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## **La fotobiomodulación puede aumentar la tolerancia al ejercicio y la activación de los músculos del antebrazo en hombres sanos: un estudio cruzado, aleatorizado y controlado con placebo**

**Photobiomodulation may increase exercise tolerance and muscle activation of forearm in healthy men: A randomized, placebo-controlled, crossover study**

**A fotobiomodulação pode aumentar a tolerância ao exercício e a ativação muscular do antebraço em homens saudáveis: um estudo cruzado, randomizado e controlado por placebo**

*Zago, Julio<sup>1,2\*</sup>; Santos, Francisco Valdez<sup>3</sup>; Vieira, Paulo, J.C.<sup>4</sup>; Rondinel, Tatiana<sup>1</sup>; Diefenthäler, Fernando<sup>5</sup>; Machado, Sergio<sup>6,7</sup>; Aprigliano, Vicente<sup>8</sup>; Silva, Weder Alves<sup>9</sup>; Inacio, Pedro Augusto<sup>9</sup>; Sá Filho, Alberto<sup>9</sup>; Chiappa, Gaspar R.<sup>9,10</sup>*

<sup>1</sup>*University of Brasília, Brasília, DF, Brazil;* <sup>2</sup>*Physiotherapy Department of University Center, UNIEURO, Brasília, Brazil;* <sup>3</sup>*Cancer Institute of São Paulo, São Paulo, SP, Brazil;* <sup>4</sup>*Cristo Redentor Hospital, Intensive Care Unit, Porto Alegre, Brazil;* <sup>5</sup>*Biomechanics Laboratory, Federal University of Santa Catarina, Brazil;* <sup>6</sup>*Laboratory of Panic and Respiration, Institute of Psychiatry (IPUB), Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil;* <sup>7</sup>*Center of Neuroscience, Neurodiversity Institute, Queimados, Brazil;* <sup>8</sup>*Escuela de Ingeniería de Construcción y Transporte, Pontificia Universidad Católica de Valparaíso, Avda Brasil 2147, Valparaíso. Postal code: 2362804, Chile;* <sup>9</sup>*Evangelical University of Goiás, UNIEVANGELICA, Anápolis, Brazil.* <sup>10</sup>*Faculty of Health Sciences, Universidad Autónoma de Chile, Providencia, 7500912, Chile.*

### **Resumen**

A pesar de los efectos positivos de la fotobiomodulación (FBM) sobre el comportamiento muscular, sus efectos sobre la tolerancia al ejercicio y la hiperemia reactiva (HR) no se conocen bien. Pretendemos evaluar los efectos agudos de la FBM con longitudes de onda de 904 y 660 nm irradiadas sobre los músculos flexores del antebrazo sobre la tolerancia al ejercicio mediante el análisis de la HR y la activación muscular. Este estudio doble ciego y controlado se realizó con 11 participante. Aplicamos aleatoriamente FBM 904 nm, PBM 660 nm y placebo en seis

Correspondencia: **Gaspar R. Chiappa**, PT, PhD; Avenida Universitária S/N, Cidade Universitária, Anápolis, Goiás, Brazil; Evangelical University of Goiás; ZIP CODE 75083-515; Phone: +55 51 9 9117 7267; E-mail: [gaspar.chiappa@gmail.com](mailto:gaspar.chiappa@gmail.com)

puntos diferentes en el área del músculo flexor dominante. El grupo placebo recibió estimulación en los mismos puntos con un dispositivo apagado. La HR se evaluó midiendo el flujo sanguíneo del antebrazo, que se calculó utilizando el flujo sanguíneo máximo después de una oclusión de 5 minutos con la técnica de plethysmografía de oclusión venosa. La electromiografía se evaluó utilizando electrodos de superficie en tres músculos flexores del antebrazo. La raíz cuadrática media (RMS) y la frecuencia mediana (MDF) se trazaron al 25, 50, 75, 100% del límite de tolerancia (Tlim). La irradiación con PBM 606 aumentó la HR en comparación con PBM660 y placebo. Además, PBM 660 y 904 aumentaron el RMS y redujeron el MDF en comparación con el placebo. La irradiación con PBM 660 fue superior a 904 y placebo en el aumento de la HR, pero la aplicación de ambas irradiaciones fue similar en la activación de los músculos del antebrazo. Registro: [www.ensaiosclinicos.gov.br](http://www.ensaiosclinicos.gov.br) RBR-7yspdx.

**Palabras clave:** Fotobiomodulación; tolerancia al ejercicio; electromiografía; salud.

## **Abstract**

Despite the positive effects of photobiomodulation (PBM) on muscle behavior, its effects on exercise tolerance and reactive hyperemia (RH) are poorly understood. Objective: assess the acute effects of PBM with 904 and 660, nm wavelengths irradiated at the forearm flexor muscles on exercise tolerance through the analysis of RH and muscle activation. This preliminary, double-blind, placebo-controlled crossover trial was performed with 11 healthy participants. We randomly applied PBM 904nm, PBM 660nm, and placebo at six different points at the dominant flexor muscle area. The placebo group received the stimulation at the same points with a turned-off device. RH was assessed by measuring forearm blood flow which was calculated using the peak blood flow after the 5-min occlusion with the technique of venous occlusion plethysmography. Electromyography was assessed through surface electrodes on three flexor forearm muscles. The root mean square (RMS) and median frequency (MDF) were plotted at 25, 50, 75, and 100% of the limit of tolerance (Tlim). PBM 660 irradiation significantly increased RH when compared to PBM 904 and placebo. Furthermore, PBM 660 and 904 increased RMS and reduced MDF when compared to placebo. PBM 660 irradiation was superior to 904 and placebo in the increase of RH but the application of both irradiations was similar in the activation of forearm muscles in healthy men. Trial registration: [www.ensaiosclinicos.gov.br](http://www.ensaiosclinicos.gov.br) RBR-7yspdx.

**Keywords:** Photobiomodulation; exercise tolerance; electromyography; health.

## **Resumo**

Apesar dos efeitos positivos da fotobiomodulação (FBM) no comportamento muscular, seus efeitos na tolerância ao exercício e na hiperemia reativa (HR) são pouco compreendidos. Objetivo: avaliar os efeitos agudos da FBM com comprimentos de onda de 904 e 660 nm irradiados nos músculos flexores do antebraço na tolerância ao exercício por meio da análise da HR e ativação muscular. Este estudo preliminar, duplo-cego, cruzado e controlado por placebo foi realizado com 11 participantes saudáveis. Aplicamos aleatoriamente FBM 904 nm, PBM 660 nm e placebo em seis pontos diferentes na área do músculo flexor dominante. O grupo placebo recebeu a estimulação nos mesmos pontos com um dispositivo desligado. A HR foi avaliada medindo o fluxo sanguíneo do antebraço, que foi calculado usando o pico de fluxo sanguíneo após a oclusão de 5 minutos com a técnica de plethysmografia de oclusão venosa. A eletromiografia foi avaliada por meio de eletrodos de superfície em três músculos flexores do antebraço. A raiz quadrada média (RMS) e a frequência mediana (MDF) foram plotadas em 25, 50, 75 e 100% do limite de tolerância (Tlim). A irradiiação com PBM 606 aumentou significativamente a HR quando comparada com PBM660 e placebo. Além disso, PBM 660 e 904 aumentaram a RMS e reduziram a MDF quando comparadas com placebo. A irradiiação com PBM 660 foi superior a 904 e placebo no aumento da HR, mas a aplicação de ambas as

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irradiações foi semelhante na ativação dos músculos do antebraço em homens saudáveis. Registro: [www.ensaiosclinicos.gov.br](http://www.ensaiosclinicos.gov.br) RBR-7yspdx.

**Palavras-chave:** Fotobiomodulação; tolerância ao exercício; eletromiografia; saúde.

### INTRODUCTION

The Photobiomodulation (PBM) has been studied as an important technique to enhance muscle performance in animal (Luo et al., 2022) and human (de Oliveira et al., 2022). Overall, PBM might reduce skeletal muscle fatigue by producing photochemical and photophysical effects through stimulation of protein synthesis and increasing antioxidant process (Toma et al., 2018). PBM acts also on skeletal muscle, affecting energy metabolism at a mitochondrial level, which contributes to the modulation of the cell's oxi-reduction potential, increasing both electrons in the respiratory chain transport and adenosine triphosphate production (Hamblin, 2018). These responses could suggest a reduction in the production of reactive oxygen species, thereby providing additional energetic support to muscle cells, and possibly reducing skeletal muscle fatigue (Leal Junior et al., 2009). In addition, PBM can also act to increase RH. RH refers to the temporary increase in blood flow following a period of restricted circulation. PBM, stimulates vasodilation, enhancing tissue oxygenation and nutrient delivery. Studies show that PBM can accelerate recovery by improving blood circulation and reducing inflammation (Dompe et al., 2020; Hamblin, 2017, 2018).

Despite conflicted results in previous clinical studies, there is some evidence of improvement in exercise tolerance (Vassao et al., 2016) with PBM intervention by reduction of muscle fatigue (C Ferraresi et al., 2012; Leal-Junior et al., 2015). In a meta-analysis (Leal-Junior et al., 2015), the authors showed that PBM improves exercise performance, as evidenced by Borsa et al. (Borsa et al., 2013). This evidence reinforces the hypothesis that PBM delays skeletal muscle fatigue (C Ferraresi et al., 2011; Leal Junior et al., 2010; Lopes-Martins et al., 2006). However, Gorgey et al. (Gorgey et al., 2008) failed to demonstrate PBM fatigue reduction for the knee extensor group, as well as another study also did not find effects in older women (Vassao et al., 2016). Thus, there seem to be discrepancies between studies, mainly regarding the effects of PBM's different wavelengths on neuromuscular performance and fatigue. Healthy individuals show greater RH after leg cycling with incremental intensity, indicating that endothelium-dependent vasodilation also seems to be changed after acute exercise (Miranda et al., 2016; Nampo et al., 2016). Despite this, the literature is still unclear whether there is a difference in the application of 660 and 904 irradiation in the behavior of forearm muscles, especially regarding RH and muscle activation, and their repercussions on exercise tolerance. Some studies have shown effects on performance, however, without clarifying the physiological mechanisms involved such as RH or muscle activation. Our hypothesis is that PBM can increase muscle blood flow, which may lead to increased muscle activation. We aim to evaluate the acute behavior of RH and muscle activation after the application of PBM 660nm, 904nm and placebo in the forearm flexor muscles of healthy men.

### METHODS

#### *Trial Design*

This study is a preliminary randomized crossover trial, double-blinded, conducted in healthy men according to CONSORT guidelines in Hospital de Clínicas de Porto Alegre, Brazil. The Institutional Ethical Committee was approved under register number CAAE 90093218.6.0000.5056, and the preliminary clinical trial was registered at [www.ensaiosclinicos.gov.br](http://www.ensaiosclinicos.gov.br) under code RBR-7yspdx. This study was conducted in accordance with the Declaration of Helsinki ("World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects," 2004), and participants voluntarily agreed to participate in the study by

signing the informed consent form. Furthermore, this study was conducted in accordance with the Ethical Standards for Research in Sports and Exercise Sciences (Harriss et al., 2019) and in accordance with Organic Law 3/2018, of December 5, on the Protection of Personal Data and Guarantee of Digital Rights.

### Participants

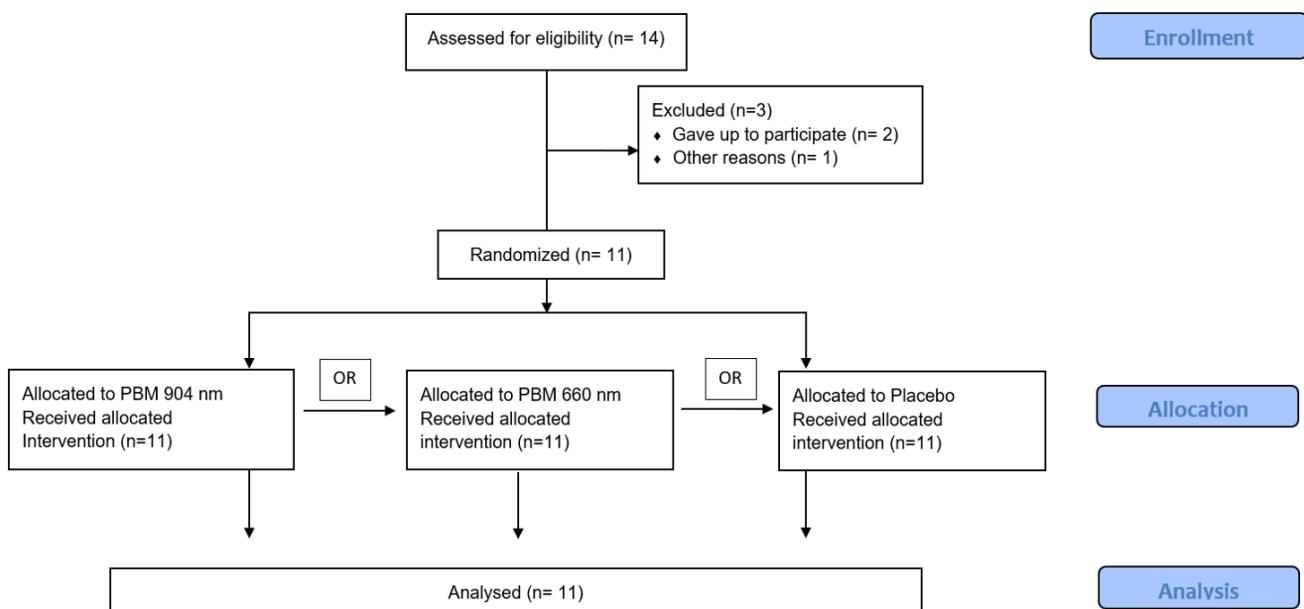
The study sample consisted of 11 healthy men, between 20–30-year-old, selected from an existing database in our extension program. All participants were physically active, not taking medications, and had no evidence of chronic diseases such as heart failure, chronic obstructive pulmonary disease. Exclusion criteria were a history of recent infection (in the past 3 months) and use of systemic corticosteroids. The CONSORT flowchart with the selection and allocation of participants is presented in the Flowchart.

### Randomization and allocation concealment

Consecutive participants were admitted after written informed consent. Computer-generated randomization lists were prepared using the website [www.random.org](http://www.random.org), which sequentially distributed the participants into the PBM 660nm and PBM 904 nm or placebo group in a 1:1:1. One researcher prepared an opaque, sealed envelope to conceal allocation, which was opened sequentially only after participant details are written. As each participant received the three interventions, randomization was performed to define the order in which each intervention was received.

### Flowchart 1

The CONSORT flowchart with the selection and allocation of participants.



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### Blinding

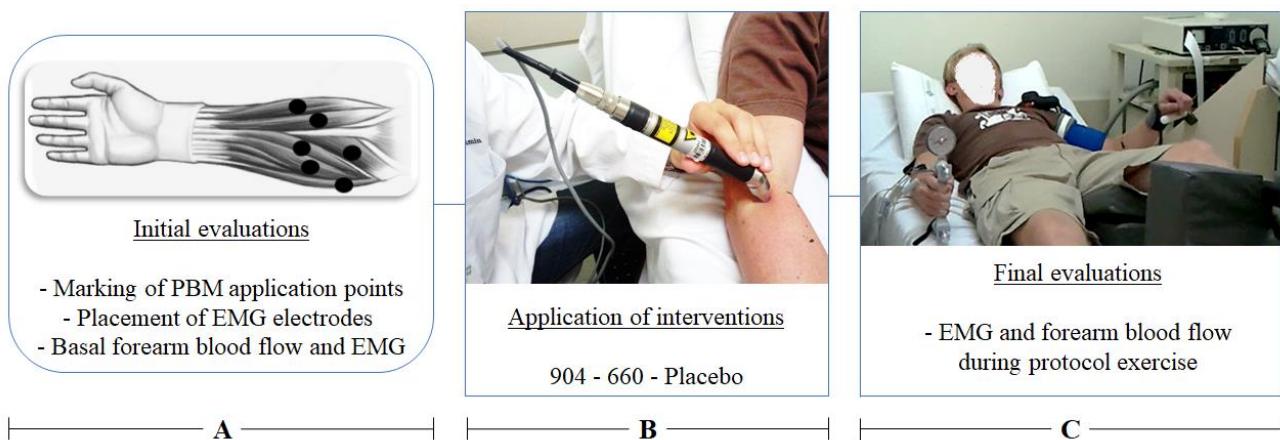
Two blinded researchers completed all assessments, and another blinded researcher performed all data processing and statistical assessments. The participant did not know which intervention they were receiving, as the laser equipment did not emit any visible light for the participant to see. Because the 660nm laser emits visible radiation, it was not possible to blind the researcher.

### Procedures

The procedures involved four visits to the laboratory. All procedures occurred in a quiet room, with the temperature between 20 and 21°C and each intervention lasted around 30 min. On visit 1, participants performed the initial evaluations, consisting of anamnesis, an anthropometric assessment, and the incremental handgrip exercise. On three subsequent visits, the participants received the interventions PBM with 660-nm, 904-nm, and placebo (PBM turned-off). The intervention was performed on different days according to the randomization. A 7-day washout was given between each intervention. Muscle activation and forearm blood flow were measured after PBM applications. Figure 1 shows the protocol of the study.

### Figure 1

#### Protocol of the study



### Interventions

The interventions were applied using a laser device (ENDOPHOTON®, KLD Biosystems, Amparo, Brazil), with an invisible pulsed pen, 100mW, 904nm, effective radiation area (ERA) of 0.01cm<sup>2</sup>, divergent, allowing modification of the dosimetry between energy density and Joule (J/cm<sup>2</sup>), using attenuation auto-correction according to the tissue depth. The 100mW, 660nm pen had an ERA of 0.035cm<sup>2</sup>, collimated, with dosimetry indication (J/cm<sup>2</sup>). The placebo condition was performed equally to PBM, but with the device turned off. Figure 2A illustrates the six application points, defined by the palpation of motor points on the muscle belly of the forearm flexors muscles: brachioradialis (BR), flexor carpi radialis (FR) and flexor carpi ulnaris (FU). In both groups, 5J was applied per point for 30 seconds. The complete description of the parameters is in Table 2.

**Table 2***Characteristics and application mode of photobiomodulation.*

Wavelength	904nm	660nm
Frequency	Continuous	Continuous
Power output	100mW	100mW
Spot size	12mm	12mm
Dose each point	5J	5J
Number of points	6	6
Total energy delivered	150J	150J
Application time (per point)	30s	30s

nm, nanometer; mW, milliwatt; mm, millimeter; J, joules; s, seconds.

*Incremental handgrip exercise*

The maximum voluntary contraction of the dominant forearm was measured with a handgrip dynamometer (Jamar®, Alimed, Boston, Mass). Handgrip strength was measured with participants sitting with their arms flexed at 90 degrees. Three attempts were performed, and the highest result was recorded. An incremental handgrip exercise was performed for a total of 6 minutes at 20%, 40%, and 60% of maximum voluntary contraction. The exercise in each percentage was performed in the same session.

*Electromyography*

A four-channel surface electromyography system (Miotool; Miotec Equipamentos Biomedicos Ltda, Porto Alegre, Brazil) was used to measure the muscle activity from the BR, FR and FU of the dominant forearm. Root mean square (RMS) and median frequency (MDF) values normalized by the maximal voluntary contraction were previously obtained and calculated using a mathematical with Matlab 7.1 software (Math Works Inc., Natick, MA).

*Reactive hyperemia*

Throughout the experimental sessions, brachial blood pressure was measured in the left arm using a calibrated oscillometric automatic device (Dinamap 1846 SX/P; Critikon, Florida, USA), and heart rate was monitored by the electrocardiogram. Baseline BP measurement was made in triplicate, and the first value was excluded. During the FBF measurements, BP was measured each minute. FBF was measured by venous occlusion plethysmography (D.E Hokanson, Washington, USA) in the nondominant forearm, as previously described (Mathiassen et al., 2006). In short, a rapid inflator cuff was used in the upper arm to occlude venous outflow (50–60mmHg), and three blood low recordings were made each minute for 3 min. Thereafter, RH was measured using an occlusion at 250mmHg for 5 min, which was released by 10s intervals for 2 min. The RH was calculated using the peak blood flow after the 5-minute occlusion. All flow recordings were manually traced by an operator who was blinded to the groups, conditions, and time. The reproducibility of FBF measurements was determined in a sample of 10 healthy young individuals with intraday and interday coefficients of variation of 6.9 and 9.2%, respectively.

*Sample size and Statistical analysis*

The sample size calculation was performed a priori using the software Gpower, 3.1.9.2 version (Universität Düsseldorf, Germany), 80% power and 20% alpha error were assigned, a total sample size of 11 participants was calculated to detect a 10% difference in RMS or MDF. Two-tailed unpaired *t*-tests were used to compare

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differences in baseline values between the groups. To compare responses between the PBM 904, PBM 660, and placebo interventions, generalized estimating equation (GEE) models for repeated measures were used. Data were analyzed with a statistical program (SPSS version 20.0) considering an alpha level of  $p < 0.05$ . All the analyses followed the principles of Intention-to-treat (i.e., patients were analyzed into the groups they were originally allocated to).

### RESULTS

#### *Participants characteristics*

Between June 2020 and September 2020 were recruited participants to study. Fourteen healthy participants were screened for the study. Three participants did not meet the inclusion or the exclusion criteria, and therefore, eleven participants were randomized totaling thirty-three samples collected (flowchart 1). There was no sample loss. Table 1 shows the main characteristics of the participants.

**Table 1**

*Characteristics of the participants.*

Variables	Values
Age, yr	$25.5 \pm 1.3$
Height, cm	$175 \pm 1.2$
Weight, kg	$74.4 \pm 1.9$
BMI, kg/m <sup>2</sup>	$24.3 \pm 0.5$
Forearm circumference, cm	$27.5 \pm 0.5$
Handgrip Force, N	$54.3 \pm 4.3$

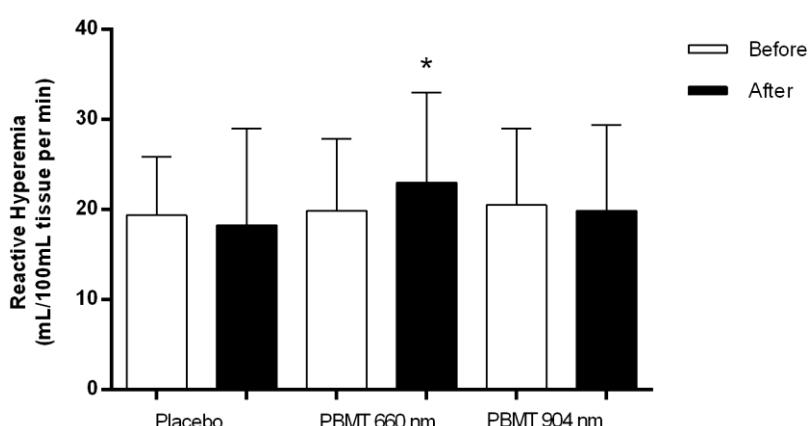
Data expressed as mean  $\pm$  SE. yr, years old; cm, centimeters; kg, kilogram; BMI, body mass index; kg/m<sup>2</sup>, kilogram per square meter; cm, centimeters; N, newtons.

#### *Reactive hyperemia*

Both the PBM 904 and PBM 660 induced a significant increase in endothelium-dependent vasodilation ( $p < 0.05$ ). The RH was significantly higher with PBM 660 compared to PBM 904 and placebo (+ 33% vs + 17% vs. + 1.2%,  $p < 0.05$ ) (figure 2).

**Figure 2**

*Reactive hyperemia results between interventions*

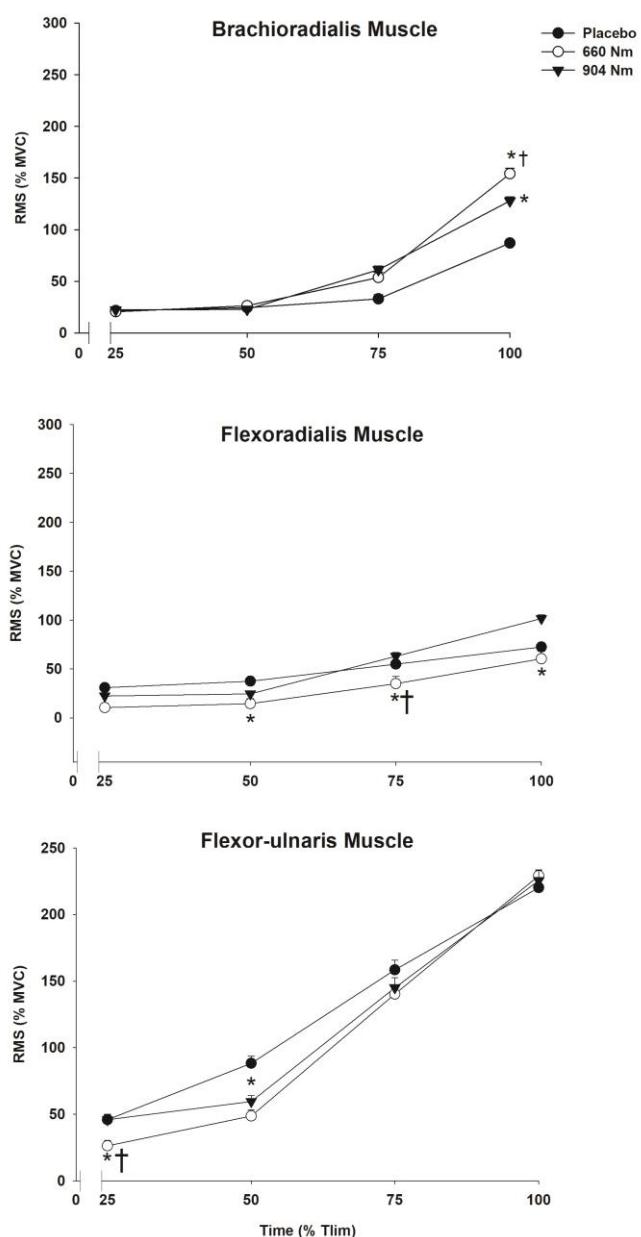


*Muscle activity*

The RMS during the incremental handgrip exercise was higher at 75% and 100%  $T_{lim}$  in the flexor radialis and brachioradialis muscles, respectively, and flexor-ulnaris at 100%  $T_{lim}$  following the PBM 660 ( $p<0.01$ ) (figure 3). Contrarily, the MDF significantly reduced following the PBM 904 ( $p<0.01$ ). The PBM 660 resulted in higher  $T_{lim}$  compared to PBM 904 and placebo ( $131 \pm 18$  vs.  $152 \pm 22$  vs.  $125 \pm 15$  s;  $p < 0.05$ ) (figure 4).

**Figure 3**

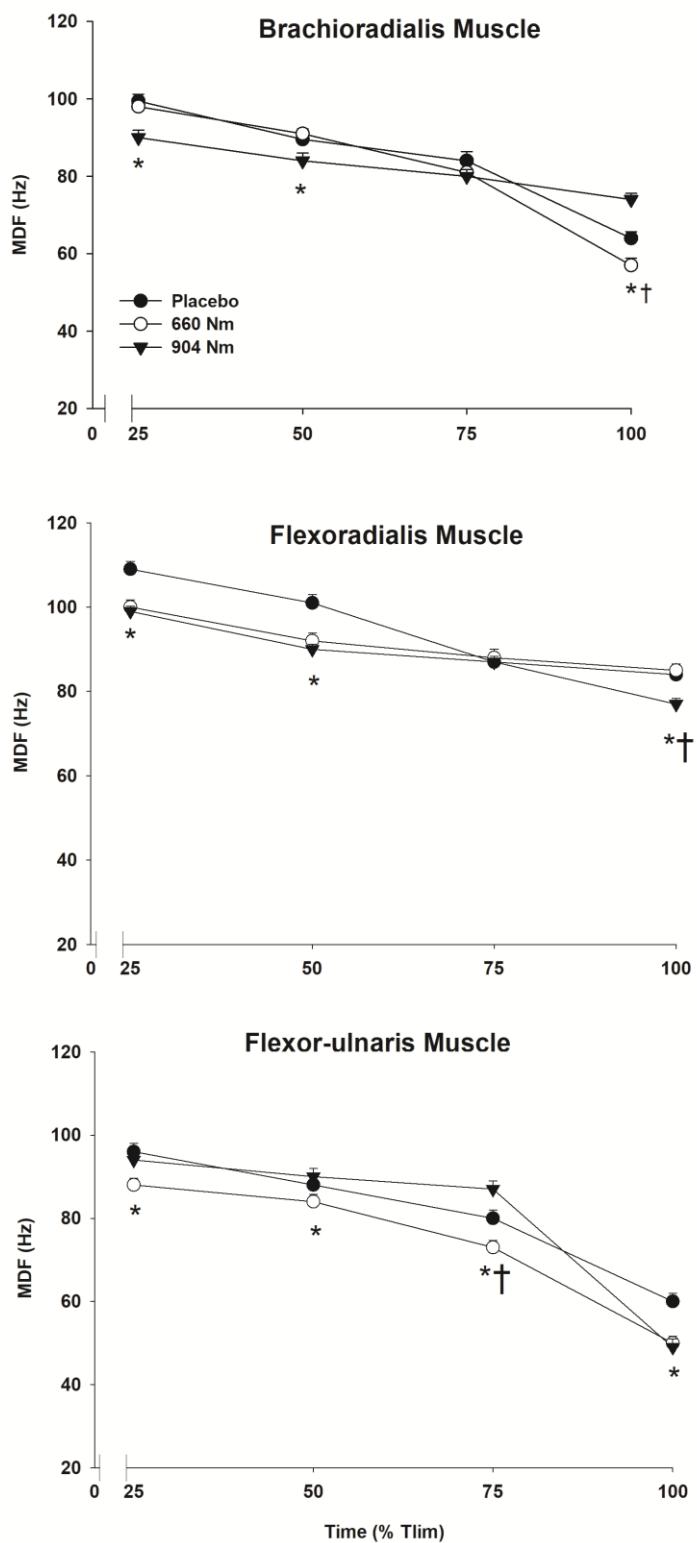
*The RMS results for  $T_{lim}$  between the flexor radialis and brachioradialis muscles, respectively and flexor-ulnaris.*



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**Figure 4**

The MDF results for Tlim between the flexor radialis and brachioradialis muscles, respectively, and flexor-ulnaris.



## DISCUSSION

In this study, we explored the acute effects of PBM on reactive hyperemia RH and muscle activation of the forearm flexor muscles in healthy men. As hypothesized, PBM at 660 nm proved superior to 904 nm and placebo in enhancing RH. This finding aligns with the literature, which associates PBM with vasodilation mediated by the release of nitric oxide (NO) (Cleber Ferraresi et al., 2012; Leal Junior et al., 2010). The increased local blood flow may improve the delivery of oxygen and nutrients to muscle tissues, enhancing metabolic efficiency and potentially increasing muscle performance.

The vasodilation observed after 660 nm PBM application can be attributed to mitochondrial stimulation, resulting in higher adenosine triphosphate (ATP) production and enhanced cellular metabolism (Hamblin, 2018), which can contribute to vascular relaxation. Additionally, the light absorption by chromophores like cytochrome c oxidase also triggers signaling that may activate signaling pathways associated with endothelial function, promoting the expression of vascular endothelial growth factor (VEGF), facilitating angiogenesis, and improving peripheral circulation (Dompe et al., 2020).

Furthermore, PBM-induced anti-inflammatory effects could reduce endothelial cell dysfunction, contributing to better post-exercise recovery (Hamblin, 2017, 2018; Leal Junior et al., 2010). Experimental studies with rats demonstrated alterations in cellular and protein expression cell biomarkers such as TNF- $\alpha$ , IL-1 $\beta$ , and IL-10. The phenomena of neuroendocrine response are not well established in the literature, but it is known that there is a systemic action after the application of a 660nm laser on superficial tissues (Aimbre et al., 2006; Marcolino et al., 2022). These combined effects may explain the superior performance of 660 nm PBM in inducing RH.

Regarding muscle activation, our results demonstrated that both 660 nm and 904 nm PBM significantly increased root mean square (RMS) values and decreased median frequency (MDF) compared to placebo. Our results were like studies that suggested that PBM increases exercise performance (Baroni et al., n.d.; da Silva Alves et al., 2014). Some mechanisms involved in the improvement in exercise capacity have been investigated, such as increased mitochondrial activity (Mantefel et al., 1997; Oron et al., 2007), oxidative activity enzymes, the concentration of ATP, and oxygen consumption (Lanferdini et al., 2018). Besides that, there is evidence that PBM may also induce increases in blood flow (Ihsan, 2005). These mechanisms, especially changes in the local blood flow response, may suggest important improvements in hemodynamic variables. These findings seem to contribute to understanding the higher muscle activation of motor units in the flexor muscle of the forearm through the RMS, with the use of PBM 904-nm. This increase is attributed to the higher recruitment and activation of type I motor units to compensate for the loss of strength that accompanies fatigue (Smith et al., 2007; Stout et al., 2007). Also, MDF response was reduced significantly in our study. Studies have demonstrated that MDF presents a direct relationship with muscle fiber conduction velocity (MFCV) (Masuda et al., 1999) . Thus, the decrease in MFCV is due to an accumulation of metabolic byproducts such as H $^+$ , which reduces intracellular pH and decreases the excitability of the muscle fiber membrane (Masuda et al., 1999). The reduction in MFCV and MDF has been reported by several studies (Arendt-Nielsen & Mills, 1988; Eberstein & Beattie, 1985). Our data demonstrated that the reduction in MDF was associated with higher RMS, which could suggest an increase in the recruitment of type I motor units and reduced recruitment of type II motor units. It is possible to speculate that the increase in blood flow was a determinant factor. Masuda et al. suggest (Masuda et al., 1999) that the intramuscular pressure during a static contraction prevents blood flow, so that metabolic byproducts, such as H $^+$ , accumulate in the muscle. However, it is believed that the blood flow response may affect MDF by changing the pH or concentration of ion K $^+$ , as described by Matsuda et al (Masuda et al., 1999).

Finally, although both wavelengths yielded positive effects, 660 nm PBM demonstrated superior efficacy over 904 nm in increasing RH. This can be attributed to the greater penetration of 660 nm radiation in superficial tissues, favoring the stimulation of vascular structures near the surface (Leal Junior et al., 2015). Conversely, 904 nm PBM appeared to be more effective in muscle recruitment, as indicated by the increased RMS. This distinction suggests that different wavelengths can be strategically applied based on therapeutic objectives.

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### LIMITATIONS

This study has some important limitations that may have influenced the results. Firstly, the small sample size may limit the generalizability of the findings. The level of physical activity of the participants was not measured, a fact that may influence the results of the EMG analysis at baseline. In the same way, the positioning of the laser on the muscles described in the study was topographic, performed by an experienced physical therapist in palpitory anatomy, without the assistance of any device, so there may not have been precision in the placement of the laser on these areas, due to anatomical variability of the participants. The trial design does not allow definitive conclusions about the effectiveness of interventions, should be interpreted with caution.

### CONCLUSION

This study concluded that PBM can increased RH in the flexor muscles of the forearm. PBM 660nm irradiation was superior to 904nm and placebo in the increase of RH, but the application of both irradiations was similar in the activation of forearm muscles in healthy men. The protocol was to be safe as no serious side effects were recorded in the study participants. We also concluded a larger clinical trial is needed to evaluate the effectiveness of interventions through PBM.

### PRACTICAL APPLICATIONS

The finding that 660nm and 904nm PBM increased muscle activation (measured by RMS) and decreased median frequency (MDF) in electromyography measurements indicates that these treatments may be useful for improving muscular endurance and the ability to sustain prolonged physical effort. Athletes may benefit from PBM to optimize their performance, allowing for higher training intensities or durations without the risk of excessive fatigue. PBM can be integrated into exercise programs to enhance physical performance and accelerate strength gains. The therapy can be applied before or after physical activity to improve muscle efficiency and recovery. For individuals undergoing functional rehabilitation programs, PBM can help restore muscle function more quickly, with synergistic effects combined with conventional physical therapy.

### ETHICAL STATEMENT

The protocol of the study was approved by the Research Ethics Committee of University (CAAE 90093218.6.0000.5056), before the enrolment of the participants. We obtained written consent from all individual participants included in the study who also received an information sheet detailing what the study involved.

### AUTHOR DISCLOSURE STATEMENT

The authors declare that they have no competing interests.

### AUTHORS' CONTRIBUTIONS RESEARCH CONCEPT AND STUDY DESIGN

JZ; PV; TR; FD; and GC Literature review: JZ; PAI; PV; TR; Data collection: JZ; PV; TR; PAI; FD; Data analysis and interpretation: JZ; PV; TR; FD; GC. Statistical analyses: WAS, VA, GC. Writing of the manuscript: JZ; PV; TR; FD; ASF; GC. Reviewing/editing a draft of the manuscript: JZ; PV; TR; FD; SM; GC. All authors read and approved the final manuscript.

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