UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
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KAREN ANDREA MOLINA BAQUERO

EFEITOS DA FOTOBIOMODULAÇÃO SOBRE A FADIGA E DANO MUSCULAR INDUZIDOS POR ESTIMULAÇÃO ELÉTRICA NEUROMUSCULAR EM INDIVÍDUOS SAUDÁVEIS

PHOTOBIOMODULATION EFFECTS ON FATIGUE AND MUSCLE DAMAGE INDUCED BY NEUROMUSCULAR ELECTRICAL STIMULATION IN HEALTHY INDIVIDUALS

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Dissertação apresentada ao Programa de Pós-Graduação em Ciências do Movimento Humano da Escola de Educação Física, Fisioterapia e Dança da Universidade Federal do Rio Grande do Sul como requisito para obtenção do título de Mestre em Ciências do Movimento Humano.

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EFEITOS DA FOTOBIOMODULAÇÃO SOBRE A FADIGA E DANO MUSCULAR INDUZIDOS POR ESTIMULAÇÃO ELÉTRICA NEUROMUSCULAR EM INDIVÍDUOS SAUDÁVEIS

RESUMO

A presente dissertação teve como objetivo avaliar os efeitos agudos da fotobiomodulação (FBM) e da FBM-sham em indivíduos saudáveis, aplicadas antes de um protocolo de fadiga evocada por estimulação elétrica neuromuscular (EENM). Esses efeitos agudos foram avaliados em parâmetros neuromusculares de fatigabilidade (torque isométrico máximo, torque do abalo supramáximo, atividade elétrica, trabalho total da integral da curva torque-tempo, redução do torque evocado), no dano muscular (ecointensidade, dor muscular de início tardio), no desconforto (escala visual analógica – EVA, algometria) e na funcionalidade (Single Hop Test - SHT, Teste de subir e descer escadas). A dissertação está dividida em três capítulos a saber: **Capítulo I:** Revisão da literatura referente à EENM e à FBM, tendo conceitos básicos, parâmetros de tratamentos, aplicação na prática clínica, e efeitos terapêuticos. Também abordamos tópicos como os tipos de fadiga existentes e as evidências encontradas na literatura dos estudos que utilizaram FBM para prevenir a fadiga e o dano muscular produzidos pela EENM. **Capítulo II:** Descrição dos Métodos: Os participantes visitaram o laboratório em nove dias, sendo que foram submetidos à familiarização com as técnicas e protocolos no primeiro dia, avaliação do primeiro membro nos quatro dias seguintes, e os quatro dias finais para a avaliação do segundo membro. A familiarização dos sujeitos foi efetuada com o ultrassom (US), com a contração voluntária máxima isométrica (CVMI), com os testes funcionais e com a EENM. No segundo dia foi realizado o protocolo de fatiga; porém, antes do protocolo de fadiga evocada pela EENM, se aplicou FBM ou FBM-sham (de forma randomizada) nos extensores do joelho de cada um dos membros. Um intervalo de sete dias foi observado entre os momentos de intervenção nos dois membros (período de "wash out"). Foram utilizados 8 pontos de aplicação da FBM e cada ponto foi tratado por 30s, com uma dose de 6J por diodo (sonda com 5 diodos), ou seja, 30J por local, totalizando 240J no membro inferior que recebeu FBM. A seguir, os sujeitos foram submetidos a um protocolo de fadiga de EENM (20 minutos de duração,

duração de pulso = 1 ms, tempos de contração-repouso de 5s:10s, e uma frequência de 100 Hz). Após o protocolo de fadiga evocada, foram avaliadas as variáveis de dor, desconforto e desempenho nos testes funcionais SHT e teste de subir e descer escadas. Para avaliar o dano muscular, foram coletadas nove imagens de ultrassom do músculo reto femoral (RF) e 3 do vasto lateral (VL). O nível de desconforto foi obtido por meio da EVA e do algômetro, o qual foi aplicado em todos os três extensores do joelho. Para os músculos RF e VL, o algômetro foi aplicado a 50% da linha entre a espinha ilíaca anterossuperior e a borda superior da patela, enquanto para o vasto medial (VM) o ponto de aplicação foi a 50% da linha entre a borda superior da patela e o ventre do músculo. A força voluntária máxima foi avaliada por meio de três CVMIs dos extensores do joelho pré protocolo de fadiga e uma CVMI pós protocolo de fadiga para avaliação do índice de fadiga. Além disso, os participantes executaram os testes funcionais pré e pós protocolo de fadiga a fim de avaliar os efeitos da fadiga e da FBM e FBM-sham na funcionalidade. Todas as variáveis mencionadas acima foram mensuradas novamente após o protocolo de fadiga evocada e pós 24h, 48h e 72h do protocolo de fadiga. Todas as mesmas etapas foram realizadas no outro membro. Na análise dos dados, inicialmente foi realizada estatística descritiva e avaliação da normalidade dos dados por meio do teste de Shapiro-Wilk. As comparações envolvendo os fatores membro (FBM e FBM-sham) e também os momentos (Pré, Pós-imediato, Pós-24h, Pós-48h e Pós-72h) foram realizadas utilizando o modelo de Equações de Estimação Generalizadas com um gama log link para resposta de escala. Quando foi identificada diferença estatisticamente significativa entre os momentos, foi utilizado o teste post hoc LSD (Least Significant Difference). Para comparações envolvendo as variáveis apenas com os momentos Pré e Pós-Imediato, foi utilizado o teste U de Mann-Whitney. Todas as informações quantitativas foram apresentadas como média e erro padrão, exceto para as variáveis de caracterização onde foram utilizados valores de média e desvio padrão. O nível de significância para todas as análises estatísticas foi fixado em p<0,05. **Capítulo III:** Resultados e Discussão. Participaram do estudo 12 jovens saudáveis (mulheres = 6 e homens = 6; idade = 28 ± 5,5 anos). Na avaliação do dano muscular no RF e VL, não foram observadas diferenças significativas entre os membros (FBM e FBM-sham) e nem entre os momentos (p>0,05). Em relação

ao desconforto, também não foram observadas diferenças significativas entre os membros (p>0,05), porém houve aumento do desconforto imediatamente após o protocolo de fadiga tanto para FBM quanto para FBM-Sham (p<0,05). O protocolo de fadiga gerou aumento do desconforto sentado e diminuição dos limiares dor dos músculos RF, VL e VM (p<0,05). Quanto às variáveis de fadiga durante o protocolo, não houveram diferenças significativas entre os membros, e a fadiga evocada pela EENM foi observada em ambos os membros. Em relação aos testes funcionais, também não houve diferença significativa entre os membros (p>0,05), porém sim entre os momentos, tendo uma queda do desempenho imediatamente após o protocolo de fadiga (p<0,05). No desconforto ao subir e descer escadas, foi observado aumento em ambos os membros (p<0,05) após a fadiga evocada. Porém, o desempenho do SHT no membro FBM não diminuiu após o protocolo de fadiga, enquanto que no membro FBM-sham isso aconteceu. **Conclusão:** Não foram observadas diferenças significativas entre os membros no desconforto, no dano e no desempenho dos testes funcionais. Esses achados sugerem que a FBM não tem um impacto significativo sobre os efeitos deletérios da EENM, o que reduz a sua utilização no aumento do desempenho e sua aplicabilidade clínica na redução da fadiga e do dano muscular.

Palavras-chaves: Fotobiomodulação; Estimulação Elétrica Neuromuscular; Fadiga Muscular; Dano Muscular; Desconforto.

PHOTOBIOMODULATION EFFECTS ON FATIGUE AND MUSCLE DAMAGE INDUCED BY NEUROMUSCULAR ELECTRICAL STIMULATION IN HEALTHY INDIVIDUALS

ABSTRACT

The present dissertation aimed to evaluate the effects of photobiomodulation (PBM) and PBM-sham in healthy individuals, applied before a fatigue protocol induced by neuromuscular electrical stimulation (NMES). These acute effects were assessed on neuromuscular parameters of fatigability (maximum isometric torque, supramaximal twitch torque, total work obtained by the torque-time integral, evoked torque reduction), muscle damage (echointensity, delayed onset muscle soreness), discomfort (visual analog scale - VAS, algometer), and functionality (Single Hop Test - SHT, stair ascend and descent Test). The dissertation is divided into three chapters as follows: **Chapter I:** Literature review on NMES and PBM, covering basic concepts, treatment parameters, clinical application, and therapeutic effects. We also addressed topics such as the types of fatigue and the evidence found in the literature regarding the use of PBM to prevent fatigue and muscle damage evoked by NMES. **Chapter II:** Methods: Participants came to the laboratory for 9 days, with the first day for familiarization, followed by the first leg evaluation in the next 4 days, and 4 days for the second leg. Familiarization was performed with ultrasound (US), maximal voluntary isometric contraction (MVIC), functional tests, and NMES. On the second day, the fatigue protocol was performed, but before the NMES fatigue protocol, either PBM or PBM-sham was applied to the knee extensors of each leg in a randomized fashion. A 7-day interval was observed between interventions to warrant a wash out period. Eight PBM application points were used, with each point treated for 30 seconds with a dose of 6 J per diode (probe with 5 diodes), totaling 30 J per location, and 240 J in the PBM limb. Subsequently, the subjects underwent a NMES fatigue protocol (20 minutes duration, pulse duration = 1 ms, contraction-rest times of 5 s:10 s, and a stimulation frequency of 100 Hz). To assess muscle damage, 9 US images of the rectus femoris (RF) muscle and 3 of the vastus lateralis (VL) muscle were collected. Discomfort level was measured using VAS and an algometer that was applied to all knee extensors. For the RF and VL muscles, the algometer was applied at 50% of a line between the anterior superior iliac spine and the upper edge of the patella, while for the vastus medialis

(VM) it was applied at 50% of the distance between the upper edge of the patella and the muscle belly. Participants performed 3 MVICs of the knee extensors pre-fatigue protocol and 1 MVIC post-fatigue protocol to assess the fatigue index. In addition, they also performed the functional tests pre- and post-fatigue protocol to evaluate the effects of fatigue and PBM (or PBM-sham) on functionality. The aforementioned variables were measured again after 24, 48, and 72 hours of the fatigue protocol. The same steps were carried out for the other leg. Regarding the data analysis, descriptive statistics were performed, as well as an assessment of data normality using the Shapiro-Wilk test. Comparisons involving the factors limb (PBM and PBM-sham) and moments (Pre, Immediately after, Post-24 hours, Post-48 hours, and Post-72 hours) were conducted using the Generalized Estimating Equations model with a gama log link for scale response. When a statistically significant difference was identified among the time moments, the LSD (Least Significant Difference) post hoc test was used. For comparisons involving the variables with only the pre and immediately after time-moments, the Mann-Whitney U test was used. All quantitative information is presented as mean and standard error, except for the characterization variables where mean and standard deviation values were used. The significance level for all statistical analysis was set at $p<0.05$. **Chapter III:** Results and Discussion: Twelve healthy young individuals participated in the study (women = 6 and men = 6; age = 28 ± 5.5 years). In the assessment of muscle damage in the RF and VL, no significant differences were observed between the limbs (PBM and PBM-sham) or between the moments ($p>0.05$). Regarding discomfort, no between-limbs differences were found ($p>0.05$), but there were differences between the moments ($p<0.05$). The fatigue protocol resulted in increased sitting discomfort and decreased pain thresholds in the RF, VL, and VM muscles ($p<0.05$). Regarding the fatigue variables during the protocol, there were no differences between the limbs, and fatigue effects were observed in both limbs. Regarding functional tests, no differences were observed between the limbs ($p>0.05$), but there were differences between the moments ($p<0.05$). Discomfort during stair climbing and descending showed an increase in both limbs after fatigue ($p>0.05$). However, the performance in the SHT did not decrease after the fatigue protocol in the PBM limb, while it did in the PBM-sham limb. **Conclusion:** No significant differences were observed among the limbs for discomfort, muscle damage, and functional

test performance. These findings suggest that PBM does not have a significant impact over the deleterious NMES effects, which reduces its use in increasing performance as well as its applicability in clinical practice to reduce fatigue and muscle damage.

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LIST OF ABBREVIATIONS AND ACRONYMS

Chapter I:

ATP - Adenosine triphosphate

BMI - Body Mass Index

Ca - Calcium

COVID 19 - Coronavirus

CCO - Cytochrome c oxidase

CFS - Chronic fatigue syndrome

CK - Creatine kinase

CNS - Central nervous system

DOMS - Delayed onset muscle soreness

ESEFID - Physical Education, Physiotherapy, and Dance School

HILT - High Intensity Laser Treatment

HFF - High-frequency fatigue

Ka+ - Potassium

LAPEX- Laboratório de Pesquisa do Exercício (Exercise Research Laboratory)

LASER - Light Amplification by Stimulated Emission of Radiation

LED - Light-Emitting Diode

LLLT - Low Level Laser Therapy

LFF - Low-frequency fatigue

MVC - Maximal Voluntary Contraction

MUs - Motor units

MTC - Muscle-tendon complex

Na - Sodium

NO - Nitric oxide

NMES - Neuromuscular Electrical Stimulation

PBM - Photobiomodulation

REC - Research Ethics Committee

ROM - Range of motion

ROS - Reactive oxygen species

UFRGS - Universidade Federal do Rio Grande do Sul (Federal University of Rio Grande do Sul)

US - ultrasound

VAS - visual analog scale

Chapter II:

BMI - Body Mass Index

ESEFID - Escola de Educação Física, Fisioterapia e Dança (Physical Education, Physiotherapy, and Dance School)

LAPEX - Laboratório de Pesquisa do Exercício (Exercise Research Laboratory)

MVC - Maximal Voluntary Contraction

NMES - Neuromuscular Electrical Stimulation

PBM - Photobiomodulation

UFRGS - Universidade Federal do Rio Grande do Sul (Federal University of Rio Grande do Sul)

VAS - visual analog scale

Summary

PRESENTATION

The present study aims to investigate whether photobiomodulation (PBM) applied prior to a neuromuscular electrical stimulation (NMES) fatigue protocol can reduce the fatigue effects and muscle damage. To achieve this goal, we developed the present research project at the Neuromuscular Plasticity Sector of the Exercise Research Laboratory (LAPEX) at the School of Physical Education (ESEFID) of the Federal University of Rio Grande do Sul (UFRGS).

PBM and NMES are two techniques used in physiotherapy and other healthcare fields to help in muscle recovery and strengthening, respectively. Both are based on the application of specific physical stimuli to the body to promote proper muscle and tissue functioning and adaptation.

PBM, also known as light therapy, involves the application of low-intensity light to specific areas of the body. The light is absorbed by the cells and helps promote tissue repair and reduce inflammation. Currently, PBM is used to treat a variety of conditions such as pain, inflammation, and muscle injuries.

NMES is a technique that involves the application of electric current to specific muscles, promoting muscle contraction. NMES can be used to treat muscle injuries, reduce muscle weakness, and is also used for analgesia to manage chronic pain and other conditions. The technique involves applying self-adhesive electrodes on the skin over the targeted muscle or muscle group and applying electric pulses to stimulate muscle contraction. It can also be used in clinical rehabilitation sessions to regain lost muscle strength in postoperative patients.

Therefore, both PBM and NMES are non-invasive techniques that can help in the treatment of injuries and other muscle conditions, as well as improve people's quality of life. On one hand, it is known that NMES induces fatigue and muscle damage very quickly, which becomes the main limitations of this technique. Since there is evidence that PBM can be used to reduce these NMES-induced deleterious effects, this project is aimed at investigating the potential effects of PBM on fatigue and muscle damage induced by NMES.

INTRODUCTION

Neuromuscular electrical stimulation (NMES) is widely used in clinical rehabilitation to prevent the physiological process of muscle loss or atrophy, the reduction in force production capacity and to improve muscle strength (MAFFIULETTI et al., 2013; DIRKS et al., 2015; HASHIDA et al., 2016). This tool has been used in different populations such as critically ill bedridden patients (DIRKS et al., 2015), postoperative anterior cruciate ligament reconstruction (KIM et al., 2010; LEPLEY et al., 2015), knee osteoarthritis rehabilitation (VAZ et al., 2013; MELO et al., 2015), older adults (LANGEARD et al., 2017), and athletes (GONDIN et al., 2011). NMES has shown positive results in rehabilitation protocols for strength gain (MAFFIULETTI et al., 2013; HERZIG et al, 2015; HONG et al., 2018), improving functionality (DOUCET et al., 2012), and for a faster return to activities of daily living (LEWEK et al., 2001; STEVENS-LAPSLEY et al., 2012; VAZ et al., 2013; PINFILDI et al., 2018) and sports participation (HAUGER et al., 2018).

Furthermore, it has been reported that NMES can be an appropriate treatment modality to be implemented in COVID-19 rehabilitation, as it can prevent muscle atrophy, improve muscle strength and function, maintain blood flow, reduce edema, and influence the weaning of patients from ventilators (BURGES et al., 2021). However, the NMES limited tolerance capacity (SCOTT et al., 2021) and the reduced exposure time to the electrical stimulus (BARSS et al., 2018; PAZ et al., 2021) have direct implications for clinical practice, as they may decrease this technique's efficacy and effectiveness. In this sense, in recent years, there has been an increased interest in the investigation of methods and resources that minimize NMES-induced neuromuscular fatigue, muscle damage and discomfort, thereby improving muscle performance (VAZ; FRASSON, 2018).

One of the strategies used to reduce fatigability is Photobiomodulation (PBM), a technique that utilizes light and has shown to be effective in treating a wide range of conditions, including chronic pain and muscle injuries. Moreover, PBM is considered safe and non-invasive, making it a good option for many patients. It has also demonstrated positive results for fatigue and muscle damage after exercise (BARONI et al., 2010).

Pre-clinical studies evaluated the PBM effects on discomfort and neuromuscular fatigue induced by NMES in an animal model, finding positive results in reducing fatigue (LOPES-MARTINS et al., 2006; SANTOS et al., 2014) and decreasing muscle damage in animals that received PBM compared to the PBM-placebo group (SANTOS et al., 2014).

However, in clinical studies involving humans, the results are conflicting with those obtained in animal studies (CUNHA et al., 2020; CIESLINSKI et al., 2018; GORGEY et al., 2008). More specifically, results indicate similarities between the PBM and PBM-placebo groups for peak torque of the knee extensors during maximal voluntary contraction after NMES (CUNHA et al., 2020; JÓWKO et al., 2019; CIESLINSKI et al., 2018; GORGEY et al., 2008). Similar results are presented for discomfort and muscle pain, where existing studies (CIESLINSKI et al., 2018; JÓWKO et al., 2019) demonstrated no significant difference between PBM and PBM-placebo applied before a NMES protocol on the knee extensors.

Considering the discrepancies among studies that have evaluated the effects of PBM on fatigue, muscle damage, and discomfort induced by NMES in humans (i.e., clinical trials) compared to preclinical studies in animal models, further research should be developed in this direction. However, before we can assess the effects of these two therapeutic modalities in the rehabilitation of patients who have experienced muscle loss due to reduced use of the neuromuscular system, it is necessary to first clearly understand their acute effects on discomfort, neuromuscular fatigue, and muscle damage in healthy subjects. Therefore, the aim of this study is to evaluate the acute effects of PBM or PBM-sham applied before a NMES-induced fatigue protocol on neuromuscular parameters of fatigability, functionality, and muscle damage in healthy individuals.

CHAPTER I: LITERATURE REVIEW

1.1. NEUROMUSCULAR ELECTRICAL STIMULATION (NMES)

Neuromuscular electrical stimulation (NMES) involves the application of electrical stimuli to superficial skeletal muscles to generate visible muscle contractions by depolarizing the axons under the stimulating electrodes and providing pre-programmed trains of stimuli to nerves, muscles, or joints. The depolarization of motor axons produces contractions by signals that travel from the stimulation site to the muscle (via the periphery), without the involvement of the central nervous system (BERGQUIST et al., 2012; GOBBO et al., 2014; MAFFIULETTI et al., 2018).

According to Maffiuletti et al. (2018), depending on the electrode location and current characteristics, NMES can be differentiated into three modalities. Transcutaneous electrical nerve stimulation involves the application of continuous low-intensity electrical current to cutaneous nerve fibers without apparent involvement of muscle contraction, and this modality is mainly used for the treatment of acute and chronic pain. Functional electrical stimulation involves the application of cyclic electrical stimulation of moderate intensity to selected muscles, and it is mainly used to generate functional movements that mimic voluntary contractions and to restore lost functions. Finally, NMES involves the application of high-intensity intermittent electrical stimuli to generate relatively strong muscle contractions, most frequently in isometric tetanic conditions. Therefore, Maffiuletti et al. (2018) stated that the electrodes should be large and adapted to the size of the thigh to minimize current density and patient discomfort and maximize muscle recruitment.

Currently, NMES has been used in clinical settings to improve muscle strength, increase range of motion, reduce edema, decrease atrophy, heal tissues, and decrease pain (BERGQUIST et al., 2011; DOUCET et al., 2012; MAFFIULETTI et al., 2013). This tool has been used in different populations such as critically ill bedridden patients (DIRKS et al., 2015), postoperative anterior cruciate ligament reconstruction (KIM et al., 2010; LEPLEY et al., 2015), knee osteoarthritis rehabilitation patients (VAZ et al., 2013; MELO et al., 2015), elderly (LANGEARD et al., 2017), and athletes (GONDIN et al., 2011). NMES has shown

positive results in rehabilitation protocols for strength gain (MAFFIULETTI et al., 2013; HERZIG et al., 2015; HONG et al., 2018), improving functionality (DOUCET et al., 2012), faster return to daily activities (LEWEK et al., 2001; STEVENS-LAPSLEY et al., 2012; VAZ et al., 2013; PINFILDI et al., 2018), and return to sports (HAUGER et al., 2018). The use of NMES has demonstrated improvement in muscle activation (LANFERDINI et al., 2015), functional resistance (VELDMAN et al., 2016), and post-exercise athlete recovery (BABAULT et al., 2011). In addition, it has been reported that NMES can be an appropriate treatment modality to be implemented in COVID-19 rehabilitation, as it can prevent muscle atrophy, improve muscle strength and function, maintain blood flow, reduce edema, and influence patient weaning from ventilators (BURGES et al., 2021; MINETTO et al., 2021).

1.1.2. MECHANISM OF MUSCLE ACTIVATION AND THE EXCITATION CONTRACTION COUPLING

To understand how NMES activates skeletal muscle, it is first necessary to review some basic concepts about the process of voluntary activation of skeletal muscle and the process of muscle contraction to generate force.

The process of voluntary muscle contraction (also known as the excitation-contraction coupling) occurs in the following sequence (ENOKA, 2000; GUYTON, HALL, 2002; PHAM et al., 2020):

1) Planning of actions and regulation of voluntary movements occur in the cerebral cortex.

2) This information is transmitted via electrical potentials to the base of the telencephalon nuclei and then to the spinal cord.

3) These action potentials are directed to the motor neurons until they reach the motor endplate.

4) The action potential on the muscle cell membrane that surrounds the myofibrils travels to the T-tubules, which are responsible for transmitting the action potential from the surface to the interior of the muscle fiber.

5) The T-tubules contain dihydropyridine receptors located close to the sarcoplasmic reticulum of the muscle fiber. Upon activation, the dihydropyridine receptor opens the ryanodine receptors in the

sarcoplasmic reticulum, releasing calcium (Ca^{2+}) into the cytoplasm of the muscle fiber.

6) Ca^{2+} ions bind to troponin-C, thereby determining a conformational change in this protein that moves the tropomyosin protein from obliterating or blocking the actin-binding sites.

7) With the actin-binding sites free, the myosin head connects to the actin site.

8) In the presence of adenosine triphosphate (ATP) in the cytoplasm, binding occurs between this molecule and the ATPase protein found in the heads of the thick myosin filament of the sarcomere.

9) The ATP molecule is broken down by the ATPase, activating the myosin head or cross-bridge.

10) When myosin is activated, it connects to actin, and its head perform the powerful rotational angular movement.

11) The powerful movement of the cross-bridges displaces the actin filaments moving the thin filament towards the center of the sarcomere, causing this structure to shorten. All these myofibril protein actions constitute the contractile process and are responsible for the active force generation.

12) To cease muscle contraction, the central nervous system stops motor neuron activation, the Ca^{2+} ions are pumped back into the sarcoplasmic reticulum by a Ca^{2+} pump and the proteins return to their pre-activation condition.

When a motor neuron is activated, it transmits the action potential to all the muscle fibers that it innervates, and this union between the motor neuron and its innervated muscle fibers is called a motor unit. Motor units are activated according to the Size Principle (SENN et al., 1997), which means that smaller motor neurons (i.e., with smaller dendrites, cellular body, and smaller diameter axons) are activated first, as they have a smaller excitation threshold. A higher release of neurotransmitters at the spinal cord will activate higher threshold motor units (i.e., higher size), which innervate a higher number of muscle fibers. Therefore, whenever a new motor unit is recruited, additional force is produced due to the increased number of contractile units that are recruited.

Parallel to motor unit recruitment, there is also an increase in the firing rates of the firstly recruited (i.e., smaller) motor units. This increased activation frequency (i.e., increased number of action potentials per second) produces force summation from the contractile units until a plateau or a tetanic contraction is achieved. When a full tetanic contraction is produced, increased activation frequency will not produce increased force production, as the contractile system will have reached its maximal force summation process. This is also known as the plateau of the force-frequency relationship (SENN et al., 1997).

Another aspect that is important to mention is that the motor neuron dimensions will have a direct effect on the contractile apparatus. More specifically, larger motor neurons can transmit a larger number of action potentials compared to small-diameter motor neurons. Therefore, a higher stimulation frequency will be produced by large compared to small motor neurons. A larger number of action potentials delivered per second will activate the abovementioned contractile proteins more times per second, thereby requiring faster contractions. Faster contractions are related to a faster capacity of the contractile proteins to respond, and apparently, the reason for the myosin head to break the ATP molecules faster is to generate a larger number of power strokes by second. This seems to be the reason why there are different muscle fiber types, slow and fast contracting, which have been related to the myosin ATPase protein that is different between these two fiber types (SENN et al., 1997).

Therefore, when a voluntary movement is generated, small (slow contracting) motor units are recruited first at a small firing frequency. To increase force production or voluntary effort, the increased release of neurotransmitter would increase the firing rates of the small motor units, while new higher (fast contracting with higher excitation threshold) motor units will be recruited. This recruitment from small to large units is what is known as the size principle, first described by Elwood Henneman (HENNEMAN et al., 1965a, 1965b).

Understanding how the voluntary contraction process occurs helps us understanding how the neuromuscular system functions. However, when we artificially activate this system, as when we use artificial electrical stimulation, the contractions generated or evoked by NMES do not follow the same process

above described. It has been suggested that NMES leads to a reversal of the size principle, recruiting larger (faster) motor units before smaller ones. This theory is based on two generally accepted results: (1) larger motor unit axons have lower excitability thresholds and (2) data show increased fatigue with NMES versus voluntary activation. Although there is sufficient data to suggest that voluntary and NMES-induced contractions are physiologically distinct, we state that motor unit recruitment during NMES reflects a non-selective, spatially fixed, and temporally synchronized pattern rather than a reversal of the physical order of voluntary recruitment (GREGORY et al., 2007; BICKEL et al., 2011).

Independent of how a muscle is activated, voluntarily or artificially, the mechanisms of muscle contraction above described function in a similar way. Therefore, when a motor unit reaches the excitability threshold and all its muscle fibers are activated, excitation-contraction coupling occurs and the motor units generate force. However, repetitive activation leads to fatigue, which can occur in any of the biochemical and biomechanical steps. In addition, repetitive activation can also lead to damage of the muscle fibers. As NMES recruits motor units in a non-selective, spatially fixed, and temporally synchronized pattern, the mechanical overload to these motor units' muscle fibers during a fatigue protocol will most likely lead to muscle damage (ENOKA et al., 2008).

1.2. MUSCLE DAMAGE

Muscle damage is a physiological phenomenon that occurs when intense or unfamiliar physical activity leads to structural disruptions and functional impairments in muscle tissue. It involves the creation of small ruptures in muscle fibers, disruption of the contractile units (i.e., the sarcomeres), and activation of inflammatory responses. Multiple factors can trigger this type of damage, including eccentric exercise, substantial mechanical forces, and excessive strain placed on the muscle fibers (CLARKSON & SAYERS, 1999). These activities cause small lesions in the muscles, affecting the structure of the contractile units. The extensive disruption of muscle fibers also occurs after short-duration eccentric exercises, in which high mechanical forces are generated. The damage continues to develop during the post-exercise period, before the tissues are

repaired (EBBELING & CLARKSON, 1989; PROSKE & ALLEN, 2005; KANZAKI 2022).

Several physiological indicators are commonly used to identify muscle damage. A prolonged reduction in muscle strength, delayed onset muscle soreness (DOMS), decreased joint range of motion (ROM), muscle swelling, elevation of creatine kinase (CK) activity and/or myoglobin (Mb) concentration in the blood, are some of these indicators. These phenomena can persist for approximately one week, impairing physical performance and increasing vulnerability to injuries during this period (CLARKSON et al., 1992; CLARKSON & HUBAL 2002; CHEN et al., 2011; BRUSCO et al., 2018).

In healthy skeletal muscle, the Z-disc exhibits a highly organized and structured appearance, resembling a well-organized woven basket or square grid. However, after eccentric exercise, complete disruption of Z-discs is frequently observed. This indicates that the Z-disc might be the most vulnerable component in the chain of myofibrillar contractile elements. In addition to Z-disc alterations, structural changes include myofibrillar and sarcolemmal disruptions, (EBBELING & CLARKSON, 1989). Moreover, there is a notable presence of myogenic satellite cells (CRAMERI et al., 2007), along with elevated levels of collagen types I and III (FOURÉ et al., 2020), primarily observed in fast-twitch or type II muscle fibers (CRAMERI et al., 2007; FOURÉ et al., 2019).

Indirect methods such as ultrasound (US), measurement of muscle proteins in the blood (e.g., CK activity, myoglobin concentration), assessment of muscle weakness (during maximal voluntary contraction), evaluation of muscle pain (measured using the visual analog scale -VAS), detection of increased muscle stiffness, and observation of muscle swelling are utilized to evaluate the extent of muscle damage (NOSAKA et al., 2011).

NMES has also been reported to produce muscle damage (NOSAKA et al., 2011). The damage of motor units by an electrical stimulation fatigue protocol refers to the process in which repetitive application of high-intensity electrical stimuli occurs. During the fatigue protocol, electrical stimulation is continuously performed, resulting in a prolonged overload on the motor units and their associated muscle fibers (NOSAKA et al., 2011). This type of damage can be

characterized by a decrease in muscle force production capacity, muscle fatigue, alterations in motor neuron action potentials, and the intensity and duration of the fatigue protocol can influence the extent of motor unit damage (WAN, et al., 2017). Therefore, it is important to remember that the application of an electrical stimulation fatigue protocol should be carefully monitored and controlled.

1.3. MUSCLE FATIGUE

Muscular fatigue is characterized as an exercise-induced reduction in maximal voluntary muscle force. It can arise not only due to peripheral changes at the muscle level, but also because the central nervous system fails to adequately drive the motoneurons (GANDEVIA, 2001; ENOKA & DUCHATEAU, 2016). Consequently, fatigue can be defined as the reduction in a muscle's capacity to perform work (GANDEVIA, 2001; SHEI & MICKLEBOROUGH, 2013). It is a transient condition that can be reversed through rest (SHEI & MICKLEBOROUGH, 2013).

If not addressed, fatigue buildup can result in various conditions such as overwork, chronic fatigue syndrome (CFS), overtraining syndrome, and even endocrine disorders, immune dysfunction, and organic diseases, posing significant risks to human health (WAN et al., 2017). One of the commonly employed methods to study the behavior of fatigued muscles is the combined analysis of force and surface electromyographic (EMG) signals (CÈ et al., 2020).

1.3.1. CENTRAL FATIGUE

Central fatigue is characterized by the involvement of the central nervous system, which includes the brain and spinal cord (DUGAN & FRONTERA, 2000). The central fatigue model refers to the reduction in muscle tension or force production caused by a decrease in the impulse action of the MUs recruited at the beginning of muscle force generation. Structural alterations in the muscle induced by repeated contractions or stretching or changes in stiffness lead to a decrease in the firing rates of muscle spindles, contributing to increased presynaptic inhibition and resulting in a limitation of alpha motoneurons firing

(SHEI & MICKLEBOROUGH, 2013; BOYAS & GUÉVEL, 2011; WAN et al., 2017).

Central fatigue can also be related to sensory feedback that reduces/inhibits the firing rate of motoneurons regulated by peripheral reflexes from mechanoreceptors. These receptors include muscle spindles, Golgi tendon organs, and type III and IV nerve endings (in response to the accumulation of certain metabolites at the muscular level during exercise) (DAVIS & BAILEY, 1997).

Some neurotransmitters that can influence central fatigue include gamma-aminobutyric acid (GABA), serotonin, and adenosine. GABA is an inhibitory neurotransmitter that can reduce neuronal excitability, contributing to the sensation of fatigue. Serotonin also plays a significant role in fatigue regulation, with increased levels associated with feelings of tiredness and decreased performance. Adenosine, on the other hand, is a neuromodulator that accumulates during exercise and is associated with fatigue. These neurotransmitters can interact and influence the neural processes involved in central fatigue (MEEUSEN et al., 2006; ROELANDS et al., 2013).

1.3.2. PERIPHERAL FATIGUE

Peripheral fatigue occurs due to a decrease in the contractile strength of muscle fibers and to changes in the underlying mechanisms of muscle action potential transmission. Therefore, it occurs either at or distal to the neuromuscular junction (GANDEVIA, 2001; BOYAS e GUÉVEL, 2011; WAN et al., 2017), because of limitations in the muscular environment (caused by the accumulation of metabolites, depletion of phosphagens and substrates). This includes disruptions in neuromuscular transmission, sarcolemma excitability, and excitation-contraction coupling (alterations in the contractile machinery of muscles) (MEEUSEN et al., 2006 SHEI & MICKLEBOROUGH, 2013).

According to Cè Emiliano et al. (2020), the changes induced by fatigue can occur locally at the cellular level and within the muscle-tendon complex (MTC). For this reason, these authors divide peripheral fatigue into electrochemical

factors and mechanical factors. Among the electrochemical factors, the main mechanisms affected by the development of peripheral fatigue are changes in (1) synaptic transmission of action potentials, (2) propagation properties of sarcolemma action potentials and release of Ca^{2+} from the sarcoplasmic reticulum, and (3) excitation-contraction coupling, cross-bridge kinetics, and Ca^{2+}. Among the mechanical factors, peripheral fatigue seems to influence the force transmission from the muscle to the tendon insertion point, altering the mechanical properties of the muscle-tendon complex (MTC) and other tissues that surround a joint. These electrochemical and mechanical events work together to generate afferent feedback to the spinal cord, which then modulates motor drive and final force production (CÈ et al., 2020; WAN et al., 2017).

Peripheral fatigue has been divided into low-frequency fatigue (LFF) and high-frequency fatigue (HFF). LFF is characterized by a selective decrease in force, particularly when muscles are stimulated at low frequencies (10-30 Hz) (JONES, 1996). It is also referred to as long-duration fatigue due to slow recovery (JONES, 1996; BAPTISTA et al., 2009). Low-frequency NMES and electrical stimulation with long-duration contractions induce LFF (JONES, 1996; BAPTISTA et al., 2009). Its origin is attributed to a failure in the excitation-contraction coupling process (JONES, 1996; LIMA et al., 2018). The main characteristics of LFF are as follows: (1) forces at low stimulation frequencies are the most severely affected; (2) recovery is slow, taking hours or, in severe cases, days for complete recovery; (3) the effect persists in the absence of metabolic or electrical disturbances in the muscle (EDWARDS et al., 1977).

According to JONES (1996), HFF is characterized by a decrease in the force generated by high-frequency stimuli (50-100 Hz) and can be rapidly reversed when the stimulation frequency ceases. It is characterized by waveform deceleration and loss of action potential amplitude along the surface membrane. Since the muscular action potential is primarily driven by electrical events on the cell membrane's surface, when the extracellular concentration of K+ is sufficiently high to hinder conduction along the surface membrane, it is suggested that high-frequency fatigue occurs near the excitation-contraction coupling process. This is caused by the accumulation of K+ in the T-tubules, leading to a failure in stimulus propagation. The conduction along the T-tubules will be blocked

because the increase in the extracellular concentration of K+ will be greater in the lumen of the tubule due to its small volume, large surface area, and low diffusion. In the T-tubules, it is still possible to observe a higher ratio of surface area to volume, lower diffusion, and lower density of Na+ and K+ pumps, which inhibits the excitation-contraction coupling mechanism (JONES, 1996; LIMA et al., 2018).

Biomarker for the diagnosis of muscle fatigue

ATP metabolism biomarkers

When ATP supply fails to meet the ATP demand during exercise, fatigue occurs. In the case of inadequate oxygen supply, oxidative phosphorylation of ADP to generate ATP fails to meet the energy demand, and ATP production shifts from aerobic processes (glucose/glycogen metabolism, lipid metabolism, or amino acid metabolism) to anaerobic glycolysis or glycogenolysis, resulting in lactate accumulation (WAN et al., 2017).

Oxidative stress biomarkers

Reactive oxygen species (ROS) levels remain low in skeletal muscle at rest but increase in response to contractile activity. ROS products lead to the oxidation of proteins, lipids, or nucleic acids accompanied by a marked decrease in antioxidant capacity, ultimately inducing fatigue (WAN et al., 2017).

Inflammatory biomarkers

In addition to ATP depletion and ROS production, exercise and fatigue also induce a local or systemic inflammatory response. Leukocytes, IL-6, and TNF-α are considered promising biomarkers for assessing inflammation in muscle fatigue. After exercise, T-lymphocytes, particularly CD4+ and CD8+ lymphocytes, are transported from peripheral lymphoid compartments to the bloodstream. Furthermore, there is a notable rise in neutrophil count immediately following physical activity (WAN et al., 2017).

1.4.   PHOTOBIOMODULATION (PBM)

Photobiomodulation (PBM) is a therapeutic modality that employs non-ionizing light sources such as LASER (Light Amplification by Stimulated Emission of Radiation) and LED (Light-Emitting Diode) to treat tissues. It exerts its effects through a phenomenon known as the photobiomodulatory effect, which influences cellular activity by either stimulating or inhibiting chemical and physiological functions (KARU, 1999; LEAL-JUNIOR et al., 2013).

To reduce exercise-induced fatigue and muscle damage, PBM involves the application of light prior to physical exercise (BARONI et al., 2010; LEAL-JUNIOR et al., 2013; ROSSATO et al., 2017; LANFERDINI et al., 2018). By applying light to tissues, PBM can alleviate muscle fatigue by emitting photons that are absorbed through chromophores present in the mitochondria (which is the primary site of energy production in muscle cells), thereby converting light energy into chemical energy within the cytoplasm. Therefore, during cellular respiration, fatty acids and carbohydrates are converted into ATP, the molecule used as an energy source for muscle contraction. As the demand for ATP is higher during exercise, mitochondria's workload increases to provide the necessary energy to sustain muscle contractions (REDDY, 2004), and PBM has been used to reduce the effects of peripheral muscle fatigue and muscle damage (IBITOYE et al., 2016) by improving mitochondrial function.

Grotthus-Draper's first law of photobiology states that, for a clinical effect to occur, the tissue needs to absorb the light, while light that is reflected, transmitted, or scattered does not have any impact (CAVALCANTI et al., 2011). The amount of energy absorbed is measured in Joules/cm^2 and is referred to as energy density or fluence. The absorption of laser light depends on the presence of chromophores in the tissue and the compatibility between the wavelength used and the absorption characteristics of those chromophores. Once absorbed, light can produce three primary effects: photothermal, photochemical, and photomechanical. The photothermal effect involves the conversion of luminous energy into heat, leading to the destruction of the targeted area. In the photochemical effect, light absorption by photosensitizing agents, whether endogenous or exogenous, triggers a chemical reaction, which forms the basis

of photodynamic therapy. Additionally, light absorption can cause rapid thermal expansion, resulting in the generation of acoustic waves and the photomechanical disruption of the tissue that absorbed the light (CAVALCANTI et al., 2011).

Two primary types of lasers exist. High-power lasers, referred to as surgical lasers or HILT (High-Intensity Laser Treatment), are employed during surgical procedures to perform tasks such as cutting, coagulating, or cauterizing tissues. On the other hand, low-power lasers, known as therapeutic lasers or Low-Level Laser Therapy (LLLT), are extensively utilized for therapeutic purposes. These lasers facilitate the acceleration or regulation of physiological processes through non-thermal effects, without causing significant heating of the targeted tissues (CAVALCANTI et al., 2011; SCHINDI et al., 2000).

LLLT involves the application of light, typically from a low-power laser or light emitting diode (LED) with a power output between 1mW and 500mW, to an area affected by a pathology, to promote tissue regeneration, reduce inflammation, and alleviating pain. These effects are attributed to various potential mechanisms, including increases in energy metabolism and ATP synthesis, stimulation of defenses against oxidative stress, and the prevention or repair of muscle damage (FERRARESI et al., 2016).

Recent findings have revealed the PBM mechanisms of action on mitochondrial function, particularly concerning the ATP production. These new insights can help us gaining a better and deeper understanding of how PBM plays a role in mediating the ATP synthesis (KARU, 1999). During aerobic metabolism, mitochondria naturally produces reactive oxygen species (ROS) as a byproduct. Under normal circumstances, mitochondria maintains ROS levels within a regulated range to safeguard cells from harm. Research has shown that PBM therapy has the potential to reduce oxidative stress and ROS levels (SANTOS et al., 2010), thus indicating its ability to modulate mitochondrial activity and counteract the detrimental effects of excessive ROS production. Mitochondrial-produced nitric oxide (NO) has the potential to hinder cellular respiration by binding to cytochrome c oxidase (CCO) and competitively displacing oxygen, particularly in stressed or hypoxic cells. However, the application of PBM can induce the photodissociation of NO from CCO, leading to an increase in ATP

production (CHUNG et al., 2011). This implies that PBM can counteract the inhibitory effects of NO on cellular respiration and promote ATP synthesis.

PBM has demonstrated positive results in attenuating fatigue induced by exercise in humans (BARONI et al., 2010; LEAL-JUNIOR et al., 2013; ROSSATO et al., 2017; LANFERDINI et al., 2018). Several studies (LEAL-JUNIOR et al., 2008; LEAL-JUNIOR et al., 2009b; MIRANDA et al., 2018; FRITSCH et al., 2020) found similar positive results in fatigue attenuation, assessing fatigue through the number of repetitions of a given exercise before and after PBM usage, or through self-reported fatigue. Similarly, the fatigue index and perceived fatigue were significantly lower in the rugby players group that received PBM compared to the placebo group (PINTO et al., 2016). These findings provide evidence that PBM can attenuate fatigue and enhance performance during physical exercise.

However, randomized clinical trials conducted with soccer and volleyball athletes did not find any difference in muscular performance between active PBM and PBM-placebo (LEAL-JUNIOR et al., 2009). In judo athletes, PBM showed similar results to placebo in terms of perceived fatigue and fatigue index after the protocol (ORSSATTO et al., 2019). The same was observed in runners, where no between-groups difference was observed in relation to perceived fatigue (LANFERDINI et al., 2021).

Similarly, studies involving NMES have shown similarities between the PBM and PBM-placebo groups for knee extensors' peak torque during maximal voluntary contraction after NMES (GORGEY et al., 2008; CIESLINSKI et al., 2018; JÓWKO et al., 2019; CUNHA et al., 2020). Similar results were observed for discomfort and muscle pain, where studies (CIESLINSKI et al., 2018; JÓWKO et al., 2019) demonstrated similarity for maximal knee extensor voluntary effort between PBM or PBM-placebo applied before a NMES protocol.

However, clinical trials involving eccentric and isometric training programs in healthy individuals have shown positive results for the group receiving PBM (BARONI et al., 2015; HEMMINGS et al., 2017). The application of PBM before exercise resulted in lower changes in creatine kinase (CK, present immediately after muscle damage) and lactate dehydrogenase (LDH, present several days after muscle damage), especially 24 and 48 hours after exercise (LEAL-JUNIOR

et al., 2009; BARONI et al., 2010; BUBLITZ et al., 2016). Although this evidence suggests a reduction in muscle damage by PBM, the number of studies in this specific area is still limited.

It is important to mention that the number of studies in the area of the PBM effects on muscle fatigue and muscle damage induced by NMES is still limited, with few human studies evaluating the PBM effects on immediate muscle damage after voluntary effort. In addition, to the best of our knowledge, there are no studies evaluating the PBM effects applied before a NMES-induced fatigue protocol on muscle fatigue and muscle damage immediately after and in later periods such as 24, 48, and 72 hours after the fatigue protocol.

Studies in animal models (i.e., pre-clinical studies) have shown positive results in NMES-induced fatigue (LOPES-MARTINS et al., 2006; SANTOS et al., 2014). More specifically, a lower increase in CK and an increase in the time to reach a 50% reduction in maximum force was observed in the PBM group compared to the control or PBM-placebo group after fatigue (LOPES-MARTINS et al., 2006; SANTOS et al., 2014). PBM also showed positive results for evoked peak torque, increased muscle work, and decreased muscle damage in animals that received PBM compared to those in the PBM-placebo group (SANTOS et al., 2014).

The reason for the PBM results discrepancy between clinical studies involving voluntary effort and the pre-clinical studies involving NMES-evoked contractions is still unknown. In addition, as previously mentioned, there are still gaps in determining whether PBM can help to reduce the deleterious effects from fatigue induced by NMES in clinical studies. Therefore, taking into consideration all the aspects mentioned earlier, our study aims to identify whether PBM can help reduce NMES-induced fatigue, muscle damage, and discomfort and improve performance in functional tests.

1.5.   CRITICAL EVALUATION OF THE EXISTENT LITERATURE

According to the literature, PBM has shown positive results in attenuating exercise-induced fatigue in humans (BARONI et al., 2010). A smaller decrease in force of the knee extensors immediately after exercise and a better recovery of muscle function 24 and 48h post exercise were observed. Baroni et al. (2010) used an infrared laser Cluster Probe, with a wavelength of 810 nm, 200 mW of output power, and applied PBM at two points in the distal region of the vastus medialis (VM), two points in the distal region of the vastus lateralis (VL), and two points in the central region of the rectus femoris (RF) muscles. Each point was treated for 30 seconds, resulting in 30 J per point and 180 J for the whole quadriceps that was applied before eccentric exercise.

In the study by Leal-Junior et al. (2013), the meta-analysis showed laser and LED used with wavelengths of 640, 655, 660, 808, 810, 830, and 850 nm. Doses ranged from 0.3 J to 41.7 J per point or area, with output power ranging from 10 to 200 mW per point. The most significant results were obtained with higher power (100 and 200 mW) and energy doses between 5 and 41.7 J per irradiated area. The improvement in performance ranged from 2% to 57%, and CK activity decreased from 11.6% to 83.2% with active phototherapy.

Rossato et al. (2017) utilized a probe with 33 diodes, applying a continuous frequency and 30 J per area, with nine locations over the quadriceps muscle group (total dose = 270 J). PBMT was applied both at 6 hours before and immediately before a fatigue test [maximal effort involving one attempt of 45 repetitions for knee extension-flexion at $180 \cdot s^{-1}$, with the range of motion at 70° (30°–100°, 0° = full knee extension)]. The MVIC peak torque for the group that received PBM 6 hours before + immediately before the fatigue test presented higher values than control (p=0.001) and placebo (p=0.004) treatments in the post-fatigue evaluation. MVIC peak torque percentage change between pre and post evaluations presented lower reduction in the 6 hours before + immediately before treatment (26%) compared with control (33%), placebo (29%), and immediately before (32%) treatments. The application of PBMT 6 hours + immediately before the concentric contractions fatigue protocol and the just immediately before condition were able to reduce the fatigue.

In the study by Lanferdini et al. (2018), PBMT was applied to the quadriceps muscle and hip extensor and plantar flexor muscles, using an infrared laser Cluster. PBMT was applied immediately before the cycling exhaustion test, with dosages of 135 J, 270 J, and 405 J per muscle. Power output and muscle activation from both lower limbs were recorded throughout the tests. Increased performance in time-to-exhaustion tests was observed with the LLLT-135J, LLLT-270J and LLLT-405J compared to PBM-placebo. In addition, LLLT-135J led to an increase in the high-frequency content of the electromyographic signal compared to PBM-placebo in both limbs at the end of the exhaustion test.

Although different dosages of PBM were applied in the above-mentioned studies, only when it was consistently administered before physical activity or before a fatigue protocol, there were positive PBM results in reducing fatigue. However, other studies have shown no significant changes in neuromechanical outcomes in various sports when evaluating the PBMT effects.

Leal Junior et al. (2009) used a Thera Laser with a wavelength of 830 nm and a continuous frequency, with a total energy delivered of 40 J for volleyball players and 30 J for soccer players. The PBM was applied at 10 points in soccer and volleyball athletes. Active LLLT or placebo LLLT was administered after the stretching protocol, but immediately before the fatigue test exercise. There was no significant difference in the work performed during the Wingate test ($p > 0.05$) between subjects given active LLLT and those given placebo LLLT in both groups. However, for the volleyball athletes, the change in CK levels from before to after the exercise test was significantly lower ($p = 0.0133$) for those given active LLLT than for those given placebo LLLT. For the soccer athletes, the change in blood lactate levels from before exercise to 15 min after exercise was significantly lower ($p < 0.01$) in the active LLLT group than in the placebo LLLT group.

In judo athletes (ORSATO et al., 2019), PBM treatments were applied using a Chattanooga Intelect Mobile Laser 2779 system. PBMT was applied before the fatigue protocol to 15 locations in each lower limb, including eight locations in the quadriceps (3 in VL, 3 in RF, and 2 in VM), four locations in the hamstrings (2 in the semitendinosus and 2 in the semimembranosus), two locations in the gastrocnemius, and one location in the soleus. The total dose was 450 J (quadriceps = 240 J, hamstrings = 120 J, and gastrocnemius = 60 J).

No differences were observed between PBMT and PBM-placebo at any time point for any of the outcomes (p>0.05), indicating no positive effect of the PBMT. Their findings suggest no PBMT effect when applied before exercise to reduce lower limb muscle fatigue and muscle damage during and following a stretch-shortening cycle protocol in judo athletes.

Similar findings were observed for perceived fatigue in runners (LANFERDINI et al., 2021), with no between-group changes on the ratings of perceived exertion and metabolic cost with the application of PBMT and PBM-placebo. PBM was applied at five locations in each lower limb, targeting the quadriceps (two locations), gluteus maximus (one location), hamstrings (one location), and gastrocnemius (one location). The treatments were administered using the LEAP Sports Pod, with a wavelength of 880 nm, continuous frequency, a dose of 300 J per location, and total dose of 1500 J. The PBMT application before and after the test did not attenuate the impairment in running economy and metabolic cost, and therefore did not reduce the rating of perceived exertion 24 h after the 3000 m running test.

In eccentric training in healthy subjects, positive results were observed in terms of strength gain and muscle thickness in the group receiving PBMT compared to the control group. Baroni et al. (2015) used an infrared laser cluster probe with a wavelength of 810 nm and an output power of 200 mW, with 30 J of energy per application, and a dose of 240 J in the quadriceps muscle. They applied PBM at two points in the distal region of VM, at three points in the distal region of VL, and three points in the central region of RF. Each point was treated for 30 seconds, and PBMT was applied immediately before each training session in the PBMT group. Eccentric training led to significant increases in muscle thickness and peak torque in the eccentric training group and in the PBMT + eccentric training group. However, subjects in the PBMT group reached significantly higher percent changes compared to subjects from the just eccentric training group for knee extensors' muscle thickness (15.4 vs. 9.4 %), isometric peak torque (20.5 vs. 13.7 %), and eccentric peak torque (32.2 vs. 20.0 %).

Hemmings et al. (2017) conducted LED therapy (LEDT) with irradiation times of 30 sec using 1.5 J, 60 sec using 3.0 J, and 120 sec using 6.0 J at each point, with PBMT applied at six points before the fatigue test. There was also a

placebo test in which no irradiation was applied for 45 sec per point. After the PBM application, the participant was positioned in the isokinetic dynamometer (Biodex) and then performed one set of 5 eccentric repetitions for familiarization, followed by a single-leg eccentric knee extensor repetitions to exhaustion test (120% of MVIC until fatigue - a drop of 20% in the MVIC value). There was significant increase in the number of repetitions performed for the 60 sec of PBMT (p=0.023) compared to placebo treatment and 120 sec (p=0.004) of PBMT compared to PBM-placebo. LEDT had a positive effect on performance when irradiating 6 points on the superficial quadriceps for 60 and 120 sec before an eccentric leg extension.

As we can see from the existent literature, there is a significant divergence in the results from the PBMT application, with studies showing positive results and other studies showing no difference between PBMT and PBM-placebo or control groups. Although Leal Junior et al. (2019) have provided PBMT dosage recommendations based on the existing literature, they have not addressed why there are positive PBMT effects in the pre-clinical but not in the clinical trials regarding the PBMT effects on muscle fatigue and muscle damage. In addition, despite several clinical studies with athletes from different modalities showed a fatigue reduction and performance improvement with PBMT, other clinical trials did not show any PBMT effect. Therefore, our study aimed at evaluating the effects of PBMT applied before a fatigue protocol on muscle fatigue, muscle damage, discomfort and functionality in healthy subjects. Despite the above-mentioned literature divergence, we hypothesized that PBMT should reduce fatigue and muscle damage, thereby reducing discomfort from a NMES-induced fatigue protocol and improving functionality.

CHAPTER II: METHODOLOGY

2.1. RESEARCH PROBLEM

Can PBMT reduce neuromuscular fatigue, discomfort, and muscle damage, and improve functionality after an NMES-induced fatigue protocol consisting of 60 isometric knee extensor evoked contractions in healthy young participants?

2.2. OBJECTIVES

2.2.1. OVERALL OBJECTIVE

The overall objective of this study was to assess the acute effects of PBM and PBM-sham, applied before a NMES-induced fatigue protocol, on neuromuscular parameters of fatigability (maximum isometric strength, torque-time curve integral, total work, evoked torque reduction), on muscle damage (echointensity, delayed onset muscle soreness), on discomfort (visual analog scale - VAS, algometer), and on functionality (Single Hop Test - SHT, stair ascent and descent test) in healthy individuals.

2.2.2. SPECIFIC OBJECTIVE

To evaluate the acute effects of PBM and PBM-sham, applied before a NMES-induced fatigue protocol (60 stimulations), on the following variables of interest measured before, during, or after the fatigue protocol (immediately after, at 24, 48, and 72 hours): a) supramaximal twitch; b) knee extensor maximum voluntary isometric torque; c) integral of the evoked torque-time curve; d) total work; e) muscle damage of the rectus femoris and vastus lateralis muscles; f) discomfort in the knee extensors; g) delayed onset muscle soreness in the knee extensors; h) performance in the SHT and stair ascent and descent test.

2.3. STUDY DESIGN

The present research project was conducted in the Neuromuscular Plasticity Laboratory of the Exercise Research Laboratory (LAPEX) at the School

of Physical Education, Physiotherapy, and Dance (ESEFID) of the Federal University of Rio Grande do Sul (UFRGS), located in Porto Alegre, Rio Grande do Sul, Brazil. This study was characterized by a quantitative approach with a randomized clinical trial design, sham-controlled with blinded subjects.

2.4. SAMPLE SIZE

The sample size calculation was performed using G-Power software (version 3.1.3; University of Trier, Trier, Germany). The sample size was determined based on a study by Cieslinski et al. (2018), which had a methodology similar to the present study. The mean and standard deviation values for the torque generated during post-NMES maximum voluntary isometric contraction were extracted for the groups that used LLLT (210.2 ± 40.7 Nm) or sham (229.3 ± 55.9 Nm) prior to NMES, and Cohen's effect size was calculated. Thus, using the mentioned values, we obtained an effect size (d) of 0.39, which was transformed into an effect size (f) of 0.19 and used for sample size calculation. Therefore, with a power of 80% and α = 0.05, the calculated sample size was 36 subjects. Assuming a possible sample loss of 20% as previously reported in the study by Jówko et al. (2019), a total of 44 subjects was determined, including (22 men and 22 women). However, technical problems in the laboratory caused by the COVID-19 pandemic affected several equipment, delaying the start of the present dissertation project. As a result, we made the decision to utilize both lower limbs from 22 subjects (11 men and 11 women) and randomized the two intervention conditions (PBM vs PBM-sham), resulting in 44 lower limbs. This approach was taken to ensure that the study could be completed within the designated period and due time of the MSc Program of the PPGCMH.

2.5. INCLUSION AND EXCLUSION CRITERIA OF THE SAMPLE

Inclusion criteria:

- Healthy adults, aged between 18 and 40 years, of both sexes.
- Individuals who engage in regular physical activity (i.e., at least twice a week).
- Not being hospitalized for COVID in the last 12 months.

- Individuals capable of tolerating NMES.

- Individuals capable of understanding the protocol.

Exclusion criteria:

- Subjects with sensory alterations.

- Consumption of coffee or other stimulants in the past 12 hours.

- Body Mass Index (BMI) greater than 30 kg/m^2.

- Any contraindication to perform maximum effort or presenting an abnormal increase in blood pressure after maximum effort.

2.6. INSTRUMENTS

Data collection instruments were used for this study to obtain sociodemographic information from participants, such as age, gender, body mass index (BMI), and female menstrual cycle data. Data collection was conducted through interviews. To assess the level of physical activity of each participant, the short version of the International Physical Activity Questionnaire (IPAQ) was used, which categorizes people's responses according to the time and days they engage in physical activities. The interpretation and analysis of the results were performed using IPAQ short-form categorical scoring (CHENG, 2016).

2.7. EXPERIMENTAL DESIGN

The evaluations were carried out on 9 different days for each participant, with 1 day for the familiarization and baseline evaluation, 4 days for each lower limb (pre-fatigue measurements, fatigue protocol, immediately after fatigue, post-24-hrs, post 48-hrs, and post 72-hrs of the fatigue test). The average duration of each evaluation was 1.5 hrs for the first day of familiarization/evaluation, 2 hrs for the day of the PBMT and fatigue protocol, and 40 min for the 24, 48 and 72 hrs post-fatigue evaluation days.

On the first day, anamnesis was conducted, meaning that the subjects answered a questionnaire regarding personal and sociodemographic data, as well as a questionnaire about the level of physical activity. Additionally, they were

familiarized with the isokinetic dynamometer used to assess knee extensor strength and with NMES. After this initial evaluation, a 4-day interval was observed, after which the participants returned to the laboratory for the evaluations corresponding to the 2nd day of evaluation. The same measurements of the variables of interest performed on the 1st day of evaluation were obtained, except for the determination of the motor point of the RF muscle that was obtained on the first day.

2.7.1. RANDOMIZATION

The dominant and non-dominant lower limbs of the participants were randomized to one of the PBM protocols, either receiving active PBM or PBM-sham. Randomization was performed using envelopes. During the familiarization stage, participants drew one of the four conditions:

• Dominant leg, active PBM

• Dominant leg, PBM-sham

• Non-dominant leg, active PBM

• Non-dominant leg, PBM-sham.

2.7.2. MUSCLE DAMAGE

To determine muscle damage, an ultrasound device (VIVID i ®, GE) and a linear array matrix probe (50mm linear array 3-10 MHz; L12-3) were used. The transducer was positioned perpendicular to the longitudinal direction of the evaluated musculature. Three images of the muscles of interest were obtained through ultrasound imaging.

Muscle damage was measured through the analysis of ultrasound images in the transverse plane of the RF and VL muscles. The procedure for determining muscle damage was based on the intensity of gray shades in ultrasound images

obtained from the maximum area of the RF and VL muscles (NOSAKA e NEWTON, 2002; CHEN et al., 2011).

To obtain images of RF and VL, participants remained seated in the Biodex System 3 Pro isokinetic dynamometer, with their lower limbs flexed at 90°. The measurement was performed with the transducer positioned transversely to the muscle fibers (Figure 1) at three locations: 60% of the distance from the anterior superior iliac spine to the upper edge of the patella (adapted from the methodology of RABELLO et al., 2019), and at 50% and 40% of that same line. Additionally, a bubble level was attached to the transducer to ensure proper perpendicular positioning (i.e., at 90°) to the skin surface without any tilt.
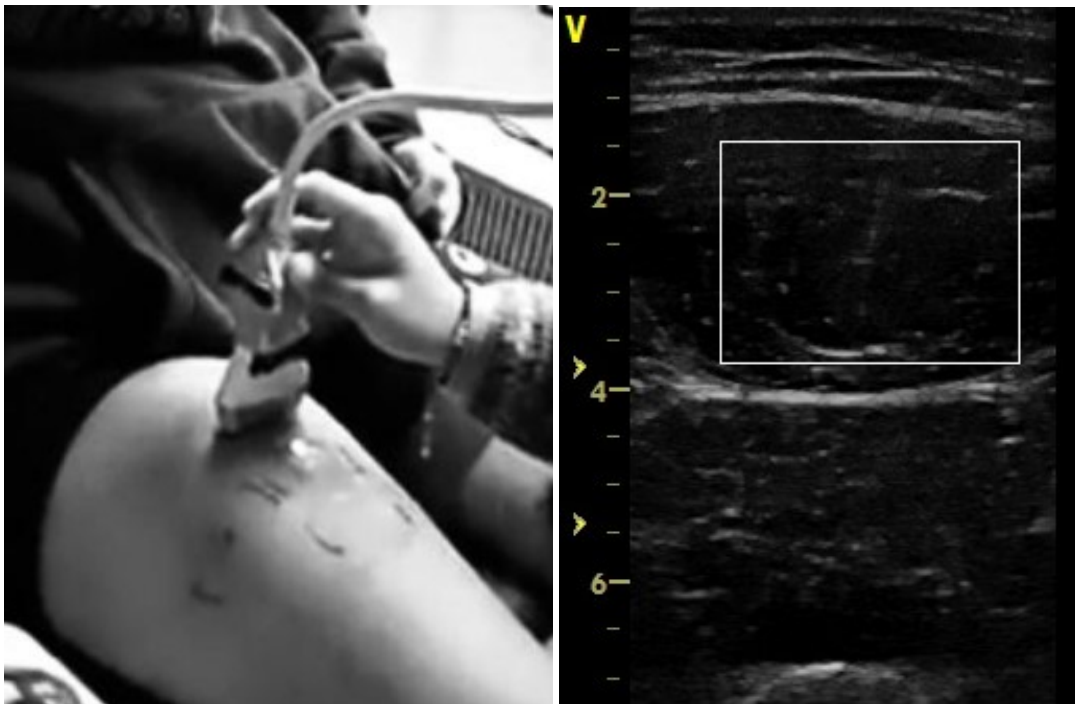
Figure 1. Example of muscle damage measurement and analysis of the RF muscle at 50% of the distance between the anterior superior iliac spine and the upper edge of the patella. Probe position (left) and region of interest in IMAGE J software (right) are shown.

All images were analyzed using Image J software (version 1.43u, National Institutes of Health, Bethesda, MD, USA). The "maximum rectangular region of interest (ROI)" option was selected, to include as much muscle as possible, while avoiding surrounding fascia and bone (LANFERDINI et al., 2019) (Figure 1). Muscle damage was determined based on a mean value of grayscale histogram

in Image J software (0 = black, 255 = white) of the ultrasound images (LANFERDINI et al., 2019; WONG et al., 2019).

2.7.3. DISCOMFORT

2.7.3.1. EVALUATION OF THE LEVEL OF SITTING DISCOMFORT

The level of discomfort was measured using a 10 cm Visual Analog Scale (VAS) (Figure 2). The Visual Analog Scale was presented to the participants while they were seated, both before starting the fatigue protocol and upon completion of it. Participants drew a mark on the scale, indicating the level of discomfort experienced. The level of discomfort was defined as the measurement in centimeters between the zero value and the pen mark made by the participant (PAZ et al., 2021).
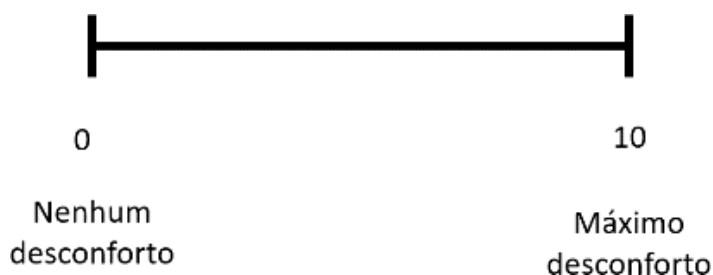
Figure 2. Visual analog pain scale.

2.7.3.2. EVALUATION OF PAIN PRESSURE THRESHOLD

The pain pressure threshold was assessed using an electronic pressure algometer with traction and compression (Instrutherm, model DD-200, with a capacity of up to 20 kgf). The algometer was calibrated, and its readings were taken in kgf/cm^2. The participants were informed that pain would be assessed through pressure stimulation. They were required to indicate the moment when the sensation of pressure turned into a sensation of pain. At that point, the test was interrupted, and the reading on the algometer was recorded. The application of pressure occurred with the participants seated, with the quadriceps muscle relaxed. The algometer, positioned at a 90° angle, was applied to all knee extensors: RF and VL at 50% of the line from the anterior superior iliac spine to

the upper edge of the patella, and VM at 50% of the line from the upper edge of the patella to the muscle belly (BAKER et al., 1997) (Figure 3).

Figure 3. Example of application of the algometer in the muscle belly of the RF muscle.

2.7.4. OVERALL FATIGUE

2.7.4.1. EVALUATION OF THE KNEE EXTENSORS MAXIMUM VOLUNTARY TORQUE

The maximum isometric force production capacity of the knee extensors was measured using the Biodex System 3 Pro isokinetic dynamometer (Biodex Medical System, Shirley – NY, USA) (Figure 4). Participants were positioned on the dynamometer and underwent warm-up of the knee extensors, which consisted of 10 concentric repetitions of flexion/extension at an angular velocity of $90°·s^{-1}$, with a submaximal effort level, followed by a 2-min rest period. After the warm-up, the assessment of MVIC torque was performed. Participants performed 3 MVICs lasting 5 s each, with the knee flexed at 90°. A rest period of 120 s was observed between the tests. The MVIC with the highest torque was used to determine the submaximal intensity of 20% of the MVIC for the protocol of fatigue evoked by NMES. The MVICs at the 90° angle were performed before

and immediately after the fatigue protocol, and Post-24, Post-48, and Post-72 hrs of the fatigue protocol, and were used for comparisons between time moments and between limbs.

Figure 4. Positioning of the participant on the isokinetic dynamometer.

2.7.4.2. SUPRAMAXIMAL TWITCH TORQUE

The participant was seated on the isokinetic dynamometer with the knee flexed at 90° (0° = full extension) for this evaluation. First, the distal electrode was positioned 5 cm above the upper border of the patella. Next, the motor point was located. The search was performed using the pen protocol of the multifunctional electrical stimulator (VAZ et al., 2021), at a frequency of 1 Hz and a current intensity sufficient to generate a visible contraction of the knee extensors. Once the point was located, the site where the electrical pulse generated the highest knee extensor torque was defined as the motor point of the RF muscle, where the second NMES electrode was positioned (Figure 5).
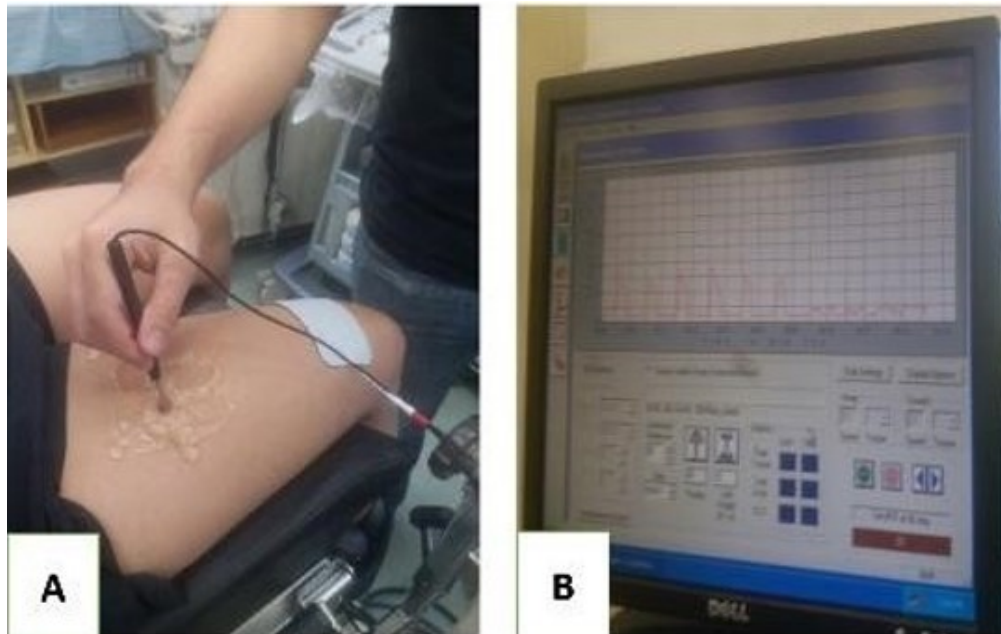
Figure 5. Example of motor point location of the RF muscle. Pen-shaped electrode (A) and evoked twitch contractions (B) are shown (FRÖHLICH & VAZ, 2018).

For the assessment of supramaximal twitch, single symmetrical rectangular biphasic electrical pulses (i.e., with a frequency of 1 Hz), with a duration of 500 µs per phase were applied. Starting from the identification of the motor threshold, the current intensity was gradually increased until no further increase in the evoked torque was observed on the dynamometer display. After that, a 10% increase in current intensity was applied to guarantee supramaximal twitches. Evoked torque (Nm) during contractions was determined by the torque produced from three supramaximal contractions (with an interval of approximately 1 s between contractions), applied with the subject at rest, before, immediately after, and 24, 48 and 72 hours after the NMES fatigue protocol. Means and standard errors of the evoked torque values of the supramaximal twitches were calculated for the two modes of PBM application.

2.7.4.3. EVOKED NEUROMUSCULAR EFFICIENCY

The evoked peak torque and the current intensity of the supramaximal twitches were used to determine the neuromuscular efficiency after PBM and PBM-sham. Means and standard errors of the torque/current intensity ratio values were calculated for the two modes of PBM application.

2.7.5.   PBM PARAMETERS AND PROTOCOL

Active PBM and PBM-sham were applied 5 min before the fatigue protocol (LEAL-JUNIOR et al., 2019). The individuals were seated on the isokinetic dynamometer, with the knee flexed at 90° and the lower limb at rest. For the application of PBM, the therapist and the participant wore protective goggles that come with the DD2 control unit (Thor Photomedicine Ltd., UK). PBM was applied using a cluster probe (GaAIAs) composed of five 810 nm laser diodes, each with an output power of 200 mW, and was used in continuous frequency (BARONI et al., 2015; FRITSCH et al., 2019; LEAL-JUNIOR et al., 2019). Eight application points of PBM were used to cover the majority of the quadriceps femoris muscle: two in the VM, three in the VL, and three in the RF (Figure 6). Each point was treated for 30 seconds with a dose of 6 J per diode, totaling 30 J per site, resulting in 240 J per lower limb (BARONI et al., 2015; FRITSCH et al., 2019; LEAL-JUNIOR et al., 2019). The probe was held stationary in contact with the skin with slight pressure at a 90° angle to the skin (LEAL-JUNIOR et al., 2019).

For the PBM-sham, the probe was positioned in the same location, but with the equipment turned off. In both conditions (PBM and PBM-Sham) the participants wore headphones for blinding them to the treatment on that day, because the laser therapy device emits a sound at the end of the PBM application, and participants would realize that the equipment was turned off in the PBM-sham condition due to no sound being generated.
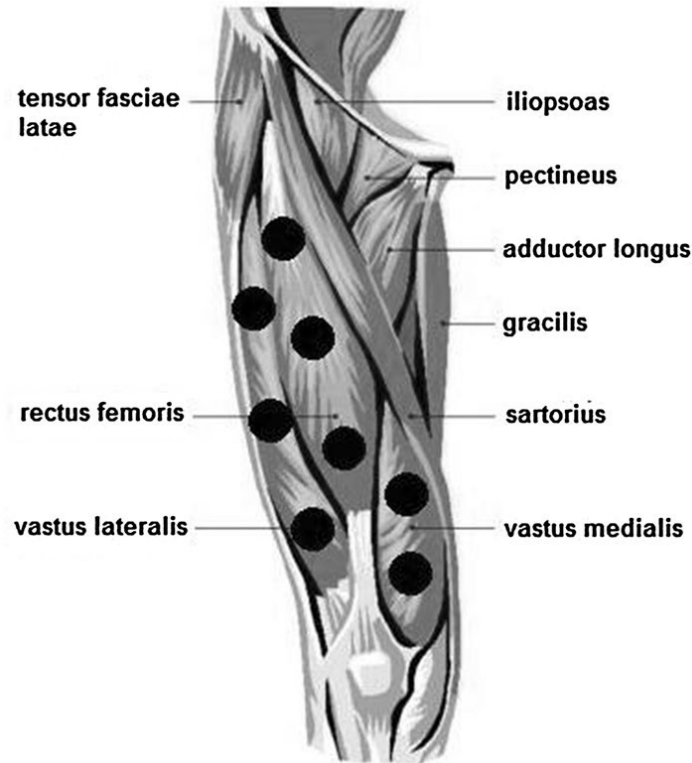
Figure 6. Application points (black circles) used for PBM (SOURCE: BARONI et al., 2015).

2.7.6. FATIGUE PROTOCOL

The multifunctional electrical stimulator, developed by the Biomedical Engineering Department at the Hospital de Clínicas de Porto Alegre, was used for the evaluation and intervention with NMES (Figure 7; VAZ et al., 2021). The fatigue protocol was induced by NMES applied at the RF's motor point. The stimulation parameters chosen for the contraction-rest time ratio (ON:OFF) were 5s:10s (WALLS et al., 2010; PAZ & VAZ, 2019). To make the protocol more comfortable for the participant, 1-sec ramp-up and ramp-down periods were used during the time ON, totalizing 7s for time ON (PAZ et al., 2021). A rectangular biphasic pulsed current, with a pulse duration of 1 ms, was used for the evoked fatigue protocol, with a stimulation frequency of 100 Hz. The NMES current intensity during the fatigue protocol was set at a fixed intensity sufficient to generate 20% of the MVIC (PAZ & VAZ, 2019). NMES was maintained for 20 minutes and comprised 69 evoked isometric contractions through EENM. However, for the analysis, only 60 contractions were considered because the

evoked torque did not change from contractions 60-69 and because fatigue becomes evident from that contraction onwards (PAZ and VAZ, 2019).
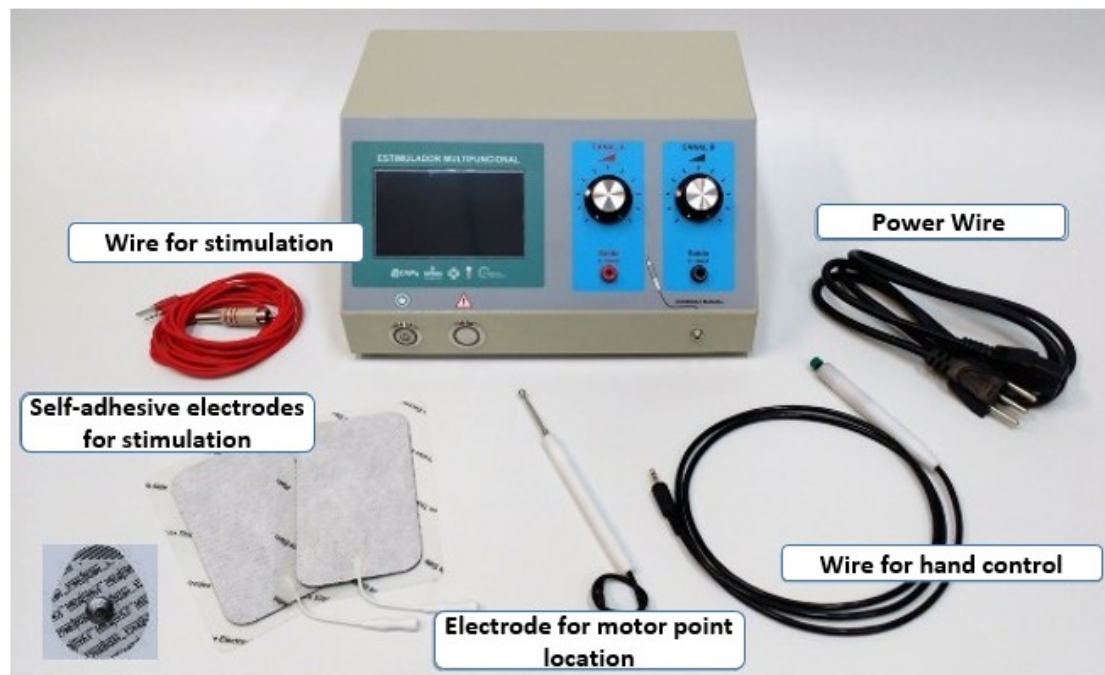
Figure 7. Multifunctional electrical stimulator (VAZ et al., 2021).

2.7.6.1. EVALUATION OF THE TOTAL WORK (TW) FROM THE TORQUE-TIME INTEGRAL (TTI)

The integral torque-time (ITT), which was measured as the area under the torque-time curve, represents the amount of isometric work performed during a specific NMES-evoked contraction (PAZ et al., 2021). The ITT of each evoked contraction was quantified in each fatigue protocol (Figure 8). The sum of the ITTs of all evoked contractions in the fatigue protocol was calculated to determine the total work (TW) elicited in each fatigue protocol (NEYROUD et al., 2014; NEYROUD et al., 2018; PAZ et al., 2021).
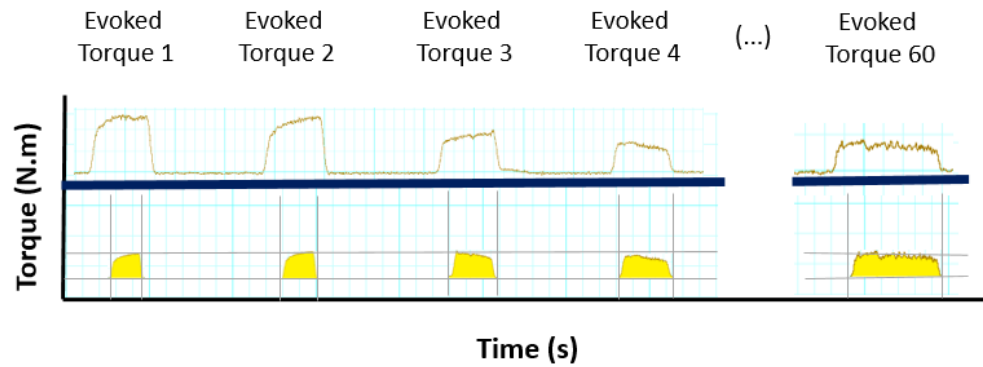
Figure 8. Evaluation of the Torque-Time Integral (ITT, yellow area) from the individual evoked contractions of the fatigue protocol.

2.7.6.2. EVALUTION OF FATIGABILITY INDEX FROM EVOKED TORQUE

The analysis of the fatigability index was performed based on the difference between the initial evoked peak torque (average of the first 5 evoked contractions) and the final evoked peak torque (average of the last 5 evoked contractions) (Figure 9). The fatigue percentage was obtained using the formula: % Decline = [(initial torque - final torque) / initial torque] x 100 (PAZ et al., 2021).
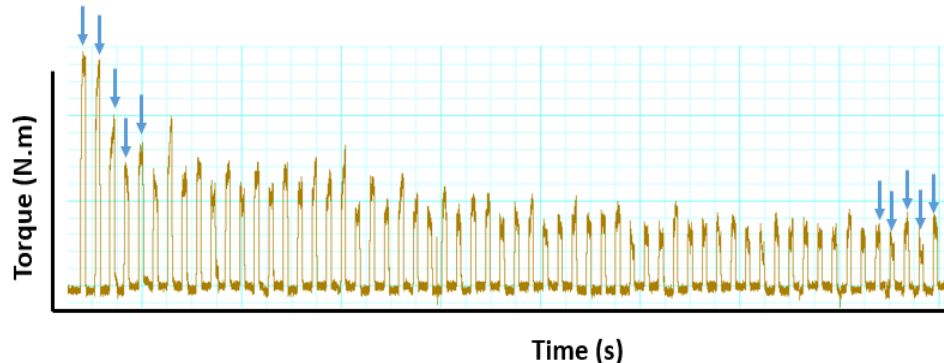
Figure 9. Evaluation of Fatigue Index from Evoked Torque

2.7.6.3. EVALUATION OF FATIGABILITY INDEX FROM WORK

Similar to the fatigability index, the work decline was obtained by calculating the difference between the average work of the first 5 contractions of the fatigue protocol and the last 5 contractions (Figure 10). It also represented a percentage of the initial values using the equation: % Decline of work = [(initial work - final work) / initial work] x 100.
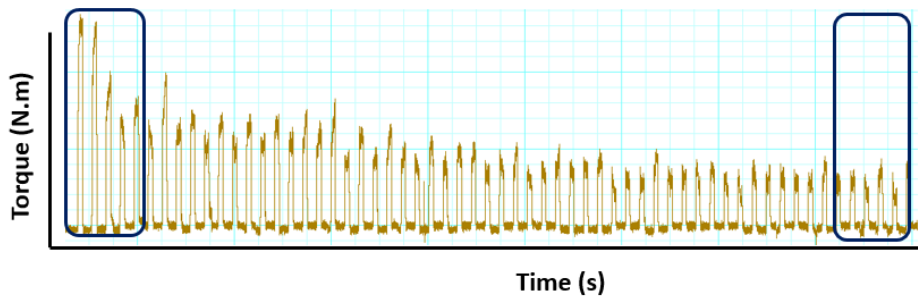
Figure 10. Evaluation of Fatigue Index from work.

2.7.6.4. EVALUATION OF THE TIME FOR THE INITIAL WORK TO DECLINE BY 10%

Fatigability was defined to be reached when the first contraction of the protocol achieved less than 10% of the work obtained in the MVIC (Figure 10). The number of contractions to reach this 10% of the MVIC work was used as the fatigability value.
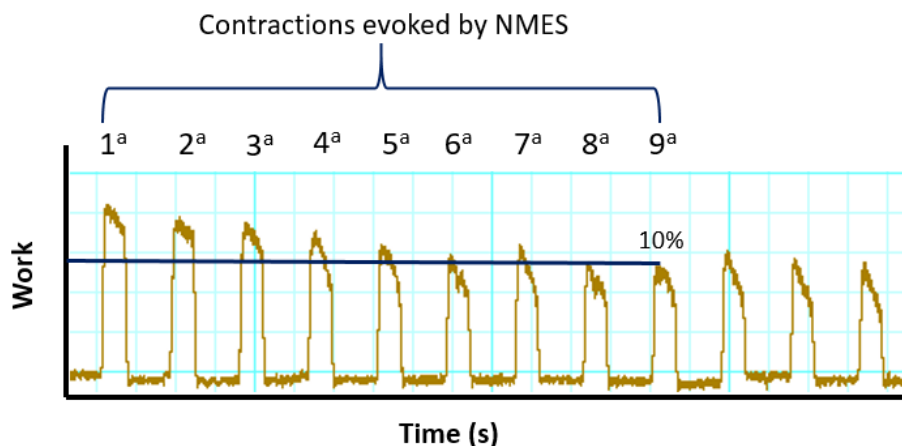
Figure 11. Reduction of work to 10% of MVIC work. Representative image of a participant who achieved a 10% reduction in work on the 9th evoked contraction.

2.7.7. FUNCTIONALITY AND PERFORMANCE TESTS

To assess the functionality and performance of the participants before and after the NMES protocol, the Single Hop Test (SHT; ROSS *et al*., 2002) and stair ascent and descent test (GUADAGNIN & VAZ, 2018) were conducted. Using a measuring tape, the starting point for the SHT was marked, with the measuring

tape fixed on the side with the 0 cm mark at the starting point. Participants were positioned with their footwear on, in a unipodal stance, with the first toe on the starting point, the knee slightly flexed, and arms free during the jump to maximize functionality in its execution. They were then instructed, through verbal command, to perform a unipodal jump as far forward as possible and land on the same leg, ensuring no subsequent imbalances or contact of the contralateral limb with the ground. The test was performed three times. The distance was measured in centimeters, and the value of the greatest distance, expressed as a percentage of the limb length, was considered for analysis. The distance was normalized by the leg length that was defined as the distance from the anterior superior iliac spine (ASIS) to the lateral malleolus. The formula used for normalization was: SHT normalized = (SHT cm / leg length) x 100.

The stair ascent and descent test was adapted from the methodology of a previous study conducted by our research group (GUADAGNIN & VAZ, 2018). During the test, the time taken to ascend and descend 10 steps while walking was recorded solely as a control variable. At the end of the test, the level of discomfort was measured using a Visual Analog Scale (VAS).

2.7.8. DATA ANALYSIS

Initially, descriptive statistics were performed, as well as an assessment of data normality using the Shapiro-Wilk test. Comparisons involving the factors Limb (PBM and PBM-sham) and moments (Pre, immediately after, Post-24 hrs, Post-48 hrs, and Post-72 hrs) were conducted using the Generalized Estimating Equations model with a gama log link for scale response. When a statistically significant difference was identified among the Time moments, the LSD (Least Significant Difference) post hoc test was used. For comparisons involving the variables with only the pre and immediately after time moments, the Mann-Whitney U test was used. All quantitative information is presented as mean and standard error, except for the characterization variables where mean and standard deviation values were used. The significance level for all statistical analysis was set at 0.05.

CHAPTER III: RESULTS AND DISCUSSION

3.1. RESULTS

Although our initial sample was estimated in 44 participants and was changed to 22 participants with both limbs being randomized for the two treatment conditions (i.e., 22 limbs for PBM and 22 for PBM-sham), we only were able to recruit 6 men and 6 women for the study. Therefore, the results here presented should be treated and interpreted as preliminary results, as when reaching the total sample size the results will most likely change.

Twelve subjects participated in the study, totaling 24 lower limbs. The participants' age (20-40 years), weight (47.6-98,7 kg), height (1.59-1.91 m), and BMI (18.29-29.76 m/kg^2) were presented as mean and standard deviation values (Table 1). The majority of our participants were right-handed (91.7%), and 6 participants received PBM on their dominant limb. Half of the women's sample took contraceptives and, although 75% of the sample had COVID, none of the participants was hospitalized. Regarding the level of physical activity measured by IPAQ, only one participant had a low level of physical activity, with 5 moderate and 6 classified as having vigorous physical activity level (Table 1).

Table 1. Characterization of the sample.

Sex	Female 6 (50%)
Dominant Member	Right-handed 11 (91.7%)
Age (years)	28.0±5.5
Mass (kg)	71.6±15.9
Stature (m)	1.73±0.1
BMI (m/kg²)	23.8±3.7
Contraceptive	Yes 3 (50%)
Had Covid	Yes 10 (75%)
Was hospitalized for Covid	No 12 (0%)
IPAQ Classification	Low 1 (8.3%) Moderate 5 (41.7%) High 6 (50%)

In the echo intensity grayscale, no differences were observed between the active PBM limb and the sham limb for RF-60, RF-50, RF-40 and VL ($p>0.05$). There were also no differences between the moments ($p>0.05$), and there was no interaction ($p>0.05$) (Table 2).

Table 2. Echo intensity (grayscale) between days

		Baseline	Immediately after	P24	P48	P72	Limb	Moments	Interaction
RF 60	PBM	11.8±0.8	11.8±0.7	11.4±0.8	11.7±0.8	11.4±0.8	0.677	0.655	0.565
	SHAM	12.2±1.0	12.3±1.0	12.4±0.9	11.7±1.0	11.8±0.8			
RF 50	PBM	13.5±0.9	14.0±0.8	13.4±1.1	13.3±1.0	13.0±1.0	0.563	0.496	0.288
	SHAM	14.7±0.9	13.7±1.0	14.4±0.9	14.3±1.1	13.8±0.8			
RF 40	PBM	15.9±1.2	16.5±0.8	15.5±1.0	15.6±1.1	15.5±0.7	0.839	0.864	0.408
	SHAM	15.8±1.1	16.0±1.2	16.8±1.4	16.1±1.2	15.8±1.2			
VL	PBM	16.7±0.9	16.7±0.9	16.1±1.1	15.6±0.8	15.6±0.6	0.667	0.116	0.810
	SHAM	17.0±0.9	16.7±1.3	16.7±0.8	16.8±0.9	15.9±1.0			

Values presented as mean ± SD.

When analyzing the VAS results obtained in the seated position, no between-limbs difference was observed. However, significant differences were observed between different moments and there was interaction, suggesting a different behavior for the VAS between the PBM-treated limb and the PBM-sham limb. Both limbs experienced an immediate increase in discomfort, which did not return to baseline values within 72 hours (Table 3).

Table 3. Discomfort and Pain pressure threshold between days

		Baseline	Immediately after	P24	P48	P72	Limb	Moments	Interaction
VAS seated	PBM	0.2±0.01[bce]	0.88±0.32[a]	0.40±0.05[a]	0.5±0.18[a]	0.35±0.06[a]	0.979	*0.001*	*0.001*
	SHAM	0.13±0.03[bcde]	1.1±0.36[ace]	0.57±0.17[abe]	0.6±0.16[ae]	0.24±0.05[abcd]			
Alg VL	PBM	5.5±0.5[b]	4.8±0.4[a]	4.6±0.5	4.3±0.5	4.7±0.6[a]	0.545	*0.001*	0.194
	SHAM	4.9±0.6[b]	4.5±0.5[a]	3.9±0.4	4.3±0.4	4.3±0.5[a]			
Alg RF	PBM	6.1±0.6[b]	5.5±0.4[a]	5.4±0.6	5.4±0.6[c]	6.1±0.7	0.509	*0.001*	0.157
	SHAM	5.7±0.6[b]	4.9±0.5[a]	4.7±0.5	5.3±0.6[c]	5.2±0.6			
Alg VM	PBM	5.2±0.4[b]	4.5±0.4[a]	4.6±0.5	4.6±0.5	4.8±0.5	0.450	*0.001*	0.157
	SHAM	4.5±0.5[b]	4.2±0.4[a]	4.1±0.4	4.2±0.5	4.4±0.5			

Values presented as mean ± SD. The between-moments changes were represented by letters: a ≠ baseline; b ≠ Immediately after; c ≠ P24; d ≠ P48; e ≠ P72. Statistically significant *p* values are in italics.

When using the algometer to assess pain pressure threshold (PPT) in the VL, RF, and VM muscles, no differences were observed between the limbs, and no interaction was observed (Table 3). Observing the results in the VL muscle, we observed a significant decrease in PPT in both limbs. This decrease persisted up to 72 hours, indicating a sustained impairment in pain sensitivity. In the RF

muscle, a significant decrease in PPT was also observed in both limbs. However, it is worth noting that PPT values returned to baseline within 48 hours, indicating a relatively faster recovery of pain sensitivity in this muscle compared to the observations in the VL. Finally, the PPT in the VM muscle decreased after the fatigue protocol in both limbs, with PPT values returning to baseline values within 24 hours. (Table 3).

In the overall fatigue analysis, the MVIC did not show differences between the limbs or interactions, with similar changes observed in participants who received PBM and PBM-sham in the different evaluation moments. Specifically, there was a reduction in MVIC from baseline (244±23) to immediately-after the NMES fatigue protocol (214±23), with an MVIC increase from immediately after (214±23) to post-24 hrs (227±23), post-48 hrs (230±24) and post-72 hrs (240±24) ($p<0.05$). This indicates that, after 48 hrs, the MVIC values returned to baseline levels, indicating a recovery in voluntary strength. In the PBM-sham limb, a similar reduction in MVIC was observed from baseline (243±26) to immediately after (206±24), with an MVIC increase from immediately after the evoked fatigue protocol (206±24) to post-48 hrs (234±26), and with further increase from post-48 hrs (234±26) to post-72 hrs (246±28) ($p<0.05$). This further suggests that, after 48 hrs from the fatigue protocol, the MVIC values returned to baseline levels, indicating a recovery in strength for both limbs (Figure 11).
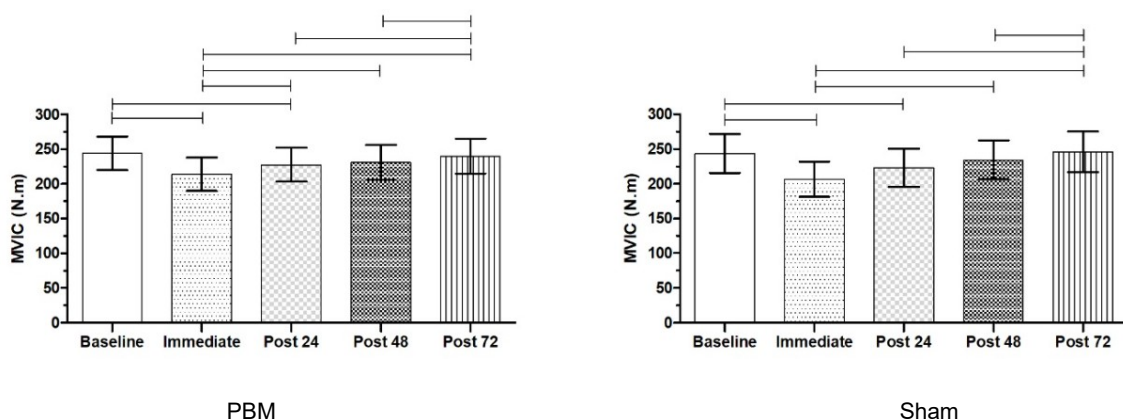
Figure 12. Behavior of MVIC between the four evaluation days. Horizontal bars represent between-moments differences.

There were no significant differences between limbs or interactions in the supramaximal twitch torque. Similar changes were observed in limbs who received PBM and PBM-sham. There was a decrease in twitch torque from baseline (38.6 ± 4.3 Nm) to immediately after fatigue (30.3 ± 3.2 Nm), with an increase from immediately after (30.3 ± 3.2 Nm) to post-24 hrs (36.1 ± 4.3 Nm), and from post-24 hrs (36.1 ± 4.3 Nm) to post-48 hrs (39.6 ± 4.0 Nm). In the PBM-sham limb, similar differences were observed, with a twitch torque decrease from baseline (35.3 ± 3.2 Nm) to immediately after fatigue (28 ± 3.1 Nm), with an increase from immediately after (28.0 ± 3.1 Nm) to post-24 hrs (34.4 ± 3.1 Nm), and from post-24 hrs (34.4 ± 3.1 Nm) to post-72 hrs (38.0 ± 3.6 Nm) ($p<0.05$) (Figure 12). Both limbs (PBM and PBM-sham) experienced a return of supramaximal evoked twitch torque to baseline values after 24 hrs after the fatigue protocol, suggesting a full recovery in their supramaximal evoked torque. These results also suggest that, although peripheral fatigue recovered 24 hrs after the NMES-evoked fatigue protocol, central fatigue recovered only 48 hrs post-fatigue.
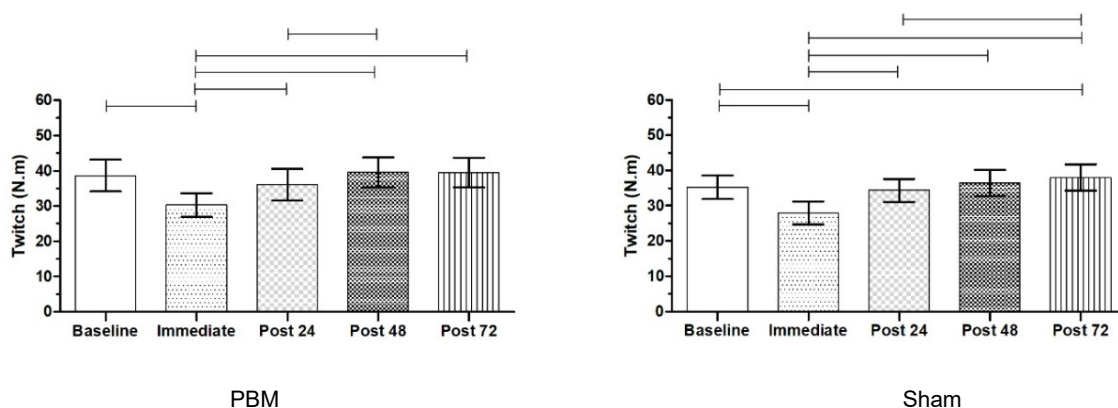
Figure 13. Behavior of supramaximal twitch torque between the moments. Horizontal bars indicate between-moments differences.

The evoked neuromuscular efficiency did not show any differences between limbs or interactions. In the PBM-limb, there was a decrease in efficiency from baseline (0.29 ± 0.03 Nm/mA) to immediately after fatigue (0.22 ± 0.03 Nm/mA), with an efficiency increase from immediately after to post-24 hrs (0.25 ± 0.03 Nm/mA), to post-48 and post-72 hrs (0.28 ± 0.03 Nm/mA). In the PBM-sham limb, similar changes were observed. There was a significant efficiency decrease from baseline (0.25 ± 0.02 Nm/mA) to immediately after fatigue (0.19 ±

0.02 Nm/mA), with an increase in efficiency from immediately after (0.19 ± 0.02 Nm/mA) to post-24 hrs (0.24 ± 0.03 Nm/mA), and to post-72 hrs (0.26 ± 0.02 Nm/mA) (p<0.05; Figure 13). Therefore, in both the PBM and PBM-sham limbs the neuromuscular efficiency decreased immediately after fatigue, with a quick recovery in the sham limb, returning to baseline values after 24 hrs, while in the PBM limb recovery occurred after 48 hrs of the fatigue protocol.
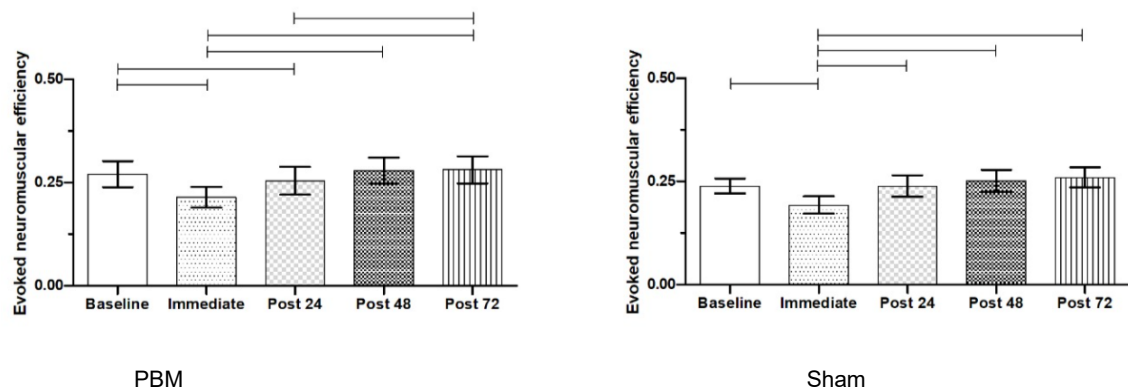
Figure 14. Behavior of evoked neuromuscular efficiency between the evaluation moments. Horizontal bars indicate between-moments differences.

When analyzing the fatigue protocol variables, we observed that the limbs were similar in all of them (Table 4).

Table 4. Between-limbs comparison of the outcome variables measured during the fatigue protocol. Values presented as mean ± SD

	PBM	SHAM	p-value
Total work (Nmxs)	10260±1802	8460±1806	0.326
N.mxs Fatigue index	-63.0±4.7	-62.0±5.9	0.862
% Work decline	-76.0±4.4	-73.6±5.8	0.999
Number contractions for 10% W MVIC	27.7±4.3	25.3±5.7	0.487

In the stair ascent and descent test, there was no difference in the VAS between the limbs, despite there being an interaction between them (Table 5). An increase in discomfort was observed in the PBM limb from baseline to immediately after fatigue during stair ascent and descent, and discomfort only returned to baseline values at post-72 hrs. In the PBM-sham limb, there was an increase in discomfort during stair ascent and descent from baseline to immediately after fatigue, and discomfort did not return to baseline values at post-

72 hours. In conclusion, PBM showed a faster recovery compared to the PBM-sham condition in reducing discomfort during stair ascent and descent.

When analyzing the performance in the SHT, no differences were observed between the limbs, and no interaction. We observed a decrease in SHT performance immediately after the fatigue protocol in both limbs, which subsequently recovered after 24 hours (Table 5).

Table 5. Functional tests performance and discomfort measured by the visual analogue scale (VAS).

		Baseline	Immediately after	P24	P48	P72	Limb	Moments	Interaction
VAS ascent	PBM	0.29 ± 0.08^{bcde}	1.32 ± 0.49^{ae}	0.87 ± 0.31^{a}	0.74 ± 0.13^{ae}	0.42 ± 0.08^{abd}	0.880	*0.001*	*0.030*
	SHAM	0.15 ± 0.02^{bcd}	1.02 ± 0.31^{a}	0.81 ± 0.18^{a}	1.10 ± 0.33^{a}	0.95 ± 0.41			
VAS descent	PBM	0.28 ± 0.05^{bcd}	1.26 ± 0.28^{ae}	0.76 ± 0.17^{a}	1.01 ± 0.24^{ae}	0.46 ± 0.11^{bd}	0.333	*0.001*	*0.008*
	SHAM	0.19 ± 0.04^{bcde}	1.34 ± 0.42^{a}	1.08 ± 0.33^{ad}	1.60 ± 0.45^{ac}	1.11 ± 0.36^{a}			
SHT	PBM	107 ± 9^{b}	104 ± 9^{a}	107 ± 10	107 ± 9	112 ± 10^{b}	0.741	*0.001*	0.149
	SHAM	111 ± 9^{b}	104 ± 9^{a}	117 ± 10	114 ± 10	113 ± 10			

Values presented as mean ± SD. The between-moments changes were represented by letters: a ≠ baseline; b ≠ immediately after; c ≠ P24; d ≠ P48; e ≠ P72. Statistically significant *p* values are in italics. SHT: Single Hop Test.

3.2. DISCUSSION

PBM is a form of light therapy that utilizes non-ionizing forms of light sources, including lasers and LEDs, in the visible and infrared spectrum. PBM is a technique that has been widely discussed for a long time, highlighting its numerous potential advantages and its potential to reduce muscle fatigue, muscle damage and enhance performance.

Here we conducted a randomized clinical trial, sham-controlled with blinded subjects, aimed at investigating if PBM can reduce neuromuscular fatigue, discomfort, and muscle damage, and improve functionality after a NMES-induced fatigue protocol consisting of 60 isometric knee extension evoked contractions in healthy young individuals. Based on the evidences obtained from the literature, we formulated the following hypotheses:

1. PBM would induce a smaller reduction in MVIC torque of the knee extensors compared to PBM-sham after the fatigue protocol;
2. PBM would lead to a smaller reduction in supramaximal twitch with fatigue;
3. PBM would increase the work done by the activated motor units as determined by a greater integral of the evoked torque-time curve;
4. PBM would lead to a greater total work during the fatigue protocol;

5. NMES-induced contractions would generate smaller muscle damage in the RF muscle when preceded by PBM;

6. The smaller fatigue induced by NMES after PBM intervention would lead to a smaller discomfort in the knee extensors after the fatigue protocol;

7. Similarly, PBM intervention would generate smaller delayed muscle soreness in the knee extensors and better performance in the SHT.

We did not observe any significant changes produced by PBM in these variables of interest. Therefore, we will try to address some of the possible explanations by contrasting our results with those existing in the literature regarding the use of PBM to reduce fatigue and muscle damage, and to improve functionality.

Preclinical studies have shown positive results in attenuating muscle damage, which were measured by a decrease in the tibial muscle's CK. Lopes-Martins et al. (2006) evaluated four groups, with a PBM continuous output power of 2.5 mW and a wavelength of 655 nm (visible red). The control group was not irradiated and the treatment groups received LLLT administered at 1.0 J/cm^2, 2.5 J/cm^2, and 5.0 J/cm^2. A greater reduction in CK was observed at 1.0 J/cm^2 and 2.5 J/cm^2. Leal Junior et al. (2010) also evaluated the tibial muscle in four groups, with a PBM frequency of 700 Hz, average output power of 15 mW, and a wavelength of 904 nm (infrared). The control group was not irradiated, and the treatment groups received LLLT administered at 0.1 J/cm^2, 0.3 J/cm^2, 1.0 J/cm^2, and 3.0 J/cm^2. The reduction in CK was observed at 904 nm (0.1 J and 1.0 J). In the study by Santos et al. (2014), the control group and the groups irradiated with LLLT were randomized and distributed according to the laser doses administered: 1, 3, or 10 J in the tibial muscle, using continuous mode and wavelengths of 660 nm (red), 830 nm (infrared), or 905 nm (infrared). A significant reduction in CK was observed at 660 nm (1.0 J) and 905 nm (1.0 J, 3.0 J, and 10 J). These results show evidence of a PBM effect in reducing muscle damage as measured by CK.

In fatigue studies (LOPES-MARTINS et al., 2006; LEAL JUNIOR et al., 2010; SANTOS et al., 2014), the time to reach 50% reduction in maximal force was longer in the PBM group compared to the control group or PBM-placebo group. PBM also produced positive results in terms of peak evoked torque, increased muscular work, and decreased muscle damage in animals receiving

PBM compared to those in the PBM-placebo group (SANTOS et al., 2014). However, it is important to consider that the methodology of animal models is significantly different from human models. More specifically, the PBM applied over the skin might lose part of its effects due to several barriers (e.g., skin, subcutaneous fat thickness) for light propagation, whereas this does not seems to occur in preclinical trials. Nevertheless, it is worth noting that preclinical studies provide valuable insights into the potential effects of PBM due to possible direct measurements that are not possible in clinical trials, but further research is needed to confirm these findings in human subjects.

In clinical trials that evaluated the PBM effects over fatigue, Cunha et al. (2020) used PBM with a wavelength of 850 nm, continuous mode, with a power of 50 mW per diode (150 mW total). The total energy per limb was 36 J, applied bilaterally to the quadriceps femoris muscles. The exposure time was 40 seconds per application, and it was applied immediately before the training session. Gorgey et al. (2008) used a continuous output power of 500 mW and a wavelength of 808 nm. The total energy provided was 3 J for 5 minutes (low dose) or 7 J for 10 minutes (high dose), applied to the knee extensor muscle group. PBM was applied immediately before electrical stimulation of the muscles. Cieśliński et al. (2018) used a wavelength of 830 nm and a power of 200 mW. PBM was applied in six areas: 5 cm above the midpoint between the anterior superior iliac spine and the base of the patella and 5 cm below that point for RF, two areas above the VM muscle belly, and two areas above the VL muscle belly. A dose of 30 J was applied to each area. Although PBM did not seem to produce a significant effect in reducing fatigue, it is important to note that these studies, different from our study, did not evaluate muscle damage, which is an important and undesired outcome from evoked contractions, as muscle damage reduces evoked torque production thereby reducing the mechanical work generated by the evoked contractions.

In our study, we used PBM with a wavelength of 810 nm, with an energy output of 200 mW in continuous frequency (BARONI et al., 2015). Eight application points were used, two in VM, three in the VL and three in RF (Figure 2). Each point was treated for 30 seconds, with 30 J per location. Despite using the PBM dose suggested as efficient by Leal-Junior et al. (2019), no differences were observed between the active PBM and PBM-sham limbs (Table 2). Our

results are consistent with those of the study by Jówko et al. (2019), who used PBM with a continuous output, wavelength of 830 nm, optical output/power of 200 mW, and energy of 30 J per area in six areas (BARONI et al., 2010). The study was divided in part I, in which group A received NMES preceded by LLLT, while group B received NMES preceded by sham LLLT. A reverse procedure was applied after an eight-day washout period (part II). They assessed muscle damage using biomarkers, and plasma CK activity did not significantly change in the LLLT compared to the sham-LLLT intervention (no main effects found for plasma CK activity). All studies administered the PBMT immediately before electrical stimulation of the muscles, and, even though our study had more PBM application points, the same energy in J was applied but with a different wavelength, but we did not observe any changes in muscle damage with PBM in the different evaluations that were applied.

The studies conducted by Cunha et al. (2020) and Gorgey et al. (2008) did not assess muscle pain, which again is a strength of our study as we evaluated it using the Visual Analog Scale (VAS) in a seated position. When assessing pressure pain threshold in the VL, RF, and VM muscles, no difference was observed between the limbs. Nevertheless, significant differences were observed between different time points, indicating an interaction. In the VL, we observed a significant decrease in pressure pain threshold in both groups, which persisted after a 72-hour period, indicating sustained impairment in pain sensitivity. In the RF, a significant decrease in pressure pain threshold was also observed in both groups, but the values returned to baseline levels within 48 hours. The pressure pain threshold in the VM muscle decreased after the fatigue protocol only in the PBM group, while it remained unchanged in the sham group. Our results do not fully agree with the findings of Cieśliński et al. (2018), as no significant differences in pain intensity were found between the groups at later stages during the pressure test ($p > 0.05$) or the squat test (VAS). Similarly, Jówko et al. (2019) reported that muscle pain after NMES preceded by LLLT and sham-LLLT was not statistically significant. In our study, significant differences were observed at different time points, suggesting a different behavior in VAS scores between the PBM-treated limb and the sham limb. This could be attributed to our study having more areas where PBM was applied compared to the aforementioned studies. Interestingly, we observed that both limbs experienced an immediate increase in

discomfort after fatigue, which did not return to baseline values within 72 hours (Table 3). This suggests that other physiological events determined by NMES during the fatigue protocol did not fully recover, despite the recovery in maximal knee-extensor force production.

In the study by Jówko et al. (2019), MVIC showed a significant decrease in both the PBM group (p<0.05) and the placebo group (p<0.05) with NMES-induced fatigue. Similar results were observed by Cieśliński et al. (2018), who found a similar decrease in strength between LLLT and sham-LLLT. Gorgey et al. (2008) did not observe a difference in torque reduction after control and both LLLT attempts. This is consistent with our findings as we observed a similar pattern of strength decrease with fatigue that was comparable between the PBM limb and the PBM-sham limb (Figure 11). In both studies, full recovery of muscle strength did not occur within 96 hours, which differs from our results as both limbs returned to pre-fatigue MVIC levels 48 hours after the fatigue protocol. The absence of a group-by-time interaction in our MVIC results suggests that PBM did not have an effect on central fatigue.

Another important aspect of our study was the evaluation of supramaximal twitch torque, where no significant differences between limbs or interactions were observed. These results also suggest that, while peripheral fatigue recovered 24 hours after NMES-induced fatigue protocol, central fatigue was only recovered 48 hours after fatigue. We also assessed evoked neuromuscular efficiency, which showed no differences between groups or interactions, both in the PBM limb and the PBM-sham limb. Neuromuscular efficiency decreased immediately after fatigue but had a faster recovery in the PBM-sham group. It is worth noting that none of the previously mentioned studies conducted this evaluation.

In the study conducted by Gorgey et al. (2008), there was no difference in fatigue between the attempts of LLLT applied at 3 J for 5 minutes and 7 J for 10 minutes (p=0.10). This is consistent with our study, as we also observed that the two groups were similar in all aspects. This raises a question because even when using a higher dosage, like in our study, we had the same findings. Therefore, what exactly is the correct dose to produce a PBM effect in human studies evaluation NMES-induced fatigue?

In the study conducted by Cunha et al. (2020), lower limb strength increased in both PBM therapy groups (mean difference = 1.1, 95% CI = 0.3 to

2). In addition, the jump capacity improved between pre-training and post-training for the PBM group (mean difference = 2.9, 95% CI = 0.5 to 5.3), which was not observed for the PBM-placebo group. This finding partially resembles our study results, as the limb that received PBM demonstrated an increase in SHT performance between immediately after and post-72 hrs of the fatigue test, indicating that there was a faster performance recovery after the fatigue protocol for the PBM limb. However, surprisingly the PBM-sham limb showed a decrease in performance immediately after the protocol, which recovered 24 hours after the fatigue test, and we do not have a clear explanation for these between-limbs difference in SHT performance.

Different studies have shown no significant changes in neuromechanical outcomes in different sports where the effects of PBM were evaluated. Leal Junior et al. (2009) used a wavelength of 830 nm, continuous frequency, total energy delivered of 40 J for volleyball players, 30 J for soccer players, and they used 10 points conducted with football and volleyball athletes. No difference in muscle work was observed between active PBM and PBM-placebo. In judo athletes, PBM showed similar results to placebo-PBM in terms of perceived fatigue and fatigue index after the protocol (ORSATO et al., 2019). Similar findings were observed for perceived fatigue in runners, where no between-group difference was found (LANFERDINI et al., 2021). These results support our findings, as we also did not find any significant differences between the limbs in terms of fatigue, recovery from fatigue and improvement in functional test performance.

However, several studies have observed beneficial PBM effects in sports performance and fatigue. In amateur football players, PBM had beneficial effects on eccentric peak torque of the hamstrings, hamstring-to-quadriceps torque ratio, and countermovement jump height (DORNELLES et al., 2019). Pinto et al. (2016) observed that perceived fatigue and fatigue index were significantly lower in rugby players who received PBM compared to placebo-PBM. De Marchi et al. (2019) applied PBM before futsal matches and observed improved performance and accelerated player recovery. In the study by De Oliveira et al. (2017) conducted with football players, PBM increased muscle strength endurance (MVIC) and reduced delayed onset muscle soreness (DOMS).

These results differ significantly from our findings, but it should be noted that the methodology and PBM dosage in these studies were quite different from

ours. Dornelles et al. (2019) used a flexible pad, so it was attached properly to the convexity of the back of the thigh. They used a wavelength of 880nm, continuous frequency, applied for 60 s, totaling an energy dose of 300 J in each of the volunteer's hamstring muscles before exercise. Pinto et al. (2016) used a wavelength of 905 nm, a frequency of 250 Hz, total dose per site of 30J, applied in 9 sites on the knee extensor muscles, with 6 sites on knee flexors, and 2 sites on the calf of both lower limbs. De Marchi et al. (2019) used a wavelength of 905 nm, a frequency of 250Hz, and a total dose per site of 30J, performed at nine different knee extensor and hip flexor muscle locations, six knee-flexor muscle and hip extensor muscle locations, and two plantar flexor muscle locations of both lower limbs. Oliveira et al. (2017) administered PBM exactly 2 min after the pre-exercise, with a wavelength of 810 nm, a continuous frequency output, energy per site of 50 J, applied at six different sites of the knee extensor muscles (two medial, two lateral, and two central points). These different parameters used in the above-mentioned studies raise questions about how dosage can significantly affect PBM outcomes and what the ideal dosage might be to achieve specific objectives. Despite Leal Júnior et al. (2019) have proposed clinical and scientific recommendations for the use of PBM therapy in exercise performance enhancement and post-exercise recovery, we think that future research should focus on finding the correct dosage and standardizing it for future studies, as apparently, very few studies have evaluated the effects of PBM dose in fatigue and muscle damage.

LIMITATIONS

We encountered several limitations in conducting this study. The main limitation was our inability to achieve the required sample size of 22 participants, as we were only able to recruit 12 participants. Therefore, when we achieve the calculated sample size, some of the results here presented may change, and therefore we consider our results as preliminary results.

The closure of the laboratory during the pandemic significantly affected our study. Many of the equipment, including the multifunctional electrical stimulator used in the project, suffered damage and required repairs. The stimulator malfunctioned three times during data collection, and when it returned from the

Biomedical Engineering Department from the Hospital de Clínicas, there was significant variability in the NMES curves. Due to this variability, we believe that we were unable to induce the desired level of fatigue in this study, and the electrical stimulator will probably need a more rigorous technical evaluation due to the long time that it remained unused and in a very humid environment.

Another limitation was the use of both limbs of the participants in the study. Initially, this was intended to be advantageous as it allowed for a within-subject comparison (i.e., the subject being his own control). However, during the course of the project, we realized that this became a limitation due to the time commitment required from participants. The laboratory sessions extended over a period of 9 days, conducted exclusively during the evening hours, which made it challenging to recruit participants, and several participants declined to participate due to the large number of experimental days.

For that reason, the limitations of this study include a smaller sample size than originally planned, equipment malfunctions, and logistical difficulties in participant recruitment and scheduling. These limitations were taken into consideration when interpreting the results and it emphasizes the need for further research with larger sample sizes and well-functioning equipment.

Based on our results, we can assert that PBMT does not demonstrate positive effects when applied before an NMES fatigue protocol. Therefore, based on these findings, we can conclude that, in the clinical setting, the use of PBM is not essential, as no significant changes were observed in either pain reduction or performance enhancement when applying NMES.

CONCLUSIONS

No significant differences were observed among the limbs (i.e., PBM and PBM-sham) in discomfort, damage, and functional test performance. These findings suggest that PBM does not have a significantly impact on its clinical applicability when the goal is to reduce fatigue, muscle damage and discomfort and to improve functionality.

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COMPLEMENTARY MATERIAL

Effect sizes of post hoc tests

		BL X IP	BL X P24	BL X P48	BL X P72	IP X P24	IP X P48	IP X P72	P24 X P48	P24 X P72	P48 X P72
					Effect size - *d* Cohen						
VAS seated	PBM	-0,737	-0,697	-0,481	-0,458	0,500	0,476	0,598	0,000	0,247	0,186
	PLACEBO	-0,762	-0,820	-0,870	-0,606	0,277	0,250	0,635	-0,039	0,578	0,627
AlgVL	PBM	0,360	0,432	0,596	0,350	0,121	0,295	0,037	0,152	-0,072	-0,222
	PLACEBO	0,252	0,135	0,083	0,275	-0,147	-0,199	0,021	-0,060	0,173	0,226
AlgRF	PBM	0,341	0,361	0,357	0,022	0,073	0,062	-0,280	-0,011	-0,309	-0,304
	PLACEBO	0,253	0,174	-0,178	-0,101	-0,084	-0,462	-0,375	-0,375	-0,291	0,079
AlgVM	PBM	0,478	0,316	0,337	0,242	-0,082	-0,071	-0,174	0,011	-0,076	-0,090
	PLACEBO	0,140	0,013	-0,048	-0,118	-0,132	-0,195	-0,258	-0,064	-0,134	-0,074
MVIC	PBM	0,361	0,194	0,154	0,047	-0,164	-0,198	-0,305	-0,037	-0,143	-0,104
	PLACEBO	0,399	0,216	0,095	-0,024	-0,178	-0,298	-0,414	-0,119	-0,236	-0,117
Twitch	PBM	0,610	0,162	-0,064	-0,059	-0,428	-0,708	-0,706	-0,231	-0,226	0,005
	PLACEBO	0,144	-0,717	-0,313	-0,505	-0,874	-0,454	-0,647	0,364	0,164	-0,186
Neuromsucular efficiency	PBM	0,574	0,155	-0,074	-0,093	-0,376	-0,646	-0,661	-0,224	-0,242	-0,020
	PLACEBO	0,003	-0,960	-0,433	-0,799	-0,905	-0,409	-0,750	0,459	0,163	-0,306
VAS ascending	PBM	-1,272	-0,989	-0,949	-0,773	0,355	0,001	0,882	-0,290	0,538	0,676
	PLACEBO	-1,007	-0,983	-1,168	-0,766	0,420	0,366	0,453	-0,090	0,069	0,154
VAS descending	PBM	-1,306	-1,110	-0,991	-0,810	0,198	0,037	0,854	-0,134	0,641	0,650
	PLACEBO	-0,806	-0,253	-0,350	-0,175	0,463	0,307	0,544	-0,118	0,071	0,184
Single HOP	PBM	0,130	0,002	-0,045	-0,092	-0,129	-0,171	-0,223	-0,048	-0,095	-0,043
	PLACEBO	0,311	0,054	0,502	-0,018	-0,256	0,183	-0,312	0,444	-0,069	-0,491

AlgVL = Algometer vastus lateralis
AlgRF = Algometer rectus femoris
ALgVM = Algometer vastus medialis
MVIC = Maximal Voluntary Contraction
BL = Baseline
Ip = Immediately post
P24
P48
P72