes cardiopulmonares de potência constante (TCCP) (TCPB x placebo-TCPB) utilizando cicloergômetro, utilizando 75% da potência máxima atingida em um teste cardiopulmonar de potência incremental máxima primária. O PBMT (GaAlAs - 808 nm – 100mW – 250 J/cm2) foi aplicado em oito pontos distribuídos na região anterior do quadríceps femoral de ambos os membros (7J de energia por ponto - 56J de energia em cada membro), antes da TCPC . As variáveis avaliadas foram VO_2 pico, cinética do VO_2 (tau e a1), Tlim, tempo de recuperação (1º e 3º minuto) e coeficiente de angulação do componente lento. Resultados: Um aumento significativo no VO_2 pico (PBMT: 41,68 ± 8,8 x placebo-PBMT: 39,17 ± 9,25; p = 0,01) foi encontrado sem efeitos no Tlim. Não houve efeito na cinética do VO2 ou da frequência cardíaca (FC) sob as variáveis de recuperação do exercício. Conclusão: Apesar do aumento do VO_2 pico encontrado, não foram observados efeitos nas respostas cardiorrespiratórias. O PBMT, conforme utilizado no presente estudo, não tem efeito no aumento das respostas aeróbicas ao exercício de alta intensidade.

Palavras-chave: Teste de esforço cardiopulmonar. Capacidade funcional. Capacidade aeróbica. Laser. Cinética do consumo de oxigênio.

INTRODUCTION

Muscle fatigue is a common feature in sports and clinical settings. In humans, muscle fatigue can be defined as the exercise-induced decrease in the ability to produce force and power in response to contractile activity (Wan et al., 2017).

Fatigue can have two origins, central and peripheral (Gandevia et al., 2001).

Peripheral fatigue is related to modifications that occur distally to the neuromuscular junction and the central fatigue originates at the central nervous system (CNS), decreasing the neural drive to the muscle. As a result of fatigue, muscle performance decreases, which can result in losses for athletes during their sport practice or older people during their daily activity (Gandevia et al, 2001).

In this context, one of the most promising resources that may delay fatigue onset by stimulating the muscle is photobiomodulation therapy (PBMT) (Marchi et al., 2012; Baroni et al., 2010; Leal Junior et al., 2010).

Photobiomodulation is a non-ionizing electromagnetic radiation that promotes therapeutic effects due to the interaction of the light with the biological tissues, with a dose-dependent response (Basford et al., 1989; Enwemeka et al., 2004).

The rationale to use PBMT on skeletal muscle relies on its stimulatory effect on cellular respiratory chain, which intensifies the activity of the oxidative system, increasing ATP production and microcirculation, providing additional energetic support to muscle cells, and therefore possibly delaying fatigue onset (Lanferdini et al., 2018).

Recent studies in animal models demonstrated that PBM can improve aerobic response and muscle performance during physical exercise, especially due to its stimulatory effects on the antioxidant enzymes activity, such as superoxide dismutase, catalase and glutathione peroxidase (Guaraldo et al., 2016; Silva et al., 2015).

Also, PBMT applied before high-intensity exercise seems to increase blood removal lactate; both effects lead to a reduction of the oxidative stress (Guaraldo et al., 2016; Silva et al., 2015), with additional energetic support to muscle cells and a decreased recovery time between exercise sessions (Leal Junior et al., 2009).

These benefits are also observed with the chronic application of PBM associated with endurance training, revealing a greater reduction in fatigue levels and improvement in muscle performance (Leal Junior et al., 2009; Leal Junior et al., 2010; Brito Vieira et al., 2012; Xu et al., 1999; Lopes-Martins et al., 2006).

In a previous case report study, Ferraresi et al., (2015) evaluated the effect of PBMT on the response of VO_2 kinetics in athletes and found a faster adjustment of cardiorespiratory dynamics after PBMT application prior exercise.

Silva Alves et al., (2014) observed a significantly increased peak O_2 uptake (with higher values of VO_2) and cardiovascular efficiency in untrained healthy adults after PBM irradiation.

Both studies suggest that PBM is effective in improving responses to exercise, but they have used different PBM parameters; as such, the optimal PBM dosage to improve endurance performance is yet to be further investigated (Marchi et al., 2012; Ferraresi et al., 2015; Silva Alves et al., 2014). Although previous studies (Guaraldo et al., 2016; Silva et al., 2015; Leal Junior et al., 2009) reported an improvement of oxidative capacity from the application of PBMT, there is a lack of evidence showing that this response can improve exercise tolerance.

From a practical standpoint, the positive results of PBM on the fatigue level decrease could result in an improvement in the adjustment time of the aerobic processes measured by VO_2, which considered determinant for sports performance (Xu et al., 1999; Kemps et al., 2009).

In this context, it was hypothesized that PBM would promote a faster adjustment in the initial phase of exercise and better interaction of oxygen delivery and skeletal muscle consumption.

Based on it, the aim of the present study was to evaluate the acute effects of PBMT before high-intensity constant power output (CPCT) in cardiorespiratory adjustment and exercise tolerance in healthy subjects.

The hypothesis of the study was that PBMT would result in better cardiorespiratory adjustment and, as such, lower levels of perceived fatigue.

MATERIALS AND METHODS

Subjects

Thirteen healthy untrained male subjects participated of this study. Participants were considered untrained according to physical fitness classified by the American Heart Association (ACSM, 2017) between very weak to good. None of the volunteers showed respiratory dysfunction in the lung function test (FEV1/FVC > 70% of predicted) evidenced by pulmonary function test (FEV_1 / FVC < 70% of predicted) (Wasserman et al., 2004).

Smokers, alcoholics, and users of illicit drugs, or those with clinical diagnosis of cardiopulmonary, musculoskeletal, neurological, immune and/or metabolic disorders were excluded. The Research Ethics Committee of the Federal University of Rio de Janeiro approved the research (CAAE: CAAE 47813415.8.0000.5257). All participants signed the Informed Consent Term.

Experimental Approach to the problem

This prospective cross-sectional study was conducted at Laboratory of Research Group in Cardiorespiratory Evaluation and Rehabilitation. The reporting was guided by the STROBE statement. This sample size was justified by a priori power analysis in G*power using a target effect size (ES) of f = 0.25, alpha of 0.05 and power of 0.80, which determined that 13 subjects were required for participation; the additional recruitment accounted for the possibility of dropouts. This study used a within-subjects repeated-measures, in which all subjects completed both protocols (active and placebo PBMT) in a random order. The study was performed at the University Hospital. The assessments occurred on two separate days. During the first evaluation day, anthropometrics data were obtained and an incremental power cardiopulmonary test (IPCT) was performed to determine the power output (PO) for the experimental protocols. In the second session, two constant PO protocols were applied, in random order: one with the PBMT and the other with the placebo-PBMT. The order in which the protocols were performed was randomized.

Incremental Power Cardiopulmonary Test

Incremental power cardiopulmonary test was performed to determine peak power output (PPO). During test, the metabolic and ventilatory responses to exercise (VE, VO_2 and VCO_2) were captured by a gas analyzer (VO_{2000} - Portable Medical Graphics Corporation) and was performed in an electromagnetic braking cycle ergometer. Initially, a warm-up was performed, consisting of three minutes at minimum PO of the cycloergometer. Then, the incremental phase started, which PO was increased every minute (W/min) and the volunteer was asked to maintain a constant cadence of 60 rpm throughout the test.

The PO level of increment was estimated using the formula of Wasserman et al., (2004); and subjectively by the reported functional capacity of each volunteer. The test was discontinued if the volunteer presented signs of exercise intolerance, such as inability to maintain cadence and/or hemodynamic instability.

During test, the HR was monitored and collected through electrocardiogram (ECG - USB Wincardio) and arterial blood pressure by the auscultatory method.

The peripheral oxygen saturation (SpO_2) was monitored by pulse oximetry and the modified scale of Borg measured the subjective sensation of respiratory and peripheral muscular effort. After discontinuation, the active recovery phase was initiated, for three minutes with 25W of PO, followed by a further three minutes of passive recovery.

Constant power output protocol

After a resting period [at least 48 hours] of the IPCT, two constant power output (CPCT) protocols were performed using 75% of the PPO.

Both conditions (PBMT and placebo-PBMT) were performed in the same day, separated by 30 minutes, in a random order. First, a warm-up phase consisted of three minutes, with minimum PO of the cycle ergometer was performed. Then, the high-intensity phase (75% of PPO) was performed until exhaustion.

During CPCT, volunteers were oriented to maintain a constant cadence of 60 rpm throughout the entire protocol. The gas analyzer was set to generate the data sampling by the average of every ten seconds collected, for a better stability of the collected signal and better identification of the kinetics of the oxygen consumption later. During tests, gas exchange

was collected to analyze the metabolic and ventilatory data of the VO_2 kinetics. The total Tlim are presented in seconds.

Photobiomodulation therapy protocol

To evaluate the acute aerobic responses triggered by the laser in physical exercise, the PBMT protocol was performed immediately before each of the two CPCT performed at second evaluation session. A laser device (DMC PHOTON LASE III, São Carlos, SP, Brazil) was used. The choice of apparatus and dosimetry of laser irradiation was based on the method proposed by Toma et al., (2016). The PBMT used was GaAlAs - 808nm, with the following parameters: Diode area - 0.028mm; Power - 100mW; Energy density - 250J/cm^2; Irradiation points - 8 in each member; Energy per point - 7J; Total energy - 112J; Irradiation per point - 70 seconds; Mode - continuous. To standardize the PBMT application, the irradiation points were distributed to cover the quadriceps, considered the primary muscle group used during cycling. The points were placed at a distance equivalent to 25%, 35%, 50% and 75% of the total measure obtained between the anterior superior iliac spine and the superior border of the patella. Starting from these points, three centimeters laterally and bilaterally was considered, totaling eight irradiation points (Figure 1).

The application of the laser was performed with the volunteer in the supine position, in punctual mode, on direct contact with the skin (90 degrees). During the application, both the research and the volunteer used protection goggles.

The device used was properly calibrated prior to the start of the study. For the placebo-PBMT, the device was placed over the same places on the muscles, and the timer was turned on, with no light emitted to the muscle during the period.

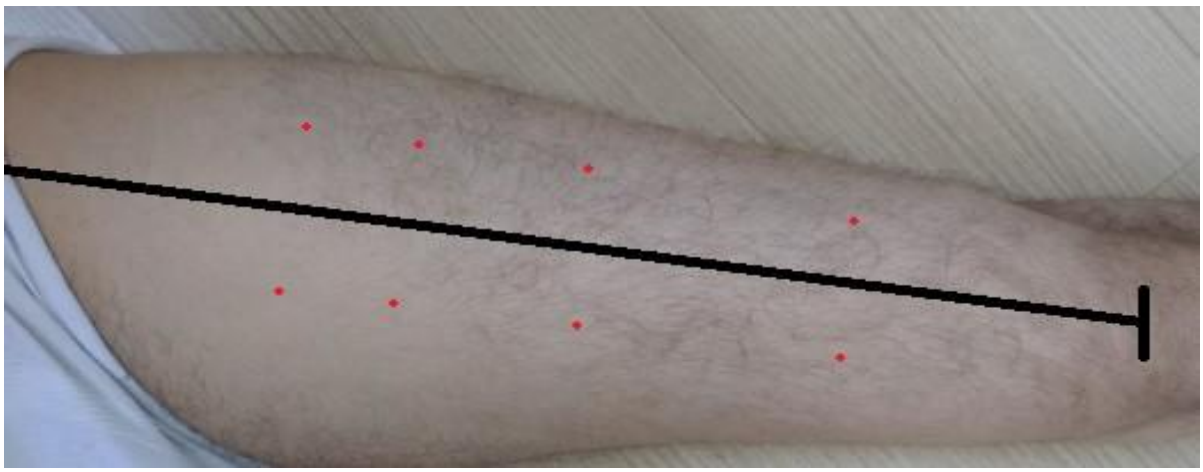

Figure 1 - Application points of the LLLT protocol. Source: author's own picture.

Analysis of the VO_2 and HR kinetics

A monoexponential analysis of the VO_2 and HR signals collected during the CPCT test was performed on the Sigmaplot 11.0 program, using the signal stretch corresponding to the rest-exercise transition ("on" period of the exercise). For this, the stretch was considered from the last thirty seconds from the warm-up phase (stable signal) until the peak of the exercise. In order to standardize the kinetic analysis, for each individual, the test with lower Tlim was verified, and in this way, the same amount of time was considered in the section to be analyzed in the test with the highest Tlim. Through the monoexponential analysis, two variables were obtained (Figure2A): tau (time equivalent to 63% of the metabolic response to the stable state) and a1 (amplitude of the response to the stable state). A reduction in these variables may demonstrate a faster cardiorespiratory adjustment (tau) and a lower energy expenditure (a1) against the metabolic demand imposed during physical exercise (Xu et al., 1999).

For the determination of the angulation coefficient (slow component), an identity line of the exercise stretch was drawn statistically starting from the value of tau until the peak of the exercise (Figure 2B).

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This analysis allows to evaluate the slow increase of the steady state in a qualitative way, so that the greater the angulation, the greater the slow component of increase of VO_2 and the HR.

Statistical Analysis

The data were tabulated in excel [office 2016 package] and analyzed on the software Sigmaplot 11.0. According to the nature of the distribution of the variables, a paired t test or Wilcoxon test was used to compare the variables between the conditions PBMT x placebo PBMT. The central tendency and dispersion measures used were mean and standard deviation. For the determination of recovery time, the median of the 1st and 3rd minutes of active recovery, immediately after the exercise peak, was considered.

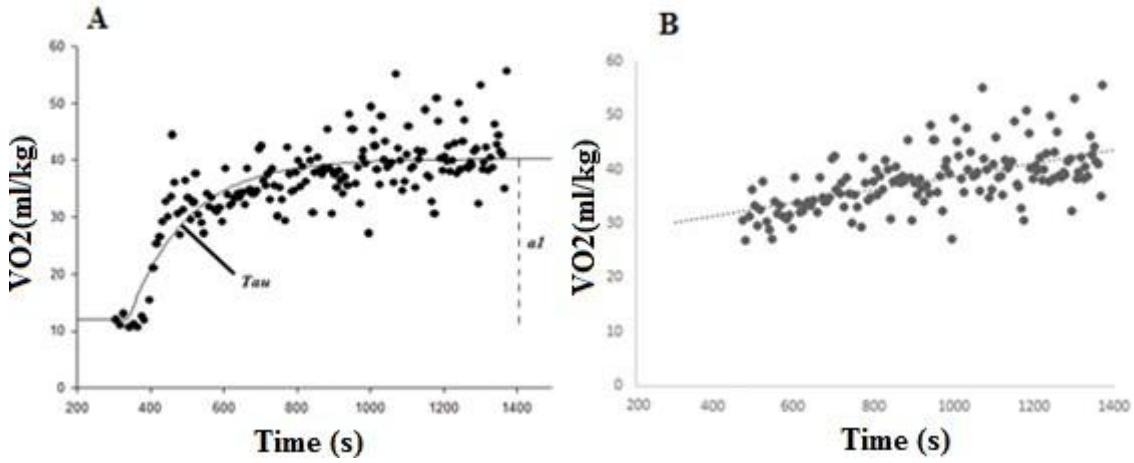

Figure 2 - (A) Analysis of the oxygen uptake kinetics (tau and a1). (B) Linear regression for qualitative analysis of the slow component of oxygen uptake. Both analyzes of the same volunteer.

RESULTS

Table 1 shows the main characteristics of the subjects and the IPCT results. All cardiopulmonary tests were interrupted by the inability of the volunteer to maintain the cadence due to muscle fatigue. PBMT significantly increased VO_{2peak} ($p = 0.010$).

Table 1 - Anthropometric characteristics, pulmonary function test and cardiometabolic variables of the Incremental Power Cardiopulmonary Test performed on the first assessment day. Data are presented in mean and standard deviation.

Variables	**Data**
Age (years)	26 ± 6
Weight (kg)	73.61 ± 10.87
Height (cm)	1.72 ± 0.07
BMI (kg/m^2)	24.74 ± 3.04
Spirometry	
FVC (L)	5.11 ± 0.83
SVC (L)	4.91 ± 0.85
FEV_1 (L)	4.35 ± 0.78
FEV_1/FVC (% predict)	99 ± 7.41
IPCT	
Resting VO_2 (L/min/kg)	4.3 ± 3.26
Resting HR (bpm)	76 ± 13
Resting SBP (mmHg)	118 ± 15
VO_2 peak (L/min/kg)	36.09 ± 10.02
Peak HR (bpm)	175 ± 9
Peak SBP (mmHg)	197 ± 31

VO_2 VT (L/min/kg)	25.49 ± 12.10
VCO_2 VT (L/min)	1.71 ± 0.84
VE/VO_2 VT	16.79 ± 1.42
VE/VCO_2 VT	19.16 ± 1.39
Respiratory Borg	8 ± 2
Muscular Borg	8 ± 2
Peak Power Output (W)	200 ± 36.46

BMI - body mass index; FVC - forced vital capacity; SVC - Slow Vital Capacity; FEV_1 - forced expiratory volume in the first second; IPCT – Incremental Power Cardiopulmonary Test; VO_2 - Oxygen consumption; HR - Heart Rate; SBP - Systolic Blood Pressure; VT -Ventilatory Threshold.

However, no significant effect was observed on the other cardiometabolic variables, such as HR and systolic blood pressure (Table 2).

There was no difference in the rating of perception exertion (RPE) reported by the volunteers (both respiratory and muscular) by Borg scale. There was no significant difference between protocols for Tlim (Figure 3).

Table 2 - Cardiometabolic responses and Borg scale of the constant power cardiopulmonary test (PBMT x placebo-PBMT).

CPCT	PBMT	placebo-PBMT	p-value
VO_2 at rest (L/min/kg)	4.94 ± 2.32	5.06 ± 2.68	0.85
HR ar rest (bpm)	73 ± 15	78 ± 10	0.20
SBP at rest (mmHg)	111 ± 17	114 ± 10	0.42
VO_2 peak (mL/min/kg)	41.68 ± 8.8	39.17 ± 9.25	0.01*
HR peak (bpm)	170 ± 10	171 ± 11	0.75
SBP peak (mmHg)	188 ± 25	187 ± 25	0.73
Respiratory Borg	7 ± 2	7 ± 2	0.72
Muscle Borg	8 ± 1	9 ± 1	0.10

PBMT – Photobiomodulation therapy; VO_2 peak - Oxygen Uptake peak; HR – Heart Rate; SBP - Systolic Blood Pressure; (*) statistically significant (paired t-test).

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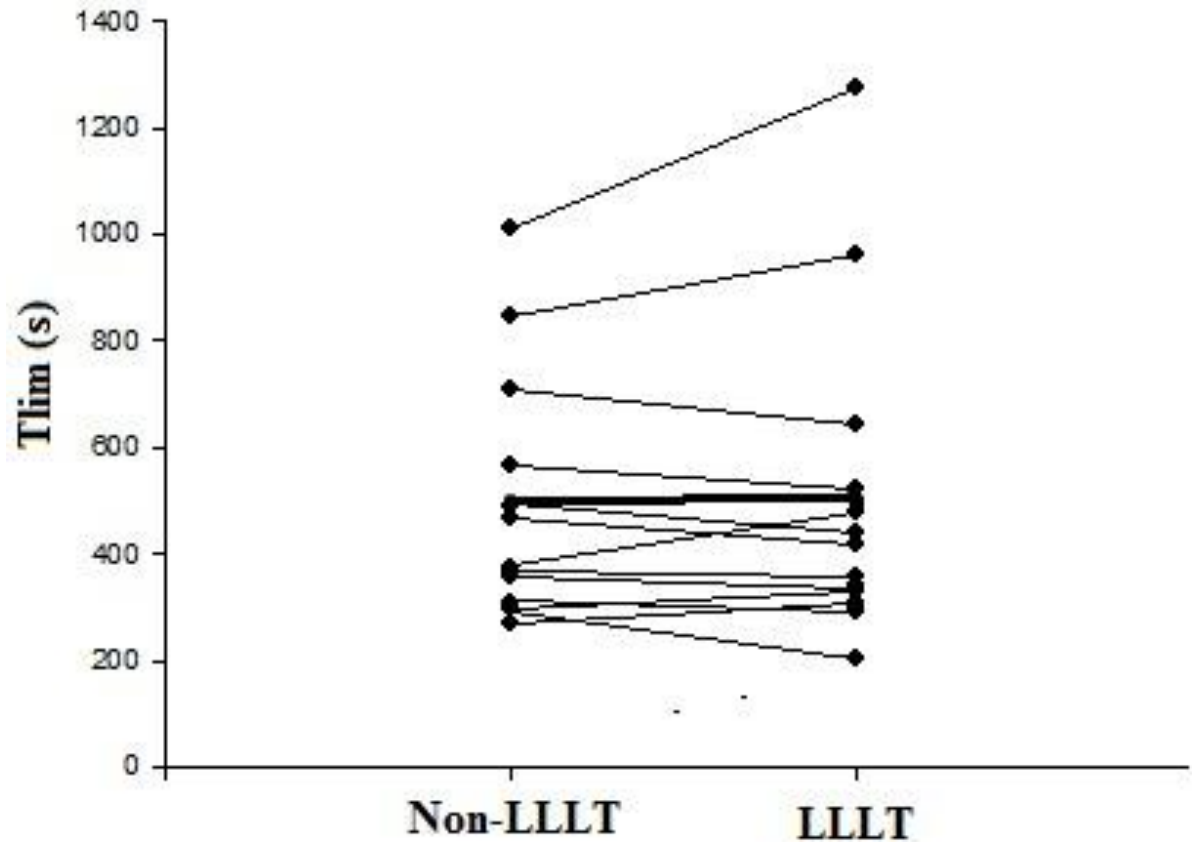

Figure 3 - Comparison of exercise tolerance time until fatigue between low level laser therapy (LLLT) and placebo-controlled (non-LLLT). Thicker line represents the average of individuals.

Oxygen uptake kinetics did not show statistical differences regarding the effect of the PBMT on tau and a1 variables in the rest to high-intensity exercise transition (table 3). Similarly, HR kinetics showed no significant difference between conditions. Slow component, measured by the coefficient of angulation of the VO_2 and HR curve, did not show significant differences as well as the analysis of the active recovery time (table 4).

Table 4 - Coefficient of angulation of the slow component and recovery time of oxygen uptake and heart rate during the Constant Power Cardiopulmonary Test.

	VO_2 (mL/kg/min)			HR (bpm)		
Recovery time	**PBMT**	**placebo-PBMT**	**p-value**	**PBMT**	**placebo-PBMT**	**p-value**
1° min	31.85 ± 8.45	32.07 ± 8.52	0.81	160 ± 11	163 ± 7	0.15
3° min	19.21 ± 5.06	18.83 ± 5.22	0.56	144 ± 11	143 ± 8	0.86
Coefficient of angulation (slow component)	0.02 ± 0.01	0.02 ± 0.01	0.30	0.09 ± 0.03	0.10 ± 0.02	0.70

VO_2 – oxygen uptake; HR – heart rate; CPCT – Constant Power Cardiopulmonary Test; LLLT – Low-Level Laser Therapy. Statistical test: Paired t-test.

DISCUSSION

PBMT was able to increase VO_{2peak} when applied before a high-intensity CPCT test, with no effects on Tlim, VO_2 and HR kinetics variables, as well as on the slow component and active recovery phase of exercise of young healthy men, partially rejecting the initial hypothesis.

Oxygen uptake during a PO protocol is considered an indirect measure of oxygen utilization by active muscles during exercise,

reflecting the oxygen delivery and use during energy process aerobic metabolism (Kemps et al., 2009). PBMT may increase the activity of the cytochrome C oxidase (COX) in the electron transport chain of mitochondrial cell respiration of skeletal muscle tissue, increasing aerobic ATP production by the increase of oxygen utilization during exercise (Leal Junior et al., 2010; Ferraresi et al., 2015; Capalonga et al., 2016; Farivar et al., 2014; Huang et al., 2009).

Our results corroborate with these findings, indicating that one application of PBMT immediately before exercise can increase VO_{2peak} values.

The same effect was found in studies with animal models (Perini et al., 2016) and humans (Marchi et al., 2012; Ferraresi et al., 2015; Silva Alves et al., 2014), with only one application prior to exercise.

A clinical study showed that the use of PBMT before progressive load exercise increases maximum oxygen uptake (VO_{2max}) without changing ventilatory thresholds in healthy individuals (Marchi et al., 2012).

Another study showed that PBMT when applied to the quadriceps and gastrocnemius before the cardiopulmonary exercise test increased VO_2 in untrained individuals, being this effect justified by the increase in O_2 extraction by the peripheral musculature (Silva Alves et al., 2014).

However, the results should be considered with caution, since an increase in VO_{2peak} is not the only parameter that reflects exercise performance (Perini et al., 2016).

In the present study, although a higher VO_{2peak} was obtained in CPCT test with PBMT, no significant differences were observed in Tlim.

Some studies describe the PBMT action due to the COX-bound nitric oxide (NO), which is produced by mitochondria on high oxidative stress situations, such as high intensity activities.

Presenting greater concentration in mitochondria, NO can inhibit cellular respiration when competing with oxygen for the binding sites. It has been described a photodissociation effect on COX-bound NO, reversing the inhibition of cellular respiration and postponing muscle fatigue due to physical exercise; however, it is not possible to ensure that this effect was triggered in our volunteers, since there was no increase in Tlim with PBMT (Farivar et al., 2014; Huang et al., 2014; Srinivasan et al., 2014).

To our knowledge, this was the first study to evaluate the effects of PBMT on VO_2 and HR kinetics in healthy subjects. The VO_2 kinetics reflects the behavior of VO_2 during all phases of the exercise (incremental phase, steady state and recovery), allowing an understanding of the aerobic muscle metabolism (Xu et al., 1999; Kemps et al., 2009).

Considering the effects of PBMT on aerobic metabolism, laser irradiation on active muscles is expected to faster adjust the aerobic transition from rest to exercise, reducing time of metabolic response (tau), and consequently with lower anaerobic demand (Xu et al., 1999; Kemps et al., 2009).

However, this effect was not observed in the present study, which can corroborate with the lack of improvement in exercise tolerance.

In addition, the present results showed the behavior of metabolic and cardiac autonomic variables during active recovery in the end of the exercise (1st minute and 3rd minute).

Considering the increase and acceleration of aerobic metabolism during the first stages of exercise, lower oxygen deficit can occur, accelerating the recovery of cardiorespiratory and metabolic variables at the end of the exercise (Armstrong et al., 2009).

However, this was not observed during active recovery, justified by the lack of effect on VO_2 and HR (tau and a1) kinetics during exercise transition.

Persisting the high-intensity exercise, the VO_2 slow component, considered the loss of steady state, associated with higher amounts of metabolites, is observed (Xu et al., 1999; Kemps et al., 2009).

No effect of PBMT was observed in the slow component, probably because there is little effect of the laser to dissipate fatigue.

Important considerations of PBMT dosimetry and equipment should be highlighted. Ferraresi et al., (2015) used a dosimetry of 450J total, distributed in different muscle groups involved in a running on treadmill.

This dosimetry is only logistically possible within a protocol using a device such a multi-diode laser, allowing greater energy radiation in a shorter time. In another study, Marchi et al., (2012) used a larger dosimetry [360J per member - 12 application areas], distributed in different muscle groups, resulting in an increased Tlim and VO_{2peak}. On the other hand, studies using a smaller dosimetry, with an

equipment similar of the present study, did not found a significant effect on the fatigue tolerance during exercise (Reis et al., 2014).

Reis et al., (2014) used a dosimetry of 50.4J distributed on the quadriceps bilaterally in 27 men [soccer players], before the knee extension exercise [75% of the maximum load], and no differences was founded in performance repetitions and duration of fatigue.

Demonstrated similar results with water polo athletes, applying PBMT before 200-m maximal swimming with 48J distributed in the long adductor and magnus adductor. No effect on performance or fatigue tolerance was found, and this result was attributed to the small muscle area irradiated in its protocol. These evidence suggest that there is a relation between dosimetry (total energy and muscles irradiated) and the effect on the aerobic metabolism of the active musculature during physical exercise.

The study presents some limitations. One of them may be related to the form of PBMT application, as the total energy delivered to the muscle tissue may not have been enough to lead to lesser muscle fatigue during the protocol.

Another point is that only one muscle group was irradiated, which may have impacted on the fatigue of other muscle groups which are also involved in the task performance.

CONCLUSION

Low level laser therapy failed to improve endurance performance in untrained subjects, despite the increase in VO_{2peak}. The absence of performance improvement indicated that the application of PBMT with equipment and dosimetry of this study are not recommended.

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