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FOTOTERAPIA NA MELHORA DO DESEMPENHO E NA RECUPERAÇÃO MUSCULAR: COMPARAÇÃO COM CRIOTERAPIA E ENTRE DIFERENTES FORMAS DE EMISSÃO DE LUZ

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Orientador: Profa. Dra. Mirian Salvador

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Prof. Dr. Rodrigo Labat Marcos

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Sumário

Lista de Abreviaturas

AMP	adenosina monofosfato
AsGaAl	arseneto de gálio e alumínio
AsGa	arseneto de gálio
ATP	adenosina trifosfato
CAT	catalase
CCO	citocromo c oxidase
CK	creatina quinase
CK-MM	isoforma da creatina quinase
CK-MB	isoforma da creatina quinase
CK-BB	isoforma da creatina quinase
COX2	ciclooxigenase 2
Cu	cobre
CVM	contração voluntária máxima
DNA	ácido desoxirribonucléico
ECSOD	superóxido dismutase extracelular
ER	espécies reativas
ERA	área de irradiação efetiva

FBM	fotobiomodulação
GPx	glutationa peroxidase
HeNe	hélio neônio
IL-1β	interleucina-1 Beta
IL	Interleucina
IV	Infravermelho
LASER	*light amplification by stimulated emission radiation*
Led	diodo emissor de luz
LBP	laserterapia de baixa potência
LDH	lactato desidrogenase
MDA	malondialdeído
NAD^+	nicotinamida adenina dinucleotídeo
NADH	nicotinamida adenina dinucleotídeo (forma reduzida)
NADP	nicotinamida adenina dinucleotídeo fosfato
NADPH	nicotinamida adenina dinucleotídeo fosfato (forma reduzida)
NF-kB	fator nuclear kappa B
PC	proteínas carboniladas
PGE_2	prostaglandina E2
PIFM	protocolo indutor de fadiga muscular

PMM	potencial de membrana mitocondrial
RL	radical livre
ROS	espécies reativas de oxigênio
SOD	superóxido dismutase
SODCuZn	superóxido dismutase cobre zinco
SODMn	superóxido dismutase maganês
TBARS	espécies reativas ao o ácido tiobarbitúrico
TNFα	fator de necrose tumoral alfa
$VO_{2\ max}$	consumo máximo de oxigênio
VO2	consumo de oxigênio
XDH	xantina desidrogenase
XO	xantina oxidase

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ESTRUTURA DA TESE

A presente tese está estruturada da seguinte forma: introdução geral, objetivos do trabalho (geral e específicos), 3 capítulos com artigos e discussão geral. As conclusões obtidas são apresentadas, seguida das perspectivas, referências bibliográficas e os anexos.

A introdução da tese apresenta generalidades sobre a fotobiomodulação, suas características e propriedades biológicas, mecanismo de ação, aspectos sobre a atividade física e tipos de luz utilizada na prevenção e reabilitação. O Capítulo I apresenta o trabalho publicado na revista "Lasers in Medical Science", o qual abordou a capacidade de recuperação muscular proporcionada pela laserterapia, pela crioterapia e pela associação de ambas terapias.

O Capítulo II se refere ao artigo publicado na revista "Journal Athletic Training", que abordou a eficiência e utilização de diferentes equipamentos disponíveis no mercado com o objetivo de prevenir a fadiga muscular e diminuir o dano muscular oxidativo ou não.

O Capítulo III se refere ao artigo publicado na revista "Lasers in Medical Science", que abordou a utilização da fotobiomodulação de melhor resposta no capítulo II para verificarmos seus efeitos em condições reais de jogo, em atletas profissionais.

A discussão geral aborda os resultados dos capítulos apresentados, a relação entre os mesmos e a importância desse estudo como contribuição científica. Finalmente é apresentada a conclusão final do trabalho desenvolvido e as perspectivas.

Os anexos I, II e III contêm publicações desenvolvidas durante o doutorado, que não estão diretamente relacionadas ao presente trabalho, mas que foram utilizadas na discussão deste.

O Anexo I contém artigo científico publicado na revista "Photomedicine Laser Surgery", no qual foram avaliados os efeitos de doses diferentes de fotobiomodulação aplicadas antes do exercício. O Anexo II compreende artigo publicado na revista "Lasers in medical Science" e propôs aprofundar a relação de efeitos da fotobiomodulação utilizando-se a laserterapia ou os diodos emissores de luz (LED's) como modo de aplicação. O anexo III compreende carta ao editor publicado na revista "Lasers in medical Science" e propôs aprofundar a relação e discussão de efeitos da fotobiomodulação utilizando-se a laserterapia de alta potência como modo de aplicação.

RESUMO

A fotobiomodulação (FBM) pode ser utilizada isoladamente ou como coadjuvante no tratamento de diversas patologias. Estudos recentes sugerem que o uso profilático de laser/luz de baixa potência tem efeitos ergogênicos no desempenho atlético e na recuperação pós-atividade. Diante disso, o objetivo deste trabalho foi avaliar os efeitos proporcionados por diferentes emissões de luz, com a combinação de diferentes fontes, e avaliar o efeito da FBM e da crioterapia como formas de recuperação muscular pós-exercício de alta intensidade, bem como verificar o efeito da FBM aplicada antes de uma situação real de jogo. Para tal, 86 voluntários foram incluídos em três estudos randomizados, controlados (placebo) e duplo-cegos. No primeiro, quarenta voluntários foram aleatoriamente distribuídos em cinco grupos: Grupo Placebo; Fototerapia, Crioterapia, Crioterapia-Fototerapia, Fototerapia-Crioterapia. Todos os indivíduos realizaram quatro sessões, intervaladas por um período de 24 horas, onde foram submetidos à avaliação isométrica (CVM) e coletas sanguíneas em momentos pré-exercício, 5 e 60 min. pós-exercício. O protocolo de indução a fadiga muscular (PIFM) ocorreu após as coletas pré-exercício. Nas sessões de 24h, 48h e 72h realizaram-se apenas as coletas sanguíneas e as CVMs. A FBM e a crioterapia foram aplicadas sempre e somente 2 minutos após a realização do teste de CVM pós 5min. No segundo estudo, quarenta indivíduos masculinos foram randomizados em quatro grupos: placebo, laser/luz contínua de alta potência, laser/luz contínua de baixa potência ou um laser/luz pulsada de baixa potência (composto de lasers e LEDs). Uma dose única de 180 J ou placebo foi administrada ao quadríceps. Foi avaliado CVM, atraso no início da dor muscular e atividade da creatina quinase (CK) desde a linha de base até 96 horas após o PIFM. Já para o terceiro estudo, a pesquisa incluiu seis atletas profissionais de futsal. Em cada partida oficial os atletas receberam a aplicação de FBM ou placebo, 40 min antes da partida. Foi coletado sangue basal, imediatamente após o final da partida e 48h após. Além disso, os jogos foram filmados e os vídeos analisados para quantificar o tempo que os atletas permaneciam em quadra e a distância percorrida durante o jogo por cada atleta. Os marcadores bioquímicos avaliados foram CK, lactato desidrogenase, lactato e danos oxidativos em lipídios e proteínas. Obtivemos como principais resultados: os exercícios propostos geraram diminuição na CVM, bem como elevaram significativamente os valores basais de creatina quinase, lactato desidrogenase, lactato e danos oxidativos a lipídios e proteínas. Quando comparamos a crioterapia com a FBM,

podemos verificar que o grupo fototerapia obteve os melhores resultados na recuperação muscular. Quando optamos por testar tipos diferentes de emissão de luz, observamos melhores resultados no grupo laser / luz pulsada de baixa potência. Assim escolhemos este tipo de luz para testarmos em condições de jogo e obtivemos uma melhora na performance e na recuperação muscular de atletas profissionais submetidos a partidas oficiais de futsal. Sendo assim, concluímos que a utilização de FBM é mais eficiente que a crioterapia na recuperação muscular, que dentre as possibilidades de FBM a luz pulsada de baixa potência é a melhor opção. Verificando-se essas observações em situações e condições reais, fora de ambiente laboratorial, é possível concluir que a aplicação prévia de FBM pode melhorar o desempenho e acelerar a recuperação de jogadores de futsal de alto nível.

Palavras-chave: Fototerapia, crioterapia, estresse oxidativo, dano muscular, Recuperação muscular, Performance musculo esquelética

ABSTRACT

Photobiomodulation (PBMT) can be used alone or as adjuvant in the treatment of various pathologies. Recent studies suggest that prophylactic use of low-power laser / light has ergogenic effects on athletic performance and post-activity recovery. Therefore, the objective of this study was to evaluate the effects of different light emissions, with the combination of different sources, and to evaluate the effect of PBMT and cryotherapy as forms of post-exercise muscle recovery of high intensity, as well as verify the effect of the PBMT applied before a real game situation. For this, 86 volunteers were included in three randomized, placebo-controlled, and double-blind studies. In the first, forty volunteers were randomly assigned to five groups: Placebo Group; Phototherapy, Cryotherapy, Cryotherapy-Phototherapy, Phototherapy-Cryotherapy. All subjects performed four sessions, 24-hour intervals, where they were submitted to isometric evaluation (MCV) and blood samples at pre-exercise moments, 5 and 60 min. post-exercise. The muscle fatigue induction protocol (MFIP) occurred after the pre-exercise collections. In the 24h, 48h, and 72h sessions, only blood collections and MCVs were performed. PBMT and cryotherapy were always applied and only 2 minutes after the MCV test after 5min. In the second study, forty male subjects were randomized into four groups: placebo, laser / high power continuous light, laser / low power continuous light or a low power laser / pulsed light (composed of lasers and LEDs). A single dose of 180 J or placebo was given to the quadriceps. MCV, delayed onset of muscle pain and creatine kinase (CK) activity were assessed from the baseline to 96 hours after MFIP. Already for the third study, the research included six professional futsal athletes. At each official match the athletes were given PBMT or placebo application 40 min before departure. Baseline blood was collected, immediately after the end of the match and 48 hours after. In addition, the games were filmed and the videos analyzed to quantify the time that the athletes remained in court and the distance covered during the game by each athlete. The biochemical markers evaluated were CK, lactate dehydrogenase, lactate and oxidative damage in lipids and proteins. We obtained as main results: the proposed exercises generated a decrease in MCV, as well as significantly elevated basal creatine kinase, lactate dehydrogenase, lactate and oxidative damage to lipids and proteins. When comparing cryotherapy with PBMT, we can verify that the phototherapy group obtained the best results in muscle recovery. When we chose to test different types of light emission, we observed better results in the low-

power laser / pulsed light group. Thus, we chose this type of light to test under conditions of play and we obtained an improvement in the performance and muscle recovery of professional athletes submitted to official futsal matches. Thus, we conclude that the use of PBMT is more efficient than cryotherapy in muscle recovery, that among the possibilities of PBMT low power pulsed light is the best option. By verifying these observations in real situations and conditions outside of the laboratory environment, it is possible to conclude that prior application of PBMT can improve performance and accelerate the recovery of high-level futsal players.

Keywords: Phototherapy, cryotherapy, oxidative stress, muscle damage, muscle recovery, skeletal muscle performance

1. Introdução

Fotobiomodulação

O termo *LASER* é um acrônimo para *light amplification by stimulated emission radiation*, ou "amplificação da luz por emissão estimulada de radiação", sendo esse o princípio que baseou sua criação (Schawlow, 1995).

A luz LASER difere da luz comum devido à suas características específicas, como a monocromaticidade, coerência e colimação (Schawlow, 1995; Hamblin, 2017). As ondas emitidas são sincronizadas em relação ao tempo e ao espaço, viajam ordenadamente e em amplitudes iguais. A colimação é obtida pela unidirecionalidade do laser, que possui um feixe de fótons paralelo ao eixo do tubo que o produz, possuindo uma divergência angular muito pequena e concentrando toda a energia emitida em um único ponto (Kitchen & Bazin, 1998). Devido a essas características, os equipamentos que emitem luz laser são capazes de transferir uma considerável quantidade de energia luminosa aos tecidos, com alta precisão e eficiência (Enwemeka, 2009; Hamblin, 2017).

Os lasers podem ser classificados em dois grupos: lasers de alta potência (superiores a 1 W), os quais são usados para finalidades cirúrgicas como cortes, carbonização ou desnaturação de proteínas através de efeito fototérmico (Chavantes & Jatene, 1990), e lasers de baixa potência (inferiores a 1 W), utilizados para reparação tecidual, alívio de dor e a obtenção de efeitos antiinflamatórios. Dentre os lasers de baixa potência existem diferentes configurações de comprimento de onda, variando do vermelho (visível) ao infravermelho (invisível). Esses raios são produzidos por misturas de gases ou compostos químicos sintéticos, incluindo hélio neônio (HeNe), arseneto de gálio e alumínio (AsGaAl), e arseneto de gálio (AsGa) (Beckerman et al., 1992). Apesar

da onda vermelha ter sido a mais estudada, a luz infravermelha tem maior poder de penetração nos tecidos (Enwemeka, 2001; Enwemeka, 2009).

A fim de obter-se bons resultados, o tratamento com a laserterapia de baixa potência (LBP) deve considerar a potência, a energia, a área de irradiação, a densidade de potência e de energia e o comprimento de onda utilizados (Enwemeka, 2009). A potência (Equação 1) é uma medida que indica a quantidade de energia aportada por unidade de tempo. Atualmente, esse parâmetro é fixo e invariável nos aparelhos terapêuticos de LBP (Enwemeka, 2009, Huang et al., 2009, Huang et al., 2011).

(1) Potência (W) = energia (J) / tempo (s)

A energia da LBP (Equação 2) corresponde à quantidade de energia empregada durante a aplicação da terapia (dose) (Enwemeka, 2009).

(2) Energia (J) = potência x tempo (s)

A área de irradiação corresponde à área de secção transversa do aplicador da fibra óptica, também conhecido como área de irradiação efetiva (ERA), desde que a técnica utilizada para a aplicação garanta o contato com a pele (Enwemeka, 2009). A densidade de potência (Equação 3) é definida como a potência de saída do equipamento pela ERA do mesmo (Enwemeka 2009, Huang et al., 2009, Huang et al., 2011).

(3) Densidade de potência (W/cm^2) = potência (W) / ERA (cm^2)

A densidade de energia (Equação 4) é a quantidade total de energia entregue ao tecido (fluência) pela ERA do equipamento (Enwemeka, 2009, Huang et al., 2009, Huang et al., 2011).

(4) Densidade de Energia (J/cm^2) = $\frac{\text{potência (W) x tempo (s)}}{\text{ERA } (cm^2)}$

Sabe-se que o comprimento de onda (nm) da LBP equivale à distância entre dois sucessivos picos do feixe luminoso. Esta variável é um fator determinante para os efeitos fisiológicos produzidos pela LBP, pois a especificidade de absorção para um dado comprimento de onda determina quais os tipos de tecidos que irão absorver preferencialmente a radiação incidente, assim como a profundidade de penetração da mesma. Dessa forma, a avaliação correta de cada situação na qual a laserterapia será empregada é de extrema importância. Devem ser consideradas, cuidadosamente, a dose a ser utilizada por sessão de tratamento, a dose cumulativa total e a freqüência do tratamento (Enwemeka, 2009; Hamblin, 2017).

A LBP pode ser utilizada isoladamente ou como coadjuvante no tratamento, podendo ser aplicada uma vez e/ou várias vezes durante uma ou mais semanas. Ela não possui efeitos térmicos e sim fotoquímicos, sendo que, esse fenômeno foi pioneiramente publicado por Ender Mester, em 1966, que conduziu um experimento para testar se a radiação de laser poderia causar câncer em camundongos. Ele dividiu os camundongos em dois grupos e um dos grupos recebeu a aplicação de LBP (694nm) nas costas. Esse grupo não desenvolveu o câncer, entretanto os pelos (que foram raspados para a aplicação de LBP) cresceram mais rapidamente do que os do grupo não tratado. Mester chamou isso de "Bioestimulação Laser" (Huang et al., 2009; Hamblin, 2017). Porém, devido ao efeito dose-dependente da LBP, tanto a estimulação quanto a inibição do metabolismo celular são possíveis de acontecer. Assim, atualmente, assume-se a existência de uma "janela terapêutica" de ação estimulatória da LBP, em que a dose a ser aplicada é definida dependendo da patologia e dos objetivos a serem alcançados (Bjordal et al., 2006; Vanin et al., 2016b).

Além disso, há alguns anos inúmeros trabalhos têm relatado o efeito cicatrizante da LBP em feridas (Al-Watban et al., 2007; Fioro et al. 2017), tecidos tendinosos

(Reddy et al., 1998, Haslerud et al. 2017) e musculares (Bibikova & Oron, 1994; Basford et al., 1999; Amaral et al., 2001; Rodrigues et al. 2014), além do seu efeito analgésico (Ferreira et al., 2005; Basford, 1993; Martins et al., 2016) e anti-inflamatório (Pessoa et al., 2004; Basford, 1995; Naterstad et al., 2017). Relatos apontam que, a LBP é capaz de estimular a atividade celular e levar a liberação de fatores de crescimento através dos macrófagos (Woodruff et al., 2004, Fernandes et al., 2015), induzindo a proliferação de queratinócitos, angiogênese, ativação dos mastócitos e degranulação (Chagas-Junior, 2004; Fioro et al. 2017), as quais podem acelerar a cicatrização (Sato et al., 2000; Mendez et al., 2004; Al-Watban et al., 2004; Nascimento et al., 2004; Fioro et al. 2017).

Hamblin (2017) afirma que a LBP é capaz de modular a dor inflamatória, reduzindo marcadores de inflamação (PGE_2 ,COX2, IL-1β, TNFα), o fluxo de células neutrófilas ao local da inflamação, o estresse oxidativo, a formação de edema e a hemorragia. Algumas pesquisas em laboratório realizadas em ratos sugerem que a LBP pode tornar-se uma alternativa à terapia antiinflamatória com uso de fármacos (Campaña et al., 1999; Albertini et al., 2004; Naterstad et al., 2017). Emanet et al. (2010), em estudo que avaliou os efeitos da LBP no tratamento (cinco vezes por semana durante três meses) da epicondilite lateral crônica em humanos, verificaram que, além da diminuição dos sintomas da patologia no tratamento a longo prazo, a LBP não apresentou nenhum efeito adverso.

Estudos pioneiros avaliaram a LBP de 655nm e 904nm, aplicada anteriormente a realização de protocolo de contrações musculares induzidas por estimulação elétrica, mostrando a eficácia desta terapia em atenuar a fadiga em ratos e diminuir a concentração de creatino quinase (CK) no músculo (Lopes-Martins et al., 2006; Leal Junior et al., 2010a).

Sabe-se que uma demonstração de fadiga pode decorrer da diminuição da força muscular máxima, bem como de uma redução na resposta muscular, acarretando perda de rendimento durante o exercício (Sesboüé & Guincestre, 2006). Terrados & Fernández (1997) relatam que a fadiga pode ocorrer tanto de maneira local, afetando um músculo ou um grupo muscular, como de maneira global, afetando todo o organismo do indivíduo.

Estudos realizados com atletas de voleibol, tendo como objetivo averiguar os efeitos da aplicação prévia de LBP ao exercício de curta duração e alta intensidade (protocolo de exercícios de contrações voluntárias máximas), verificaram que quando os atletas receberam a aplicação da LBP, o número de contrações realizadas aumentou, bem como o tempo total de duração do exercício, não havendo alterações das concentrações de lactato sanguíneo (Leal Junior et al., 2008, 2010b).

Leal Junior et al. (2009b, 2009d) observaram que a aplicação de LBP em humanos, antes da realização do teste de Wingate (protocolo de exercício de alta intensidade), inibiu o aumento dos níveis de CK pós-exercício, bem como acelerou a remoção de lactato sanguíneo. Esses resultados indicam que a LBP aplicada antes do exercício pode proteger os músculos contra danos e acelerar o processo de recuperação muscular após exercício de alta intensidade.

Já em exercícios excêntricos, Baroni et al., (2010) observaram os efeitos do tratamento prévio com LBP, em humanos, com exercício de extensores de joelho, sobre o dano muscular e a capacidade funcional do músculo exercitado até 48 horas após o exercício. Verificou-se que a LBP foi capaz de diminuir os efeitos deletérios do dano muscular, além de reduzir o incremento dos níveis séricos de CK e lactato desidrogenase (LDH), enzima marcadora de dano muscular (el-Mallakh et al., 1992).

Até dado momento não existiam estudos acerca dos efeitos da LBP sobre o exercício, quando aplicada a um conjunto de grupo musculares, sendo assim, De Marchi et al. (2012) propuseram-se a avaliar tal situação no desempenho do exercício, estresse oxidativo e estado muscular em humanos. Foi possível observar que o uso de LBP antes do exercício de corrida de intensidade progressiva aumenta o desempenho, diminui o estresse oxidativo e dano muscular induzido pelo exercício, sugerindo que a modulação do sistema redox pela LBP pode estar relacionado ao atraso na fadiga do músculo esquelético observada após o uso desta terapia.

Nesta mesma época, iniciaram-se estudos para investigar se outras formas de emissão de luz eram capazes de gerar efeitos parecidos como os encontrados com a LBP, além disso, outros comprimentos de luz (azul e verde) também começaram a ser estudados (Hamblin, 2017). Sendo assim, e comprovando-se que com a utilização de diodo emissor de luz (LED) para aplicação da terapia, também é possível atingir efeitos benéficos (Antonialli et al., 2014; Leal Junior, 2015), uma decisão de consenso (Anders et al., 2015) foi tomada para usar a terminologia fotobiomodulação (FBM), uma vez que o termo LBP tornou-se muito subjetivo, pois é conhecido que os laser não são necessários, LEDs não-coerentes funcionam igualmente bem (Hamblin, 2017).

Em grande parte desse tempo o mecanismo de ação de FBM não estava claro, porém atualmente muitos progressos foram feitos na tentativa de elucidar a absorção de luz pelos cromóforos e outras vias de sinalização. Os primeiros trabalhos neste campo foram realizados utilizando-se de vários tipos de lasers, sendo assim, acreditou-se que a luz laser tinha algumas características especiais que os demais tipos de luz, como, a luz solar, lâmpadas fluorescentes ou incandescentes e LEDs não possuíam. No entanto, todos os estudos que foram feitos comparando lasers com fontes de luz equivalentes

com comprimento de onda, potência e densidade semelhantes nas suas emissões, não encontraram essencialmente nenhuma diferença entre elas (Wang et al., 2016).

Muitos comprimentos de onda no espectro vermelho (600-700 nm) e infravermelho (770-1200 nm) demostram resultados positivos, no entanto, existe uma região entre (700-770 nm) onde em termos gerais, os resultados provavelmente serão decepcionantes. Comprimentos de onda azuis e verdes também começaram a ser explorados (Wang et al., 2016), mas eles têm grandes problemas com a profundidade de penetração. É aceito que a penetração da luz no tecido é governada pela absorção e dispersão por moléculas e estruturas presentes no tecido. Tanto a absorção quanto a dispersão tornam-se significativamente menores à medida que o comprimento de onda fica mais longo, de modo que a profundidade de penetração do infravermelho (IV) é máxima em cerca de 810 nm, e em longos períodos da onda a água torna-se um absorvente importante e a profundidade de penetração fica mais curta novamente (Wang et al, 1995).

Ainda assim, a fototerapia é dose dependente. Situação que descreve a ocorrência de um valor ótimo da "Dose" de FBM, que é mais frequentemente definida pela densidade de energia (J / cm2) (Huang et al, 2009; Huang et al., 2011). Constatou-se que quando a dose de FBM é elevada, uma resposta máxima é atingida em algum valor, e se a dose é aumentada além desse valor máximo, a resposta diminui, desaparece e é mesmo possível que efeitos negativos ou inibitórios sejam produzidos com fluências muito altas. Neste conceito se aplicam os termos “dose resposta bifásica” ou “curva Arndt-Schulz” (Hamblin, 2017).

Atualmente, considera-se o citocromo c oxidase (CCO) como a principal responsável pela FBM, ela é unidade IV (quatro) da cadeia de transporte de elétrons na

mitocôndria, sendo a mesma encarregada de transferir um elétron de cada uma das quatro moléculas da citocromo c, para uma única molécula de oxigênio, produzindo duas moléculas de água. Ao mesmo tempo, quatro prótons são translocados através da membrana mitocondrial, produzindo um gradiente de prótons que a enzima ATP sintase necessita para sintetizar o ATP (Karu, 1999; Karu et al., 2005).

O CCO tem dois centros de heme (a e a3) e dois centros de cobre (Cu_A e Cu_B), estes centros metálicos podem existir em um estado oxidado ou reduzido, e estes têm diferentes espectros de absorção, o que significa que o CCO pode absorver luz na região até 950 nm (Mason et al, 2014). Tiina Karu et al. (2005 e 2010) foram os primeiros a sugerir, que o espectro de ação dos efeitos da FBM combinavam com o espectro de absorção de CCO, e esta observação foi confirmada por Wong-Riley et al. (2005). O pressuposto de que o CCO é um dos principais alvos do FBM também explica o amplo uso de vermelho e infravermelho, uma vez que estes comprimentos de onda mais longos têm uma penetração de tecido muito melhor do que a luz azul ou verde que são absorvidas pela hemoglobina. A teoria mais popular para explicar por que a absorção de fótons pela CCO pode levar ao aumento da atividade enzimática, aumento do consumo de oxigênio e da produção de ATP baseia-se na fotodissociação e inibição do óxido nítrico (NO) (Pannala et al., 2016). O NO por não ser ligado de maneira covalente aos centros Heme e Cu do CCO, pode de maneira competitiva, bloquear o oxigênio em uma proporção de 1:10, sendo assim um fóton de energia (FBM) relativamente baixo pode expulsar o NO e permitir que ocorra uma grande quantidade de respiração (Fernandes et al., 2013).

Estresse oxidativo e fotobiomodulação

Radicais livres (RL) são espécies químicas (átomos ou moléculas) que possuem um elétron desemparelhado na sua camada mais externa. Essa situação lhe confere alta reatividade química, especialmente como agente oxidante, com o intuito de adquirir o segundo elétron para estabilizar o seu orbital de valência (Halliwell & Gutteridge, 2007).

Em nosso organismo são produzidos RL de carbono, enxofre, nitrogênio e oxigênio, mas o que ganha mais destaque devido à reatividade e aos danos que podem causar são os radicais derivados do oxigênio. Além dos RL, existem espécies reativas (ER), um termo coletivo frequentemente usado para incluir não apenas RL, mas também alguns não radicais capazes de gerá-los, como por exemplo, o peróxido de hidrogênio, o ácido hipocloroso, entre outros. Quando há um aumento das ER e/ou uma diminuição da capacidade antioxidante celular, pode ocorrer uma situação denominada de estresse oxidativo, a qual está associada à vários processos fisiológicos e patológicos, como mutagênese, diabetes mellitus, catarata, câncer, aterosclerose, doenças degenerativas, fadiga, envelhecimento, entre outras (Halliwel & Gutteridge, 2007).

O estresse oxidativo tem seus danos minimizados pelo sistema de defesa antioxidante não enzimático e/ou enzimático. Entre os antioxidantes não enzimáticos, podem-se citar vitaminas e os compostos polifenólicos, entre outros. A principal linha de defesa enzimática é constituída pelas enzimas superóxido dismutase (SOD) e catalase (CAT) (Bonnefoy et al., 2002).

A SOD dismuta o radical superóxido a peróxido de hidrogênio (Reação 5), que é menos reativo e pode ser degradado por outras enzimas, como a CAT ou glutationa peroxidase (GPx). Em células eucariotas, há várias isoformas do tipo SOD, geralmente responsáveis por compartimentos celulares distintos (Fridovich, 1998). A SOD1 ou

SODCuZn encontra-se quase que exclusivamente no espaço citoplasmático intracelular (Zelko et al., 2002). A Sod2 ou SODMn, pode ser encontrada na mitocôndria da maioria dos animais (Fridovich, 1998). A Sod3 ou ECSOD possui um peptídeo sinalizador que a direciona exclusivamente para o espaço extracelular. Essa enzima existe como um tetrâmero de 135kDa de massa molecular e já foi detectada no plasma, linfa e fluido cerebroespinhal (Zelko et al., 2002).

$$(5)\ O_2^{\cdot -} + O_2^{\cdot -} + 2H^+ \xrightarrow{\text{SOD}} H_2O_2 + O_2$$

A atividade da SOD pode ser medida por método espectrofotométrico indireto negativo, isto é, através de uma reação onde a presença da enzima inibe a formação do produto colorido resultante da interação entre o indicador (adrenalina, por exemplo) e o radical superóxido (Bannister & Calabrese, 1987).

A enzima CAT é uma ferrilhemoenzima cuja função principal é dismutar o peróxido de hidrogênio formando água e oxigênio molecular, conforme a reação 6 (Halliwel & Gutteridge, 2007). A CAT é um tetrâmero formado por unidades idênticas, sendo que cada monômero contém um grupo prostético heme no centro catalítico (Ursini et al., 1997; Halliwel & Gutteridge, 2007). Em animais, a catalase está presente em todos os órgãos essenciais do corpo, principalmente no fígado. Porém, alguns órgãos por não possuírem peroxissomos, estão mais expostos a danos provocados pela produção de ER, como coração, pulmões e cérebro. Nesses órgãos, como mecanismo de defesa, pode ocorrer a difusão de peróxido de hidrogênio para o sangue, onde reage com a CAT eritrocitária (Inoue, 1994). A atividade da CAT pode ser avaliada, espectrofotometricamente, através do consumo de peróxido de hidrogênio adicionado a reação (Aebi, 1984).

$$\text{CAT}$$
$$(6)\ 2H_2O_2 \rightarrow 2H_2O + O_2$$

Quando o sistema de defesa é insuficiente, o estresse oxidativo pode gerar um aumento dos níveis de peroxidação lipídica e de carbonilação de proteínas (Halliwel & Gutteridge, 2007). Entre os produtos finais da peroxidação lipídica estão compostos de baixo peso molecular, como hidrocarbonetos (etano e pentano) e aldeídos, como o malondialdeído (MDA). O MDA pode lesar proteínas e também reage com o DNA, sendo a guanina a base mais danificada (Esterbauer & Cheeseman, 1990; Halliwell & Gutteridge, 2007).

A determinação dos produtos de reação com o ácido tiobarbitúrico (TBARS) é um importante indicativo dos níveis de dano oxidativo lipídico. Esse método detecta não somente o MDA, mas também outros aldeídos produzidos na lipoperoxidação (Halliwell & Gutteridge, 2007). A determinação de proteínas oxidadas pode ser considerada um importante e sensível marcador de oxidação protéica (Levine et al., 1990; Chakravarti & Chakravarti, 2007). O processo de carbonilação protéica pode ocorrer pela oxidação direta das cadeias laterais dos aminoácidos; pela interação das proteínas com produtos finais da peroxidação lipídica como 4-hidroxinonenal e o MDA e também através de reações de glicação (Chakravarti & Chakravarti, 2007). A reação dos grupos carbonílicos com 2,4-dinitrofenilhidrazina formando 2,4-dinitrofenilhidrazona tem sido bastante utilizada como método de avaliação do conteúdo de proteínas carboniladas (Levine et al., 1990).

Estudos apontam efeitos da FBM como recurso de reabilitação, observando seus efeitos após lesões induzidas em ratos. Silveira et al. (2011) verificaram os efeitos da FBM aplicada em lesões de pele induzidas experimentalmente. Para isso a FBM foi aplicada sete vezes (2, 12, 24, 48, 72, 96 e 120 horas) após a formação da lesão, utilizando-se de equipamentos distintos (AsGa, 904nm, pulsado, 70 mW, 60 segundos

de aplicação/sessão e He-Ne, 660nm, contínuo, 30 mW, 60 segundos de aplicação/sessão) e doses distintas. Observou-se que a aplicação da FBM foi capaz de diminuir a peroxidação lipídica, os danos às proteínas e a atividade de SOD e CAT, potencializando a cicatrização da ferida, principalmente nas doses de 1 e 3 J/cm² do laser de He-Ne e na dose de 3 J/cm² do laser de AsGa.

Avni et al. (2005) avaliaram os efeitos da aplicação de FBM em lesões induzidas em ratos, por 3 horas de isquemia seguida de reperfusão. A aplicação de FBM (AsGa, 810nm) ocorreu imediatamente e após 1 hora de oclusão do suprimento sanguíneo. Observou-se que a aplicação de FBM protegeu o músculo contra os efeitos deletérios gerados pela lesão isquêmica, proporcionando uma diminuição significativa nos marcadores utilizados (creatina fosfoquinase, capacidade antioxidante total sérica) quando comparados com o grupo que não recebeu a aplicação. Os autores relataram também um aumento na capacidade antioxidante total sérica quando a FBM foi aplicada em ratos sem a indução de lesão por isquemia/reperfusão.

Fillipin et al. (2005) e Rizzi et al. (2006), investigaram os efeitos da FBM (AsGa 904nm, contínuo, 45 mW e 5 J/cm^2, 35 segundos de aplicação) sobre o estresse oxidativo em modelo experimental de trauma no tendão de Aquiles e músculos de ratos, respectivamente. Verificou-se que a FBM reduziu a perda da arquitetura normal (histologia) e a resposta inflamatória, bem como os níveis de TBARS, quando comparado ao grupo que não recebeu laser.

Em um estudo que objetivou investigar o efeito da FBM aplicada por dois lasers distintos (He-Ne, 632,8nm, contínuo, 5mW, 1min de aplicação/sessão e AsGa, 904nm, pulsado, 12mW, 47 segundos de aplicação/sessão) em um modelo experimental de miopatia, que foi induzida através da infiltração de adrenalina (0,05 mg/rato/dia) no músculo durante 5 dias, observou-se que no grupo em que a lesão foi desenvolvida,

houve um aumento significativo dos marcadores de dano e uma diminuição de ON. A aplicação de FBM diminuiu significativamente estes danos, aumentou a atividade da SOD e a concentração de ON (Servetto et al., 2010).

Liu et al. (2009) induziram lesão muscular em ratos através de exercícios excêntricos em esteira (corrida em dowhill), em que os animais receberam aplicação de FBM em 3 momentos: imediatamente após, 18 horas após e 42 horas após o protocolo de exercícios. Observou-se que os grupos que receberam a aplicação de FBM apresentaram uma melhora significativa na avaliação histológica (menor quantidade de infiltrados inflamatórios), diminuição nas concentrações de CK e da peroxidação lipídica e aumento na atividade de SOD.

Pioneiramente, De Marchi et al., (2012) avaliaram o efeito da FBM na prevenção ao dano oxidativo gerado por corrida de intensidade progressiva em humanos, dessa forma e aplicando a FBM antes do exercício, foi possível observar que a FBM foi capaz de diminuir o estresse oxidativo a lipídeos e proteínas, bem como o dano muscular gerado pelo exercício, mantendo as concentrações basais de SOD e CAT.

Fotobiomodulação vs espécies reativas de oxigênio

Quando FBM estimula a atividade de CCO em células saudáveis, ocorre um aumento, acima dos níveis basais, do potencial de membrana mitocondrial (PMM) o que resultará em uma elevação bastante modesta na geração de ER (Chen et al., 2009). No entanto, este breve aumento de ER causada por 3 J / cm2 de laser de 810 nm mostrou-se suficiente para ativar o fator de transcrição que possui sensibilidade redox, NF-kB em fibroblastos embrionários (Chen et al., 2011). A adição da N-acetil-cisteína (antioxidante) às células bloquearam a ativação do NK-kB, mas não o aumento do ATP celular causado pela estimulação mitocondrial. Nos neurônios corticais de cultura primária (Sharma et al., 2011), o laser de 810 nm produziu uma resposta de dose

bifásica na produção de ATP e PMM com um máximo de 3 J / cm2. Em uma dose elevada (30 J / cm2), o PMM diminuiu em relação à condição basal. Curiosamente, a curva dose-resposta entre fluência (J / cm2) e produção de ER mostrou dois máximos diferentes. Um desses máximos ocorreu a 3 J / cm2, onde o PMM mostrou seu aumento máximo. O segundo máximo na produção de ER ocorreu a 30 J / cm2, onde o PMM diminuiu em relação à condição basal. Com um valor entre estas duas fluências (10 J / cm2), uma dose na qual o PMM foi aproximadamente igual à condição basal, não houve muita geração de ROS. Esses dados são exemplos muito bons da "resposta à dose bifásica" ou "curva Arndt-Schulz", que é muitas vezes discutida na literatura FBM (Huang et al., 2009; Huang et al., 2011).

Assim, parece que as ER podem ser geradas dentro das mitocôndrias quando o PMM é aumentado acima dos valores normais e também quando é diminuído abaixo dos valores normais. Resta saber quais ER são gerados pelas diferentes doses de FBM. Uma possibilidade intrigante é que as ER geradas pela FBM podem ser benéficas ou prejudiciais dependendo da taxa em que é gerado. Se o superóxido for gerado nas mitocôndrias a uma taxa que permita a SOD formar peróxido de hidrogênio, então o H2O2 pode ser difundido para fora da mitocôndrias para ativar caminhos de sinalização benéficos, enquanto que se o superóxido for gerado a uma taxa ou em níveis além da capacidade de SOD para lidar com isso, o superóxido produzido pode se acumular dentro das mitocôndrias e danificá-las (Hamblin, 2017).

Não obstante, a capacidade de FBM para produzir um breve aumento ER em células normais, é bem aceito que a FBM utilizada como tratamento de lesão tecidual ou dano muscular, é capaz de reduzir marcadores de estresse oxidativo (De Marchi et al., 2012, Tatmatsu-Rocha et al., 2016; De Marchi et al., 2017, De Marchi et al., 2018). Como reconciliar esses achados aparentemente contraditórios? Um estudo tentou

responder a esta pergunta (Huang et al., 2013). Os neurônios corticais de cultura primária foram tratados com uma das três intervenções diferentes, todas as quais foram escolhidas a partir de métodos literários para induzir artificialmente o estresse oxidativo na cultura celular. O primeiro foi o cloreto de cobalto (CoCl2), que é usado como um mimético para hipoxia e funciona por uma reação de Fenton produzindo radicais hidroxila (Hervouet et al., 2008). O segundo foi o tratamento direto com peróxido de hidrogênio. O terceiro foi o tratamento com o inibidor do complexo mitocondrial I, a rotenona (Madungwe et al., 2016) . Todos os três diferentes tratamentos aumentaram as ERO mitocondriais intracelulares e, ao mesmo tempo, diminuíram o PMM medido pelo éster metílico de tetrametil-rodamina. A aplicação de FBM (3 J / cm2 de 810 nm laser) elevou o PMM de volta para a linha de base, ao mesmo tempo, reduziu a geração de ERO em células com estresse oxidativo (enquanto aumentava ligeiramente ERO em células normais). Nas células controle (sem estresse oxidativo), a FBM aumentou a PMM acima da condição basal e ainda produziu um modesto aumento nas ERO.

Uma vez que a maioria dos estudos laboratoriais de FBM como terapia examinaram vários modelos animais, de doença ou lesão, não é surpreendente que a maioria dos trabalhos tenham medido a redução em marcadores de estresse oxidativo após FBM (De Marchi et al., 2012; Martins et al., 2016; Vanin et al., 2018). Houve muitos estudos sobre os músculos nos seres humanos, especialmente nos atletas, o exercício de alto nível produz efeitos nos músculos caracterizados por dor muscular tardia, dano muscular (creatina quinase), inflamação e estresse oxidativo (Leal Junior et al., 2015, De Marchi et al., 2017).

Um estudo celular de Macedo et al., 2015 usou células musculares isoladas de camundongos com distrofia muscular (mdx LA 24) e descobriram que 5 J / cm² de 830 nm aumentaram os níveis de expressão da cadeia pesada de miosina e cálcio

intracelular, além disso, diminuiu a produção de H2O2, os níveis de 4-HNE, de glutationa e as atividades de glutationa redutase e SOD. As células mdx mostraram aumento significativo nos níveis de TNF-α e NF-κB, que foram reduzidos por FBM.

Espécies reativas de oxigênio e dano muscular no exercício físico

O músculo esquelético é desenhado para suportar sobrecargas mecânicas e metabólicas até um determinado limite. Quando estimulado, atinge rapidamente sua carga máxima de contração e aumenta o fluxo de oxigênio em até 100%, o que pode levar a um aumento de ER e estresse oxidativo (Alessio, 2000). Sabe-se que esse fenômeno acompanha a atividade contrátil esquelética (Elosua et al., 2003) e pode provocar a diminuição da função contrátil dos grupamentos musculares envolvidos e produzir fadiga (Reid et al., 1992).

O grau de produção de ER (oxigênio singlete, ânion superóxido, peróxido de hidrogênio radical hidroxila e NO), no exercício, costuma estar relacionada com o aumento do funcionamento da cadeia de transporte de elétrons (Chevion et al., 2003), a ação do sistema xantina oxidase/desidrogenase (McAnulty & McAnulty, 2003), por aumento no número circulante de neutrófilos (Scharhag et al., 2002) e pelo processo de isquemia-reperfusão (Seiguel et al., 1999).

Em relação a xantina oxidase (XO) e a xantina desidrogenase (XDH), ambas são enzimas que catalisam a oxidação da hipoxantina e xantina à ácido úrico durante o catabolismo das purinas em mamíferos (Gomez-Cabrera et al., 2008). Como a XDH preferencialmente transfere os elétrons liberados durante o processo de oxidação para o NAD^+, a XO utiliza o oxigênio molecular, causando assim a geração de radical superóxido (Harris et al., 1999).

A carga muscular imposta durante o exercício causa modificação na ultraestrutura do músculo, com infiltração de neutrófilos e liberação de mioglobina (Fielding et al., 1993). A ação dos neutrófilos inclui o processo microfagocítico, atributo que lhe é característico. Neutrófilos são ricos em NAD(P)H oxidase, a qual pode converter o oxigênio molecular em superóxido. Na presença de SOD e mieloperoxidase, forma-se o ácido hipocloroso. Sendo assim, o processo inflamatório é uma importante fonte de ER induzidas pelo exercício físico de alta intensidade (Viña et al.,2000).

Um mecanismo alternativo, através do qual o músculo pode produzir ER, envolve o processo de isquemia-reperfusão. O ciclo de funcionamento dos esfíncteres pré-capilares, o mais importante regulador do fluxo tissular, envolve períodos de contração e relaxamento (Seiguel et al., 1999). No exercício intenso, vários tecidos sofrem processo transitório de isquemia com o intuito de desvio seletivo do fluxo. Além disso, fibras submetidas a um esforço supramáximo podem desenvolver episódios de isquemia, pois a demanda de oxigênio torna-se momentaneamente insuficiente (Koyama et al., 1999). Desse modo, a reoxigenação que se estabelece após o término da atividade física é capaz de gerar ER, principalmente superóxido (Powers & Jackson, 2008).

Como descrito anteriormente, a prática de atividade física de alta intensidade provoca danos estruturais em células musculares e as avaliações destes danos podem ser feitas de forma direta e indireta. A avaliação direta do dano muscular em humanos é complexa, visto que sua análise é possível somente através de dois métodos: por biópsia muscular ou por ressonância magnética. Sendo assim, os problemas inerentes na análise por biópsia muscular são óbvios, pois uma pequena amostra é usada para estimar o dano no músculo todo. Além disso, devido ao fato de o dano muscular não estar presente em todo tecido, mas sim focalizado, é possível ocorrer uma estimativa errônea, sendo maior

ou menor que o dano muscular real. As técnicas de imagem utilizando a ressonância magnética vêm sendo utilizadas para avaliar o dano (edema) dentro do músculo. Embora seja uma técnica não invasiva, não são claras as mudanças indicadas nas imagens, além do custo econômico alto (Clarkson & Hubal, 2002).

Nos últimos anos, é crescente o interesse científico na busca pela resistência extrema no esporte (Banfi et.al., 2004; Zavorsky et al., 2017). Diante disso, medições da atividade de CK e LDH têm sido utilizadas cada vez mais para determinar lesões musculares (Mair et al., 1992; el-Mallakh et al., 1992; Souglis et al., 2018).

A CK catalisa a defosforilação da creatina fosfato ou sua fosforilação e é uma enzima globular que consiste em duas subunidades de massa molecular de 43 kDa. Até o momento, foram isoladas cinco isoformas diferentes, sendo três isoenzimas no citoplasma (CK-MM, CK-MB, e CK-BB) e duas isoenzimas (sarcoméricas e não-sarcoméricas) na mitocôndria (Nigro et al., 1983). A medida da atividade da CK pode ser feita através de kit colorimétrico (Labtest® - Brazil). Os valores de referência da atividade sérica de CK são ainda controversos (Tabela 1). A maior razão para haver essas discrepâncias está relacionada, possivelmente, à variação do nível de atividade física dos indivíduos testados (Strømme et al., 2004; Chacko et al., 2017).

Tabela 1. Valores de referência da atividade sérica da creatino quinase em homens e mulheres não atletas.

Homens	Mulheres	Referências
26 - 350 $U.L^{-1}$	26 - 200 $U.L^{-1}$	Wong et al. (1983), Schumann & Klauke (2003) e Wu (2006)
26 - 240 $U.L^{-1}$	26 - 207 $U.L^{-1}$	Miller et al. (1984) e Strømme et al. (2004)

26 - 532 $U.L^{-1}$	26 - 248 $U.L^{-1}$	Lev et al.. (1999)

A LDH, outra enzima marcadora de dano muscular, catalisa a redução do piruvato, produzindo lactato e NAD^+ e vice-versa. A sua atividade pode ser determinada a partir da velocidade de decomposição do NADH, medida pela queda da absortividade a 340 nm. Os valores de referência em U/L para esse método, obtidos em populações sadias do sexo masculino e feminino, em soro ou plasma, são de 200 a 480 U/L (Young, 1997).

A atividade destas enzimas musculares serve como marcador da função do músculo e do tecido tanto nas condições patológicas quanto nas fisiológicas. O seu aumento pode representar necrose celular e dano tecidual (oxidativo ou não) seguido de lesão muscular crônica ou aguda (Szumilak et al., 1998). Essas mudanças na atividade de enzimas musculares ocorrem em indivíduos normais e em atletas após exercícios extenuantes, muitas vezes extrapolando os valores de referências encontrados na literatura (Wolf et al., 1987). As atividades séricas da CK e LDH mostram um comportamento diferente antes e depois do exercício (Lawler et al.,1993; Macdougall et al.,1998, Leal Junior 2015), sendo que sua atividade varia de acordo com o tipo de treinamento, o protocolo utilizado (Szabo et al., 2003), a idade, gênero, raça, massa muscular, atividade física e condição climática (Brancaccio et al., 2007).

A diminuição dos níveis de atividade das enzimas musculares depende também, do período de repouso após o exercício ou do tempo de inatividade física (Havas et al., 1997). Alguns fatores podem auxiliar na diminuição da atividade da CK e LDH sérica, como a drenagem linfática e o uso de suplementação com aminoácidos (Coombes & Mcnaughton, 2000, Seifert et al., 2017).

Crioterapia

O uso do frio – crioterapia – é uma das formas mais baratas, recomendadas e utilizadas na reabilitação e tratamento de lesões esportivas agudas, dor de origem musculoesquelética, lesões traumáticas, pós operatórias, processos inflamatórios e contraturas musculares (Knight, 1995, Oliveira et.al., 2007, Espinoza et. Al., 2010). De acordo com Knight (1995), o termo crioterapia significa terapia com frio, portanto qualquer utilização de frio ou gelo para fins terapêuticos é definido como crioterapia.

A crioterapia é amplamente utilizada no esporte competitivo para recuperação muscular após o treino, competição ou a lesões traumáticas (Nemet et.al. 2009; Krueger et. al., 2018), mas também pode ser utilizada para diminuir a dor da lesão musculo esquelética, de forma que diminui a temperatura tissular local, promove uma vasoconstrição local, diminui inflamação e edema (Jakeman et. al. 2009).

O objetivo imediato da crioterapia é prevenir lesão posterior no qual inclui a dor, edema, e espasmo muscular (Oliveira et.al., 2007; Krueger et. al., 2018). Na lesão aguda o frio tem indicação para minimizar o processo inflamatório, diminuir metabolismo e a hipóxia secundaria à lesão, dor e edema, enquanto durante a reabilitação minimizar a dor e o espasmo muscular permitindo mobilização precoce (Lopes 2003; Espinoza et. al., 2010).

Os efeitos da crioterapia podem ser divididos em sete categorias principais 1) diminuir temperatura; 2) diminuir metabolismo; 3) efeitos na inflamação (diminuir ou aumentar); 4) efeitos circulatórios (diminuir ou aumentar); 5) diminuir a dor; 6) diminuir espasmo muscular; 7) aumentar a rigidez tissular. (Knight, 1995)

Com a diminuição do metabolismo, o gelo consegue restringir a área da lesão, visto que com o trauma ocorre lesão em diversos vasos que deveriam suprir outras regiões adjacentes a lesão, caso contrário estes vasos também começam a sofrer por hipóxia, devido ao fornecimento insuficiente de O_2 (Lopes 2003; Espinoza et. al., 2010).

De acordo com Oliveira et. al. (2007) os benefícios da crioterapia podem ser atribuídos à vasoconstrição ou a diminuição do metabolismo local. A vasoconstrição induzida pelo frio diminui a formação de edema, reduz a hemorragia local, e consequentemente, o dano celular e as demandas metabólicas (Matheus et. al. 2008).

Enwemeka et al.(2002) diz que a diminuição na temperatura do tecido estimula os receptores cutâneos fazendo com que as fibras simpáticas do vaso se contraiam, o que diminui o edema e inflamação devido a diminuição do metabolismo.

Portanto, acredita-se que o efeito analgésico seja devido a esta diminuição do fluxo sanguíneo e a subsequente diminuição do edema, o qual diminuiria a compressão mecânica de estruturas vasonervosas sensíveis a pressão e desta forma gerando um alívio da dor (Cochrane 2004; Espinoza et. al. 2010). Em 1999, Easton and Peters, concluíram que a crioterapia após exercício excêntrico reduziu o dano e rigidez muscular .

Para a aplicação da crioterapia, Janwantanakul (2006) verificou a diminuição da temperatura superficial da pele após aplicação de gelo com e sem compressão. Os resultados demonstraram que a compressão leva a uma maior diminuição da temperatura, no entanto não houve diferenças entre diferentes forças de compressão.

Em um estudo realizado por Nemet et. al. (2009) foram avaliados doze atletas de handebol. Eles realizaram 4 tiros de 250m em esteira numa velocidade de 80% da capacidade máxima, seguido de um período com e sem aplicação de bolsa de gelo. Foi observado uma diminuição de citocinas pro e antinflamatórias quando os indivíduos receberam gelo.

Banfi et. al. (2009) realizaram um estudo com 10 jogadores da seleção Italiana de Rugby, no qual avaliaram o efeito da crioterapia em corpo inteiro na recuperação muscular. Foram avaliadas mudanças em parâmetros imunológicos e inflamatórios (C3,

IgA, IgM, IgG, Proteina C-Reativa, PGE2), citocinas (IL-2, IL-8, IL-10), moléculas de adesão (sICAM-1), e enzimas musculares (CK e LDH). Dentre os resultados encontrados não foram observadas alterações nos parâmetros imunológicos, no entanto, houve quedas significativas nos níveis de CK e LDH. Ainda, observou-se aumento das citocinas anti-inflamatórias e uma diminuição das citocinas pró-inflamatórias, dessa forma, os autores concluem que houve uma melhora na recuperação muscular após dano muscular induzido pelo exercício.

Em outro estudo, Ingram et. al. (2009) comparou o tratamento de contraste e crioimersão com um grupo controle. Com uma amostra de 11 atletas que realizaram um protocolo de exercícios até a exaustão, variáveis como dor muscular, marcadores inflamatórios e de fadiga foram mensurados da linha de base até 48h pós-exercício. Os resultados demonstraram que a crioimersão foi mais efetiva que o grupo contraste e controle na melhora destes marcadores.

Sendo assim, os possíveis efeitos da FBM e da crioterapia na prevenção e tratamento de danos musculares, oxidativos ou não, gerados por exercícios complexos nos encaminham para um novo patamar de aplicação da FBM, que vem sendo utilizada no momento como forma de reabilitação. A diminuição/prevenção de lesões musculares induzidas pelo exercício físico poderá contribuir para os atletas amadores e de alto rendimento, podendo minimizar o tempo de repouso e de recuperação muscular após a prática esportiva.

2.0 Objetivos

2.1 Objetivo geral

Identificar os efeitos proporcionados por equipamentos de fototerapia, com a combinação de diferentes fontes de luz, e avaliar o efeito da fototerapia e da crioterapia como formas de recuperação muscular pós-exercício de alta intensidade, bem como verificar o efeito da fototerapia aplicada antes de uma situação real de jogo.

2.2 Objetivos específicos

I. Determinar a velocidade de remoção dos marcadores de dano muscular em indivíduos submetidos a cinco protocolos diferentes de recuperação: fototerapia, crioterapia, fototerapia mais crioterapia, crioterapia mais fototerapia e placebo.

II. Estudar o efeito da fototerapia e crioterapia sobre os marcadores de dano oxidativo a lipídios e proteínas em indivíduos submetidos a cinco protocolos diferentes de recuperação; fototerapia, crioterapia, fototerapia mais crioterpia, crioterapia mais fototerapia e placebo.

III. Verificar a queda da capacidade de geração de torque isométrico voluntário máximo após um protocolo indutor de fadiga muscular (PIFM) de alta intensidade em indivíduos submetidos a cinco protocolos diferentes de recuperação: fototerapia, crioterapia, fototerapia mais crioterpia, crioterapia mais fototerapia e placebo.

IV. Quantificar a diminuição da dor muscular pós-tardia após um PIFM por exercício de alta intensidade em indivíduos submetidos a cinco protocolos diferentes de recuperação: fototerapia, crioterapia, fototerapia mais crioterpia, crioterapia mais fototerapia e placebo.

V. Avaliar os efeitos de longo prazo utilizando-se de diferentes equipamentos de fototerapia com a combinação de diferentes fontes de luz na recuperação muscular pós-exercício excêntrico;

VI. Avaliar os efeitos proporcionados pela fototerapia, com a combinação de diferentes fontes de luz, aplicado em atletas profissionais de futsal antes de uma partida oficial;

VII. Mensurar a performance e recuperação de atletas de futsal após partida profissional de futsal.

3 Resultados

3.1 Capitulo I

Does photobiomodulation therapy is better than cryotherapy in muscle recovery after a high-intensity exercise? A randomized, double-blind, placebo-controlled clinical trial.

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ORIGINAL ARTICLE

Does photobiomodulation therapy is better than cryotherapy in muscle recovery after a high-intensity exercise? A randomized, double-blind, placebo-controlled clinical trial

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Abstract This study aimed to determine the effectiveness of photobiomodulation therapy (PBMT) and cryotherapy, in isolated and combined forms, as muscle recovery techniques after muscle fatigue-inducing protocol. Forty volunteers were randomly divided into five groups: a placebo group (PG); a PBMT group (PBMT); a cryotherapy group (CG); a cryotherapy-PBMT group (CPG); and a PBMT-cryotherapy group (PCG). All subjects performed four sessions at 24-h intervals, during which they submitted to isometric assessment (MVC) and blood collection in the pre-exercise period, and 5 and 60 min post-exercise, while the muscle fatigue induction protocol occurred after the pre-exercise collections. In the remaining sessions performed 24, 48, and 72 h later, only blood collections and MVCs were performed. A single treatment with PBMT and/or cryotherapy was applied after only 2 min of completing the post-5-min MVC test at the first session. In the intragroup comparison, it was found that exercise led to a significant decrease ($p < 0.05$) in the production of MVC in all groups. Comparing the results of MVCs between groups, we observed significant increases in the MVC capacity of the PBMT, CPG, and PCG volunteers in comparison with both PG and CG ($p < 0.05$). We observed a significant decrease in the concentrations of the biochemical markers of oxidative damage (TBARS and PC) in all groups and muscle damage (creatine kinase—CK) in the PBMT, PCG, and CPG compared with the PG ($p < 0.01$). The clinical impact of these findings is clear because they demonstrate that the use of phototherapy is more effective than the use of cryotherapy for muscle recovery, additionally cryotherapy decreases PBMT efficacy.

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Keywords Phototherapy · Cryotherapy · High-intensity exercise · Oxidative stress · Muscle damage

Introduction

The practice of physical activity promotes health and quality of life, but there is a wide range of inherent risks associated with each type of sport as well as the physical demands that each sport imposes on its practitioners. Every athlete, at either the professional or the amateur level, is subject to injuries; thus, a rapid recovery is always desired to accelerate the return to sports activities. Therefore, therapies such as cryotherapy and phototherapy are used to rehabilitate and prevent injuries. Both therapies aim to decrease the duration of the muscle recovery period in between the game and/or the training sessions.

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The use of cold therapy (cryotherapy) is one of the cheapest, most commonly recommended and used forms in the rehabilitation and treatment of acute injuries, pain of musculoskeletal origin, traumatic sports and other types of injuries, postoperative pain and edema, inflammatory processes, and muscle contractures [1]. According to Knight [2], the term cryotherapy means cold therapy, so any use of cold or ice for therapeutic purposes is defined as cryotherapy. Cryotherapy is widely used for muscle recovery after high-intensity exercise, but it can also be used to decrease the pain of musculoskeletal injuries, by reducing local tissue temperature, promoting local vasoconstriction, and decreasing both inflammation and edema [3]. In acute injuries, cold is indicated to minimize the inflammatory process, decrease metabolism and hypoxia secondary to injury, pain, and edema; during rehabilitation, cold decreases pain and muscle spasm enabling early mobilization [2].

Photobiomodulation therapy (PBMT) low-level laser therapy (LLLT) is the application of laser light (1–500 mW) to a pathologic condition and is applied by means of a light (usually low-powered laser and/or light emitting diodes—LEDs, with power between 1 and 500 mW) to a pathological or preventive clinical condition [4–7] and, unlike other procedures with a medical (surgical) laser, PBMT does not have ablative or thermal effects, but rather has photochemical effects in which light is absorbed and induces a chemical change in the tissues [8, 9]. PBMT is generally used to promote tissue regeneration, reduce swelling and inflammation, and relieve pain [5, 10]. The first randomized clinical trial to investigate its effects on disorders of the musculoskeletal system was carried out in the 1980s, in patients with rheumatoid arthritis [9]. Since the publication of this first clinical trial, additional positive effects of phototherapy have been identified in several other pathologies, such as osteoarthritis [11], tendinopathies [12, 13], back pain [14, 15], and neck pain [16, 17]. Thus, phototherapy presented a new form of therapy that has been used to treat muscular pain; however, the mechanisms responsible for the effects observed in clinical trials remain partially unclear [18].

Metabolism during contractile activity produces reactive oxygen species, which can cause the muscle to develop oxidative stress. This may be a factor associated with a reduction in contractile function and the development of muscle fatigue [7]. Skeletal muscle fatigue is characterized by a deficiency of the muscle's ability to both generate and maintain force produced during muscle activity. In submaximal activities, skeletal muscle fatigue is denoted as a failure to continue the activity at its initial intensity. The development of muscle fatigue is a complex and multifaceted process involving many physiological and biomechanical elements, including the muscle fiber type, oxidative stress, and both the intensity and duration of the activity [19].

The use of PBMT before and after exercise has shown positive results in slowing skeletal muscle fatigue and improving skeletal muscle recovery in both athletes and non-athletes [4]. Some studies have compared the effects of cryotherapy (ice immersion and application) and PBMT in both rats [20, 21] and humans [22]. However, to our knowledge, no studies have compared the ability of these therapies to act in combination or the effectiveness of such a joint therapeutic intervention for upper limbs.

To address these issues, this study aims to determine the effectiveness of PBMT and cryotherapy, when used in both isolated and combined forms following muscle fatigue, induced by performing high-intensity exercise protocols having a predominance of eccentric contraction.

Methods

Ethical aspects

The study was approved by the Ethics Committee of the University of Caxias do Sul. In accordance with the Declaration of Helsinki, all subjects were advised about the procedure and they signed an informed consent prior to participation in the study (CAEE 31344214.3.3001.5341).

Subjects

Forty volunteers were selected for this study. The number of the participants was calculated using a statistical power of 80% and a significance level of $p < 0.05$ (or 5%). The individuals were recruited from among healthy physically active male volunteers aged between 19 and 29 years, from the University of Caxias do Sul. Exclusion criteria were any previous musculoskeletal injury in the previous 3 months and the use of any kind of nutritional supplements or pharmacological agents.

Randomization and blinding procedures

Prior to the study, volunteers were randomly divided into five groups: (1) placebo group (PG); (2) PBMT group (PBMT); (3) cryotherapy group (CG); (4) cryotherapy-PBMT group (CPG); and (5) PBMT-cryotherapy group (PCG). Individuals of all groups attended the Institute of Sports Medicine for four sessions, with 24-h intervals. On the first day, they were subjected to a muscle fatigue-inducing protocol (MFIP) and blood collection during the pre-exercise period, 5 min post-exercise, and 60 min post-exercise. In the remaining sessions performed 24, 48, and 72 h later, blood collection and isometric evaluation in the isokinetic dynamometer were repeated. To ensure the blind nature of the study, the researchers responsible for verbal stimulation during the performance of the isokinetic dynamometry protocol had no knowledge about the allocation of the volunteers in the groups, thereby ensuring impartiality during the evaluations. A single

Table 1 Parameters for PBMT

Number of LEDs	69 (34 red LEDs and 35 infrared LEDs)
Wavelength	660 nm (red) and 850 nm (infrared)
Frequency	Continuous output
Optical output	10 mW (red) and 30 mW (infrared)
LED spot size	0.2 cm^2 (for both—red and infrared), total spot sizes 13.8 cm^2
Power density	0.05 W cm^{-2} (for red) and 0.15 W cm^{-2} (for infrared)
Energy	41.7 J (0.3 J from each red LED, 0.9 J from each infrared LED)
Energy density	1.5 J cm^{-2} (for red) and 4.5 J cm^{-2} (for infrared)
Treatment time	30 s
Number of irradiation points per muscle	1
Total energy delivered per muscle	41.7 J
Total area irradiated	13.8 cm^2
Application mode	Cluster held stationary in skin contact with a 908 angle and slight pressure

researcher, responsible for randomization and programming the PBMT equipment, was aware of the correct allocation of the volunteers in the groups.

Exercise protocol

Initially, the volunteers were subjected to kinanthropometric measurements (body mass and height) and information about DOMS through the 100-mm visual analog scale (VAS). Next, the volunteers were properly positioned with their non-dominant upper limb in the position of evaluation by the isokinetic dynamometer *Biodex System 4 Pro* (Biodex Medical Systems, USA) according to the resolutions provided by the manufacturer for evaluating the forearm flexion movements. In the other sessions (evaluation points of muscle recovery), the measurements and positioning of the equipment remained the same.

The first part of the protocol on the isokinetic dynamometer consisted of determining the maximum isometric torque of the elbow flexors (biceps brachii). To this end, three maximal voluntary contractions (MVCs) were performed in the isometric mode, in the position of 45° of elbow flexion, having a 5-s duration and a 5-s interval between contractions. During the MVCs, a constant and standardized verbal stimulus was presented by the researchers. The maximum isometric torque value reached in the MVCs was considered the maximum capacity of power generation of the volunteer prior to exercise (PRE-MVC).

After determining the PRE-MVC, a period of 180 s of rest before the MFIP by eccentric exercise was allowed. After the rest period, the MFIP was initiated, consisting of five sets of 10 eccentric/concentric contractions of the elbow flexors separated by 30 s. The contractions were performed with an amplitude of 90° and speed of 90°.seg^{-1} for the eccentric contractions and 180°.seg^{-1} for concentric contractions. The guidance provided to the volunteers was to employ the highest strength possible to execute the elbow flexion movement and resist the elbow extension movement imposed by the dynamometer from the first to the last repetition.

Exactly 30 s after the MFIP, volunteers were subjected to a new MVC following the parameters of the MVCs prior to MFIP in relation to the limb's position, the duration, and the verbal stimulation provided by the researchers. The value found in this isolated MVC will be considered the maximum capacity of power generation of the volunteer after the exercise (POST-MVC). The MVC will be evaluated 24 (MVC24), 48 (MVC48), and 72 (MVC72) hours after the execution of MFIP.

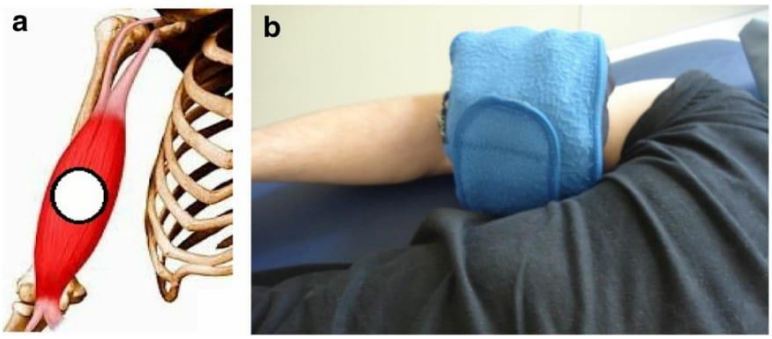

Fig. 1 **a** Application of PBMT. **b** Application of cryotherapy

Table 2 Performance in maximal voluntary contraction ($N \times m$)

	Placebo group	Cryotherapy	PBMT	Cryotherapy + PBMT	PBMT + cryotherapy
Pre	67.11 ± 10.39	64.02 ± 17.02	71.66 ± 16.03	73.31 ± 8.27	72.75 ± 16.54
Post	41.63 ± 9.13	43.44 ± 15.51	49.04 ± 10.94	55.49 ± 21.35	45.65 ± 13.68
60′	47.06 ± 5.43	46.52 ± 11.47	64.14 ± 9.83*	62.68 ± 13.56*	60.93 ± 14.14*
24 h	56.86 ± 7.22	57.01 ± 10.11	70.73 ± 10.04*	69.67 ± 5.49*	71.21 ± 15.54*
48 h	58.08 ± 5.67	52.91 ± 9.28	72.09 ± 10.71*	70.76 ± 11.97*	72.30 ± 13.80*
72 h	58.14 ± 9.44	59.66 ± 13.13	76.66 ± 6.45*	75.98 ± 9.75*	75.94 ± 11.63*

*Statistical difference between the treated groups compared with the placebo group and cryotherapy ($p < 0.05$)

PBMT and cryotherapy

A single treatment with PBMT and/or cryotherapy was applied 2 min after the completion of the post-exercise MVC test. For the application of PBMT (Table 1), we used a cluster of 69 LEDs (34 red LEDs and 35 infrared LEDs), with 660 and 850 nm, 10 mW (red) and 30 mW (infrared) output power (each diode), manufactured by THOR® Photomedicine (London, UK). The application of PBMT was held with the cluster in direct contact with the skin, on the muscle belly of the biceps, as illustrated in Fig. 1. Thus, volunteers received phototherapy with the 41.7-J dose (30 s of irradiation) or 0 J—placebo (3 seconds of irradiation, but without effective irradiation). The choice of these parameters was based in a previous study that used this same PBMT device and observed positive outcomes in performance enhancement and in biochemical markers of recovery [23]. Cryotherapy was performed on the muscle belly of the biceps, with the patient lying down, using thermal bags containing ice cubes, and fixed on the segment with compression. As for duration of cryotherapy, there is good [24, 25] and fair evidence [26] that support cryotherapy should not exceed 20 min. Therefore, the application of ice was limited to 20 min total. The PBMT device was calibrated before and after data acquisition and the equipment showed the same power output in both calibrations. The optical power was measured using a Newport multifunction optical meter model 1835 C. The stability of the laser during the laser irradiation was measured collecting light with a partial reflect (4%).

Blood samples and biochemical assays

Blood samples were collected by a qualified nurse blinded to group allocation and were obtained from an antecubital vein before exercise and exactly 5 min, 60 min, 24 h, 48 h, and 72 h after the end of the exercise protocol. Blood was centrifuged at 2700×g for 10 min at 4 °C. Serum was immediately pipetted into Eppendorf tubes and stored at −80 °C until analysis. Lipid damages were measured spectrophotometrically (Shimadzu spectrophotometer Model UV-1700, Shimadzu®, Japan) by determining thiobarbituric acid reactive substances (TBARS), as previously described by Wills [27]. Results were expressed as nanomole per milliliter. The oxidative damage to proteins was assessed by determining carbonyl groups based on the reaction with 2,4-dinitrophenylhydrazine (DNPH), as previously described by Levine et al. [28]. Results were expressed as DNPH nanomole per milligram of protein. Total protein levels were evaluated using the Total Protein kit from Labtest® (Protein Kit, Labtest Diagnostica S.A., Brazil). Creatine kinase (CK) activity was measured by using a commercial kit (CK—Labtest®, Brazil). CK catalyzes the dephosphorylation of creatine phosphate to produce adenosine if thiotriphosphate, which reacts with glucose in the presence of hexokinase forming glucose-6-phosphate. Glucose-6-phosphate is acted on by glucose-6-phosphate dehydrogenase,

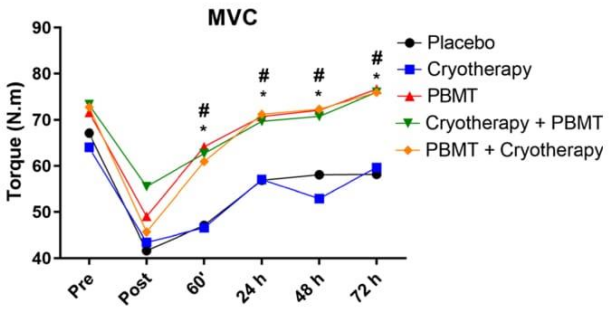

Fig. 2 Pre and post-exercise MVC. *Values* are mean and *error bars* are SEM. #Different of placebo ($p < 0.05$); *different of cryotherapy ($p < 0.05$)

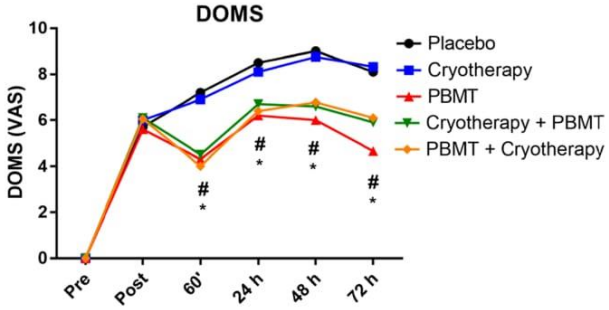

Fig. 3 Pre and post-exercise DOMS. *Values* are mean and *error bars* are SEM. #Different of placebo ($p < 0.05$); *different of cryotherapy ($p < 0.05$)

Table 3 Pre and post-exercise levels of the oxidative damage to lipids (TBARS nmol/ml)

	Placebo group	Cryotherapy	PBMT	Cryotherapy + PBMT	PBMT + cryotherapy
Pre	3.56 ± 0.16	3.59 ± 0.16	3.55 ± 0.19	3.57 ± 0.15	3.51 ± 0.18
Post	4.14 ± 0.16	4.01 ± 0.27	4.15 ± 0.17	4.14 ± 0.16	4.16 ± 0.12
60′	4.08 ± 0.18	3.20 ± 0.19*	3.45 ± 0.40*	3.22 ± 0.36*	3.19 ± 0.14*
24 h	4.10 ± 0.21	4.06 ± 0.71	3.50 ± 0.27*	3.30 ± 0.19*	3.40 ± 0.26*
48 h	4.42 ± 0.32	3.93 ± 0.63**	3.72 ± 0.32*	3.44 ± 0.27*	3.54 ± 0.24*
72 h	4.27 ± 0.35	3.70 ± 0.44*	3.55 ± 0.15*	3.37 ± 0.25*	3.54 ± 0.13*

*Statistical difference between the treated groups compared with the placebo group ($p < 0.01$); **statistical difference between the treated groups compared with the placebo group ($p < 0.05$)

is oxidized to phosphogluconate, and reduces NADP+ to NADPH. The rate of increase in absorbance at 340 nm is proportional to CK activity in the sample. Results were expressed as units per liter.

Statistical analysis

Data from the exercise protocol, oxidative stress, and muscle damage markers were expressed as mean and standard deviation (±SD) and tested statistically by an ANOVA and post hoc Tukey-Kramer and the significance level was set a $p < 0.05$. The software used was SPSS 18.0 for Windows.

Results

Volunteers in this study were 25.30 years old (±3.32), weighed 77.98 kg (±11.43), with a height of 176.55 cm (±5.55). The results of the MVCs (mean ± SD) containing the muscle recovery protocols are presented in Table 2 and Fig. 2. Initially, we observed that there were no significant differences between the groups in the pre-exercise evaluations in all the variables analyzed (MVC, TBARS, PC, CK, and DOMS). In an intragroup statistical test (pre and post comparison), it was found that exercise led to a significant decrease ($p < 0.05$) in the production of MVC after the fatigue protocol in all groups. Comparing the results of MVCs and DOMS between the groups, we observed that after treatment (from 1 to 72 h after), we obtained significant increases in the MVC capacity and decrease in DOMS (Fig. 3) of the volunteers who received treatment with PBMT, CPG, and PCG, compared with the PG and CG ($p < 0.05$). The CG showed no differences compared to the PG.

The concentrations of the biochemical marker of oxidative damage to lipids, as shown in Table 3, indicate that after treatment (from 1 to 72 h after), we obtained a significant decrease in TBARS concentrations in PBMT, CPG, and PCG, compared with the PG ($p < 0.01$). In the CG, we observed a significant decrease in TBARS concentrations at 1 h ($p < 0.01$), 48 h ($p < 0.05$), and 72 h ($p < 0.01$) after treatment. In addition, our results of the concentrations of the biochemical marker of oxidative damage to proteins indicate that after treatment (from 1 to 72 h after), we obtained a significant decrease in PC concentrations in the PBMT, CG and PCG, compared with the PG ($p < 0.01$) as shown in Table 4. In the CPG, we observed a significant decrease in PC concentrations in 24 to 72 h ($p < 0.01$) after treatment.

From the results found in the concentrations of the biochemical marker of muscle damage (CK) presented in Table 5 and Fig. 4, we can see that after treatment (from 1 to 72 h after), we obtained a significant decrease in CK concentrations in the PBMT, compared with the PG ($p < 0.01$). The PCG and CPG groups presented a significant decrease in CK concentrations in 48 and 72 h after treatment ($p < 0.05$ and $p < 0.01$, respectively).

Table 4 Pre and post-exercise levels of the oxidative damage to proteins (carbonylated proteins nanomole of DNPH/gram/deciliter of proteins)

	Placebo group	Cryotherapy	PBMT	Cryotherapy + PBMT	PBMT + cryotherapy
Pre	3.18 ± 0.42	3.01 ± 0.49	3.15 ± 0.28	3.08 ± 0.55	3.11 ± 0.44
Post	3.82 ± 0.90	3.48 ± 0.80	3.50 ± 0.56	3.44 ± 0.60	3.49 ± 0.35
60′	4.51 ± 1.00	3.06 ± 0.71*	3.33 ± 0.45*	3.65 ± 0.57	2.81 ± 0.48*
24 h	4.77 ± 0.80	3.43 ± 0.56*	3.30 ± 0.39*	3.26 ± 0.48*	3.17 ± 0.39*
48 h	5.48 ± 1.09	3.48 ± 0.44*	3.61 ± 0.30*	3.47 ± 0.46*	3.22 ± 0.91*
72 h	5.09 ± 0.89	3.61 ± 0.47*	3.63 ± 0.31*	2.95 ± 0.36*	2.93 ± 0.33*

*Statistical difference between the treated groups compared with the placebo group ($p < 0.01$)

Table 5 Pre and post-exercise levels of the muscle damage (creatine kinase—$U.l^{-1}$)

	Placebo group	Cryotherapy	PBMT	Cryotherapy + PBMT	PBMT + cryotherapy
Pre	63.95 ± 5.44	76.64 ± 14.12	66.91 ± 8.70	72.08 ± 6.43	73.66 ± 13.75
Post	132.37 ± 45.34	154.02 ± 50.45	109.61 ± 34.48	103.16 ± 45.46	128.37 ± 58.43
60′	131.57 ± 84.45	202. 95 ± 28.32	82.67 ± 38.02*	143.00 ± 45.66	130.01 ± 49.84
24 h	294.53 ± 120.60	212.91 ± 33.09	111.00 ± 69.00*	185.40 ± 68.17**	147.61 ± 47.91*
48 h	291.82 ± 182.05	299.83 ± 44.74	101.49 ± 69.01*	128.44 ± 45.08*	163.28 ± 45.35*
72 h	226.02 ± 101.12	145.72 ± 43.52	73.48 ± 27.00*	227.80 ± 90.33	184.31 ± 80.82

*Statistical difference between the treated groups compared with the placebo group ($p < 0.01$); **statistical difference between the treated groups compared with the placebo group ($p < 0.05$)

Discussion

In the rehabilitation process, many therapeutic options are used in an associated manner, one after another, with virtually no recovery interval between their uses. The verification of its actual effects is rare and we found only one study when we searched the effects of PBMT and cryotherapy association in humans. To our knowledge, this is the first time that the synergistic effects of cryotherapy and PBMT have been tested in order to improve the performance of exercise and post-exercise muscle recovery for upper limbs. Some authors [7, 23] have shown the efficacy of both the PBMT dose and the duration of cryotherapy used in this study.

It is known that high-intensity exercises are associated with hyperthermia, energy depletion, muscle injury, oxidative stress, inflammation, and fatigue that lead to decreased performance due to both fatigue and the start of delayed-onset muscle soreness [29]. Prevention and treatment of such afflictions are important tools for the maintenance of exercise programs. The use of non-steroidal anti-inflammatory drugs, stretching, compression therapy, ultrasound, acupuncture, deep massage, nutritional supplements, antioxidants, and electrical stimulation have all been tested, with varying degrees of success, to reduce the symptoms of muscle injury, fatigue, and delayed-onset muscle soreness [29–34]. However, there is no consensus regarding the most appropriate method to prevent delayed-onset muscle pain and muscle injury effectively. Many studies [4, 7] have demonstrated the protective effects of PBMT when applied prior to exercise; thus, we used phototherapy and cryotherapy with resources to assist the muscle recovery process, and applied the modalities subsequent to performing MFIP.

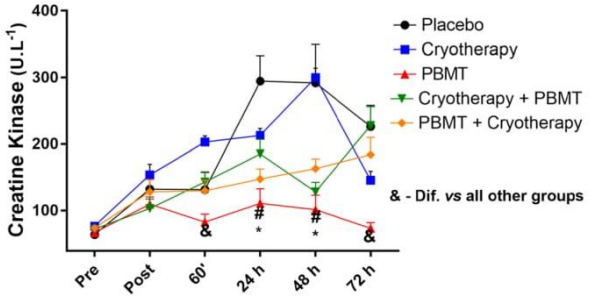

Fig. 4 Pre and post-exercise CK activity. *Values* are mean and *error bars* are SEM. #Different of placebo ($p < 0.05$); *different of cryotherapy ($p < 0.05$). &Different of the all other groups ($p < 0.05$)

We noted that PBMT has considerable potential not only for the prevention of muscle fatigue and damage caused by high-intensity exercises, but also can also improve performance conditions when applied post-exercise, in order to attain the goal of muscle recovery. Skeletal muscle is designed to withstand mechanical and metabolic overloads up to a certain limit. When stimulated, it rapidly reaches its maximum contraction load and increases oxygen flow up to 100%, which may lead to increased oxidative stress [35]. It is known that this phenomenon accompanies skeletal contractile activity [36] and may cause a decrease in the contractile function of the muscle groups involved and produce fatigue [37].

Cryotherapy has been widely used in sports to both prevent muscle injury and improve recovery [30, 38]. Therefore, it is not surprising that cryotherapy, despite not having demonstrated any effect on maintaining or increasing MVC after use in isolation, has demonstrated some effect on the reduction of markers of oxidative damage to lipids and proteins, probably through their known effects on vasoconstriction, reduction in muscle temperature, and inflammatory activity [39, 40]. This implies that the oxidative damage to lipids and proteins generated by the ischemia-reperfusion process may have been reduced by cryotherapy.

Associated with these findings, it is important to highlight that cryotherapy had no influence on maintaining the MVC capacity, having a behavior similar to the placebo group from the time of its application. Previous studies report that cryotherapy can reduce nerve conduction velocity by not only changing the perception of pain but also interfering with the recruitment of motor units [41]. In contrast, the groups that received the PBMT application exhibited a significant improvement in MVC after 60 min after the application of the muscle recovery protocol. The results obtained by cryotherapy in reducing markers of oxidative damage to lipids and

proteins have also been reached in the group that received only the application of PBMT; moreover, this group had a significant decrease in the marker of muscle damage (CK), which was not observed in the cryotherapy group. Similar results have been reported in studies conducted in animals [20, 21, 42], where the use of cold water immersion and cryotherapy proved ineffective in providing effective muscle recovery, while the use of PBMT was capable of improving muscle condition 24 h after exercise.

Another interesting factor is the fact that joint application of therapeutic interventions has not shown much relevance, as indicated by the results achieved with joint application. For example, regardless of the application order, combined application of therapeutic interventions is not seen to be more effective than the individual application of PBMT and it corroborates with recent literature [43].

Recently, Albuquerque-Pontes et al. [44] have demonstrated that one single irradiation with PBMT is capable of increasing the activity of cytochrome c-oxidase in intact skeletal muscle tissue up to 24 h after irradiation, and this up-regulation is dependent of dose and wavelength. Furthermore, the combined use of three wavelengths is beneficial for this aim [45–50]. This study [44] shows that PBMT plays a leading role in the self-regulation of mitochondrial activity by increasing the mitochondrial respiratory chain. This, in turn, consequently increases the production of ATP in muscle cells and leads to the reduction of oxidative stress and subsequently to the production of reactive oxygen species (ROS). It is important to emphasize that, in this study, uninjured animal muscles were irradiated.

The results observed lead to the discussion of using cryotherapy as a tool to speed recovery; therefore, more studies need to be conducted to confirm this hypothesis. However, the clinical impact of these findings is obvious because they demonstrate that the use of PBMT is more effective than the use of cryotherapy for muscle recovery. It is worth remembering that only one session of each mode was performed, that is, the potential shown by PBMT may be even higher if treatment is continued throughout the same week.

Conclusion

Based on the above results and discussion, this study demonstrates that the application of cryotherapy associated with PBMT does not improve the effects of the application of PBMT, so the isolated application of PBMT seems to be the best option to improve muscle recovery in both the short and long term. In contrast, the use of cryotherapy in isolation was unable to provide muscle recovery. Additional field studies should be performed to optimize dose parameters for differences in elite and recreational athletic recovery and to examine the long-term effects of PBMT.

Compliance with ethical standards

Competing interests Professor Ernesto Cesar Pinto Leal-Junior receives research support from Multi Radiance Medical (Solon, OH - USA), a laser device manufacturer. Multi Radiance Medical had no role in the planning of this study, and the laser device used was not theirs. They had no influence on study design, data collection and analysis, decision to publish, or preparation of the manuscript. The remaining authors declare that they have no conflict of interests.

Ethical aspects The study was approved by the Ethics Committee of the University of Caxias do Sul. In accordance with the Declaration of Helsinki, all subjects were advised about the procedure and they signed an informed consent prior to participation in the study (CAEE 31344214.3.3001.5341).

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3.2 Capitulo II

Phototherapy for Improvement of Performance and Exercise Recovery: Comparison of 3 Commercially Available Devices

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original research

Phototherapy for Improvement of Performance and Exercise Recovery: Comparison of 3 Commercially Available Devices

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Context: Recent studies suggest the prophylactic use of low-powered laser/light has ergogenic effects on athletic performance and postactivity recovery. Manufacturers of high-powered lasers/light devices claim that these can produce the same clinical benefits with increased power and decreased irradiation time; however, research with high-powered lasers is lacking.

Objective: To evaluate the magnitude of observed phototherapeutic effects with 3 commercially available devices.

Design: Randomized double-blind placebo-controlled study.

Setting: Laboratory.

Patients or Other Participants: Forty healthy untrained male participants.

Intervention(s): Participants were randomized into 4 groups: placebo, high-powered continuous laser/light, low-powered continuous laser/light, or low-powered pulsed laser/light (comprising both lasers and light-emitting diodes). A single dose of 180 J or placebo was applied to the quadriceps.

Main Outcome Measure(s): Maximum voluntary contraction, delayed-onset muscle soreness (DOMS), and creatine kinase (CK) activity from baseline to 96 hours after the eccentric exercise protocol.

Results: Maximum voluntary contraction was maintained in the low-powered pulsed laser/light group compared with placebo and high-powered continuous laser/light groups in all time points ($P < .05$). Low-powered pulsed laser/light demonstrated less DOMS than all groups at all time points ($P < .05$). High-powered continuous laser/light did not demonstrate any positive effects on maximum voluntary contraction, CK activity, or DOMS compared with any group at any time point. Creatine kinase activity was decreased in low-powered pulsed laser/light compared with placebo ($P < .05$) and high-powered continuous laser/light ($P < .05$) at all time points. High-powered continuous laser/light resulted in increased CK activity compared with placebo from 1 to 24 hours ($P < .05$).

Conclusions: Low-powered pulsed laser/light demonstrated better results than either low-powered continuous laser/light or high-powered continuous laser/light in all outcome measures when compared with placebo. The increase in CK activity using the high-powered continuous laser/light compared with placebo warrants further research to investigate its effect on other factors related to muscle damage.

Key Words: skeletal muscle performance, low-level laser therapy, light-emitting diode therapy, high-intensity laser therapy, photobiomodulation therapy

Key Points

- Phototherapy (or photobiomodulation therapy) had ergogenic and protective effects on skeletal muscles only if applied with the correct settings.
- The combination of low-powered pulsed laser and red and infrared light-emitting diodes was more effective than low-powered continuous infrared laser or high-powered continuous infrared laser.
- Increased power did not result in increased efficacy.

Achieving optimal athletic performance is the desire of all athletes from the recreational to the professional. Performance is influenced by a combination of physiological, psychological, and sociocultural factors. *Fatigue* is described as a failure to maintain the expected force, or the inability to maintain a given exercise intensity or power output level.[1] It results when muscle activity exceeds tissue substrate and oxygenation capacity. Previous researchers[2,3] have also shown that injury rates increase with the accumulation of fatigue, and fatigue has been identified as a limiting factor in performance in almost every individual in every sport. Fatigued participants demonstrated reduced voluntary force production in fatigued muscles (measured with concentric, eccentric, and isometric contractions).[4,5]

The positive evidence for the role of phototherapy or photobiomodulation (PBM) in improving exercise performance and markers related to exercise recovery has expanded its potential for widespread use to address fatigue-related injuries. Recent systematic reviews[6,7] demonstrated the ergogenic effects of phototherapy using lasers and/or light-emitting diodes (LEDs) administered immedi-

ately before resistance exercise, suggesting that preexercise exposure with PBM may protect exposed muscles from exercise-induced damage and speed recovery.

With the current focus on preventive measures to reduce the risk of injuries in sports, PBM offers a unique, noninvasive, nonpharmacologic means of reducing muscular fatigue. In turn, physical performance and recovery rate have improved postexercise. The positive effects seen in recent studies were obtained with red[8,9] and infrared wavelengths[9–13] generated by both laser[8–14] and LED[14–17] devices. Various exercises that represent sport-specific activities have been tested: repeated contractions,[8,10,11,13,15] isometric sustained contraction,[9,16,17] cycling,[14] and running.[12]

Only a few investigators have compared PBM with other physical agents[18,19] and addressed the effectiveness of laser versus LEDs.[14,20] Studies of commercially available devices are lacking, which complicates clinical decision-making processes and direct product comparisons. Several mixes of settings (wavelengths, powers, sources) have resulted in positive effects on performance and recovery. To our knowledge, no direct comparison of commercially available devices exists. Despite some manufacturers' claims that high-powered lasers produce similar or greater effects than low-level lasers, we believed that the same dose delivered to a target area using increased power output (and consequently with less irradiation time) would not increase clinical effects.

With this perspective in mind, our aim was to evaluate the effects of phototherapy (or PBM) on skeletal muscle performance and postexercise recovery using 3 commercially available devices to determine how the ergogenic and protective effects on skeletal muscle tissue would be affected by different device settings. This area of research provides the greatest benefit to clinicians by ensuring optimal device and setting identification.

METHODS

Study Design and Ethics Statement

A double-blind, placebo-controlled, randomized clinical trial was conducted at the Sports Medicine Institute at the University of Caxias do Sul. The study received approval from the research ethics committee (protocol number 642.595).

Participants

Forty healthy untrained males recruited from the university staff and student body participated in the study. All participants signed the informed consent statement. A priori, an intention-to-treat protocol would be followed; however, it was not needed because there were no dropouts. The Consolidated Standards of Reporting Trials (CONSORT) flowchart summarizing experimental procedures and participants is displayed in Figure 1.

Inclusion Criteria and Exclusion Criteria

The inclusion criteria were male participants between 18 and 35 years old who had been performing up to 1 session of exercise weekly for the previous 6 months. Any volunteer who presented with a preexisting musculoskeletal injury to the hips or knees in the previous 2 months, used any pharmacologic agents or nutritional supplements regularly, or was injured during the study was excluded. All of these aspects were evaluated during an initial interview used to recruit participants.

Because the available literature showed damaging thermal effects due to certain PBM settings (wavelengths, power outputs, etc) in participants with dark skin pigmentation, only participants with light and intermediate skin pigmentation (assessed through the Von Luschan chromatic scale) were accepted into the study to maximize safety and minimize discomfort.[21–23]

Composition of Groups and Randomization Process

The 40 participants had an average age of 23.14 ± 2.34 years, height of 176.08 ± 11.03 cm, and body mass of 71.08 ± 6.09 kg. We used the same study design as previous authors in this field.[24–26] For the sample-size calculation, we set the β value at 20% and α at 5%. In a study[25] used as a reference for sample-size calculation, phototherapy led to increased maximum voluntary contraction (MVC; our primary outcome) of 336.88 N·m (± 27.92) at 96 hours postexercise (Cohen d = 1.485 145), compared with baseline (286.63 ± 38.86). Thus, 10 volunteers per group and 40 volunteers in total were needed.

The participants were randomly allocated to 4 experimental groups (n = 10 per group) according to the phototherapy dose. A blinded researcher drew lots for randomization.

For placebo treatments, all 3 devices were used. Three participants were treated with the placebo mode of the high-powered continuous laser/light device, 3 with the placebo mode of the low-powered continuous laser/light device, and 4 with the placebo mode of the low-powered pulsed laser/light device. Randomization labels were created using a randomization table at a central office, where a series of sealed, opaque, numbered envelopes ensured confidentiality. A researcher who programmed the devices for either active or placebo mode based on the randomization results was instructed to not inform the participants or other researchers regarding the settings and was blinded to the group allocation.

Experimental Protocol

Blood Samples and Biochemical Analyses. Blood samples (10 mL) were taken from the antecubital vein of each participant before and 1 minute after the eccentric-contraction protocol by a qualified nurse blinded to the allocation of the participants in the 4 experimental groups. One hour after collection, each sample was centrifuged at 3000 rpm for 20 minutes. Pipettes were used to transfer the serum to Eppendorf tubes (Eppendorf AG, Hamburg, Germany), which were stored at −80°C until analysis. Additional blood samples were collected 1, 24, 48, 72, and 96 hours after the exercise protocol.

Creatine kinase (CK) activity was determined using spectrophotometry and specific reagent kits (model No. 117; Labtest, São Paulo, Brazil). The CK activity analysis was performed by a blinded researcher.

Evaluation of Delayed-Onset Muscle Soreness. With the assistance of a blinded researcher, participants used a visual analogue scale (VAS) of 100 mm to self-rate

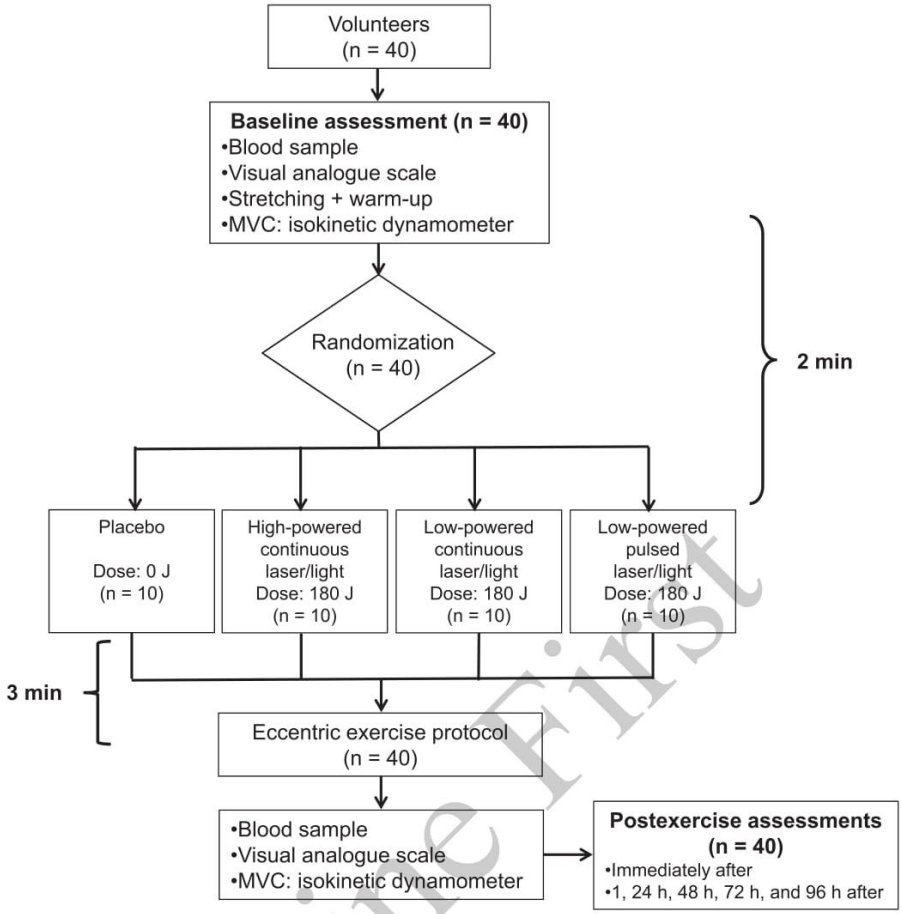

Figure 1. Consolidated Standards of Reporting Trials (CONSORT) flowchart. Abbreviation: MVC, maximum voluntary contraction.

delayed-onset muscle soreness (DOMS). The DOMS assessments were obtained at baseline and immediately and 1, 24, 48, 72, and 96 hours after the eccentric-exercise protocol (1 minute).

Stretching and Warm-up. Before the isokinetic protocol, each participant actively stretched the nondominant knee extensors 3 times for 60 seconds each. Pedaling of a stationary bicycle (Inbramed, Porto Alegre, Brazil) set at 100 rpm and without load for 5 minutes was used as a general warm-up activity.

Maximum Voluntary Contraction. After warm-up, MVC tests were performed using an isokinetic dynamometer (model System 4; Biodex Medical Systems, Inc, Shirley, NY) to assess muscle function; this is currently considered the method with the greatest reliability for studying musculoskeletal performance.[24–26] Each participant was positioned in the dynamometer with an angle of 100° between the trunk and hip and instructed to cross his arms. The nondominant leg was positioned at 60° of knee flexion (0° corresponds to complete knee extension) and the dominant leg at 100° of hip flexion.

The MVC test consisted of three 5-second isometric contractions of the knee extensors of the nondominant leg. The highest peak torque was used for the statistical analysis. The MVC was also performed immediately (1 minute) and 1, 24, 48, 72, and 96 hours after the eccentric-contraction protocol. The researcher performing the MVC assessment was blinded to randomization and allocation.

Phototherapy. The 3 devices we selected represented those commercially available to clinicians. The devices were a high-powered continuous laser/light device (model LiteForce; LiteCure, Newark, DE), a low-powered continuous laser/light device (model LX2; Thor Photomedicine Ltd, Chesham, United Kingdom), and a low-powered pulsed laser/light device (model MR4 Console with a LaserShower 50 4D emitter; Multi Radiance Medical, Solon, OH). The dose (180 J) was selected based on current literature in this field.[6,7,24,25,27,28] Both low-powered devices were applied in direct contact with the skin at 6 sites on the quadriceps femoris (2 centrally: rectus femoris and vastus intermedius; 2 laterally: vastus lateralis; and 2 medially: vastus medialis; Figure 2).

Although the same dose was also applied to the high-powered continuous laser/light group, the application was performed using skin contact and slight pressure in a scanning method. This was according to the manufacturer's specific instructions to avoid any potentially damaging thermal effects. The full description of the phototherapy settings is provided in Tables 1 through 3.

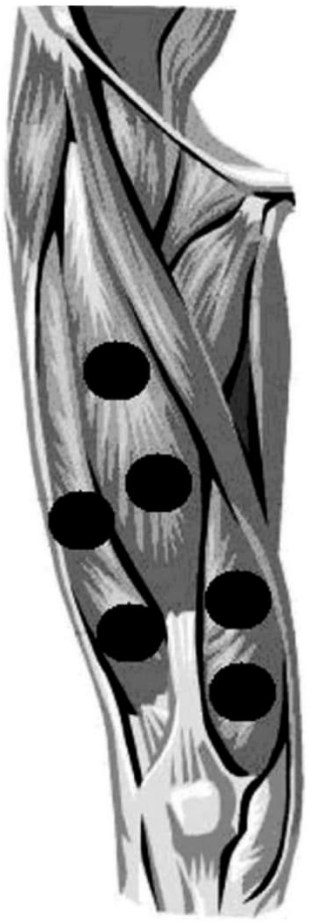

Figure 2. Sites of phototherapy irradiation on quadriceps for low-powered pulsed laser/light group and low-powered continuous laser/light group.

To ensure blinding, the active and placebo modes of each device emitted the same sounds regardless of the programmed mode or dose, and opaque goggles were worn by participants for safety and to maintain the double-blinded condition. Optical power was calibrated before irradiation for each participant using a thermal power meter (model S322C; Thorlabs, Newton, NJ). The researcher who performed the phototherapy was blinded to the randomization and allocation of participants.

Eccentric-Contraction Protocol

After treatment, participants performed the protocol of 75 eccentric isokinetic contractions of the knee extensors of the nondominant leg (5 sets of 15 repetitions, 30-second rest interval between sets) at a velocity of $60°{\cdot}s^{-1}$ both eccentrically and concentrically in a 60° range of motion (between 90° and 30° of knee flexion). For each contraction, the dynamometer automatically (passively) positioned the knee at 30°; the dynamometer then flexed the knee to 90°. The participants were instructed to resist with maximum force the knee-flexion movement imposed by the dynamometer. This eccentric-contraction protocol was based on prior optimization studies[24,25] with the low-powered continuous laser/light and low-powered pulsed laser/light devices that used the same exercise and study protocol. The researcher performing the protocol was blinded to the randomization and allocation of participants.

Table 1. Settings for High-Powered Continuous Laser/Light

Parameter	Value or Description
Class	4
No. of laser diodes	1
Wavelength, nm, mean ± SD	980 ± 10
Frequency, Hz	Continuous output
Optical output, mW	9000
Spot size, cm^2	15.90
Power density, W/cm^2	0.566
Energy density, J/cm^2	11.32
Irradiation time, s	20
Total dose applied in muscular group, J	180
Application mode	Scanning probe in contact with skin at a 90° angle and using slight pressure

Statistical Analysis

A priori, an intention-to-treat analysis would have been followed; however, there were no dropouts. The primary outcome was the peak torque obtained from MVC at the different time points. Secondary outcomes were CK activity and VAS rating. A blinded researcher performed the statistical analysis. Data were expressed as mean ± standard deviation and were first tested for normal distribution using the Shapiro-Wilk test. Analysis of variance with repeated measures for time was performed to test between-groups differences (followed by a Bonferroni-corrected post hoc test). The significance level was set at $P < .05$.

RESULTS

All recruited participants completed all assessments. The functional and biochemical performance and recovery outcomes of all groups are detailed in Table 2. As shown in Figure 3 only the low-powered pulsed laser/light group was able to maintain the MVC compared with the placebo ($P < .05$) and high-powered continuous laser/light ($P < .05$) groups immediately after the active treatment and up to 96 hours later, and MVC increased at the 48-, 72-, and 96-hour time points. The low-powered continuous laser/light group was also better than the placebo ($P < .05$), but only in the time frame between 24 and 72 hours after eccentric exercise, and was also better than the high-powered continuous laser/light group ($P < .05$) at all time points.

Regarding DOMS measured by VAS, only the low-powered pulsed laser/light group was able to minimize pain compared with the placebo ($P < .05$), low-powered continuous laser/light ($P < .05$), and high-powered continuous laser/light ($P < .05$) groups beginning at the 24-hour time point until the end of data collection at 96 hours. The results are summarized in Figure 4.

The low-powered pulsed laser/light group was able to prevent the exercise-induced increase in CK activity starting at 24 hours until 96 hours postexercise ($P < .05$) compared with the placebo and at all experimental times

Table 2. Settings for Low-Powered Continuous Laser/Light

Parameter	Value or Description
Class	3B
No. of laser diodes	5
Wavelength, nm	810
Frequency, Hz	Continuous output
Optical output, mW each	200
Spot size, cm^2 each	0.0364
Power density, W/cm^2 each	5.495
Energy density, J/cm^2 each	164.85
Dose, J each	6
Irradiation time per site, s	30
Total dose per site, J	30
Total dose applied in muscular group, J	180
Application mode	Cluster probe held stationary in contact with skin at a 90° angle and using slight pressure

compared with the high-powered continuous laser/light group ($P < .05$). The low-powered continuous laser/light device was able to decrease CK activity compared with the placebo ($P < .05$) at 48 hours postexercise and also when compared with the high-powered continuous laser/light group ($P < .05$) at 24 and 48 hours.

Finally, the high-powered continuous laser/light group did not demonstrate a significant effect ($P > .05$) on CK activity compared with any of the low-powered laser groups. In fact, the high-powered continuous laser/light group demonstrated a statistically significant increase in CK activity ($P < .05$) when compared with the placebo group at 1 and 24 hours postexercise. Results of the CK analysis are summarized in Figure 5.

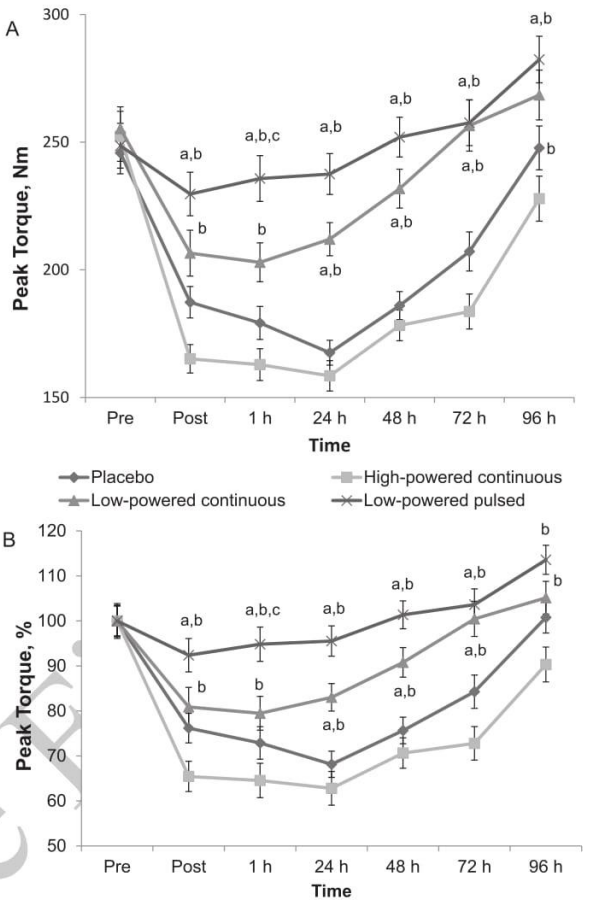

Figure 3. Maximum voluntary contraction in A, absolute, and B, percentage values. Values are means and error bars are standard errors of the mean. [a] Indicates difference compared with placebo ($P < .05$). [b] Indicates difference compared with high-powered continuous laser/light group ($P < .05$). [c] Indicates difference compared with low-powered continuous laser/light group ($P < .05$).

DISCUSSION

Fatigue is an often-forgotten aspect of an athletes' risk of injury. Fatigued muscles in the lower extremity require less force to reach muscle failure under high-intensity eccentric-loading conditions[29,30] and to display negative effects on lower extremity biomechanics and neuromuscular fatigue.[31,32]

Phototherapeutic effects linked to reinforcement of microcirculation,[33] enhanced adenosine triphosphate synthesis,[34] and mitochondrial function[35] have been observed after exposure to light. Reduced reactive oxygen species release and creatine phosphokinase activity and increased production of antioxidants and heat shock proteins have also been reported after PBM.[36,37] Albuquerque-Pontes et al[38] demonstrated that PBM in intact skeletal muscle can increase cytochrome c oxidase activity, up-regulate mitochondrial activity to increase adenosine triphosphate production, and decrease oxidative stress and reactive oxygen species production. These findings support the ergogenic effects seen in healthy individuals.

The device settings we chose were based on scientific evidence in the currently published literature.[6,7] We sought to minimize manufacturer bias by using only doses and wavelengths described in the literature. Two recent systematic reviews,[6,7] one including a meta-analysis,[7] demonstrated positive outcomes on physical performance using single-diode and multidiode laser, multidiode LEDs, and combinations of both devices.

The low-powered pulsed laser/light group demonstrated preservation of muscle performance compared with the placebo group at all time points measured; the low-powered continuous laser/light group did so after the time points beyond 24 hours. The low-powered pulsed laser/light group experienced less muscle fatigue than the low-powered continuous laser group, although the difference was significant only at the 1-hour time point. Interestingly, the MVC results were similar to those previously observed using the low-powered continuous laser/light device[24] and those seen by Antonialli et al[25] using the low-powered pulsed laser/light device.

Previous researchers[32] have also shown that injury rates increase with the accumulation of fatigue and have negative effects on biomechanics. Full recovery can take several days.[39] Fatigue is an often-neglected aspect of the decision to return an athlete to sport or the assessment of an athlete's risk for injury. Preservation of strength, as seen in both low-

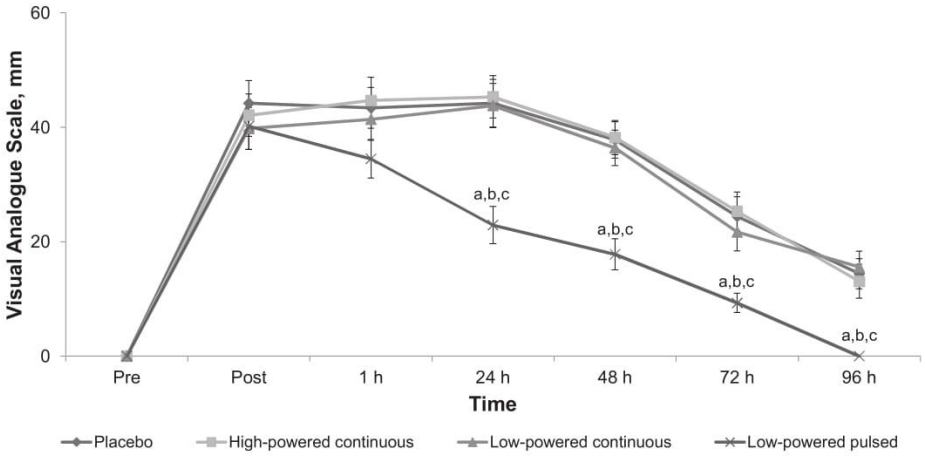

Figure 4. Delayed-onset muscle soreness assessment using 100-mm visual analogue scale. Values are means and error bars are standard errors of the mean. [a] Indicates difference compared with placebo ($P < .05$). [b] Indicates difference compared with high-powered continuous laser/light group ($P < .05$). [c] Indicates difference compared with low-powered continuous laser/light group ($P < .05$).

powered groups (low-powered pulsed laser/light and low-powered continuous laser/light), results in a reduction in fatigue and in the ability of the quadriceps muscle to exert maximal or near-maximal force. Sport-specific movements may be performed with better neuromuscular control, which can reduce the risk for both acute and overuse injuries.

Fatigue or a decline in performance may occur more rapidly at high temperatures.[40] Muscle temperature depends on many factors, including activity, blood flow, core temperature, proximity to the skin surface, and environmental temperature. Participants reported feeling heating during the high-powered continuous laser/light treatment even though the evaluators "scanned" the tissue as directed in the operator's manual. When higher-powered lasers are used to deliver energy, a corresponding increase in the surface temperature is recorded. This can create up to 6 times more heat in darker-pigmented skin than in lighter-pigmented skin-color groups.[21] The temperature increase may be related to the mean output of power, the mode of delivery, and the wavelength used in the high-powered continuous laser/light group.

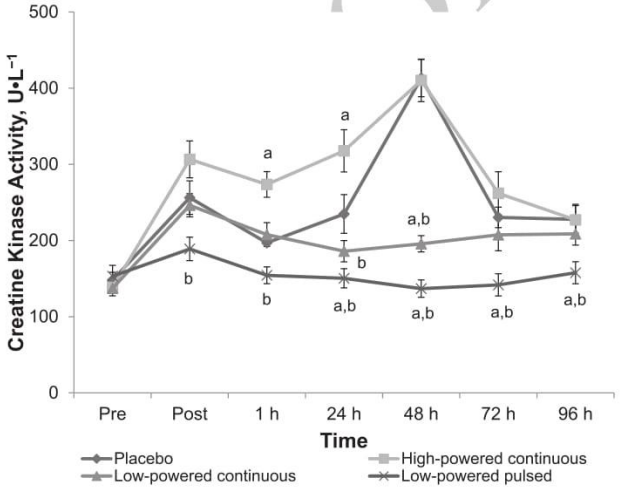

Figure 5. Creatine kinase activity. Values are means and error bars are standard errors of the mean. [a] Indicates difference compared with placebo ($P < .05$). [b] Indicates difference compared with high-powered continuous laser/light group ($P < .05$).

Table 3. Settings for Low-Powered Pulsed Laser/Light

Parameter	Value or Description
Class	1M
No. of lasers	4 Superpulsed infrared
Wavelength, nm, mean ± SD	905 ± 1
Frequency, Hz	250
Peak power, W each	12.5
Average mean optical output, mW each	0.3125
Power density, mW/cm² each	0.71
Energy density, J/cm² each	0.162
Dose, J each	0.07125
Spot size of laser, cm² each	0.44
No. of red LEDs	4
Wavelength of red LEDs, nm	640 ± 10
Frequency, Hz	2
Average optical output, mW each	15
Power density, mW/cm² each	16.66
Energy density, J/cm² each	3.8
Dose, J each	3.42
Spot size of red LED, cm² each	0.9
No. of infrared LEDs	4 Infrared
Wavelength of infrared LEDs, nm	875 ± 10
Frequency, Hz	16
Average optical output, mW each	17.5
Power density, mW/cm² each	19.44
Energy density, J/cm² each	4.43
Dose, J each	3.99
Spot size of LED, cm² each	0.9
Magnetic field, mT	35
Irradiation time per site, s	228
Total dose per site, J	30
Total dose applied in muscular group, J	180
Aperture of device, cm²	20
Application mode	Cluster probe held stationary in contact with skin at a 90° angle and using slight pressure

Abbreviation: LED, light-emitting diode.

Table 4. Functional and Biochemical Markers of Performance and Recovery, Mean ± SD (95% Confidence Interval)

	Time						
Variable	Pre-exercise	Postexercise	1 h	24 h	48 h	72 h	96 h
Maximum voluntary contraction, N·m							
Placebo	245.84 ± 25.94 (227.30, 264.40)	187.31 ± 19.50 (173.40, 201.30)	179.19 ± 20.41 (164.60, 193.80)	167.54 ± 15.40 (156.50, 178.60)	186.00 ± 17.35 (173.60, 198.40)	207.19 ± 24.20 (189.90, 224.50)	247.76 ± 27.15 (228.30, 267.20)
HPC	252.31 ± 30.98 (230.10, 274.50)	165.16 ± 17.62 (152.60, 177.80)	162.87 ± 19.69 (148.80, 177.00)	158.48 ± 18.80 (145.00, 171.90)	178.23 ± 19.02 (164.60, 191.80)	183.68 ± 21.64 (168.20, 199.20)	227.89 ± 27.92 (207.90, 247.90)
LPC	255.34 ± 26.91 (236.10, 274.60)	206.54 ± 28.38[a] (186.20, 226.80)	202.94 ± 24.11[a] (185.70, 220.20)	212.01 ± 20.52[a,b] (197.30, 226.70)	231.82 ± 24.11[b,c] (214.60, 249.10)	256.51 ± 31.65[b,c] (233.90, 279.20)	268.49 ± 30.85[b] (246.40, 290.60)
LPP	248.62 ± 27.80 (228.70, 268.50)	229.70 ± 26.97[a,b] (210.40, 249.00)	235.77 ± 28.39[a,b,c] (215.50, 256.10)	237.53 ± 25.30[a,b] (219.40, 255.60)	252.00 ± 24.65[b,c] (234.40, 269.60)	257.64 ± 28.39[b,c] (237.30, 277.90)	282.38 ± 28.86[b,c] (261.70, 303.00)
Visual analogue scale, mm							
Placebo	0.00 ± 0.00 (0.00, 0.00)	44.20 ± 12.47 (35.28, 53.12)	43.40 ± 11.24 (35.36, 51.44)	44.20 ± 13.13 (34.81, 53.59)	37.80 ± 10.12 (30.56, 45.04)	24.40 ± 11.04 (16.50, 32.30)	14.40 ± 8.28 (8.48, 20.32)
HPC	0.00 ± 0.00 (0.00, 0.00)	42.10 ± 11.69 (33.74, 50.46)	44.70 ± 12.86 (35.50, 53.90)	45.30 ± 11.70 (36.93, 53.67)	38.20 ± 9.46 (31.43, 44.97)	25.30 ± 10.66 (17.67, 32.93)	13.10 ± 9.43 (6.35, 19.85)
LPC	0.00 ± 0.00 (0.00, 0.00)	39.80 ± 11.66 (31.46, 48.14)	41.40 ± 11.61 (33.09, 49.71)	43.80 ± 12.16 (35.10, 52.50)	36.40 ± 9.85 (29.35, 43.45)	21.69 ± 10.40 (14.25, 29.13)	15.60 ± 8.63 (9.43, 21.77)
LPP	0.00 ± 0.00 (0.00, 0.00)	40.20 ± 12.80 (31.04, 49.36)	34.50 ± 10.72 (26.83, 42.17)	22.90 ± 10.33[a,b,c] (15.51, 30.29)	17.80 ± 8.55[a,b,c] (11.68, 23.92)	9.30 ± 5.32[a,b,c] (5.49, 13.11)	0.00 ± 0.00[a,b,c] (0.00, 0.00)
Creatine kinase, $U \cdot L^{-1}$							
Placebo	148.06 ± 32.12 (125.10, 171.00)	256.30 ± 69.63 (206.50, 306.10)	196.95 ± 6.56 (192.30, 201.60)	234.85 ± 80.26 (177.40, 292.30)	413.20 ± 77.36 (357.90, 468.50)	230.35 ± 42.51 (199.90, 260.80)	227.75 ± 57.21 (186.80, 268.70)
HPC	138.30 ± 34.49 (113.60, 163.00)	306.51 ± 76.41 (251.80, 361.20)	273.62 ± 53.14[b] (235.60, 311.60)	317.70 ± 87.65[b] (255.00, 380.40)	410.18 ± 88.38 (347.00, 473.40)	261.97 ± 89.53 (197.90, 326.00)	227.08 ± 64.64 (180.80, 273.30)
LPC	137.29 ± 17.04 (125.10, 149.50)	246.28 ± 48.24 (211.80, 280.80)	207.76 ± 49.58 (172.30, 243.20)	185.97 ± 44.23[a] (154.30, 217.60)	195.71 ± 33.84[b,c] (171.50, 219.90)	207.47 ± 66.35 (160.00, 254.90)	208.78 ± 46.94 (175.20, 242.40)
LPP	153.23 ± 44.60 (121.30, 185.10)	188.98 ± 48.72[a] (154.10, 223.80)	154.35 ± 34.89[b] (129.40, 179.30)	150.41 ± 39.76[a,b] (122.00, 178.90)	136.84 ± 36.02[b,c] (111.10, 162.60)	141.76 ± 46.02[b,c] (108.80, 174.70)	157.69 ± 45.73[b,c] (125.00, 190.40)

Abbreviations: HPC, high-powered continuous laser/light; LPC, low-powered continuous laser/light; LPP, low-powered pulsed laser/light.

[a] Different from HPC ($P < .05$).

[b] Different from placebo ($P < .05$).

[c] Different from LPC ($P < .05$).

When using a high-powered laser, Kim and Jeong[23] noted that if the hyperthermia lasts for several minutes, significant thermal damage may occur in biological tissues. The increase in human skin temperature can be significantly underestimated if the dependence of the optical properties of human skin on temperature is ignored during PBM treatments.[21] Total irradiation time was substantially lower; however, participants discerned appreciable heat during the application.

Both low-powered laser devices (pulsed laser/light and continuous laser/light) improved recovery times. The low-powered pulsed laser/light group demonstrated accelerated recovery to baseline that was nearly 100% faster than the placebo group, and the low-powered continuous laser/light group demonstrated a 50% acceleration. The low-powered pulsed laser/light group was able to return participants to baseline at 48 hours compared with 72 hours for the low-powered continuous laser/light group and 96 hours for the placebo group. The low-powered pulsed laser/light group maintained strength at almost 100% from immediately after to 48 hours after eccentric exercise; from 48 to 96 hours after eccentric exercise, participants were able to perform with 5% to 15% more strength over the baseline measurement. Tissue heating may have negatively affected the phototherapeutic outcome in the high-powered continuous laser/light group, as indicated by the increase in CK activity. The "pulsing" of the low-powered pulsed laser/light device and the low power of the low-powered continuous laser/light device may explain the superior results compared with the high-powered continuous laser/light treatment, because both devices generate only a small amount of superficial heat.[21,22]

The MVC results of the high-powered continuous laser/light group are similar to those of Larkin-Kaiser et al,[28] who applied 360 J using a high-powered laser and demonstrated a small, nonsignificant difference between placebo and the active groups at 24 hours and no difference at 48 hours after the treatment. However, we delivered nearly 50% of that dose and found similar reductions in MVC, which were not reversed during the course of the study.

Although the dose we selected for the high-powered continuous laser/light group (the power density was between both low-powered pulsed laser/light and low-powered continuous laser/light groups) did not exhibit a positive effect on muscle performance or pain compared with placebo, it did demonstrate an effect on CK activity ($P < .05$). Therefore, the selected dose for the high-powered continuous laser/light group cannot be considered too low to achieve biological effects. Laboratory studies have shown that more is not necessarily better and that the positive effects may, in fact, be lost when overdosing PBM.[41]

Normal recovery occurs between 24 and 48 hours. However, participants in the high-powered continuous laser/light group did not fully recover to baseline; in fact, they recovered only 60% to 70% of their original MVC. Individuals being treated with this type of high-powered device may exhibit a decreased level of performance. Moreover, the incidence of overuse injuries may increase. These would be alarming findings because in many sports, daily practices including multiple events are common.

The low-powered pulsed laser/light group maintained CK activity levels near baseline from 24 to 96 hours, even though the placebo group increased nearly 50% at 24 hours and experienced a marked increase of 215% at 48 hours using the same eccentric-exercise protocol. The low-powered continuous laser/light group demonstrated decreased CK activity at 24 hours, which was 35% less than the decrease seen in the low-powered pulsed laser/light group at the same time point. Compared with placebo, an increase in CK activity was evident, indicating additional muscle damage from the high-powered continuous laser/light treatment.

Although the literature suggests a range between 125 and 180 J, the dose delivered by the high-powered continuous laser/light did not exhibit the same prophylactic and stimulatory effects on muscle performance and recovery.[6,7] The additional CK activity in the high-powered continuous laser/light group correlates with the decreased muscle strength noted in MVC. These participants fatigued faster than those in the other groups, which may have caused the muscles to work harder and experience catabolic effects. No participants dropped out of our study, but Larkin-Kaiser et al[28] had 1 participant drop out because of excessive arm pain. This corresponds with our finding that the high-powered continuous laser/light treatment did not improve muscle performance or modulate the pain associated with DOMS. Further studies of the effects and mechanisms behind high-powered laser/light may provide optimal settings; insufficient data have been published to date for high-powered lasers.[42] Finally, it is important to highlight that for time points when differences ($P < .05$) were observed in favor of the low-powered pulsed laser/light compared with both placebo and high-powered continuous laser/light conditions, confidence intervals among treatments tested did not overlap. This leads us to believe that the observed differences are clinically meaningful and can help health care professionals to make better clinical decisions.

We conclude that low-powered pulsed laser/light (with a combination of different wavelengths and light sources) showed better effects on performance enhancement and postexercise recovery than low-powered or high-powered continuous laser/light. Additionally, high-powered continuous laser/light did not have any effect on performance enhancement or postexercise recovery. Our findings can help clinicians make better decisions regarding device choice in this field.

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3.3. Capitulo III

Photobiomodulation therapy before futsal matches improves the staying time of athletes in the court and accelerates post-exercise recovery

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ORIGINAL ARTICLE

Photobiomodulation therapy before futsal matches improves the staying time of athletes in the court and accelerates post-exercise recovery

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Abstract
This study aimed to analyze PBMT effects on futsal player's performance and recovery in a non-controlled field environment. It is a randomized, triple-blinded, placebo-controlled, crossover clinical trial. The research included six professional athletes and in each match phototherapy treatments were performed before matches (40 minutes), blood samples were collected before treatments, and samples immediately after the end of the matches and 48 h after. Furthermore, videos were analyzed to quantify the time athletes spent on the pitch and the distance they covered. PBMT was performed at 17 sites of each lower limb (40 mins before matches), employing a cluster with 12 diodes (4 laser diodes of 905 nm, 4 LEDs of 875 nm, and 4 LEDs of 640 nm, 30 J per site). The performance of the athlete could be quantified considering the time on the pitch and the distance covered; the biochemical markers evaluated were creatine kinase, lactate dehydrogenase, blood lactate, and oxidative damage to lipids and proteins. PBMT significantly increased the time of staying in the pitch and a significant improvement in all the biochemical markers evaluated. No statistically significant difference was found for the distance covered. Pre-exercise PBMT can enhance performance and accelerate recovery of high-level futsal players.

Keywords Low-level laser therapy · Light-emitting diodes · Sport · Exercise · Phototherapy

Introduction

Futsal is an indoor adaptation of conventional soccer. Also known as indoor soccer, it was invented in 1934 at the Young Men Christians' Association in Montevideo, Uruguay, where it was first named "indoor-foot-ball." It has been recognized worldwide by the Fédération Internationale de Football Association (FIFA) since 1989 [1]. A futsal match is composed of two 20-min periods and a 10-min break. Some matches may take up to 80 min because the timer in futsal is stopped every time the ball leaves the pitch boundaries. This sport consists of intense physical activity with frequent bursts of high-intensity activity, such as attacking and defending movements/sprints, rapid direction changes with very short, low-intensity intervals (4–8 s), such as walking or running. Game preparation requires training in a combination of strength, power, agility, speed, aerobic and anaerobic, and endurance. Such intense acceleration/deceleration, brisk

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direction change, and physical contact with other athletes expose players to muscular fatigue. Consequently, players may be prone to osteoarticular lesions [2].

Milioni et al. [3] claim that fatigue symptoms occur immediately after a match, which usually persist for a few days. Damage to large muscles, physiological stress, and muscle function impairment are commonly found among players, resulting in decreased performance and ability. Strategies should be employed to improve and speed up recovery after the games to better prepare the athletes for the next match.

Metabolism during contractile activity produces reactive oxygen species (ROS), which may lead muscles to develop oxidative stress. This may be a factor associated with reduced contractile function and muscular fatigue development [4]. Currently, injury prevention in competitive sport has been attracting more attention from the scientific community, committees, and athletes themselves. Using resources, such as photobiomodulation therapy (PBMT) [5], has gained increasing interest when employed to prevent and treat various diseases, such as muscular injuries, tendon injuries, and osteoarthritis, etc.

The first randomized clinical trial (RCT) investigating the use of photobiomodulation therapy (PBMT) for athletic performance enhancement was published 10 years ago by Leal-Junior et al. [6] only 2 years after the first animal study in this field was published by Lopes-Martins et al. [7]. This pioneer RCT showed for the very first time that the treatment with PBMT before an exercise session could enhance the performance of high-level volleyball athletes decreasing the onset muscle fatigue and preventing the expected increase of blood lactate levels. Currently, PBMT with lasers or light-emitting diodes (LEDs) has been proven to prevent skeletal muscle fatigue and speed up recovery [8–10].

A series of studies have shown that PBMT can reduce muscle fatigue and increase its contraction force and performance [4, 8–12]. PBMT may delay onset of fatigue, thereby improving athletic performance [8–10]. PBMT is a commercially available non-thermal modality [13] that may be used in a variety of clinical and athletic settings. The effects of PBMT are related to photochemical and photobiological effects within the tissue, which are not related to heat [13]. Non-thermal therapy modulates the biological processes of cells at the mitochondrial level. This increases oxygen consumption and adenosine triphosphate (ATP) production [13].

Several studies have shown the positive effects of PBMT on biochemical markers related to muscle damage and recovery [8], including blood lactate levels. In addition, PBMT decreases the necessary recovery time between exercise sessions [4, 11]. More recently, the literature has shown beneficial effects on muscle recovery if PBMT is applied using a combination of different wavelengths synergistically [14, 15]. This suggests that the combined use of different wavelengths may optimize cytochrome c oxidase modulation, which may increase PBMT effects [15].

Currently, most randomized clinical trials (RCTs) demonstrating the effectiveness of PBMT in increasing exercise performance and recovery acceleration have been conducted in a controlled environment such as laboratories or semi-controlled environment such as field tests [16]. To demonstrate the application and translation to the real world, this therapy must be tested in real competition settings and in different sports' modalities to confirm the findings previously observed in controlled laboratory tests. Thus, this study aimed to analyze PBMT effects on futsal players' performance and recovery in a non-controlled field environment.

Methods

Ethical aspects

This study was approved by the Committee of the Faculdade Cenecista of Bento Gonçalves, opinion number 055270/2015. In accordance with the Declaration of Helsinki, all subjects were advised regarding the procedure, and they signed an informed consent before participation in the study.

This study is a randomized, triple-blinded, placebo-controlled, crossover clinical trial. Six professional athletes of team Bento Gonçalves Futsal, who were competing on the gold series state championship, were selected for this study. Convenience sampling was employed based on the number of voluntary athletes. This was due to save research time and equal training routines among all participating athletes. The study was conducted in two different team matches in a time interval of approximately 2 weeks between the first and second interventions. The research team was present in each match to collect baseline blood samples, employ phototherapy protocol based on randomization, and perform other collections immediately after the end of the match and 48 h after. Furthermore, videos were analyzed to quantify the time that athletes spent on the pitch and the distance they covered (Fig. 1). All volunteers received information on all study procedures before the study was conducted. They also signed the consent form before be enroled in this trial.

The study included professional male athletes aged between 18 and 35 years, normotensive, eupneic, with normal heart rate. They did not have a history of musculoskeletal injury in the hip and knee regions 2 months before the study. They remained on the pitch for a minimum of 9 min, and they did not use pharmacological agents and/or nutritional supplements and participated with a minimum frequency of 80% of the team training sessions. Athletes who did not meet the aforementioned criteria, those who had musculoskeletal injury during the study, and those who may have their training routine changed during the study for any reason were also excluded from this study.

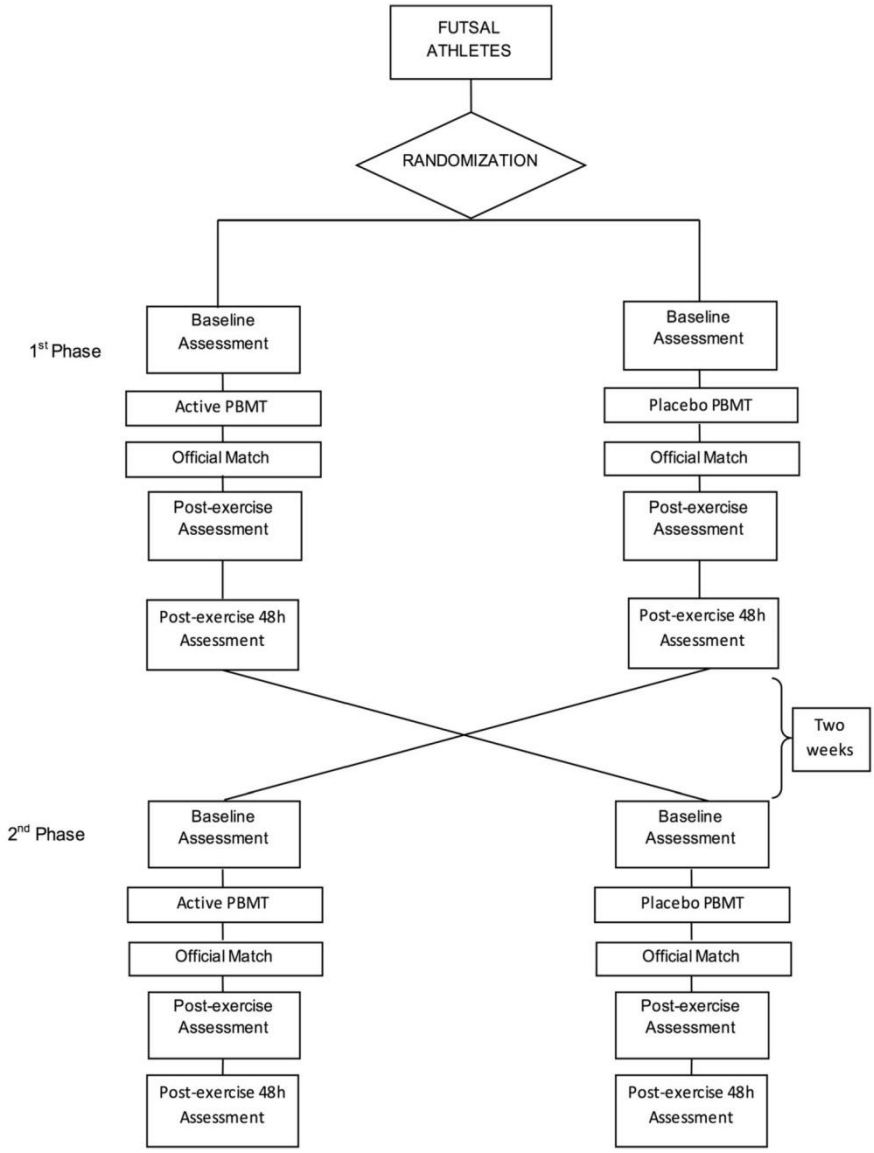

Fig. 1 Consort flowchart summarizing study procedures

Randomization and blinding procedures

The six team volunteers were randomly separated into two experimental groups of three volunteers each. Randomization was performed through a simple drawing of lots (A or B) prepared in opaque envelopes, which determines whether a volunteer receives active phototherapy in the first or second match. The volunteers who drew lot A received effective and placebo phototherapy in the first and second matches, respectively, whereas the volunteers who drew lot B received placebo and effective phototherapy in the first and second matches, respectively. The laser unit used in the study emitted the same sounds regardless of the programmed mode (effective or placebo). Randomization was conducted by a participating researcher who was in charge of programming the laser device in accordance with the randomization result. This researcher was instructed not to communicate the treatment type administered to any of the other researchers involved in the study until its end. Similarly, the researcher responsible for employing phototherapy did not know the phototherapy type (effective or placebo) that was being used with the volunteers.

Photobiomodulation therapy

The phototherapy protocol was conducted in the same way in both interventions. After randomization, athletes underwent phototherapy 40 min before they entered the pitch. Twelve clusters with 12 diodes were used for phototherapy. Four

905-nm diodes (0.3125-mW mean power, 50-W peak power for each diode), four 875-nm diodes (17.5-mW mean power for each diode), and four 640-nm diodes (15-mW mean power for each diode) were used. They were manufactured by Multi Radiance Medical® (Solon, OH, USA). This device was chosen by the research team due to its high quality and reliability [15]. Both effective application and placebo were performed using the cluster in direct contact with the skin, with light pressure and in a stationary way in an isolated environment. This was performed in the presence of the volunteer and the researcher responsible for implementation only (Table 1). A protection with opaque googles was used by the athletes not to identify whether administration was placebo or effective. It must be pointed out the device does not produce any thermal sensation in the patient's skin, and the sounds were emitted regardless of the way phototherapy was administered. Phototherapy was 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. Administration areas were selected based on studies previously performed using this same device [15–18]. The irradiation locations are illustrated in Fig. 2, and 17 sites of each lower limb were irradiated. Each site of irradiation received a 30-J dose delivered in 228 s (3 min and 48 s per site).

Table 1 Parameters for PBMT

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

Video analysis

Video recording of the games played by the team were used by the research team to measure the time and distance in pitch, by numbering the shirt each athlete used on the pitch. The distance covered by each athlete on the pitch was measured based on Withers et al. [19], who used footage of the athletes through 9 m in five different types of movement: walking, trotting, running, and lateral and backward movements. After filming, the space traveled was divided by the number of steps to determine each athlete's length of step in different forms of travel. Pitch time was collected by measuring the time each athlete was on the pitch, with the ball in play [19]. The researcher involved in the video analysis was blind to all other methodological steps.

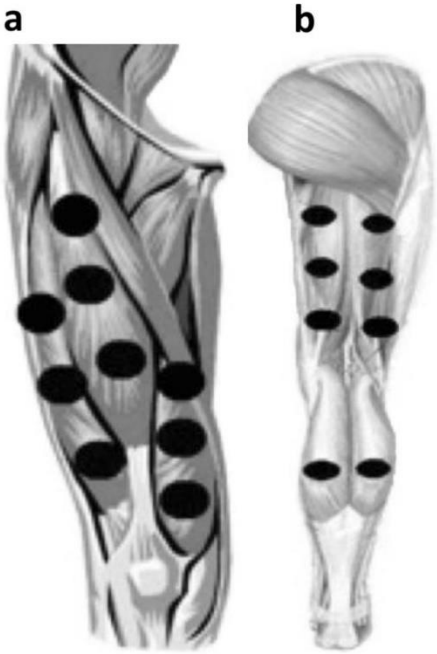

Fig. 2 **a** Treatment points in knee extensor muscles. **b** Treatment points in knee flexor and ankle plantiflexor muscles

Blood samples and biochemical assays

Blood samples were collected by a qualified nurse blinded to group allocation and were obtained from an antecubital vein before exercise and exactly 5 min, 60 min, 24 h, 48 h, and 72 h after the end of the exercise protocol. Blood was centrifuged at 2700×*g* for 10 min at 4 °C. Serum was immediately pipetted into Eppendorf tubes and stored at − 80 °C until analysis. Lipid damages were measured spectrophotometrically (Shimadzu spectrophotometer Model UV-1700, Shimadzu®, Japan) by determining thiobarbituric acid reactive substances (TBARS), as previously described by Wills [20]. Results were expressed as nmol/ml. The oxidative damage to proteins was assessed by determining carbonyl groups based on the reaction with 2,4-dinitrophenylhydrazine (DNPH), as previously described by Levine et al. [21]. Results were expressed as DNPH nmol/mg of protein. Total protein levels were evaluated using the total protein kit from Labtest® (Protein Kit, Labtest Diagnostica S.A., Brazil). The concentration of L-lactate was measured by enzymatic activity of lactic dehydrogenase (LDH), which forms NADH. It was measured in UV at 340 nm. LDH concentration was determined by a kinetic reaction that assessed NADH decomposition speed. A decrease in resorption was assessed at 340 nm. The activity of creatine kinase-MB fraction was evaluated by inhibiting M-fraction activity using a specific antibody. Consequently, it inhibited MM fractions and the fraction M of MB. Starting from the assumption that the BB dimer is virtually nonexistent in peripheral blood, the residual enzymatic activity corresponds to fraction B of CK-MB only. With regard to the NADP+ reduction speed, NADPH is proportional to the activity of CK in the sample. All laboratory analyses were performed at the laboratory of Clinical Analyses of the Non-Profit Bento Gonçalves College using a Mindray BS-120 chemistry analyzer employing protocols and BioClin Quibasa reagents and controls.

Statistical analysis

Data are expressed as mean and SD in text and as mean and SEM in the figures. To analyze data, levels of CK, TBARS, CP, LDH, and lactate were considered in blood samples collected before, immediately after, and 48 h after a match. The time on pitch and the distance covered by the athletes on the pitch were also taken into account. The values obtained for each variable will undergo the Shapiro–Wilk normality test. Based on randomization, variables were compared using *t* test (time on pitch and distance covered) and analysis of variance, with repeated measurements for the factors of time of collections, as well as testing between- and within-group differences (followed by a post hoc Bonferroni test). The SPSS 17.0 software was used for the statistical analysis, with a significance level of 5% ($p < 0.05$). Magnitude-based inference analyses were also used to examine practical significances. The magnitude of differences (Cohen-d) between groups was calculated using the mean and SD of placebo and PBMT treatments (using Gpower 3.1). We adopted the criteria of Cohen for the analysis (0.2: small; 0.50: moderate; 0.80: large).

Results

Six healthy male, futsal athletes that met the inclusion criteria participated in this study. The athletes' mean age was 26.16 ± 6.91 years.

In this study, the performance of the athlete could be quantified considering the time on the pitch. A statistically significant difference ($p < 0.05$) was found between placebo treatment (23.44 ± 5.71 min) when compared to PBMT (29.15 ± 10.92 min), as shown in Fig. 3a. No statistically significant difference was found for the distance covered by the athlete on the pitch. This variable was analyzed by comparing placebo treatment (2317.89 ± 786.79 m) with PBMT (2409.92 ± 613.44 m). Considering placebo tratment performance as 100%, we have a performance increase of 24.71% in time on the pitch and 3.97% in distance covered.

The lactate analyses are shown in Fig. 3b. A statistically significant difference ($p < 0.01$) was found among pre- (15.33 ± 2.88 mmol/l^{-1}), post, and post 48-h collections (30.63 ± 11.29 and 22.73 ± 6.87 mmol/l^{-1}) in placebo treatment. Moreover, a statistically significant difference ($p < 0.01$) was

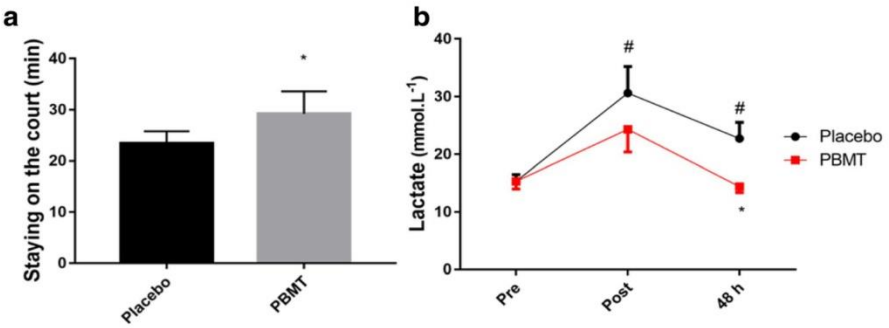

Fig. 3 **a** *Statistically significant difference in comparison between the placebo and photobiomodulatoin treatments ($p > 0.05$). **b** #Statistically significant difference in comparison with the pre ($p < 0.05$),

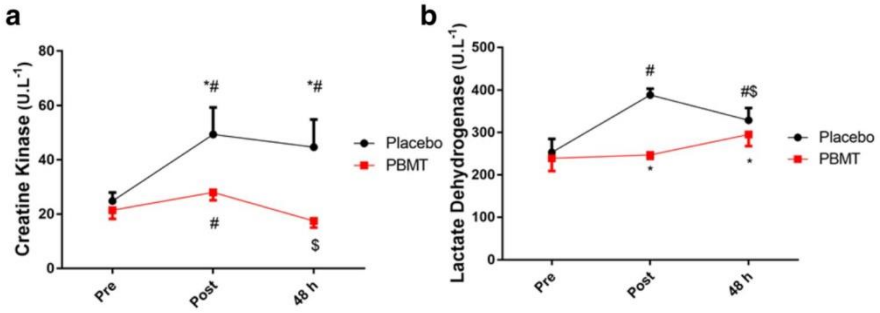

Fig. 4 **a, b** #Statistically significant difference in comparison with the pre ($p < 0.05$), $ statistically significant difference in comparison with the post and 48 h ($p < 0.05$), *statistically significant difference in comparison between the placebo and photobiomodulation groups

found after comparing post 48-h collection in placebo treatment (22.73 ± 6.87 mmol/l^{-1}) and post 48-h collection in PBMT (14.40 ± 2.58 mmol/l^{-1}). Considering the concentration of lactate pre-collection in the placebo treatment as 100%, we have an increase of 99.80% in post-match and 48.27% in 48 h for the placebo treatment. In the PBMT, an increase of 58.71% in post-match and a decrease of 6.07% in 48 h are observed.

With regard to CK analysis results (Fig. 4a), a statistically significant difference ($p < 0.05$) was found among pre-collection (24.8 ± 7.75 U/l), post-collection, and post 48 h (49.33 ± 24.37 U/l and 44.69 ± 24.91 U/l, respectively) in the placebo treatment. In addition, statistically significant differences ($p < 0.05$) were found between post-collection and post 48 h (27.95 ± 7.17 and 17.46 ± 5.96 U/l, respectively) in the photobiomodulation group (PBMT). A statistically significant difference ($p < 0.05$) was found after comparing enzyme levels of post-match and post 48 h of placebo treatment (49.33 ± 24.37 and 44.69 ± 24.91 U/l, respectively) and PBMT (27.95 ± 7.17 and 17.46 ± 5.96 U/l). Considering the concentration of CK pre-collection in the placebo treatment as 100%, we have an increase of 98.91% in post-match and 80.20% in 48 h for the placebo treatment. In the PBMT group, an increase of 30.61% in the post-match and a decrease of 18.41% in 48 h are observed.

The LDH analysis (Fig. 4b) revealed a statistically significant difference ($p < 0.05$) among pre-collection (253.00 ± 78.19 U/l), post-match, and post 48-h collections (388.40 ± 36.52 and 328.67 ± 70.85 U/l) in placebo treatment. Another statistically significant difference ($p < 0.05$) was found between the post-match and post 48-h collections of placebo treatment. A statistically significant difference ($p < 0.05$) was also found between the comparison of LDH enzyme levels of post- and post 48-h samples of placebo treatment and PBMT (247.08 ± 22.75 and 295.20 ± 65.88 U/l). Considering the concentration of LDH pre-collection in the placebo treatment as 100%, we have an increase of 53.52% in post-match and 29.91% in 48 h for the placebo treatment. In the PBMT, an increase of 3.45% in the post-match and 23.60% in 48 h is observed.

The biochemical marker concentrations of oxidative damage to lipids, as shown in Fig. 5a, indicate that a significant decrease in the concentration of TBARS in PBMT post-collection (3.30 ± 0.43) was found after treatment compared with pre-collection (3.58 ± 0.35) and placebo treatment (3.96 ± 0.44) ($p < 0.05$). Considering the concentration of TBARS pre-collection in the placebo treatment as 100%, we have an increase of 2.78% in post-match and a decrease of 9.60% in 48 h for the placebo treatment. In the PBMT, a decrease of 7.82% in the post-match and 3.35% in 48 h is observed. In

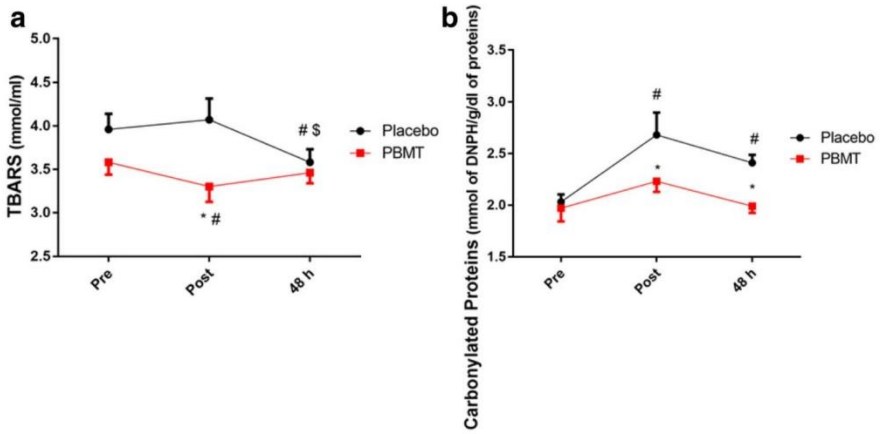

Fig. 5 **a** #Statistically significant difference in comparison with the pre ($p < 0.05$), $ statistically significant difference in comparison with the post and 48 h ($p < 0.05$), *statistically significant difference in comparison between the placebo and photobiomodulation groups. **b** #Statistically significant difference in comparison with the pre ($p < 0.05$), *statistically significant difference in comparison between the placebo and photobiomodulation groups

addition, our results of biochemical marker concentrations of oxidative damage to proteins indicate a significant decrease in concentrations of CP in the PBMT compared with the placebo treatment ($p < 0.05$) as shown in Fig. 5b. Considering the oxidative damage to proteins in pre-exercise samples in the placebo treatment as 100%, we have an increase of 32.02% in post-match and 18.72% in 48 h for the placebo treatment. In the PBMT, there is an increase of 13.20% in the post-match and 1.02% in 48 h. In addition, Table 2 shows the effect sizes for all analyzed variables.

Discussion

The aim of this study was to verify the effectiveness of phototherapy (PBMT) as a preventative resource against fatigue and muscle damage when administered before an official futsal match. Considering this is a sport increasing in popularity and played in competitive environment, and ever-increasing levels, numerous factors may interfere in the outcomes of matches [2, 3]. Technical issues and peculiar choices made by the technical staff for some athletes require them to be in optimum shape to cope with sustained exertion with minimal rest periods. Furthermore, emotional and psychological factors, such as personal demands, goals, and family pressure may directly interfere with an athlete's performance on the pitch [1]. This study's results reveal that phototherapy may be an alternative to prevent athletes from experiencing fatigue and muscle damage caused by this highly competitive sport.

Current studies [8–10, 15, 22, 23] suggest that previous use of PBMT has positive effects on maintaining muscle condition during physical activity and improving muscle recovery after exercise. However, most of these clinical trials were performed in a controlled environment laboratory. In this study, the possibilities of practical implementation of this tool were investigated, considering the sport scenario.

In a study conducted by Ferraresi et al. [24], results showing the effectiveness of preventative phototherapy in male volleyball athletes in real competition situations were found to inhibit the expected increase of CK enzyme activity in the bloodstream 24 h after the match. This study used four different doses, and one of them was a placebo. The study found that the doses 210 J and J 315 were effective in preventing the increase in CK enzyme activity in the bloodstream. However, this study does not monitored any aspect related to athletes' activity during the matches such as the number of jumps or spikes performed or the pitch time for each athlete during each match analyzed. Without data related to athletes' activity, it is not possible to infer that the variation in CK is reliable. Furthermore, a letter to the editor [25] about this article raised serious doubts and concerns about the methodological design and the statistical analysis, invalidating its findings.

Compared with these results, this study shows that the effective application of PBMT was also able to promote a reduction in the blood concentrations of muscle damage and oxidative markers comparing placebo and PBMT, and all athletes participated assiduously in matches. In addition, to measuring the real benefits of preventative phototherapy as a way of reducing muscle fatigue in athletes, this study found aspects wherein PBMT played a role in the improvement of athletes' performance. This statement is based on the comparison between placebo and PBMT regarding time on the pitch of athletes during the matches.

Some authors state that the response to oxidative damage in athletes is higher than in sedentary individuals [26]. In this study, such placebo treatment response is found in post 48-h assessments due to the similarity with the pre-exercise situation. However, the same athletes who participated in the

Table 2 The magnitude of differences between groups

		Percentage of change compared to placebo	Cohen-d	Effect rating
CK	Post-match	–43.34	1.1902	Large
	Post 48 h	–60.93	1.5029	Large
LDH	Post-match	–36.39	4.6449	Large
	Post 48 h	–10,18	0.4892	Moderate
TBARS	Post-match	–18.92	1.4751	Large
	Post 48 h	–3.35	0.3562	Small
CP	Post-match	–16.79	1.0859	Large
	Post 48 h	–17.43	2.3912	Large
Lactate	Post-match	–20.57	0.6170	Moderate
	Post 48 h	–36.65	1.6052	Large
Time on pitch	–	24.71	0.6553	Moderate
Distance covered	–	3.97	0.1304	Small

CK creatine kinase, *LDH* lactate dehydrogenase, *TBARS* oxidative damage to lipid, *CP* oxidative damage to protein

effective PBMT displayed faster defense responses to oxidative damage caused by exercise. Therefore, considering previous results associated at the laboratory level [4], it is evident that PBMT plays an important role in redox regulation of muscle metabolism, whether it is basal or after adverse stimuli generated by inflammatory processes, among other micro- or micro-damage consequences.

The non-thermal PBMT has a modulating effect on cytochrome c oxidase activity, and it may explain how PBMT improves performance while protecting the skeletal muscle from exercise-induced damage. This has been considered as the key mechanism in the light-tissue interaction, which increases cellular metabolism by increasing mitochondrial function [27]. Recently, Albuquerque-Pontes et al. [28] have shown that a single PBMT irradiation can increase cytochrome c oxidase activity in intact skeletal muscle up to 24 h after irradiation, and this is dependent on the dose and the wavelength used. In fact, the use of three wavelengths synergistically can lead to optimized outcomes in exercise performance enhancement since different wavelengths have different time-response windows in intact (non-injured) skeletal muscles [28]. Therefore, only the simultaneous use of three wavelengths in different bands of spectrum (from red to near infrared) can enhance cytochrome c oxidase activity, and consequently mitochondrial function and ATP production, from 5 min to 24 h after irradiation, which gives us the rationale to use a device that allows the simultaneous use of three wavelengths.

Lactate is another frequently used marker to indirectly monitor muscle recovery performance. The concentration of lactate in the blood is considered an important marker of muscular acidosis, and it is often monitored in sports environments, particularly in high-intensity sports [29–31]. Higher levels of this marker should be and were expected in PBMT group since athlete's performance was significantly better. Thus, it is reasonable to say that active PBMT prevented the expected increase in blood lactate levels, reduced muscle fatigue, and led to quicker recovery after exercise, supporting previous findings [4, 32, 33].

Athletes' enhanced stay on the pitch may be directly correlated with the fact that PBMT was able to delay the process of muscle fatigue in athletes during a futsal match. Despite this, with longer activity time, the damage caused by strenuous physical exercise was reduced 48 h after the match. Thus, the head coach might possibly take technical and tactic actions with more decisive athletes for longer periods during matches, leading to a better performance to the futsal team in a general way. In Table 2, we can observe positive effects in favor of the use of PBMT for all the variables analyzed, even in the variable distance traveled, which we did not find statistically significantly difference. However, its is important to highlight that in high-level sports activities an average improvement of 3.97% in performance can be the difference between win or lose.

This study has a complex organization because it investigated an official competition, and the researchers attempted to respect the official team planning as much as possible. Some limitations need to be considered, such as the final number of participating athletes based on the proposed methodological choices and the freedom of choice of the team's technical committee. In addition, only one PBMT treatment session was performed (40 min before matches), and previous studies [12, 23, 34] showed that treatment sessions subsequent to activity may be beneficial in muscle recovery. As a result, the combination of sessions (pre and post) may still enhance the effects found, considering the athletes' muscle recovery level. Finally, this study's results demonstrate the potential use of PBMT as a prophylactic strategy for the performance and improvement of high-level athletic recovery, and this seems to be a relevant advancement in the evidence to the clinical use of this therapeutic tool.

Conclusion

In summary, preventative implementation of PBMT in professional futsal players appears to be effective in preventing fatigue and muscle damage. Consequently, it accelerates muscle recovery and improves the performance of athletes who remain on the pitch for the longest period of time. Finally, further studies are needed to verify the effects of PBMT in different sports modalities in order to make this therapy wide used as an ergogenic agent.

Compliance with ethical standards

Competing interests Professor Ernesto Cesar Pinto Leal-Junior receives research support from Multi Radiance Medical (Solon, OH, USA), a laser device manufacturer. Multi Radiance Medical had no role in the planning of this study. They had no influence on study design, data collection and analysis, decision to publish, or preparation of the manuscript. The remaining authors declare that they have no conflict of interests.

Ethical aspects The study was approved by the Ethics Committee of the Faculdade Cenecista of Bento Gonçalves. In accordance with the Declaration of Helsinki, all subjects were advised about the procedure and they signed an informed consent prior to participation in the study (CAEE 46096015.4.0000.5571).

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4. Discussão geral

A fadiga, o dano muscular e a dor muscular tardia são algumas consequências que podem ocorrer após o exercício, principalmente, excêntrico. Tanto a fadiga quanto a falta de uma recuperação muscular adequada podem predispor a lesões, impedindo que o atleta esteja apto para uma nova sessão de treinamentos ou competições. A reabilitação física e a recuperação muscular ainda são baseadas em muitos conhecimentos empíricos, com a utilização de vários recursos eletrofototermoterapêuticos, aplicados de maneira conjunta, um após o outro, sem a ciência e o conhecimento de seus mecanismos de ação. Entre eles, a FBM tem sido utilizada, habitualmente, como forma de reabilitação de lesões físicas, incluindo torções e lesões musculares. Atualmente, nosso grupo de pesquisa vem demonstrando que a utilização de FBM pode retardar a fadiga muscular, o dano muscular e a inflamação (De Marchi et al., 2012; Leal Junior et. al., 2015). Além da FBM, a crioterapia é uma das formas mais baratas utilizadas para recuperações musculares após treinos, seja por atletas ou não, e normalmente sua utilização tem como objetivo a diminuição do dano e melhora na recuperação muscular.

No entanto, até o momento, não são totalmente conhecidos os mecanismos de ação destas duas terapias, nem os possíveis efeitos de sua aplicação conjunta. A verificação de seus reais efeitos é rara e encontramos apenas um estudo quando pesquisamos os efeitos do FBM associado à crioterapia em humanos. Em nosso conhecimento, esta foi a primeira vez que a sinergia destas terapias foi testada visando a melhora do desempenho no exercício e na recuperação muscular pós-exercício.

Muitos estudos (Baroni et al., 2010; De Marchi et al., 2012; Leal Junior et al., 2015; Vanin et al., 2016a), demonstraram os efeitos protetores da FBM quando aplicado antes do exercício; assim, usamos fototerapia e crioterapia como recursos para auxiliar o

processo de recuperação muscular, e aplicamos as modalidades subsequentes ao desempenho no protocolo de indução a fadiga muscular.

Observamos (capítulo I) que a FBM tem um potencial considerável não só para a prevenção da fadiga muscular e danos causados por exercícios de alta intensidade, mas também pode melhorar as condições de desempenho quando aplicada após o exercício, a fim de atingir uma real recuperação muscular em menos tempo. O músculo esquelético humano é projetado para suportar sobrecargas mecânicas e metabólicas até certo limite. Quando estimulado, ele atinge rapidamente a carga máxima de contração, assim como seu fluxo de oxigênio aumenta em até 100% o que pode levar ao aumento do estresse oxidativo (Alessio et al., 2000). Sabe-se que esse fenômeno acompanha toda atividade contrátil esquelética (Fukuda et al., 2016) e pode causar uma diminuição da função contrátil dos grupos musculares envolvidos e produzir fadiga (Reid et al, 1992). A crioterapia tem sido amplamente utilizada nos esportes para prevenir a lesão muscular e melhorar a recuperação pós exercícios (Ascensão et al., 2011; Leeder et al., 2012). Portanto, não é surpreendente que a crioterapia, apesar de não ter demonstrado qualquer efeito na manutenção ou aumento do CVM após uso isolado, demonstrou algum efeito na redução de marcadores de dano oxidativo a lipídios e proteínas, provavelmente através dos seus conhecidos efeitos na vasoconstrição, redução na temperatura muscular e atividade inflamatória (Bailey et al., 2007; Pournot et al., 2011). Dessa forma, o dano oxidativo gerado aos lipídios e proteínas pelo processo de isquemia-reperfusão podem ter sido reduzidos pela crioterapia. Associado a essas descobertas, é importante ressaltar que a crioterapia não teve influência na manutenção da capacidade de CVM, tendo um comportamento semelhante ao grupo placebo. Estudos anteriores relatam que a crioterapia pode reduzir a velocidade de condução nervosa e não só mudar a percepção da dor, mas também interferir com o recrutamento de unidades motoras (García-Manso

et al., 2011). Em contraste, os grupos que receberam a FBM apresentaram uma melhoria significativa no CVM após 60 minutos da aplicação do protocolo de recuperação muscular. Os resultados obtidos pela crioterapia na redução de marcadores de dano oxidativo a lipídios e proteínas também foram atingidos no grupo que recebeu apenas a aplicação de FBM. Além disso, esse grupo teve uma diminuição significativa no marcador de dano muscular (CK), o que não foi observado no grupo crioterapia. Resultados semelhante foram relatados em estudos realizados em animais (Camargo et al., 2012; da Costa Santos et al., 2014; de Almeida et al., 2014), onde o uso de imersão em água fria e crioterapia provou ser ineficaz para proporcionar recuperação muscular eficiente, enquanto o uso de FBM foi capaz de melhorar a condição muscular 24 horas após o exercício.

Outro fator interessante é o fato de que a aplicação conjunta das intervenções terapêuticas (grupos cryotherapy + PBMT e PBMT + cryotherapy) não se mostraram muito relevante, como indicado pelos resultados obtidos com a aplicação conjunta (capítulo I). Por exemplo, independentemente da ordem da terapia aplicada, o modo combinado de intervenções terapêuticas não é mais eficaz do que a aplicação individual de FBM e esse achado corrobora com literatura recente (de Paiva et al., 2016).

A partir destes achados surgiram outros tipos de questionamentos, pois a FBM mostra-se cada vez mais presente e importante no dia a dia de profissionais da fisioterapia espalhados pelo mundo, bem como de seus pacientes, entretanto não se conhece qual o perfil de equipamento é mais eficiente pensando no potencial demostrado pela FBM na prevenção do dano muscular de origem redox ou não. Os primeiros trabalhos desenvolvidos utilizando a FBM foram realizados com vários tipos de lasers, e assim pensou-se que a luz laser tinha algumas características especiais não possuídas pela luz de outras fontes como a luz solar, lâmpadas fluorescentes, ou

incandescentes e agora LEDs. No entanto, a maioria dos estudos que foram feitos comparando lasers com fontes de luz equivalentes com comprimento de onda, potência e densidade de emissões semelhantes, não encontrou essencialmente nenhuma diferença entre elas (Hamblin, 2017). Dessa forma, e pela variabilidade de perfis de equipamentos encontrados no mercado, optou-se por testar diferentes tipos de equipamentos (equipamento laser de aplicação contínua, equipamento laser/LED de aplicação pulsada e equipamento laser de alta potência com aplicação contínua) visando identificar qual configuração é mais eficiente na prevenção da fadiga e dano muscular. Sendo assim, as opções de configurações dos dispositivos que testamos, a escolha da dose e do comprimento de onda foram baseados em evidências científicas encontradas na literatura atual (Borsa et al., 2013; Leal Junior et al., 2015; Vanin et al., 2016a) possibilitando assim, minimizar os possíveis vieses entre os equipamentos e fabricantes.

Observamos (capítulo II) que o grupo de laser/luz pulsada de baixa potência demonstrou preservação do desempenho muscular em comparação com o grupo placebo em todos os pontos de tempo medidos; o grupo laser/ luz contínua de baixa potência fez isso depois de 24 horas. O grupo de laser/luz pulsada de baixa potência experimentou menos fadiga muscular do que o grupo laser/luz contínua, embora a diferença tenha sido significativa somente na avaliação de 1 hora pós PIFM. Curiosamente, os resultados do CVM foram semelhantes aos observados anteriormente usando o dispositivo laser/luz contínua de baixa potência (Baroni et al., 2010) e aqueles vistos por Antonialli et al., 2014 usando o laser/luz pulsada de baixa potência.

Ainda assim, encontramos na literatura uma revisão (Nampo et al., 2016) que coloca em dúvida os efeitos proporcionados pela FMB aplicada no musculo esquelético, devido ao pequeno número de estudos atualmente encontrados, a disparidade e o tamanho das amostras dos mesmos. Porém, em contraponto, três recentes revisões

sistemáticas (Borsa et al., 2013; Leal Junior et al., 2015; Vanin et al., 2018) e uma revisão integrativa (Hamblin 2017), demonstraram resultados positivos no desempenho físico usando lasers de diodo único e multidiodos, LEDs multidiodos, e combinações de ambos os dispositivos, corroborando com nossos achados.

Pesquisas anteriores (Lord, 2014) mostraram que as taxas de lesão aumentam com o acumulo de fadiga e a mesma possui efeitos negativos na biomecânica dinâmica e estática. A recuperação total pode levar até 96h para acontecer (Edwards et al., 1977; Allen et. al., 2008). A fadiga é um aspecto muitas vezes negligenciado na decisão de devolver um atleta ao esporte ou na avaliação de um atleta quanto ao risco de desenvolver lesões. A preservação da força, como se observou em ambos os grupos de baixa potência (laser/luz pulsada de baixa potência e laser/luz contínua de baixa potência), resulta em uma redução na fadiga e melhora a capacidade do músculo quadríceps em exercer força máxima ou submáxima. O grupo laser/Luz pulsada de baixa potência demonstrou acelerar a recuperação muscular para a condição basal quase 100% mais rápida do que o grupo de placebo, o grupo laser/luz contínua de baixa potência demonstrou uma aceleração de 50%. O laser/luz pulsada de baixa potência conseguiu retornar os participantes as condições basais em 48 horas em comparação com 72 horas para o grupo de laser/luz contínua de baixa potência e 96 horas para o grupo placebo. O laser/luz pulsada de baixa potência manteve a força em quase 100% imediatamente após e 24 horas após o exercício de alta intensidade. De 48 a 96 horas após o exercício excêntrico os participantes puderam realizar com 5% a 15% mais força sobre a condição basal, sendo assim o aquecimento de tecidos pode ter afetado negativamente o resultado FBM no grupo de laser/ luz contínua de alta potência, como indicado pelo aumento da atividade CK. Os lasers com emissão de luz pulsada de baixa potência e luz contínua de baixa potência geram apenas uma pequena quantidade de

calor superficial, este fato pode auxiliar a explicar os achados superiores em comparação com o laser continuo de alta potência (Camargo et al., 2012; da Costa Santos et al., 2014).

Em relação aos resultados do CVM do grupo laser/luz contínua de alta potência os dados corroboram com os achados de Larkin et al (2015), que aplicou 360 J usando um laser de alta potência e demonstrou uma pequena diferença não significante entre os grupos placebo e ativos a 24 horas e sem diferença 48 horas após o tratamento. No entanto, em nosso estudo entregamos quase 50% dessa dose e encontramos reduções similares em CVM, que não foram revertidos durante o curso do estudo.

Embora a dose que selecionamos não tenha demostrado diferenças entre os grupos placebo e laser/luz contínua de alta potência na comparação da CVM, podemos observar diferenças na análise de CK, portanto a dose não pode ser considerada muito baixa para gerar efeitos biológicos, estudos atuais mostram que nem sempre mais dose representa melhor resposta (Hamblin, 2017; De Marchi 2018). Além disso, a FBM por laser de alta potência não teve nenhum efeito na melhoria do desempenho ou na recuperação muscular pós-exercício, ainda assim nossas descobertas podem auxiliar fisioterapeutas a tomarem decisões em relação aos equipamentos utilizados nesta área.

Os trabalhos desenvolvidos anteriormente foram controlados em ambientes laboratoriais e nos trouxeram respostas relevantes quanto aos parâmetros a serem utilizados e as formas de aplicação, levando isso em consideração objetivou-se no capítulo III, verificar a eficácia da FBM como recurso preventivo da fadiga e danos musculares quando administrada antes de uma partida oficial de futsal.

Considerando que este é um esporte em ascensão que é jogado em um ambiente de competição profissionalizado e em níveis cada vez maiores, vários fatores

podem interferir nos resultados (Lefchak, 2014; Milioni et al., 2016). Questões técnicas e as escolhas peculiares feitas pelo comitê técnico por alguns atletas em detrimento de outros, exigem que eles estejam em forma adequada para receber grandes cargas de exercícios sem um período de descanso correto. Além disso, fatores emocionais e psicológicos, como demandas pessoais, metas e pressão familiar podem interferir diretamente com o desempenho de um atleta (Miloski et al., 2014). Os resultados deste estudo revelam que a FBM pode ser uma alternativa para evitar que os atletas sofram fadiga e dano muscular causado por esse esporte altamente competitivo.

Estudos atuais (Leal Junior et al., 2015; De Marchi et al., 2017a; De Marchi et al., 2017b) sugerem que o uso prévio de FBM tem efeitos positivos na manutenção da condição muscular durante a atividade física e na melhoria da recuperação muscular após o exercício. No entanto, a maioria desses ensaios clínicos foi realizada em um laboratório de meio ambiente controlado. Dessa forma, as possibilidades de implementação prática deste conhecimento foram investigadas, considerando condições científicas necessárias.

Em um estudo realizado por Ferraresi et al. (2015), os resultados demostram a eficácia da fototerapia preventiva em atletas de voleibol feminino em situações reais de jogo, mostram também uma inibição da elevação dos níveis de CK na corrente sanguínea 24 h após a partida. Este estudo usou três doses diferentes mais a aplicação placebo. Os autores verificaram que as doses 210 J e J 315 eram eficazes na prevenção do aumento nos níveis de CK na corrente sanguínea. No entanto, o número de pontos de aplicação não foi especificado no estudo. Além disso, não foram relatados os números de saltos ou o tempo de partida para cada atleta. A randomização não está clara porque os atletas que foram submetidos à aplicação efetiva de fototerapia não participaram necessariamente da partida, e seus níveis de CK podem não mudar por esse motivo.

Levando em conta esses resultados, nosso estudo (capitulo III) mostra que a aplicação efetiva de FBM foi capaz de promover uma redução nos marcadores de dano muscular e oxidativos comparando os grupos placebo e ativo, considerando que todos os atletas participaram assiduamente dos jogos. Além disso, para tentar comprovar os benefícios reais da fototerapia preventiva como forma de reduzir a fadiga muscular nos atletas, este estudo visou avaliar o desempenho comparando o tempo de permanência em quadra e a distância percorrida pelos atletas durante os jogos, buscando manter a qualidade metodológica aceitável para tais verificações.

Alguns autores afirmam que a resposta ao dano oxidativo em atletas é maior que em indivíduos sedentários (Djordjevic et al., 2012). Em nosso estudo (capítulo III), essa resposta é verificada no grupo placebo nas avaliações pós48h devido à semelhança com a situação pré-exercício. No entanto, os mesmos atletas, quando participaram do grupo FBM efetivo mostraram respostas de defesa aos danos oxidativos causado pelo exercício em 24h. Portanto, considerando os resultados de pesquisas anteriores realizadas com rigorosos controles laboratoriais (Fillipin et al., 2005; De Marchi et al., 2012; Huang et al., 2013; De Marchi et al., 2017b), é evidente que a FBM desempenha um papel importante na regulação redox do metabolismo muscular, seja basal ou após estímulos adversos gerados por processos inflamatórios, entre outras consequências de micro ou macro danos.

O lactato é outro marcador usado com frequência para monitorar indiretamente o desempenho e recuperação muscular. A concentração de lactato no sangue é considerada um marcador importante de acidose metabólica e é frequentemente monitorada em ambientes esportivos, particularmente em esportes de alta intensidade (Fitts, 1994; Menzies et al., 2010; Jastrzębski et al., 2015) . Níveis mais elevados deste marcador devem ser, e foram encontrados no grupo placebo porque o desempenho do

atleta foi significativamente maior. Portanto é razoável afirmar que a FBM evitou o aumento do lactato sanguíneo, reduziu a fadiga e promoveu recuperação mais rápida após partida oficial de futsal corroborando com dados laboratoriais encontrados anteriormente (Leal Junior et al., 2009b Leal Junior et al., 2010).

A permanência dos atletas em quadra por um maior espaço de tempo, pode estar diretamente correlacionada com o fato de que a FBM foi capaz de atrasar o processo de fadiga muscular, além disso, proporciona aos atletas mais importantes do elenco, que normalmente são os que possuem maior qualidade técnica, uma maior permanência em quadra. Mesmo assim, com maior tempo de atividade os danos causados pelo exercício físico extenuante foram minimizados em 48h após a partida. Assim é possível que a comissão técnica efetue ações técnicas e táticas com atletas mais decisivos por um maior espaço de tempo de jogo, representando uma maior qualidade no desempenho da equipe. Contudo as circunstâncias que cada partida apresenta são únicas, e inúmeros fatores emocionais, psicológicos, técnicos e táticos podem estar relacionados ao desempenho individual dos atletas durante as partidas. Muito embora estes resultados tenham sido observados, a distância percorrida pelos atletas quando receberam a aplicação de FBM e quando receberam placebo não evidenciaram uma diferença significante, podendo ser justificada pelos fatores supracitados.

Cabe salientar que este tipo de estudo possui uma complexa organização, pois se tratou de competição oficial e os pesquisadores procuraram respeitar o máximo possível o planejamento da equipe. Nesse sentido, algumas limitações precisam ser consideradas, como o número final de atletas participantes em função das escolhas metodológicas propostas e da liberdade de escolha da comissão técnica, além disso, apenas uma aplicação foi realizada (40 min antes da partida) e estudos anteriores (Leal Junior et al., 2011; De Marchi et al., 2017b) mostram que a aplicação de FBM posterior

a atividade também se mostra benéfica na recuperação muscular, sendo assim a combinação de aplicações (pré e pós) podem ainda potencializar os efeitos encontrados a nível de recuperação muscular de atletas. Por fim, nossos resultados demonstram o uso potencial de FBM como uma estratégia profilática para o desempenho e a melhora da recuperação de atletas de alto nível, bem com representam um avanço importante para o uso clínico amplo desta ferramenta terapêutica.

Embora seja altamente provável que os efeitos da FBM na prevenção a fadiga estejam envolvidos com a modulação de ERO e a ação anti-inflamatória, seria perigoso concluir que essas são as únicas vias de explicação. Os mecanismos da FBM ainda não estão totalmente elucidados, mas algumas hipóteses estão sendo discutidas na literatura atual como: melhora na microcirculação (Chagas-Junior, 2004; Fioro et al. 2017); modulação da atividade da cadeia respiratória mitocondrial (Silveira et al., 2009); aumento na produção de ATP associada a absorção de luz pela citocromo c oxidase (Hayworth et al., 2010; Karu, 2010); absorção da luz infra-vermelha pelas moléculas de água (980 – 1200 nm), permitindo a modulação dos níveis de cálcio intracelulares (Inoue & Kabaya, 1989; Chai at al., 2009; Ho, 2015; Damodaran, 2015); modulação redox (Chen et al, 2009; Tatmatsu-Rocha at al., 2011; De Marchi et al., 2012); efeitos anti-inflamatórios, redução de fatores de transcrição e citocinas pró inflamatórias (Yamaura et al., 2009; Chen et al., 2011; Hwang et al., 2015) e modulação no fenótipo de macrófagos (von Leden et al., 2013; Fernandes et al., 2015; Silva el al., 2016).

Em suma, além dos resultados apresentados e o evidente efeito da FBM em modular a fadiga, diferentes vias de ação devem ocorrer de maneira conjunta tanto em situações de homeostasia quanto em situações metabólicas adversas, conferindo a FBM uma ampla gama de possiblidades e efeitos no tecido humano.

5. Conclusões

O conjunto de resultados desta tese permite concluir que a FBM é definitivamente uma ótima ferramenta para prevenir a fadiga e potencializar a recuperação muscular pós exercício, mediando as regulações redox das células e potencializando a atividade muscular.

Este trabalho permitiu obter as seguintes conclusões específicas:

I. A velocidade de remoção dos marcadores de dano muscular foram diferentes, bem como dependentes da terapia utilizada, sendo assim, a utilização de FBM isolada, mostrou-se mais efetiva que a crioterapia ou a combinação das duas terapias.

II. A FBM e a crioterapia mostraram-se eficientes na diminuição de marcadores de dano oxidativo a lipídios e proteínas, porém a FBM foi capaz de melhorar performance, enquanto a crioterapia não.

III. A performance foi afetada pela aplicação de FBM de forma preventiva e também como recuperação muscular, a combinação da FBM com a crioterapia também foi capaz de auxiliar no processo de recuperação muscular, porem os resultados foram melhores quando a FBM foi usada isoladamente.

IV. A diminuição da dor muscular pós-tardia após um PIFM por exercício de alta intensidade foi observada nos três grupos que receberam a aplicação de FBM, mesmo o gelo possuindo grande potencial analgésico, este efeito não se manteve após 1 hora do protocolo de recuperação.

V. Os protocolos de fadiga utilizados (capítulos I e II), bem como as avaliações de performance (capitulo II) mostraram-se efetivos e metodologicamente adequadas.

VI. Tanto a FBM de baixa potência contínua quanto à pulsada mostraram-se efetivas em recuperar a musculatura após exercício de alta intensidade, a FBM de alta potência mostrou-se ineficiente.

VII. A FBM foi capaz de melhorar o tempo de permanecia em quadra dos atletas, bem como melhorar a recuperação muscular em 48h após partida oficial de futsal.

6. Perspectivas

I – Desenvolver pesquisas visando o entendimento da FBM nas defesas antioxidantes.

II- Dar continuidade em estudos de FBM com atletas de diversas modalidades, sem controle laboratorial;

III- Desenvolver pesquisas utilizando a FBM como prevenção e recuperação associada ao treinamento físico em atletas e indivíduos fisicamente ativos;

IV- Associar os efeitos conhecidos da FBM com a reabilitação em processos patológicos que acometam os músculos;

V- Relacionar os efeitos da FBM a condições de fraqueza musculoesquelética.

VI- Identificar as vias de ação (mecanismos) da FBM na melhora da produção energética aeróbia e anaeróbia.

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8. Anexos

Anexo I

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Pre-Exercise Infrared Low-Level Laser Therapy (810 nm) in Skeletal Muscle Performance and Postexercise Recovery in Humans, What Is the Optimal Dose? A Randomized, Double-Blind, Placebo-Controlled Clinical Trial

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Abstract

Aim: This study aimed to evaluate the medium-term effects of low-level laser therapy (LLLT or photobiomodulation) in postexercise skeletal muscle recovery and performance enhancement and to identify the optimal dose of 810 nm LLLT. ***Materials and methods:*** A randomized, double-blind, placebo-controlled trial was performed, with voluntary participation of 28 high-level soccer athletes. We analyzed maximum voluntary contraction (MVC), delayed onset muscle soreness (DOMS), creatine kinase (CK) activity, and interleukin-6 (IL-6) expression. The assessments were performed before exercise protocols, after 1 min, and 1, 24, 48, 72, and 96 h after the end of eccentric exercise protocol used to induce fatigue. LLLT was applied before eccentric exercise protocol with a cluster with five diodes, and dose of 10, 30, or 50 J (200 mW and 810 nm) in six sites of quadriceps. ***Results:*** LLLT increased ($p < 0.05$) MVC from immediately after exercise to 24 h with 50 J dose, and from 24 to 96 h with 10 J dose. Both 10 J then 50 J dose decreased ($p < 0.05$) CK and IL-6 with better results in favor of 50 J dose. However, LLLT had no effect in decreasing DOMS. No differences ($p > 0.05$) were found for 30 J dose in any of the outcomes measured. ***Conclusions:*** Pre-exercise LLLT, mainly with 50 J dose, significantly increases performance and improves biochemical markers related to skeletal muscle damage and inflammation.

Introduction

THE SKELETAL MUSCLES show a progressive decline of performance during strenuous physical activity/exercises, but the muscles recover fairly quickly after a period of rest. This reversible phenomenon is called muscle fatigue.[1] Muscle fatigue can be commonly divided into a central component and a peripheral component.

There are several different types of muscle fatigue, and the contribution of each type to the overall decline in muscle performance depends on the muscle fiber type, intensity, and duration of the activity.[2] However, it is not possible to confirm the etiology of the fatigue by classic tests such as maximal voluntary contraction (MVC), since this requires appropriated experimental conditions to verify the influence of the neural and peripheral components.[3] Enoka and Duchateau[3] defined fatigue as ''a disabling symptom in which physical and cognitive function is limited by interactions between performance fatigability and perceived fatigability,'' and therefore, it is hard to suggest where the main changes are occurring.[3]

Muscle damage can occur in sports or in other activities as a result of skeletal muscle fatigue development.[4] The evaluation of muscle damage in humans is difficult and

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complex. Direct analysis is possible only through muscle biopsy or magnetic resonance imaging; however, both methods are expensive and have questionable diagnostic accuracy.[5] The monitoring of serum activity of skeletal muscle enzymes is currently widely used to assess muscle damage.[6] The most common changes in protein and enzyme activity after exercises are creatine kinase (CK), lactate dehydrogenase, aspartate transaminase, and myoglobin.[7] However, the plasmatic activity of CK appears to be the best indicator of exercise intensity and the effects on muscle tissue.[8]

High-intensity and repetitive skeletal muscle contractions can also induce a protective inflammatory response, which is normally related to skeletal muscle damage.[9–11] The initiation of primary muscle damage induced by exercise may be fatiguing but is not painful. However, the ensuing inflammatory response leads to delayed onset muscle soreness (DOMS) beginning 8–24 h after the damage is initiated.[12,13] Primary muscle tissue damage promotes infiltration by inflammatory cells, which in conjunction with local muscle, endothelial, and satellite cells, produce an array of cytokines to regulate the inflammatory process, including tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and IL-6.[13–15]

Currently, there are many therapeutic modalities used after sports activities to improve skeletal muscle recovery. The most commonly modalities used are as follows: active recovery,[4,16–18] cryotherapy,[4,19,20] massage,[17,21] contrast heat therapy (use of hot and cold water immersion),[22,23] hydrotherapy,[24] stretching,[25] and electrostimulation.[26] However, the scientific evidence behind these modalities is limited.

It has been hypothesized that low-level laser therapy (LLLT) promotes tissue regeneration, reduces inflammation, and relieves pain.[27] Skeletal muscle fatigue and recovery is a novel area of research in LLLT. Recent studies of our research group with LLLT and light emitting diode therapy have shown positive results delaying skeletal muscle fatigue in both animals and humans and improving the status of biochemical markers related to skeletal muscle recovery when these therapies were applied before exercise.[28–35] Despite positive effects observed, several factors still remain unknown, such as mechanisms, optimal dose, effects in long-duration exercises, and long-term effects in skeletal muscle recovery.[35]

It is known that LLLT has a biphasic dose–response pattern, which follows the Arndt–Schulz Law. Biostimulatory effects can be achieved using doses within a dose range, also known as therapeutic window. Inhibitory effects are observed when doses above this therapeutic window are used, and in the same way, no effects are observed when doses below the therapeutic window are used. Therefore, the establishment of optimal doses and therapeutic windows for different pathologies and conditions becomes crucial for optimization of LLLT. With this perspective in mind, the aim of this study was to identify the optimal dose for preexercise irradiation with LLLT looking for performance enhancement and improvement of postexercise recovery, through functional and biochemical markers related to muscle damage.

Materials and Methods

Study design and ethics statement

A double-blind, placebo-controlled, randomized clinical trial was carried out in two phases. The study was conducted in the Laboratory of Phototherapy in Sports and Exercise at Universidade Nove de Julho in the city of São Paulo, Brazil. The project has received approval from the Research Ethics Committee of University Nove de Julho (Protocol No. 397774/2011). The protocol for this study is registered with the Protocol Registry System (clinicaltrials.gov; NCT01844271).

Characterization of sample

Twenty-eight male professional soccer athletes from the same team participated in the study. They had an average age of 18.81 ± 0.80 years old, height of 172.94 ± 4.48 cm, and body weight of 63.58 ± 4.46 kg. The decision to recruit volunteers from the same team was made to enhance the homogeneity of the sample. Moreover, the tests for this study were performed with the athletes during preseason preparation. Therefore, the whole sample performed all procedures at the same physical activity level.

Calculation of sample size

The sample size was calculated based on a previous study carried out in same research field,[36] in which a similar experimental model and exercise protocol were employed. The sample size calculation considered a β of 20% and α of 5%. We used as reference for this calculation the study performed by Baroni et al.,[36] where LLLT led to the postexercise recovery of CK (muscle injury marker) to 435.95 ± 238.04 U/L, whereas placebo treatment led to an increase in CK to 1327.58 ± 949.82 U/L. Using these parameters, a total of seven volunteers were needed for each of the four groups in study (total of 28 volunteers). The intention-to-treat analysis was followed *a priori*. CONSORT flowchart summarizing experimental procedures and subjects is shown in Fig. 1.

Inclusion criteria

The following inclusion criteria were used:

- Professional football athletes;
- Age between 18 and 35 years;
- Male gender;
- Minimum of 80% participation in team practice sessions;
- Light or intermediate skin color, following Von Luschan's chromatic scale[37];
- Agreement to participate through signed statement of informed consent.

Exclusion criteria

Participants with the following were excluded from the study:

- History of musculoskeletal injury to hips or knees in the previous 2 months;
- Use of pharmacological agents or nutritional supplements;
- Smokers and alcoholics;
- Occurrence of musculoskeletal injury during the study;
- Any change in practice routine in relation to the rest of the team during the study.

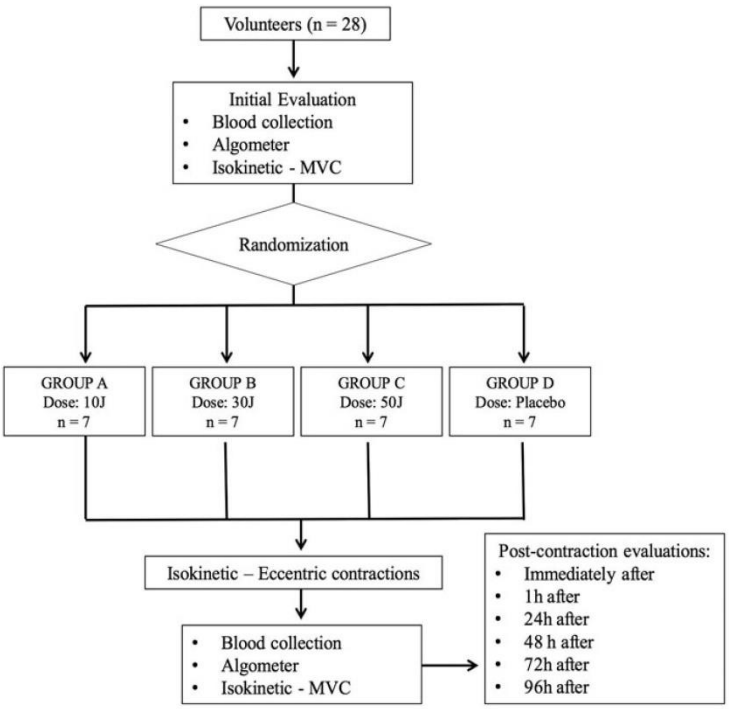

FIG. 1. CONSORT flowchart.

Composition of groups and randomization process

The volunteers were randomly allocated to four experimental groups ($n = 7$ per group) according to the LLLT dose. Randomization was carried out by a simple drawing of lots (A, B, C, or D). The laser unit emitted the same sound regardless of the programmed dose. Randomization labels were created using a randomization table at a central office, where a series of sealed, opaque, and numbered envelopes were used to ensure confidentiality. A participating researcher who programmed the laser device based on the randomization results conducted randomization. This researcher was instructed not to inform the participants or other researchers regarding the LLLT dose.

The researcher in charge of the administration of the LLLT was blinded to the dose applied to the volunteers, and therefore, one of the researchers involved in study programmed laser device unit according to randomization while another one performed the administration of the light therapy. Researchers who did not have knowledge about randomization performed the assessments and exercise protocol. Blinding was further maintained by the use of opaque goggles by the participants.

Experimental protocol

Evaluations and informative procedures. Evaluations were carried out before and at the end of the isokinetic protocol by a researcher blinded to the LLLT dose and mode (placebo or active). The volunteers were then informed about the procedures and signed a statement of informed consent in compliance with Resolution 196/96 of the Brazilian National Board of Health before the execution of the study.

Blood samples and biochemical analyses. Following the informative process and randomization, blood samples (10 mL) were taken from the antecubital vein before and 1 min after the eccentric contraction protocol. Blood samples were also collected 1, 24, 48, 72, and 96 h after the protocol. The samples were taken by a qualified nurse blinded to the allocation of the volunteers to the four experimental groups. One hour after collection, each sample was centrifuged at 3000 rpm for 20 min. Pipettes were used to transfer the serum to Eppendorf® tubes, which were stored at −80°C until analysis.

Blood analysis involved the determination of CK activity as an indirect marker of muscle damage using spectrophotometry and specific reagent kits (Labtest®, São Paulo, Brazil); and IL-6 levels as inflammatory marker using ELISA method and specific reagents (BD, San Diego, CA). The researcher who performed analysis of biochemical markers was blinded to randomization and allocation of volunteers in experimental groups.

Evaluation of DOMS. DOMS was evaluated based on the pressure pain threshold using an analog algometer (Baseline®, Parma, Italy). This device consists of a rod with a rounded rubber tip coupled to a pressure (force) meter. The display presents values in pounds (lbs). As the surface of the rubber tip measures 1 cm^2, the reading is expressed in pounds per square centimeter (lbs/cm^2). Values range from

0 to 100 lbs with a precision of 0.1 lbs. The most sensitive areas of the knee extensors (medial, lateral, and central) of the nondominant lower limb were located through palpation by an examiner blinded to the allocation of the volunteers to the different groups and were marked with a dermographic marker. The cylindrical end of the equipment was positioned perpendicularly to the demarcated area. Pressure was applied to the surface of the skin with a gradual increase in increments of 0.1 lbs.

The volunteers were instructed to say "yes" when the pressure exerted becomes painful. Three measures were taken with the algometer on the same demarcated point of the aforementioned muscle sites. The mean pressure pain threshold was determined from the three readings of each of the three sites and the mean values were used in the statistical analysis. Readings were taken before stretching and warm up, 1 min after the eccentric contraction protocol as well as 1, 24, 48, 72, and 96 h after the execution of the protocol.

To evaluate DOMS, we also used a visual analog scale (VAS) of 100 mm. VAS consisted in a 100 mm empty line with the word "no pain" on the left side (at the beginning of the line) and "worst pain imaginable" on the right side (at the end of the line). The line was always presented horizontally to the volunteers and they were asked to indicate the pain intensity in the line. After that, a researcher measured the distance between the beginning of the line until the volunteer indication, to quantify the pain intensity. The researcher who performed assessment of DOMS was blinded to randomization and allocation of volunteers in experimental groups.

Stretching and warm up. Before the isokinetic protocol, the volunteers performed three 60-sec sets of active stretching of the knee extensors of the nondominant lower limb. The volunteers then performed a warm-up exercise consisting of pedaling a stationary bike (Ibramed®, Porto Alegre, Brazil) at 100 rpm for 5 min without load.

Isometric protocol test: maximum voluntary contraction. An isokinetic dynamometer was used for the evaluation of muscle function and the execution of the exercise protocol. This instrument is currently considered the method with the greatest reliability for the measure of the musculoskeletal performance.

Immediately after the stretching and warm-up exercises, the MVC test was performed. For such, the volunteers sit on the seat of the isokinetic dynamometer (System 4; Biodex®, Shirley, NY) with an angle of 100° between the trunk and hip and the nondominant leg positioned with the knee at 60° of flexion (0° corresponds to complete knee extension) and attached to the seat of the dynamometer by straps. The dominant leg was positioned at 100° of hip flexion and was also attached to the seat by a strap. The volunteers were also attached to the seat of the dynamometer through the use of two straps crossing the trunk.

The volunteers were instructed to cross their arms over the trunk and the axis of the dynamometer was positioned parallel to the center of the knee. The MVC test consisted of three 5-sec isometric contractions of the knee extensors of the nondominant leg. The highest torque value of the three contractions (peak torque) was used for the statistical analysis. The choice of this parameter is due to the fact that this variable reflects the maximum generation of force by the muscle. Instructions on how to execute the test were given first and the volunteers received verbal encouragement during the execution of the test.

This test has demonstrated reliability and reproducibility in a previous studies carried out by our research group.[36,38] The MVC was performed also immediately (1 min) after the eccentric contraction protocol as well as 1, 24, 48, 72, and 96 h after the eccentric contraction protocol to evaluate postexercise muscle recovery. The researcher who performed assessment of MVC was blinded to randomization and allocation of volunteers in experimental groups.

Low-level laser therapy. A five-diode cluster laser device (manufactured by Thor Photomedicine®, London, United Kingdom) was used for LLLT. To ensure blinding, the device emitted the same sounds regardless of the programmed mode (active or placebo). The optical power was calibrated before irradiation in each volunteer using a Thorlabs thermal power meter (Model S322C; Thorlabs®, Newton, NJ).

LLLT was applied 2 min before the pre-exercise MVC test with the cluster in direct contact with the skin at six distinct sites of the knee extensor musculature of the nondominant limb (two medial, two lateral, and two central sites; Fig. 2). As the cluster has 5 diodes and 6 different sites were irradiated, a total of 30 points were irradiated in the musculature. The use of a cluster in this irradiation procedure is important since this allowed us to cover larger areas of irradiation. Based on the results of the randomization, the volunteers of the four experimental groups received the following doses:

FIG. 2. Sites of low-level laser therapy irradiation on quadriceps.

- Group A—60 J of total irradiated energy on knee extensors (six sites in the muscle with a five-diode probe and 2 J/diode, 10 J in each site) with 10 sec of irradiation at each site (60 sec of total irradiation time);
- Group B—180 J of total irradiated energy on knee extensors (six sites in the muscle with a five-diode probe and 6 J/diode, 30 J in each site) with 30 sec of irradiation at each site (180 sec of total irradiation time);
- Group C—300 J of total irradiated energy on knee extensors (six sites in the muscle with a five-diode probe and 10 J/diode, 50 J in each site) with 50 sec of irradiation at each site (300 sec of total irradiation time);
- Group D—0 J of total irradiated energy on knee extensors (six sites in the muscle with a five-diode probe and 0 J/diode, 0 J in each site), with 20 sec of irradiation at each site, 120 sec of total time, but without effective irradiation.

The researcher who irradiated LLLT was blinded to randomization and allocation of volunteers in experimental groups. The irradiation sites are illustrated in Fig. 2 and the parameters for LLLT are shown in Table 1.

Isokinetic protocol: eccentric contractions. Precisely 3 min after the end of LLLT, the volunteers performed the eccentric contraction protocol, which consisted of 75 eccentric isokinetic contractions of the knee extensor musculature in the nondominant leg (5 sets of 15 repetitions, 30-sec rest interval between sets) at a velocity of $60°.seg^{-1}$ in both the eccentric and concentric movements with a 60° range of motion (between 90° and 30° of knee flexion). At each contraction, the dynamometer automatically (passively) positioned the knee at 30°; the dynamometer then flexed the knee until reaching 90°.

The volunteers were instructed to resist against knee flexion movement imposed by the dynamometer with maximum force. Instructions on how to execute the maneuver were given first and the volunteers received verbal encouragement throughout the protocol. Volunteers performed five submaximal repetitions as familiarization procedure before tests. The dominant leg was determined by asking volunteers about the preferred leg to kick a ball, and then, tests were performed with the nonpreferred leg (nondominant). Despite the diversity of protocols proposed for the execution of eccentric exercises on isokinetic dynamometers, the protocol described here was chosen based on two previous studies carried out in the same research field,[36,38] in which this protocol proved effective and reproducible for the exercise-induced muscle damage.

The researcher in charge to eccentric contractions protocol was blinded to randomization and allocation of volunteers in experimental groups.

Table 1. Low-Level Laser Therapy Parameters

Wavelength, nm	810 (infrared)
Number of diodes	5
Power output per diode, mW	200 (total of 1000)
Power density per diode, W/cm^2	5.495
Energy per diode, J	2, 6, or 10
Energy per site, J	10, 30, or 50
Energy density per diode, J/cm^2	54.95, 164.84, 274.73
Spot size, cm^2—each diode	0.0364
Treatment time per point or site, sec	10, 30, or 50
Total treatment time, sec	60, 180, or 300
Total energy delivered, J	60, 180, or 300
Number of treated points/sites	30 points/6 sites
Application mode	Probe held stationary in skin contact at a 90° angle with slight pressure

Statistical analysis

Data were first tested regarding normal distribution using the Shapiro–Wilk test and are expressed as mean and standard deviation since it has normal distribution. The ANOVA test with repeated measurements for the time factor was performed to test between-group differences (followed by Bonferroni *post hoc* test). The significance level was set at $p < 0.05$. Data in graphs are expressed as mean and standard error of the mean. The researcher who performed statistical analysis was blinded to randomization and allocation of volunteers in experimental groups. *A priori,* an intention to treat basis would be followed, however, it was not performed since there were not dropouts.

Results

All athletes recruited completed all assessments performed in the study, and therefore, there were no dropouts. Table 2 shows all outcomes regarding functional aspects of performance and recovery that we observed in our study. As we can observe, there were no significant differences ($p < 0.05$) between experimental groups regarding DOMS both in algometry and in VAS.

On the contrary, 10 J LLLT dose significantly increased ($p < 0.05$) MVC compared to placebo both in absolute and in percentage values at 24, 48, 72, and 96 h. In addition, 50 J LLLT dose significantly increased ($p < 0.05$) MVC compared to placebo both in absolute and in percentage values immediately after eccentric exercise protocol and at 1 and 24 h. Figures 3 and 4 show results regarding MVC in absolute and in percentage values.

Our CK analysis shows that 10 J LLLT dose significantly decreased ($p < 0.05$) CK activity compared to both placebo and 30 J LLLT dose at 24, 48, 72, and 96 h after eccentric contractions protocol. Interestingly, 50 J LLLT dose significantly decreased ($p < 0.05$) CK activity compared to both placebo and 30 J LLLT dose at 1, 24, 48, 72, and 96 h after eccentric contractions protocol. In contrast, 30 J LLLT dose did not show significant difference ($p > 0.05$) compared to placebo LLLT in all time points tested. Results regarding CK analysis are summarized in Fig. 5.

Regarding inflammation, 30 J LLLT dose significantly decreased ($p < 0.05$) IL-6 levels compared to placebo immediately after eccentric exercise protocol and at 1, 24, 48, and 72 h. Similarly, 50 J LLLT dose significantly decreased ($p < 0.05$) IL-6 levels compared to placebo at 1, 24, 48, and 72 h. However, only 10 J LLLT dose significantly decreased ($p < 0.05$) IL-6 levels compared to placebo at all time points tested (immediately after eccentric exercise protocol, and at 1, 24, 48, 72, and 96 h). Figure 6 summarizes results regarding IL-6 levels.

TABLE 2. FUNCTIONAL MARKERS OF PERFORMANCE AND RECOVERY (MEAN ± SD)

	Pre	*Post*	*1 h*	*24 h*	*48 h*	*72 h*	*96 h*
VAS, mm							
Placebo	0.00 ± 0.00	68.30 ± 17.20	21.39 ± 20.31	45.00 ± 30.20	25.00 ± 13.80	18.30 ± 11.70	29.78 ± 30.75
10 J	0.00 ± 0.00	43.33 ± 15.05	32.17 ± 19.92	41.66 ± 32.50	26.66 ± 30.11	28.33 ± 30.60	50.78 ± 29.79
30 J	0.00 ± 0.00	48.00 ± 8.40	58.00 ± 14.80	46.00 ± 20.70	46.00 ± 24.10	30.00 ± 21.20	28.00 ± 27.70
50 J	0.00 ± 0.00	48.00 ± 13.03	52.00 ± 19.23	44.00 ± 24.08	48.00 ± 33.46	28.00 ± 19.23	22.50 ± 22.17
Algometry, lbs							
Placebo	30.00 ± 5.52	28.53 ± 7.66	27.06 ± 10.64	30.93 ± 10.46	31.26 ± 12.86	33.40 ± 11.98	34.80 ± 14.19
10 J	27.85 ± 29.78	25.65 ± 10.76	24.38 ± 5.73	26.09 ± 6.85	29.62 ± 11.34	27.90 ± 8.13	28.14 ± 7.01
30 J	28.67 ± 5.28	26.07 ± 6.28	20.00 ± 1.20	20.87 ± 5.66	25.53 ± 4.05	31.13 ± 6.88	33.00 ± 7.68
50 J	24.13 ± 12.21	25.06 ± 11.02	25.13 ± 11.17	24.31 ± 10.43	26.60 ± 8.98	29.60 ± 12.44	26.25 ± 10.13
MVC, N.m							
Placebo	249.90 ± 22.65	228.14 ± 13.57	213.86 ± 29.00	247.40 ± 11.40	249.72 ± 28.28	243.86 ± 12.41	256.86 ± 8.52
10 J	253.32 ± 24.53	226.67 ± 15.35	238.41 ± 10.00	286.77 ± 22.78[ab]	294.31 ± 21.75[abc]	292.08 ± 20.71[ab]	305.57 ± 23.30[ab]
30 J	246.79 ± 23.61	220.83 ± 24.00	215.91 ± 6.36	223.44 ± 9.23	242.11 ± 7.90	228.44 ± 12.73	240.79 ± 18.72
50 J	249.78 ± 15.71	259.04 ± 19.43[abd]	262.17 ± 20.08[ab]	275.97 ± 12.21[ab]	261.92 ± 27.32	270.07 ± 13.43[b]	281.22 ± 22.14[b]

[a]Different of placebo ($p < 0.05$).
[b]Different of 30 J ($p < 0.05$).
[c]Different of 50 J ($p < 0.05$).
[d]Different of 10 J ($p < 0.05$).
MVC, maximum voluntary contraction; VAS, visual analog scale.

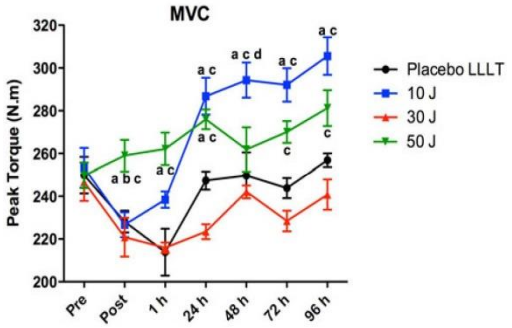

FIG. 3. MVC in absolute values. Values are mean and error bars are SEM. [a]Different of placebo ($p < 0.05$); [b]Different of 10 J ($p < 0.05$); [c]Different of 30 J ($p < 0.05$); [d]Different of 50 J ($p < 0.05$). MVC, maximum voluntary contraction; SEM, standard error of the mean.

Discussion

As far as we know, this is the first time that several LLLT doses with infrared 810 nm wavelength are tested in same experiment to evaluate effects on exercise performance and postexercise recovery in high-level athletes. We decided to evaluate three different doses to help establish a ''therapeutic window'' for LLLT in performance and recovery enhancement.

We choose to irradiate muscles before exercise, since several studies have shown that when pre-exercise LLLT is used, it has ergogenic effects and protects muscles against damage. Recently, a systematic review with meta-analysis has stated the same in its conclusions.[35]

Interestingly, two doses tested (10 and 50 J) showed significant results in improvement of MVC, but at different times. Dose 10 J resulted in a significant increase in muscle strength compared to the placebo group from 24 to 96 h after eccentric contractions protocol, and dose of 50 J resulted in a significant increase in muscle strength compared to placebo group from immediately postexercise to 24 h after eccentric contractions protocol. However, 30 J dose does not show any significant effect. We believe that different doses

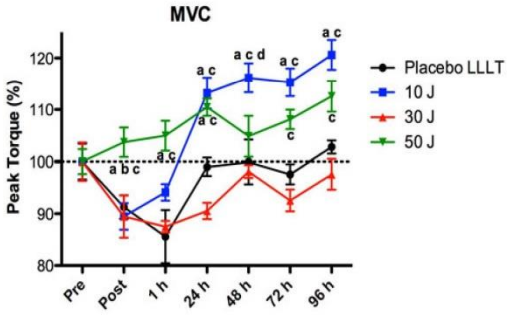

FIG. 4. MVC in percentage values. Values are mean and error bars are SEM. [a]Different of placebo ($p < 0.05$); [b]Different of 10 J ($p < 0.05$); [c]Different of 30 J ($p < 0.05$); [d]Different of 50 J ($p < 0.05$).

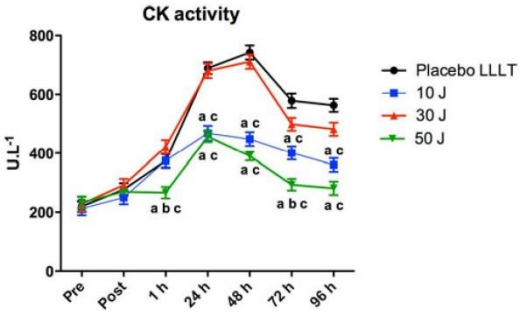

FIG. 5. CK activity. Values are mean and error bars are SEM. [a]Different of placebo ($p<0.05$); [b]Different of 10 J ($p<0.05$); [c]Different of 30 J ($p<0.05$); [d]Different of 50 J ($p<0.05$). CK, creatine kinase.

used in our study can lead to different time-windows (which can explain the immediate and delayed responses promoted by different doses) and/or different mechanisms of action. However, these points warrant further investigation.

These results could be very helpful thinking about sports modalities, for instance, in sports, such as swimming, judo, and short-distance running, an immediate or short-term recovery is required, and therefore, 50 J dose would be the best dose to be used in athletes. On the contrary, in sports, such as football, basketball, and volleyball, the medium-term recovery (from 48 to 96 h) is needed, and therefore, the best dose to be used would be 10 J. Curiously, our results regarding MVC are very different than those observed by Baroni et al.[36] Authors used an 810 nm LLLT, 200 mW, and only tested a single dose of 30 J with the same other parameters (power density, energy density and irradiation time) used in the current study; however, they observed a significant improvement in muscle performance immediately after and at 24 and 48 h after exercise.

Antonialli et al.[38] also tested the same three doses we tested (10, 30, and 50 J) but using a different device that simultaneously uses different light sources and wavelengths (super-pulsed laser of 905 nm, red LED of 640 nm, and infrared LED of 875). Untrained volunteers were recruited and

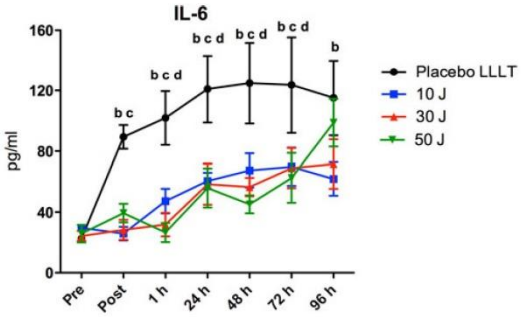

FIG. 6. IL-6 levels. Values are mean and error bars are SEM. [a]Different of placebo ($p<0.05$); [b]Different of 10 J ($p<0.05$); [c]Different of 30 J ($p<0.05$); [d]Different of 50 J ($p<0.05$). IL-6, interleukin-6.

it was found that there were better results regarding MVC enhancement, decrease in DOMS, and decrease in CK activity with 30 J dose applied per site before exercise.

This difference could be related to some aspects such as the sample selected, high-level male football athletes versus healthy male volunteers (nonathletes),[36,38] or the device used, single wavelength versus multiple wavelengths.[38] However, further studies are also needed to investigate these aspects.

Our results also show that 10 and 50 J doses significantly decreased CK activity, with best results in favor of 50 J. However, 30 J dose again showed no effect compared to placebo. LLLT also decreased the serum CK in the study performed by Dos Reis et al.[39] The effect was more pronounced when LLLT was applied after the fatigue protocol used by authors. They recruited professional football players, but the device and parameters used were very different from those chosen in our study. Furthermore, the irradiation time used by Dos Reis et al.[39] was very limited (10 sec) to achieve significant effects for LLLT before and/or after exercise.

Also, our results are very different than those observed by Baroni et al.,[36] and we believe that this difference may be due subjects' characteristics (high-level athletes vs. nonathletes). It is known that athletes normally present different plasma CK activities than nontrained individuals[40] and that postexercise CK activity also increases differently in athletes compared to nontrained subjects,[41] which could explain this difference in results regarding CK compared with the results of Baroni et al.[36]

Interestingly, all doses tested significantly decreased IL-6 expression. This result is in line with previous studies performed by our research group,[31,34] where we observed that pre-exercise phototherapy significantly decreased c-reactive protein levels. Over the years, several animal and human trials have shown that LLLT with both red and infrared wavelengths has modulatory effects on inflammatory marker release (PGE_2, TNF-α, IL-1β, plasminogen activator)[42] and several phases of the inflammatory process itself (edema, hemorrhagic formation, necrosis, neutrophil cell influx) and leukocyte activity (macrophages, lymphocytes, neutrophils).[43–47] This includes inhibition of the NF-kappa pathway[48] and modulation of inducible nitric oxide synthase.[49]

It is important to highlight that assessments performed in this study do not allow us to explore mechanisms of action nor if effects on performance enhancement are related to delayed central or peripheral fatigue. Therefore, to avoid overstatements or speculation on observed outcomes, we believe that these aspects should be investigated in further studies in this field.

Hayworth et al.[50] demonstrated that a single irradiation with LLLT is able to increase the cytochrome c-oxidase activity in intact skeletal muscle tissue 24 h after irradiation. In addition, authors demonstrated that there is a dose- and fiber-type-dependent increase in cytochrome c-oxidase in skeletal muscle fibers. It means that LLLT leads to upregulation of mitochondrial activity through increasing mitochondrial respiratory chain, which consequently increases ATP production into muscle cells and decreases oxidative stress and reactive oxygen species production. These effects can explain the mechanism through LLLT enhances performance and protects skeletal muscle against damage and inflammation.

Very recently, Albuquerque-Pontes et al.[51] investigated the effects of different doses (1, 3, and 10 J) and wavelengths (660, 830, and 905 nm) in cytochrome c oxidase activity in intact skeletal muscle. They concluded that parameters which increased the cytochrome-c oxidase were mainly 660 nm at 1 J, 830 nm at 3 J, and 905 nm at 1 J. The increase in cytochrome-c oxidase was observed from 5 min up to 24 h after irradiation, depending upon irradiation parameters used. This demonstrates that phototherapy can be used in different time-windows between irradiation and beginning of muscular activity, and it is dependent of wavelengths and doses used.

This agrees with the previous observation by Santos et al.,[52] who observed that ergogenic and protective effects of LLLT on skeletal muscle are also dependent of wavelengths and doses used. These results help us to elucidate how pre-exercise phototherapy improves performance,[35,38,53,54] delays fatigue development, and can protect muscle against damage even in difficult diseases such as muscular dystrophies.[55,56]

Despite positive results observed in muscle strength and biochemical markers of muscle damage and inflammation, none of LLLT doses tested showed significant results in decreasing DOMS. Interestingly, the same was observed in a previous study using the same device and single wavelength.[36] In contrast, the combination of multiple wavelengths showed positive results in decreasing DOMS.[38] Therefore, the effect of LLLT with single wavelength on DOMS is still an open issue and deserves further investigation.

Conclusions

Pre-exercise LLLT significantly increases performance and improves biochemical markers related to skeletal muscle damage and inflammation. Better results were observed with 10 and 50 J doses. The overall analysis of results shows that better results are reached with 50 J dose.

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Author Disclosure Statement

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Anexo II

Lasers Med Sci
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ORIGINAL ARTICLE

Phototherapy in skeletal muscle performance and recovery after exercise: effect of combination of super-pulsed laser and light-emitting diodes

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Abstract Recent studies with phototherapy have shown positive results in enhancement of performance and improvement of recovery when applied before exercise. However, several factors still remain unknown such as therapeutic windows, optimal treatment parameters, and effects of combination of different light sources (laser and LEDs). The aim of this study was to evaluate the effects of phototherapy with the combination of different light sources on skeletal muscle performance and post-exercise recovery, and to establish the optimal energy dose. A randomized, double-blinded, placebo-controlled trial with participation of 40 male healthy untrained volunteers was performed. A single phototherapy intervention was performed immediately after pre-exercise (baseline) maximum voluntary contraction (MVC) with a cluster of 12 diodes (4 of 905 nm lasers—0.3125 mW each, 4 of 875 nm LEDs—17.5 mW each, and 4 of 670 nm LEDs—15 mW each- manufactured by Multi Radiance Medical™) and dose of 10, 30, and 50 J or placebo in six sites of quadriceps. MVC, delayed onset muscle soreness (DOMS), and creatine kinase (CK) activity were analyzed. Assessments were performed before, 1 min, 1, 24, 48, 72, and 96 h after eccentric exercise protocol employed to induce fatigue. Phototherapy increased ($p<0.05$) MVC was compared to placebo from immediately after to 96 h after exercise with 10 or 30 J doses (better results with 30 J dose). DOMS was significantly decreased compared to placebo ($p<0.05$) with 30 J dose from 24 to 96 h after exercise, and with 50 J dose from immediately after to 96 h after exercise. CK activity was significantly decreased ($p<0.05$) compared to placebo with all phototherapy doses from 1 to 96 h after exercise (except for 50 J dose at 96 h). Pre-exercise phototherapy with combination of low-level laser and LEDs, mainly with 30 J dose, significantly increases performance, decreases DOMS, and improves biochemical marker related to skeletal muscle damage.

Keywords Skeletal muscle performance · Super-pulsed laser · Light-emitting diodes · Exercise recovery

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Introduction

Since the first clinical trial was published by Goldman et al. [1] showing that low-level laser therapy (LLLT) improved erythema, pain, and grip strength in patients with rheumatoid arthritis of the hands, several studies have been reporting beneficial effects of phototherapy in several musculoskeletal disorders such as osteoarthritis [2] tendinopathies [3, 4], back pain [5, 6], and neck pain [7, 8]. Very recently, a systematic review with meta-analysis published in *Lancet* journal [9] provided evidence that LLLT reduces pain immediately after

treatment in acute neck pain and up to 22 weeks after completion of treatment in patients with chronic neck pain.

Skeletal muscle fatigue is a novel area of research in phototherapy field. Since publication of first clinical trial in 2008 [10], positive effects have been reported with red [10, 11] and infrared wavelengths [11–15] and both with lasers [10–15] and LED [16–19]. Interestingly, different kinds of exercises were employed in these studies such as repeated contractions [10, 12, 13, 15, 17], isometric sustained contraction [11, 18, 19], cycling [16], and running [14], which increase the evidence about the positive effects of phototherapy in improvement of exercise performance and markers related to exercise recovery, and its potential use in clinical practice.

More recently, two systematic reviews [20, 21] showed that phototherapy with lasers and/or LEDs administered immediately before a bout of resistance exercise provide ergogenic effects to skeletal muscle by improving physical performance. These reviews also provided evidence that phototherapy may preserve tissue against exercise-induced muscle damage and speed up recovery when applied before exercises.

Since red and infrared wavelengths, and lasers and LEDs have been showing positive effects in improvement of performance and recovery, it is reasonable to think that the combined use of different light sources (lasers and LEDs) with different wavelengths (red and infrared) could represents a therapeutic advantage, and consequently better clinical outcomes could be achieved using this combination. But interestingly, the effects of combination of different wavelengths and light sources used synergistically aiming improvement of exercise performance and recovery were still not investigated.

With this perspective in mind, the aim of this study was to evaluate the effects of phototherapy with the combination of super-pulsed laser and light-emitting diodes on skeletal muscle performance and post-exercise recovery, and also to establish the optimal dose for this therapeutic application.

Materials and methods

Study design and ethics statement

A double-blind, placebo-controlled, randomized clinical trial was carried out. The study was conducted in Laboratory of Phototherapy in Sport and Exercise at Nove de Julho University in the city of São Paulo, Brazil. The project has received approval from the research ethics committee of Nove de Julho University (protocol number 273.257).

Characterization of sample

Forty male healthy untrained subjects participated in the study. Volunteers were recruited from university staff and students and all volunteers that agreed to participate signed the informed consent statement. The intention-to-treat analysis was followed. CONSORT flowchart summarizing experimental procedures and subjects are showed in Fig. 1.

Inclusion criteria and exclusion criteria

The following inclusion criteria were employed: age between 18 and 35 years, male gender, less than two exercise practice per week, light or intermediate skin color [22]. Volunteers were excluded when they presented musculoskeletal injury to hips or knees in the previous 2 months, use pharmacological agents or nutritional supplements regularly, have occurrence of musculoskeletal injury during the study.

Composition of groups and randomization process

Forty male healthy untrained subjects were recruited in our study. They had an average age of 24.10 years old (±1.52), height of 171.44 cm (±6.22), and body weight of 67.05 kg (±5.38). The volunteers were randomly allocated to four experimental groups (n=10 per group) according to the phototherapy dose. The sample size was determined based in previous studies performed by our research group in this field [10–13]. Randomization was carried out by a simple drawing of lots (A, B, C, or D). The phototherapy unit emitted the same sound regardless of the programmed dose. Randomization labels were created using a randomization table at a central office where a series of sealed, opaque, and numbered envelopes were used to ensure confidentiality. A participating researcher who had the function of programming the phototherapy device based on the randomization results conducted randomization. This researcher was instructed not to inform the participants or other researchers regarding the phototherapy dose. Thus, the researcher in charge of the administration of the phototherapy was blinded to the dose applied to the volunteers.

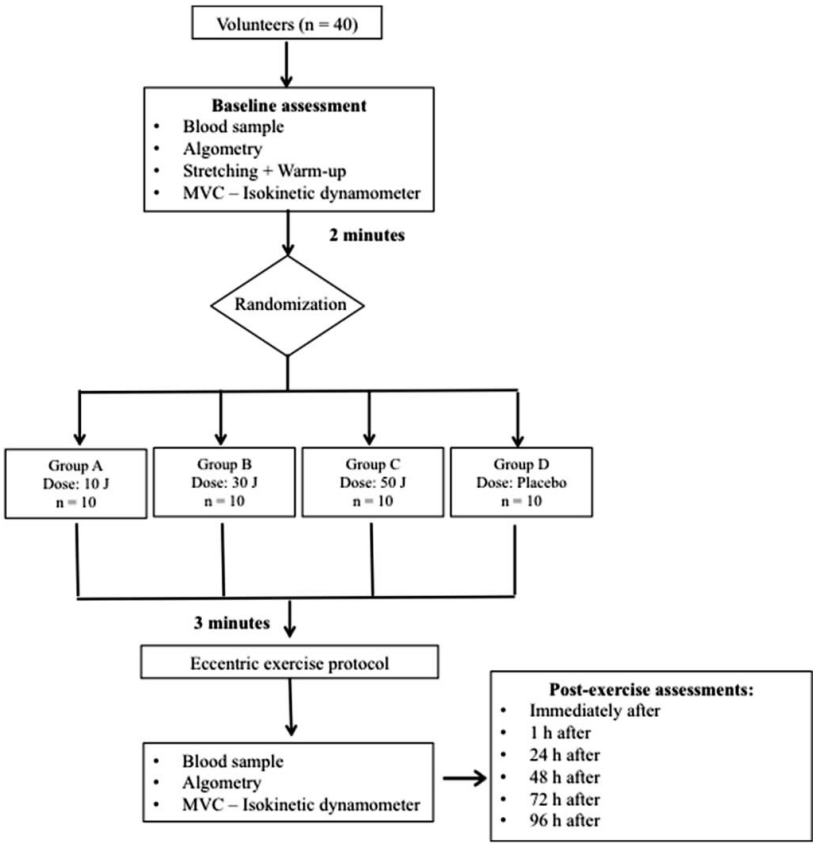
Experimental protocol

Blood samples and biochemical analyses

Following the informative process and randomization, blood samples (10 ml) were taken from the antecubital vein before and 1 min after the eccentric contraction protocol. Blood samples were also collected 1, 24, 48, 72, and 96 h after the protocol. The samples were taken by a qualified nurse blinded to the allocation of the volunteers to the four experimental groups. One hour after collection, each sample was centrifuged at 3,000 rpm for 20 min. Pipettes were used to transfer the serum to Eppendorf® tubes, which were stored at −80 °C until analysis.

Blood analysis involved the determination of creatine kinase (CK) activity as an indirect marker of muscle

Fig. 1 CONSORT flowchart



damage using spectrophotometry and specific reagent kits (Labtest®, São Paulo - SP, Brazil). The researcher that performed analysis of CK was also blinded to randomization and allocation of volunteers in experimental groups.

Evaluation of delayed onset muscle soreness

Delayed onset muscle soreness (DOMS) was evaluated based on the pressure pain threshold using a digital algometer (Kratos® Brazil). This device consists of a rod with a rounded metal tip coupled to a pressure (force) meter. The display presents values in kilogram force. As the surface of the metal tip measures 1 cm^2, the reading is expressed in kilogram force per square centimeter. The most sensitive areas of the knee extensors (medial, lateral, and central) of the non-dominant lower limb were located through palpation by an examiner blinded to the allocation of the volunteers to the different groups and were marked with a dermographic marker. The circular end of the equipment was positioned perpendicularly to the demarcated area. Pressure was applied to the surface of the skin with a gradual increase in increments of 1 kgf. The volunteers were instructed to say "yes" when the pressure exerted becomes painful. Three measures were taken with the algometer on the same demarcated point of the aforementioned muscle sites. The mean pressure pain threshold was determined from the three readings of each of the three sites and the mean values were used in the statistical analysis. Readings were taken prior to stretching and warm up, 1 min after the eccentric contraction protocol as well as 1, 24, 48, 72, and 96 h after the execution of the protocol. In order to evaluate DOMS, we also employed a VAS scale of 100 mm. The researcher that performed assessment of DOMS was blinded to randomization and allocation of volunteers in experimental groups.

Stretching and warm up

Prior to the isokinetic protocol, the volunteers performed three 60-s sets of active stretching of the knee extensors of the non-dominant lower limb. The volunteers then performed a warm-up exercise consisting of pedaling a stationary bike

(Inbramed®, Porto Alegre, RS, Brazil) at 100 rpm for 5 min without load.

Isokinetic protocol—maximum voluntary contraction

An isokinetic dynamometer was used for the evaluation of muscle function and the execution of the exercise protocol. This instrument is currently considered the method with the greatest reliability for the measure of the musculoskeletal performance. Immediately after the stretching and warm-up exercises, the maximum voluntary contraction (MVC) test was performed. For such, the volunteers sit on the seat of the isokinetic dynamometer (System 4, Biodex®, USA) with an angle of 100° between the trunk and hip and the non-dominant leg positioned with the knee at 60° of flexion (0° corresponds to complete knee extension) and attached to the seat of the dynamometer by straps. The dominant leg was positioned at 100° of hip flexion and was also attached to the seat by a strap. The volunteers were also attached to the seat of the dynamometer through the use of two straps crossing the trunk. The volunteers were instructed to cross their arms over the trunk and the axis of the dynamometer was positioned parallel to the center of the knee. The MVC test consisted of three 5-s isometric contractions of the knee extensors of the non-dominant leg. The highest torque value of the three contractions (peak torque) was used for the statistical analysis. The choice of this parameter is due to the fact that this variable reflects the maximum generation of force by the muscle. Instructions on how to execute the test were given first and the volunteers received verbal encouragement during the execution of the test. This test has demonstrated reliability and reproducibility in a previous study carried out by our research group [23]. The MVC was performed also immediately (1 min) after the eccentric contraction protocol as well as 1, 24, 48, 72, and 96 h after the eccentric contraction protocol to evaluate post-exercise muscle recovery. The researcher that performed assessment of MVC was blinded to randomization and allocation of volunteers in experimental groups.

Phototherapy

Phototherapy was performed exactly 3 min before eccentric exercise protocol. The therapy was applied employing a MR4 LaserShower 50 4D emitter (manufactured by Multi Radiance Medical, USA) in six sites of quadriceps femoris in direct contact with the skin (two centrally–rectus femoris and vastus intermedius; two laterally—vastus lateralis; and two medially—vastus medialis) (Fig. 2). Phototherapy was performed employing three different doses (10, 30, or 50 J per site; 60, 180, or 300 J per muscle, respectively) or placebo among experimental groups. The description of phototherapy parameters is provided in Table 1.

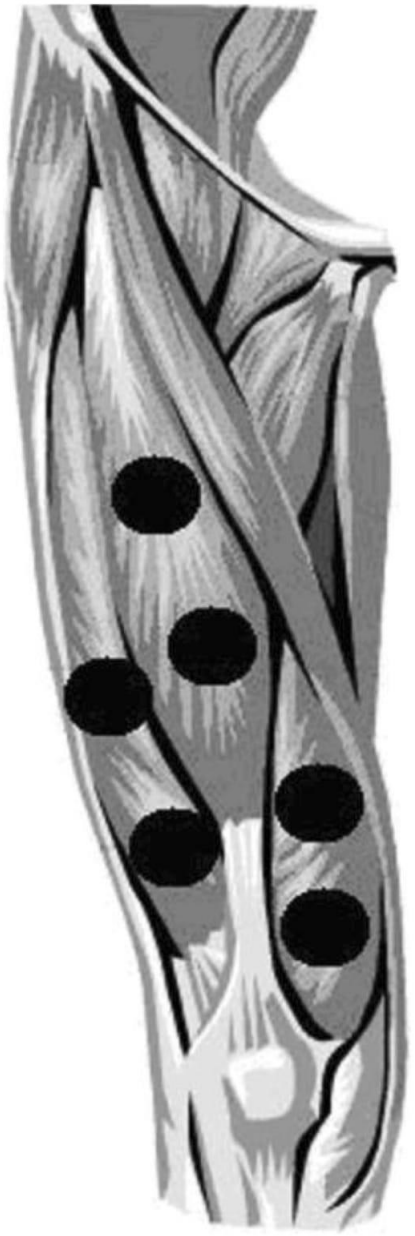


Fig. 2 Sites of phototherapy irradiation on quadriceps

To ensure blinding, the device emitted the same sounds regardless of the programmed mode (active or placebo). The optical power was calibrated before irradiation in each volunteer using a Thorlabs thermal power meter (Model S322C, Thorlabs®, Newton, NJ, USA). Based on the results of the randomization, the volunteers of the four experimental groups

Table 1 Phototherapy parameters

MR4 Base Control Unit	LaserShower
	
Number of lasers	4 super-pulsed infrared
Wavelength (nm)	905
Frequency (Hz)	250
Peak power (W) - each	12.5
Average mean optical output (mW) - each	0.03125
Power density (mW/cm^2) - each	0.07
Dose (J) - each	0.02375, 0.07125 or 0.1190625
Spot size of laser (cm^2) - each	0.44
Number of red LEDs	4 Red
Wavelength of red LEDs (nm)	640
Frequency (Hz)	2
Average optical output (mW) - each	15
Power density (mW/cm^2) - each	16.66
Dose (J) - each	1.14, 3.42 or 5.715
Spot size of red LED (cm^2) - each	0.9
Number of infrared LEDs	4 Infrared
Wavelength of infrared LEDs (nm)	875
Frequency (Hz)	16
Average optical output (mW) - each	17.5
Power density (mW/cm^2) - each	19.44
Dose (J) - each	1.33, 3.99 or 6.6675
Spot Size of LED (cm^2) - each	0.9
Magnetic Field (mT)	35
Irradiation time per site (sec)	76, 228 or 381
Total dose per site (J)	10, 30 or 50
Total dose applied in muscular group (J)	60, 180 or 300
Aperture of device (cm^2)	20

received the following doses: group A—10 J in each site (76 s of irradiation at each site), 60 J of total irradiated energy on the muscle (456 s of total irradiation time); group B—30 J in each site (228 s of irradiation at each site), 180 J of total irradiated energy on the muscle (1,368 s of total irradiation time); group C—50 J in each site (381 s of irradiation at each site), 300 J of total irradiated energy on the muscle (2,286 s of total irradiation time); and group D—0 J in each site (placebo) (100 s of irradiation at each site, 600 s of total time, but without effective irradiation). The researcher that performed phototherapy was blinded to randomization and allocation of volunteers in experimental groups.

Isokinetic protocol—eccentric contractions

Precisely 3 min after the end of phototherapy, the volunteers performed the eccentric contraction protocol, which consisted of 75 eccentric isokinetic contractions of the knee extensor musculature in the non-dominant leg (five sets of 15 repetitions, 30-s rest interval between sets) at a velocity of 60° seg^{-1} in both the eccentric and concentric movements with a 60° range of motion (between 90° and 30° of knee flexion). At each contraction, the dynamometer automatically (passively) positioned the knee at 30°; the dynamometer then flexed the knee until reaching 90°. The volunteers were instructed to resist against knee flexion movement imposed by the dynamometer with maximum force. Instructions on how to execute the maneuver were given first and the volunteers received verbal encouragement throughout the protocol. Despite the diversity of protocols proposed for the execution of eccentric exercises on isokinetic dynamometers, the protocol described here was chosen based on a previous study carried out by our research group [23] in which this method proved effective and reproducible for the exercise-induced muscle damage. The researcher in charge to eccentric contractions protocol was blinded to randomization and allocation of volunteers in experimental groups.

Statistical analysis

The intention-to-treat analysis was followed. The primary outcome was the peak torque obtained from MVC at different time-points. Secondary outcomes are CK, VAS, and pain threshold. The researcher that performed statistical analysis was blinded to randomization and allocation of volunteers in experimental groups. Data were expressed as mean and standard deviation and were firstly tested regarding normal distribution using Shapiro–Wilk test. ANOVA test with repeated measurements for the time factor were performed to test between-groups differences (followed by Bonferroni post hoc test). The significance level was set at $p<0.05$.

Table 2 Markers of performance and recovery (mean±SD)

		Pre	Post	1 h	24 h	48 h	72 h	96 h
VAS (mm)	Placebo	0.00 (±0.00)	44.25 (±11.76)	44.00 (±11.14)	46.50 (±14.12)	38.25 (±6.03)	19.50 (±7.06)	15.25 (±9.22)
	10 J	0.00 (±0.00)	39.00 (±11.95)	36.60(±19.89)	38.80 (±19.04)	23.00 (±11.55)	3.40 (±3.78)	0.60 (±1.34)
	30 J	0.00 (±0.00)	37.80 (±11.28)	33.80 (±11.05)	12.60 (±8.53)	12.00 (±4.55)	2.80 (±2.26)	0.00 (±0.00)
	50 J	0.00 (±0.00)	28.75 (±7.23)	30.75 (±15.40)	13.00 (±3.64)	5.25 (±7.09)	5.00 (±3.00)	2.50 (±7.00)
Algometry (kgf)	Placebo	9.70 (±3.34)	6.71 (±1.05)	8.19 (±1.35)	7.88 (±1.17)	8.41 (±1.37)	9.60 (±0.82)	8.68 (±2.05)
	10 J	11.25 (±2.78)	10.71 (±2.52)	10.68 (±2.47)	10.11 (±2.27)	10.54 (±2.19)	12.79 (±2.67)	12.80 (±2.46)
	30 J	12.00 (±2.15)	11.55 (±2.58)	11.48 (±2.08)	11.91 (±2.74)	10.95 (±2.04)	10.71 (±2.43)	9.30 (±2.68)
	50 J	8.67 (±1.51)	9.25 (±2.17)	8.07 (±2.08)	6.91 (±1.07)	8.88 (±0.80)	10.00 (±2.56)	7.41 (±2.20)
MVC (N m)	Placebo	271.30 (±28.71)	187.95 (±31.68)	191.48 (±37.83)	220.18 (±12.09)	226.76 (±10.25)	252.82 (±14.64)	265.06 (±24.79)
	10 J	279.50 (±14.33)	241.90 (±25.35)	241.37 (±15.19)	276.14 (±23.82)	280.17 (±36.38)	299.32 (±34.35)	325.25 (±37.00)
	30 J	286.63 (±38.86)	271.20 (±26.55)	278.57 (±23.78)	281.52 (±26.87)	281.62 (±20.79)	317.90 (±26.12)	336.88 (±27.92)
	50 J	254.38 (±28.24)	219.62 (±26.88)	231.68 (±24.46)	240.02 (±22.29)	262.51 (±29.97)	282.68 (±30.62)	304.73 (±26.23)
CK	Placebo	504.12 (±54.69)	581.55 (±68.97)	748.37 (±84.92)	1,168.32 (±170.80)	1,297.60 (±163.18)	1,173.09 (±404.15)	1077.81 (±372.23)
	10 J	489.67 (±46.02)	448.50 (±64.58)	472.17 (±41.30)	674.33 (±44.26)	531.00 (±80.36)	526.67 (±58.59)	877.67 (±111.72)
	30 J	521.00 (±84.50)	537.50 (±78.53)	567.33 (±100.80)	576.00 (±104.69)	502.67 (±53.23)	414.00 (±90.39)	604.17 (±64.76)
	50 J	475.17 (±112.59)	530.83 (±134.17)	507.00 (±108.12)	709.33 (±105.08)	509.83 (±120.99)	540.33 (±194.00)	1,078.50 (±41.25)

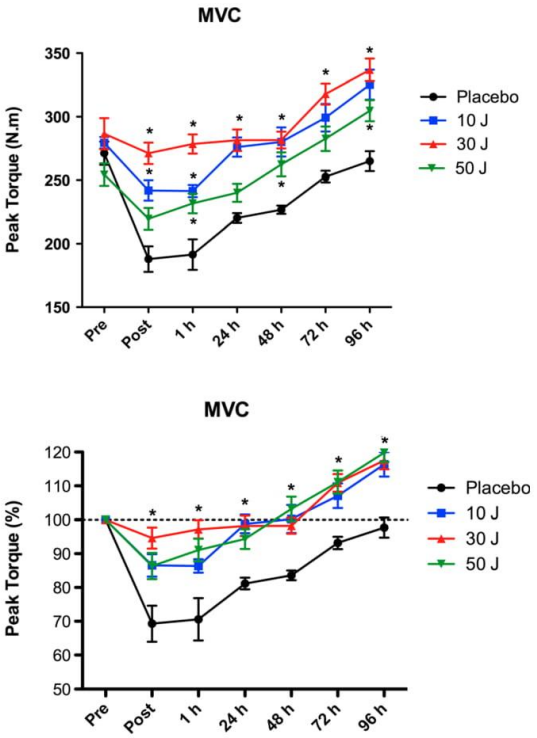


Fig. 3 MVC in absolute and percentage values. *Values* are mean and *error bars* are standard error of the mean (SEM). $^{*}p<0.05$ indicates significant difference compared to placebo

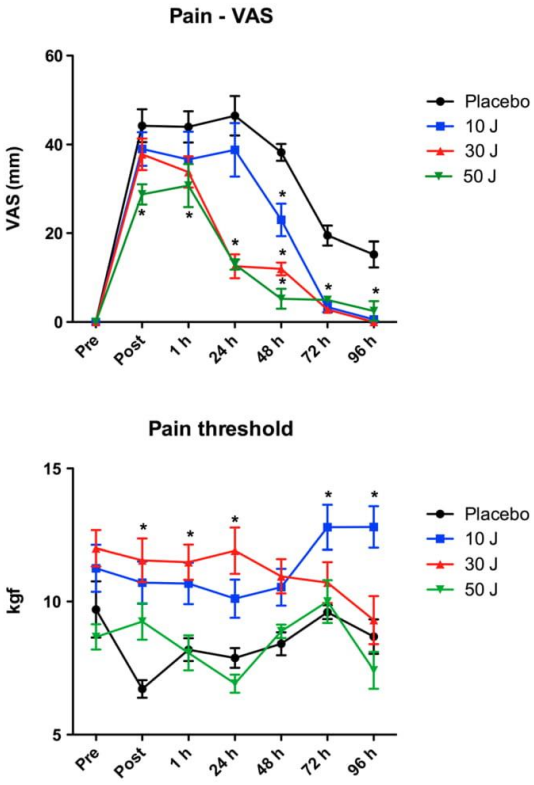


Fig. 4 DOMS assessment using 100 mm VAS and algometry. *Values* are mean and *error bars* are standard error of the mean (SEM). $^{*}p<0.05$ indicates significant difference compared to placebo

Results

Table 2 shows all outcomes regarding functional and biochemical aspects of performance and recovery that we observed in our study. As we can see in Fig. 3, 30 J dose significantly increased ($p<0.05$) MVC compared to placebo both in absolute than in percentage values from immediately after eccentric exercise to 96 h after eccentric exercise. On the other hand, 10 and 50 J doses significantly increased ($p<0.05$) MVC in percentage values compared to placebo in all time-points tested; however, 10 and 50 J doses do not show the same consistency over the time-points tested regarding MVC in absolute values compared to placebo group.

Significant differences ($p<0.05$) between phototherapy groups and placebo-control group regarding DOMS both in VAS than in algometry (pain threshold) were found, but interestingly distinct dose–response patterns were observed between pain measured through VAS and algometry. Results are summarized in Fig. 4.

CK analysis shows that 30 J dose significantly decreased ($p<0.05$) CK activity compared to placebo group from 1 to 96 h after eccentric contractions protocol, and the same was observed for 10 J dose. However, 50 J dose significantly decreased ($p<0.05$) CK activity compared to placebo group

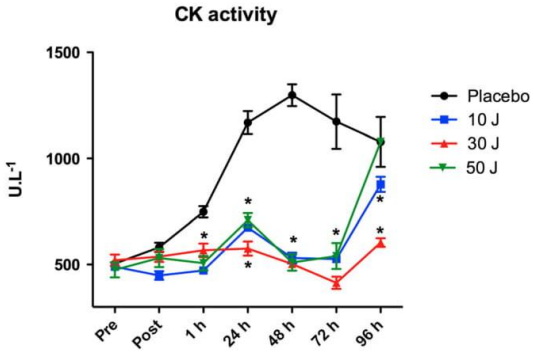


Fig. 5 CK activity. *Values* are mean and *error bars* are standard error of the mean (SEM). $^{*}p<0.05$ indicates significant difference compared to placebo

from 1 to 72 h after eccentric contractions protocol. Results regarding CK analysis are summarized in Fig. 5.

Discussion

As far as we know, this is the first time that synergistic effects of super-pulsed laser combined with red and infrared LEDs are tested aiming to enhance exercise performance and post-exercise recovery. We decided also to evaluate three different doses trying to help in establishment of a "therapeutic window" for phototherapy with this aim.

One more time we decided to irradiate muscles before exercise, since several studies and scientific evidence show that when pre-exercise phototherapy is performed, phototherapy is showing ergogenic and protective effects on skeletal muscle tissue [20, 21].

Our eccentric exercise protocol lead to decrease in muscle strength, increase in CK activity, and increase in pain in placebo-control group. Regarding muscle strength, all doses tested in this trial worked very well; however, 10 and 30 J doses were able to increase strength in all post-exercise time-points tested. It is important to highlight that volunteers irradiated with 30 J dose kept strength almost at 100 % from immediately after to 48 h after eccentric exercise and that from 72 to 96 h after eccentric exercise volunteers were able to perform 10–20 % more strength than in baseline. It perfectly illustrates the potential of phototherapy to promote ergogenic effects. Interestingly, our results regarding MVC were better than previously observed [24]. MVC even 48 h after eccentric exercise was not 100 % recovered with 30 J energy dose irradiated per site (also applied in six locations in quadriceps muscle belly). It is important to highlight that above-mentioned study was performed using a laser device with a single wavelength. In this perspective, it seems that combination of different wavelengths and light sources acting synergistically represented a therapeutic advantage in enhancement of muscle strength.

Regarding CK activity, our findings showed that all doses tested worked very well in decreasing biochemical marker of muscle damage (CK). Actually, all doses decreased CK activity from 1 to 96 h after eccentric exercise protocol and only 50 J dose does not decreased CK activity at 96 h after exercise. Again, 30 J dose showed the best results among all doses tested since this dose kept CK activity very closed to baseline values in all post-exercise time-points tested. One more time, our results were superior than previously observed [24]. It was shown that 30 J dose decreased CK activity compared to placebo; however, CK activity increased 87.78 % at 24 h and 201.29 % at 48 h after same eccentric exercise protocol employed in our study. This leads us to think that combination of different wavelengths and light sources acting synergistically represented a therapeutic advantage also in protecting muscle against exercise-induced damage.

Finally, regarding pain assessment, doses tested showed different patterns for two methods performed. For VAS score, 50 J dose showed the best effects decreasing VAS to immediately post to 96 h after exercise. Positive effects were observed for 30 J from 24 to 96 h after exercise. However, regarding pain threshold (algometry), only 30 J dose showed positive results increasing pain threshold of volunteers acutely (from immediately post to 24 h after exercise) and only 10 J increased pain threshold in long term (at 72 and 96 h after eccentric exercise). These distinct patterns observed among doses between VAS and algometry (pain threshold) clearly illustrates the biphasic dose–response pattern of phototherapy reported several times in literature [21, 25–28].

Interestingly our best dose (30 J) is the same tested previously with other phototherapy device available in the market [24]. Both studies employed the same eccentric exercise protocol and assessed similar aspects like MVC, VAS, and CK activity. However, our results were superior showing that combination of super-pulsed laser with red and infrared LEDs can represents a therapeutic advantage as suggested previously in an experiment with rats performed by our research group [28].

Some physiological effects attributed to LLLT are related to soft tissue metabolism and can explain our findings. Across different disorders, increased microcirculation [29], enhanced ATP synthesis [30], and stimulation of mitochondrial respiratory chain [30] and mitochondrial function [31] have been observed after LLLT. Reduction of ROS release and creatine phosphokinase activity, and increased production of antioxidants and heat shock proteins, has also been reported after LLLT [32, 33].

Recently, Hayworth et al. [34] demonstrated that a single irradiation with LLLT is able to increase cytochrome c-oxidase activity in intact skeletal muscle tissue 24 h after irradiation. Additionally, authors demonstrated that there is a dose and fiber type-dependent increase in cytochrome c-oxidase in skeletal muscle fibers. It means that LLLT lead to up-regulation of mitochondrial activity through increasing mitochondrial respiratory chain, which consequently increases ATP production into muscle cells and decrease oxidative stress and reactive oxygen species (ROS) production. It is important to highlight that in this study intact (non-injured) skeletal muscle of animals were irradiated with red wavelength (660 nm) LED and with a very low irradiance of 9 mW/cm^2 for 20, 40, or 60 min. This finding help to shift the paradigm that phototherapy can just be applied in tissues with injuries and can explain the mechanism through phototherapy applied before exercises can increase performance and decrease skeletal muscle fatigue and exercise-induced muscle damage.

Conclusion

Combination of super-pulsed laser with red and infrared LEDs is beneficial in enhancement of exercise performance and post-exercise recovery when irradiated before exercise. Irradiation of 30 J in six locations of knee extensors lead to better outcomes increasing muscle strength, and at same time decreasing pain and levels of biochemical marker related to muscle damage.

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

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Anexo III

Lasers in Medical Science
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LETTER

Comment on "Photobiomodulation delays the onset of skeletal muscle fatigue in a dose-dependent manner"

Thiago De Marchi[1,2]

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Dear Editor in Chief,

I am writing regarding the manuscript "Photobiomodulation delays the onset of skeletal muscle fatigue in a dose-dependent manner" authored by Larkin-Kaiser et al. [1]. The authors state that the work done was adding new information to the field of photobiomodulation (PBM). However, the finding of this manuscript only confirms that the use of high-powered lasers is not effective for enhancing sports performance or accelerating recovery.

The use of a large dose (240 J to enhance performance of a very small muscle—first dorsal interosseous) seems excessive when low-powered lasers need only 180 J to improve performance of large muscle groups such as knee extensors [2–6] in fact, very recently, a head-to-head comparison between three commercially available devices showed that high-powered lasers show worst outcomes when compared to low-powered lasers/LEDs. There was an obvious absence of articles cited in manuscript [1] that detail optimal parameters, including dose ranges [4, 7] for the use of PBM for muscle enhancement. More curiously, authors did not include two recently published systematic reviews, one of them authored by the senior author of this article [8, 9], which could be valuable in a discussion regarding parameters and dose ranges.

Authors use the term "dose dependent manner", however, the study design did not include a minimum of three active doses and a placebo comparator. The lack of adequate data points would not provide any plausible notion of the action of the delivered dose along the ArndtShutz curve.

The article title contains the MeSH term "Photobiomodulation". It should be noted that Joensen et al. [10] have demonstrated that light devices that deliver more than 200 mW of power can significantly increase skin temperature. How is it then possible that a high-powered class 4 laser applying 2 and 4 W for a 2-min duration not increase a subjects' skin temperature? According to the editorial published by Anders et al. [11] regarding photobiomodulation: "The use of this term is key, as it distinguishes photobiomodulation therapy, which is nonthermal, from the popular use of light based devices for simple heating of tissues [...]", therefore, this term should not have been included in this manuscript.

The introduction is problematic. The authors state, "Photobiomodulation (PBM) therapy is the medicinal use of both low and high intensity light sources [...]". Several editorial published by experts in the field do not support the use of high intensity sources [11, 12]. The inclusion of wavelengths beyond 1000 nm was included in the manuscript; however, the references used to endorse this statement (refs. [4], [5] and [11] of Larkin-Kaiser et al. [1] manuscript) used wavelengths below 1000 nm. The intentional use of overstatements and false statements is meant to intentionally misled readers into thinking high-powered laser is included in photobiomodulation.

Several additional biases exist in the paper:

1. The sample size description is lacking and does not provide any objective data for its calculation.
2. Details regarding the randomization procedure are insufficient. The lack of adequate randomization creates a serious risk of bias due learning effects of the employed exercise protocol.
3. The rationale for the use of the selected doses is weak. A greater explanation is necessary for those parameters since the authors did not include doses identified in published systematic reviews.
4. This study cannot be considered double-blinded. In our own laboratory, we studied the identical device and

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recorded a noticeable skin increased at least 5 °Celsius with 2-W power output compared to sham (device turned off) with a thermographic camera. The subjects would be aware of heating and therefore of an active intervention, since the placebo did not produce heat.

5. It would be very difficult to translate the work in their experiment to a real-world application. The use of a single small muscle would not likely improve athletic performance of an athlete.
6. In the discussion, the fact that high-powered lasers need much more energy to reach some small effectiveness when compared with low-powered lasers is not discussed.

The findings of this article are at best inconclusive and do not provide any new evidence for the use of ergogenic use of PBM. The authors lacked a proper perspective on the currently evidence for this field (PBM and performance enhancement/exercise recovery) and disregarded outcomes published previously because it did not support their study design or conclusions.

Compliance with ethical standards

Competing interests The author declares that he has no conflict of interest.

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