UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
CÂMPUS DE PRESIDENTE PRUDENTE

ARYANE FLAUZINO MACHADO

EFEITOS DA FOTOTERAPIA NO PROCESSO DE RECUPERAÇÃO PÓS-EXERCÍCIO E PERFORMANCE

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Tese de Doutorado apresentada ao Programa de Pós-Graduação em Fisioterapia da Faculdade de Ciências e Tecnologia da Universidade Estadual Paulista "Júlio de Mesquita Filho" (FCT/UNESP)

Orientador: Prof. Dr. Carlos Marcelo Pastre

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TÍTULO DA TESE: EFEITOS DA FOTOTERAPIA NO PROCESSO DE RECUPERAÇÃO PÓS-EXERCÍCIO E PERFORMANCE

AUTORA: ARYANE FLAUZINO MACHADO
ORIENTADOR: CARLOS MARCELO PASTRE

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Presidente Prudente, 01 de fevereiro de 2019

Dedicatória

Dedico esse trabalho a minha família.

Meus pais, Vânia e Jurandir e meu irmão, Gabriel.

Nosso esforço, nossa vitória!

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Epígrafe

“Agir, eis a inteligência verdadeira. Serei o que quiser. Mas tenho que querer o que for. O êxito está em ter êxito, e não em ter condições de êxito. Condições de palácio tem qualquer terra larga, mas onde estará o palácio se não o fizerem ali?”

Livro do Desassossego – Fernando Pessoa

SUMÁRIO

Apresentação

Essa Tese de Doutorado está apresentada em consonância com as normas do modelo alternativo do Programa de Pós-Graduação *Stricto Sensu* em Fisioterapia da Faculdade de Ciências e Tecnologia da Universidade Estadual Paulista "Júlio de Mesquita Filho".

O conteúdo desse trabalho contempla o material originado a partir da pesquisa "Efeitos da fototerapia na recuperação de desfechos clínicos, psicológicos, funcionais e crescimento endotelial vascular: um ensaio clínico randomizado por amostra estratificada, duplo-cego, placebo-controlado" financiada pela Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP (Bolsa de Doutorado no País, processo 2015/25219-0).

O presente material está dividido nas seguintes sessões:

- Contextualização do tema pesquisado;
- Artigo I: Machado AF, Micheletti JK, Lopes JSS, Vanderlei FM, Leal-Junior ECP, Netto Junior J, Pastre CM. *Phototherapy on Management of Creatine Kinase Activity in General Versus Localized Exercise: A Systematic Review and Meta-Analysis*, publicado no periódico *Clinical Journal of Sport Medicine* (doi: 10.1097/JSM.0000000000000606);
- Artigo II: Machado AF, Micheletti JK, Vanderlei FM, Nakamura FY, Leal-Junior ECP, Netto Junior J, Pastre CM. *Effect of low-level laser therapy (LLLT) and light-emitting diodes (LEDT) applied during combined training on performance and post-exercise recovery: protocol for a randomized placebo-controlled trial*, publicado no periódico *Brazilian Journal of Physical Therapy* (doi: 10.1016/j.bjpt.2017.05.010);
- Artigo III: Machado AF, Leal-Junior ECP, Batista NP, Espinoza RMCPP, Hidalgo RBR, Micheletti JK, Vanderlei FM, Pastre CM. *Phototherapy applied during combined training does not promote additional effects on performance and post-exercise*

recovery: a randomized controlled trial, placebo-controlled, submetido ao periódico *British Journal of Sports Medicine*.

- Conclusão;
- Referências.

Contextualização

A fisioterapia no âmbito esportivo agrega processos de avaliação funcional ou de risco, recuperação pós-exercício e prevenção e/ou tratamento de lesões e integra junto a profissionais de áreas diversas equipes que atuam em diversos momentos da prática esportiva[1]. Ações de campo durante a prática sistemática de modalidades têm sido cada vez mais comuns para o profissional em diferentes níveis de performance. No treinamento físico, uma das estratégias da fisioterapia desportiva é intervir por meio de técnicas que visem a recuperação pós-exercício, com o objetivo final de acelerar o retorno dos sistemas do corpo a níveis basais e otimizar o rendimento funcional[2]. Dentre as técnicas investigadas para este fim destaca-se a fototerapia.

Também denominada como fotobiomodulação ou fotobioestimulação[3], essa técnica consiste na aplicação de luz monocromática capaz de influenciar a atividade celular[4]. Considerando os efeitos fisiológicos citam-se alterações mitocondriais[5, 6], modulação e ativação precoce de genes[7, 8] e aumento da microcirculação local[9]. Clinicamente, tais efeitos repercutem na melhora da performance[4], tanto em número de repetições e tempo de exaustão[10-14], como em testes de força máxima[15-19] e VO2máx[13, 20]. Apontam-se ainda efeitos positivos em desfechos clínicos, como dor/desconforto muscular e seu limiar[16] e na resposta autonômica[21, 22].

Acredita-se que alguns fatores possam influenciar a magnitude dos efeitos da fototerapia como o comprimento de onda, a densidade da energia e da potência, o tipo de dano e o seu momento de aplicação[4]. Pastre et al.[23] apontam que a falta de padronização para utilização das técnicas recuperativas e o controle de suas variáveis pode dificultar a comparação de resultados. Em concordância, Leal-Junior et al.[4] destacam que importantes questões referentes ao tema ainda devem ser investigadas e sugerem ainda o desenvolvimento de estudos

que identifiquem os mecanismos fisiológicos da fototerapia, bem como a padronização de protocolos de exercícios empregados e desfechos a serem avaliados, com o objetivo de otimizar o uso da fototerapia na prática clínica.

Nesse sentido, essa pesquisa objetiva identificar o melhor método de aplicação da técnica para a recuperação pós-exercício em dois principais cenários: 1-) eficácia da fototerapia em um marcador sanguíneo de dano muscular (atividade da creatina quinase) em diferentes tipos de estresse; 2-) eficácia da aplicação da fototerapia ajustada a um treinamento combinado em desfechos funcionais, clínicos e psicológicos e no fator de crescimento endotelial vascular.

Phototherapy on Management of Creatine Kinase Activity in General Versus Localized Exercise: A Systematic Review and Meta-Analysis

Aryane Flauzino Machado, MSc,* Jéssica Kirsch Micheletti, MSc,* Jaqueline Santos Silva Lopes, MSc,* Franciele Marques Vanderlei, PhD,* Ernesto Cesar Pinto Leal-Junior, PhD,† Jayme Netto Junior, PhD,* and Carlos Marcelo Pastre, PhD*

Abstract

Objective: The main focus of this systematic review was to determine the efficacy of phototherapy in the management of creatine kinase (CK) activity after exercise and furthermore to identify for which exercise model protocol phototherapy provides the best results. **Design:** Meta-analysis comparing phototherapy with a control condition. **Setting:** The MEDLINE, EMBASE, SPORTDiscus, PEDro, and CENTRAL databases were searched from their earliest records to October 03, 2016. Data were pooled in a meta-analysis and described as standardized mean difference (SMD) with 95% confidence intervals (CIs) using a random effects model. **Participants:** Healthy subjects (no restrictions were applied, eg, age, sex, and exercise level). **Intervention:** Phototherapy (low-level laser therapy and/or light-emitting diode therapy) before or after exercise and a placebo or control condition. **Main Outcome Measures:** Creatine kinase activity (no restriction to any analysis, eg, serum, plasma, or capillary blood). **Results:** Fourteen studies were included for review. The results revealed that phototherapy has a more positive effect than control condition in management of CK activity [SMD = 0.77, 95% CI (0.32 to 1.22); $P = 0.0007$; $I^2 = 72\%$]. In exploratory analysis, the results showed that phototherapy was effective only in the exercise protocol with localized exercise with large effect size [localized exercise: SMD = 0.89, 95% CI (0.26 to 1.51); $P = 0.0002$; $I^2 = 76\%$; general exercise: SMD = 0.61, 95% CI (−0.05 to 1.26); $P = 0.07$; $I^2 = 67\%$]. **Conclusions:** The available evidence suggest that phototherapy has beneficial effects on the management of CK activity and demonstrate a possible relationship based on damage caused by exercise, providing a greater effect in studies that used localized exercise.

Key Words: phototherapy, recovery of function, athletic performance, creatine kinase

(*Clin J Sport Med* 2018;0:1–8)

INTRODUCTION

Exercise-induced muscle damage has been commonly used in scientific research.[1] Brancaccio et al[2] show that exercises with low to moderate intensity are not able to cause changes in muscle tissue. However, exercise exceeding this range, such as high intensity, unaccustomed, or using eccentric contractions, can cause alterations in mechanical and muscle fiber function and local inflammatory response.[2–4] These alterations may also lead to changes in permeability of the cell membrane with consequent release of enzymes, characterized by muscle damage markers. Determination of these enzymes in the blood is one of the most commonly used tools for measuring muscle damage, followed by delayed onset muscle soreness and muscle strength.[5] Felismino et al[4] indicate that creatine kinase (CK) activity in the blood is related to the muscle damage process,[5] and its levels can provide an information about the current state of membrane and consequently muscle integrity and the athlete's recovery process.[6]

To minimize the changes caused by exercise, recovery techniques have been used in the sports field.[7,8] In recent years, there have been an increasing number of studies discussing the use of light interventions as a recovery strategy.[9] The terms phototherapy or photobiomodulation have been used to define these interventions that can differentiate as to their light source [low-level laser therapy (LLLT) and light-emitting diode therapy (LEDT)].[9–11] Recent systematic reviews have reported positive effects on muscle soreness,[12] improved fatigue response,[12–14] with increased repetition numbers[13,14] and delayed time to exhaustion,[13,14] improved performance,[13,14] and reduced blood lactate[13–15] and CK levels.[3,13]

The effects of phototherapy are associated to the other variables and not to light source.[11] The magnitude of results can be influenced by the method of application, such as the

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wavelength, energy density and power, and time of application,[13,16] as like as the magnitude and type of damage caused by exercise.[5] Studies point out the difficulty of comparing studies using different exercises and suggest the standardization of exercise protocols to improve the use of phototherapy in clinical practice.[5,13]

Therefore, a systematic review addressing the effects of phototherapy from different types of exercise could clarify whether the technique is more effective in specific types of stress. Thus, the purpose of this systematic review was to determine the efficacy of phototherapy in the management of CK activity after exercise compared with a control condition and furthermore to identify for which exercise model protocol phototherapy provides the best results.

METHODS

This systematic review was registered in an international database of systematic reviews in health and social care (registration number: CRD42017056877; http://www.crd.york.ac.uk/PROSPERO/) and followed the Cochrane Collaboration Handbook[17] and PRISMA Statement recommendations.[18]

Search Strategies

The studies were selected from 5 databases [MEDLINE, EMBASE, SPORTDiscus, PEDro (Physiotherapy Evidence Database), and CENTRAL (Cochrane Central Register of Controlled Trials)] from the earliest record of each database to October 03, 2016. The search strategy used a combination of the following Medical Subject Headings (MeSH) terms: phototherapy and exercise (see details in **Appendix 1, Supplemental Digital Content 1**, http://links.lww.com/JSM/A172). The reference list of each included study was checked to find further studies that could be included.[16,19] There was no language restriction.

Study Selection

The selected studies compared the effects of phototherapy (LLLT and/or LEDT) before or after exercise with a placebo or control condition. Clinical trials were considered eligible if they included healthy subjects and assessed CK activity (no restriction to any analysis, eg, serum, plasma, or capillary blood).

The study selection process was conducted in steps (title, abstract, and full text) by 2 independents authors (A.F.M., and J.K.M.). In case of disagreement, consensus was used, checking the data of 2 authors to identify possible errors. If any disagreement is not resolved, a third author is contacted, and the data are reported in the study. These steps for consensus are recommended by Cochrane Handbook for Systematic Reviews of Interventions[17] (Figure 1).

Data Extraction

Outcome data including mean, SD, and sample size were extracted by 2 authors (A.F.M. and J.K.M.). When final values were not available, change values were used. When there was insufficient information, the authors were contacted. As SD values are not always reported by researchers, where necessary, these data were calculated using methods recommended in the Cochrane Handbook for Systematic Reviews of Interventions.[17] As an example, in the study by Aver Vanin et al,[20] SD was obtained from the SE of the mean multiplied by the square root of the sample size. This information is reported in **Supplemental Digital Content 2** (see **Appendix 2**, http://links.lww.com/JSM/A173).

The extraction process was performed using a standardized form that included details such as characteristics of participants, exercise protocol, phototherapy and its details (muscle treated and time of application), comparator, time of analysis, and methodological characteristics.

Some studies included multiple observations. In these cases, the data were extracted at a clinically relevant time point (the peak of CK activity of the phototherapy group).

Quality and Risk of Bias Assessment

The methodological quality was based on the PEDro Scale (0-10). Each study was assessed for random and concealed allocation, baseline comparability, blinding of subjects, therapists and assessors, adequate follow-up, intention-to-treat analysis, between-group comparisons, and point estimates and variability. For studies assessed and listed in the PEDro database (http://www.pedro.org.au), these scores were adopted. In other cases, the evaluation process was performed by 2 independent authors (J.K.M. and J.S.S.) using the scale recommendations. Consensus was used to solve disagreements, as reported before. A PEDro score of 7 or greater was considered "high quality," studies with a score of 5 or 6 were considered "moderate quality," and those with a score 4 or less were considered "poor quality."[16,19,21] The methodological quality was not considered as an inclusion criterion.

An adapted version of the GRADE approach was used to evaluate the overall quality of evidence and strength of the recommendation, as advocated by the Cochrane Back Review Group.[22] In brief, the GRADE classification was downgraded by 1 level for each of the 4 factors we considered: design limitations [$\leq$75% of participants from studies with high methodological quality (PEDro score 7 points)], inconsistent results ($\leq$75% of participants from studies with findings in the same direction), imprecision (300 participants for each pooled), and reporting bias (funnel plot for analysis with less than 10). The funnel plot was analyzed quantitatively (Egger test[23]; P value <0.100) and visually (funnel plot asymmetry, see **Supplemental Digital Content 3**, http://links.lww.com/JSM/A176). Overall quality of evidence was defined as high quality (further research is very unlikely to change our confidence in the estimate of effect), moderate quality (further research is likely to have an important effect on our confidence in the estimate of effect and may change the estimate), low quality (further research is very likely to have an important effect on our confidence in the estimate of effect and is likely to change the estimate), and very low quality (very uncertain about the estimate).[19,21,24]

Data Analysis

All meta-analysis calculations were conducted using Review Manager—RevMan software (version 5.3, Copenhagen: The Nordic Cochrane Center, The Cochrane Collaboration, 2014). The analysis of pooled data was calculated using a random effect model, because of the heterogeneity of the studies. Data were pooled in meta-analyses and described as standardized mean differences (SMDs) with 95% confidence intervals (95% CIs) and the effect size interpreted as 0.2 representing a small effect, 0.5 a moderate effect, and 0.8 a large effect.[17]

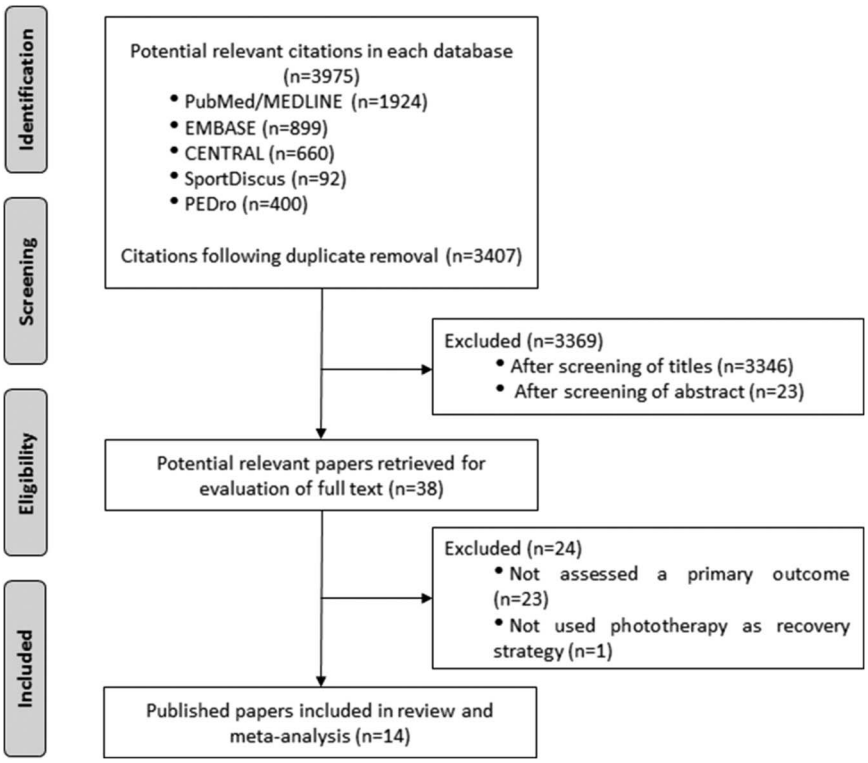

Figure 1. Flowchart for selection of studies.

The overall pooled values were calculated to analyze the effects of phototherapy, independent of type of exercise. In addition, a stratified analysis based on exercise protocol was performed using the same procedures as the main analysis, comparing studies that performed general exercise and localized exercise.

RESULTS

The database search identified, after removal of duplicates, 3407 studies, only 38 being eligible for full text review, after exclusion by title and abstract. Of these studies, 14 met all inclusion criteria. Figure 1 shows the selection process based on the PRISMA flowchart (see **Supplemental Digital Content 4** for PRISMA checklist, http://links.lww.com/JSM/A177).

All randomized controlled trials were provided from Brazil, 8[6,9,25–30] studies used a crossover design and 6[4,5,20,31–33] parallel groups. Publication date of the included studies ranged from 2009 to 2016. These studies comprised a total of 338 healthy male participants and fluctuated between untrained individuals[30,31,33] and professional volleyball,[6,9,27–29] water polo,[32] soccer,[6,25,34] and futsal players.[26]

There were variations in the interventions. Eight[4–6,20,25,27,30,32] of the included studies used LLLT, whereas 4 used LEDT[9,26,28,29] and 2[31,33] used a combination of both light sources (LLLT and LEDT). The wavelength varied between 640 nm and 905 nm,[31,33] both used with other wavelengths and light sources. The dose (energy) varied between 4J[4] and 630J[9] and the duration of therapy between 30[28,32] and 2286 seconds.[31] The studies showed a preference for application before exercise (n = 10),[5,6,9,20,25,27–31] whereas 3[26,32,33] studies applied the phototherapy after exercise and one during exercise, applying between sets of exercise.[4]

It was observed that 9[6,9,25–30,32] of the 14 studies presented CK peak immediately after exercise, one found peak at 1 hour,[33] one at 48 hours,[5,20] one at 72 hours,[4] and one[31] 96 hours after exercise. Eleven studies analyzed serum CK levels,[5,6,20,25–31,33] 2 studies analyzed plasma CK levels,[4,32] and one study analyzed CK levels by capillary blood.[9]

The characteristics of the included studies are summarized in Table.

The methodological quality assessment of studies revealed a mean of 7 points on the PEDro Scale. Nine[6,9,25,27–29,31,33,34] studies were considered "high quality" and 5[4,5,26,30,32] were considered "moderate quality." Among the assessment items, it was observed that all studies included in the review demonstrated random allocation. The items that were most satisfactory were the blinding, in which 100% of the studies showed blinding of subjects, 85% blinding of assessors, and 78% blinding of therapists (see details in **Appendix 3, Supplemental Digital Content 5**, http://links.lww.com/JSM/A174).

Phototherapy in Management of Creatine Kinase Activity

Fourteen studies were included in the analysis that compared the effects of phototherapy with a control intervention (control or placebo). The results of the overall analysis showed that there was "moderate-quality evidence" (according to GRADE classification), and the meta-analysis demonstrated that phototherapy was significantly superior in terms

TABLE. Characteristics of the Included Studies

Study	Study Design	Characteristics of Participants*	Exercise Protocol	Classification of Exercise Protocol	Phototherapy Group†‡	Comparator Group	Muscle Treated	Application Time	Time of Analysis and CK Activity Values	PEDro Score
Antonialli et al[31]	Parallel groups	N = 40 male; healthy untrained 24.1 ± 1.52 yrs	75 eccentric repetitions—60 degrees/s Knee extension	Localized exercise	LLLT and LEDT LLLT: 905 nm; 0.125 mW LEDT: 640/875 nm; 60/70 mW Total dose: 300J; irradiation time: 2286 s	Placebo	Quadriceps femoris (6 sites)	3 minutes before exercise	96 hours after exercise (group 1) (PG: 1078.50 ± 41.25; CG: 1077.81 ± 372.23)	8
Baroni et al[5]	Parallel groups	N = 36 male; healthy, physically active PG: 25.35 ± 3.41 yrs; CG: 24.28 ± 5.48 yrs	75 eccentric repetitions—60 degrees/s Knee extension	Localized exercise	LLLT 810 nm; 200 mW; total dose: 180J; irradiation time: 180 s	Placebo	Quadriceps femoris (6 sites)	2 minutes before exercise	48 hours after exercise (PG: 435.95 ± 238.04; CG: 1327.58 ± 949.82)	6
De Marchi et al[30]	Crossover	N = 22 male; healthy untrained 22.02 ± 3.02 yrs	Progressive-intensity running protocol on treadmill	General exercise	LLLT 810 nm; 200 mW; total dose: 360J; irradiation time: 360 s	Placebo	Lower limb (6 sites in quadriceps femoris; 4 in hamstrings; and 2 in gastrocnemius)	5 minutes before exercise	Postexercise (PG: 178.26 ± 82.36; CG: 290.42 ± 127.11)	6
de Paiva et al[33]	Parallel groups	N = 50 male; healthy untrained 24.98 ± 5.90 yrs	75 eccentric repetitions—60 degrees/s Knee extension	Localized exercise	LLLT and LEDT LLLT: 905 nm; 1.25 mW; LEDT: 640/875 nm; 15/17.5 mW Total dose: 236.2J; irradiation time: 1800 s	Placebo	Quadriceps femoris (6 sites)	3 minutes after exercise	1 hour after exercise (PG: 56.69 ± 16.03; CG: 56.92 ± 16.86)	10
Felismino et al[4]	Parallel groups	N = 22 male; healthy, physically active PG: 26.1 ± 4.1 yrs; CG: 25.9 ± 4.6 yrs	10 × 10 repetitions—50% 1RM Elbow flexion-extension	Localized exercise	LLLT 808 nm; 100 mW; total dose: 4J; irradiation time: 40 s	Placebo	Biceps brachii (4 sites)	Between each set of exercise	72 hours after exercise (PG: 357 ± 318.4; CG: 841 ± 623.5)	6
Ferraresi et al[9]	Crossover	N = 12 male; professional volleyball players 25.5 ± 5.3 yrs	Official volleyball matches	General exercise	LEDT 850/630 nm; 130/80 mW; total dose: 630J; irradiation time: 60 s	Placebo	Lower limb (quadriceps femoris; hamstrings; triceps surae)	40-60 before matches	Postexercise (group 1) (PG: 499.60 ± 232; CG: 406.10 ± 150.50)	7
Leal-Junior et al[29]	Crossover	N = 8 male; volleyball players 18.5 ± 0.93 yrs	Wingate test	General exercise	LEDT 660/850 nm; 10/30 mW; total dose: 83.4J; irradiation time: 60 s	Placebo	Quadriceps femoris (4 sites)	Immediately before exercise	Postexercise (PG: −20 ± 40; CG: 26.7 ± 15.3)	7

TABLE. Characteristics of the Included Studies *(Continued)*

Study	Study Design	Characteristics of Participants*	Exercise Protocol	Classification of Exercise Protocol	Phototherapy Group†‡	Comparator Group	Muscle Treated	Application Time	Time of Analysis and CK Activity Values	PEDro Score
Leal-Junior et al[6]	Crossover	N = 20 male (N = 9, volleyball players; N = 11, soccer players) 20.67 ± 2.96 yrs	Wingate test	General exercise	LLLT 830 nm; 100 mW; total dose: 20J; irradiation time: 400 s (volleyball) and 300 s (soccer)	Placebo	Rectus femoris (5 sites)	Immediately before exercise	Postexercise (PG: 2.52 ± 7.04; CG: 28.49 ± 22.62)	8
Leal-Junior et al[28]	Crossover	N = 10 male; professional volleyball players 23.6 ± 5.6 yrs	Repetitions until exhaustion 75% 1RM Elbow flexion	Localized exercise	LEDT 660/850 nm; 10/30 mW; total dose: 41.7J; irradiation time: 30 s	Placebo	Biceps muscle (1 site)	Immediately before exercise	Postexercise (PG: −3.04 ± 4.47; CG: 4.33 ± 8.65)	8
Leal-Junior et al[27]	Crossover	N = 9 male; professional volleyball players 18.6 ± 1.0 yrs	Repetitions until exhaustion 75% 1RM Elbow flexion	Localized exercise	LLLT 810 nm; 200 mW; total dose: 60J; irradiation time: 60 s	Placebo	Biceps muscle (2 sites)	3 minutes before exercise	Postexercise (PG: 263.6 ± 134.2; CG: 525.7 ± 386.5)	8
Leal-Junior et al[26]	Crossover	N = 6 male; professional futsal players 20.67 ± 2.96 yrs	Wingate test	General exercise	LEDT 660/850 nm; 10/30 mW; total dose: 208.5J; irradiation time: 150 s	Control	Rectus femoris (2 sites); Hamstring (2 sites); Triceps surae (1 site)	5 minutes after exercise	Posttreatment (PG: −8.55 ± 7.04; CG: −1.72 ± 22.62)	6
Dos Reis et al[25]	Crossover	N = 27 male; professional soccer players Age: DNR (15-30 years)	Repetitions until exhaustion 75% 1RM Knee extension	Localized exercise	LLLT 839 nm; 60 mW; total dose: 25.2J; irradiation time: 70 s	Placebo	Quadriceps femoris (42 sites)	Immediately before exercise	Postexercise (PG: 215 ± 33; CG: 412.5 ± 107.5)	8
Aver Vanin et al[20]	Parallel groups	N = 28 male; professional soccer players 18.81 ± 0.80 yrs	75 eccentric repetitions—60 degrees/s Knee extension	Localized exercise	LLLT 810 nm; 200 mW; total dose: 180J; irradiation time: 180 s	Placebo	Quadriceps femoris (6 sites)	3 minutes before exercise	48 hours after exercise (PG: 745.6 ± 22.4; CG: 712 ± 24)	10
Zagatto et al[32]	Parallel groups	N = 20 male; water polo players 15.4 ± 1.2 yrs	Water polo training	General exercise	LLLT 810 nm; 100 mW; total dose: 24J; irradiation time: 30 s	Placebo	Adductor magnus and adductor longus (8 sites)	5-40 minutes after each training session	Posttreatment (PG: 114.06 ± 178.4; CG: 107.66 ± 161.97)	6

** Age data are mean ± SD.*
† Light source; wavelength (nm); power output (W); energy (J); and duration of therapy (s).
‡ Data of group selected for analysis.
1RM, repetition maximum; CG, comparator group; DNR, data not reported; MVC, maximal voluntary contraction; PG, phototherapy group; yrs, years.

of management of CK activity [14 studies, n = 338; SMD = 0.77, 95% CI (0.32 to 1.22); $P = 0.0007$; $I^2 = 72\%$]. The effect size was considered to be moderate.

A stratified analysis was performed to compare the type of exercises (general vs localized exercise). The results of pooling data showed that phototherapy was effective in the management of CK activity only in the exercise protocol with localized exercise, presenting a large effect size [general exercise: "low-quality evidence," 6 studies, n = 134; SMD = 0.61, 95% CI (−0.05 to 1.26); $P = 0.07$; $I^2 = 67\%$ and localized exercise: "moderate quality," 8 studies, n = 204; SMD = 0.89, 95% CI (0.26 to 1.51); $P = 0.0002$; $I^2 = 76\%$] (Figure 2).

DISCUSSION

The results from this systematic review and meta-analysis showed that phototherapy was more effective compared with a control condition in the management of CK activity. The findings regarding stratification demonstrated that phototherapy provided greater effects in protocols with localized exercise than general exercise.

The findings involving overall analysis are in accordance with previous systematic reviews.[3,12,13] Leal et al[13] and Borsa et al[12] identified positive results in the management of CK activity through individual analysis of studies and effect size, respectively, with no meta-analysis. Nampo et al[3] found similar results by grouping studies (meta-analysis) but only considered studies that applied LLLT before exercise.

Our analysis considered studies with LLLT and LEDT, conceptualized as noninvasive therapies by light.[10,11] Despite the LLLT is considered as coherent and LEDT as noncoherent light, both light sources encompass the concept of phototherapy and share the same principle of photobiomodulation.[10,11,14] As pointed before, factors such as wavelength and doses should be given a higher importance than light source.[11] Studies that compared LLLT and LETD have found a nonsuperiority of one of these light sources.[29,35] Regarding application time, Leal-Junior et al,[13] through a systematic review, observed that 92% of included studies chose phototherapy before exercise, although the justification for this choice was not mentioned. However, evidence has shown positive effects from application before and after exercise in the ability to prevent injuries related to muscle fatigue and also to improve the postexercise recovery process.[13,31,34] Thus, it was considered appropriate to group studies in a single analysis.

The effects of phototherapy are mainly related to the prevention of exercise-induced damage and ergogenic effects, promoting faster recovery with an increase in performance.[9] Some mechanisms can explain these therapeutic effects[4] such as the ability to increase microcirculation and anti-inflammatory action, reduce oxidative stress, and modulate gene expression with regeneration of musculoskeletal tissue.[36] Studies in vitro indicate the formation of giant mitochondria through modulation[37] that promotes, as a secondary response, increased synthesis of adenosine triphosphate.[38] Accordingly, studies suggest that the mechanism of protection against exercise-induced muscle damage promoted by phototherapy is also a secondary response; however, the relationship between absorption and this effect is not known.[9]

These responses could be influenced by the different protocols used to induce muscle damage and can increase the CK levels.[9] This increase seems as consequence of metabolic and mechanical factors related to exercise. Metabolically exhausted muscle fibers exhibit a decrease in membrane resistance after an increase in calcium ions, promoting the activation of potassium channels.[2] The increase of membrane permeability allows for the liberation of CK on interstitial fluid that enters on circulation through the lymphatic system.[39]

The CK level is dependent on the age, sex, ethnicity, muscle mass, and physical activity level of each subject[2,40,41] and

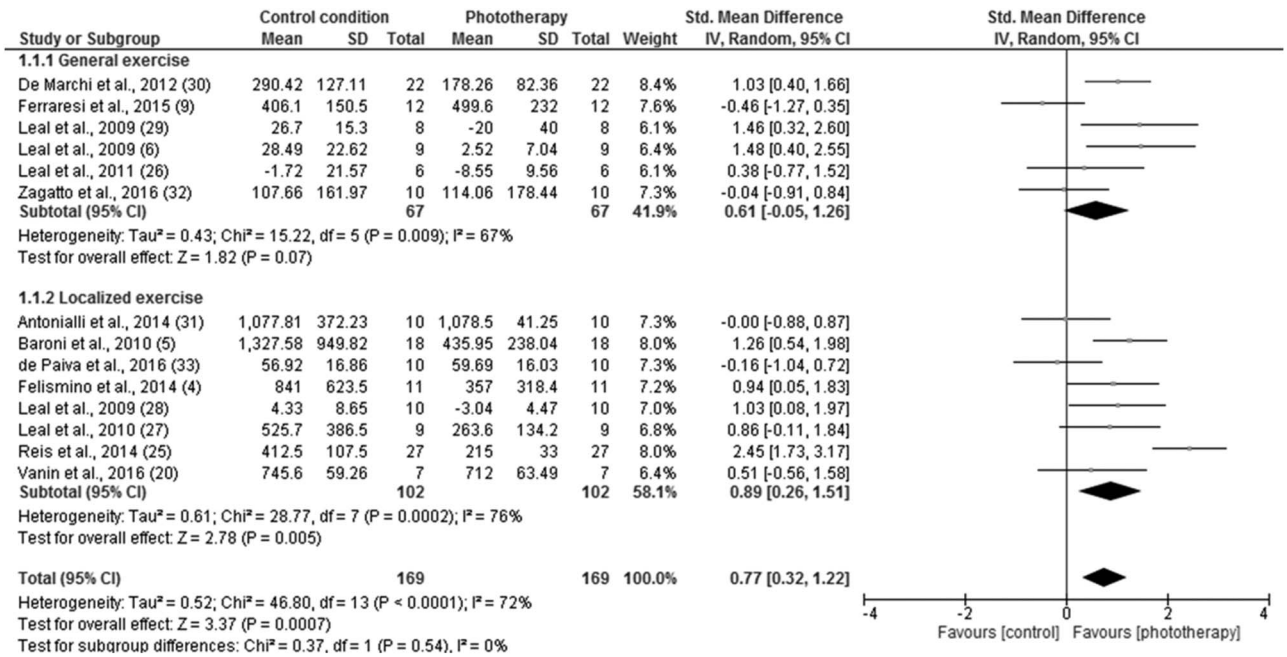

Figure 2. Forest plot illustrating the effects of phototherapy versus control condition on management of CK activity (stratified analysis). Std, standardized.

demonstrated a large intersubject variability.[42] Several factors can determine the level of CK activity during and after exercise and its moment of release and removal from the blood, such as level of training, type, and intensity and duration of exercise. The findings revealed that higher values of CK levels was found after localized exercise and that phototherapy provided greater effects when applied as a recovery strategy for localized exercise rather than general exercise. So, apparently, phototherapy has a relationship with the magnitude of damage caused by exercise. However, the mechanisms responsible for this management after phototherapy application remain unclear.[3] In the analysis of the "general exercise" subgroup, studies were observed that performed sports activities already undertaken by the participants, and in this case, most studies did not show elevated CK levels above the normal range, demonstrating possible adaptation to the proposed stress model. Thereby, an additional gap can be presented, regarding sample adaptation to the stress induced when performing general exercises.

When we analyzed the subgroup "localized exercise," it was noted that 4 trials[20,27,31,33] did not find positive results for phototherapy, some of which are justifiable. Although it did not present differences between the placebo and phototherapy groups, the study of Leal Junior et al[27] demonstrated a favorable trend for phototherapy, as can be observed in the CI [SMD = 0.85, 95% CI (−0.11 to 1.84)]. The studies of Antonialli et al[31] and Aver Vanin et al[20] aimed to identify the best dosage of application and also did not find favorable effects for phototherapy. Antonialli et al[31] highlighted the superiority of phototherapy in postexercise recovery for all doses analyzed in the study (10J, 30J, and 50J), except the 50J dose at the time 96 hours after exercise. This dosage and time represent the CK peak in the phototherapy group, justifying the inclusion of such data. The same occurred in the study of Aver Vanin et al,[20] which identified the best results when applying 50J; however, the CK peak was in the 30J group. Thus, an exploratory analysis was performed considering peak CK in the phototherapy group with the best dosage indicated by studies (96 hours after exercise, 30J, and 24 hours after exercise, 50J), and the findings indicated a large effect favoring phototherapy [localized exercise: 8 studies, n = 204; SMD = 1.39, 95% CI (0.72 to 2.06); $P < 0.0001$; $I^2 = 75\%$].

To the authors' knowledge, this is the first systematic review with meta-analysis to investigate the effects of phototherapy in different exercise protocols. One limitation of the study was the high heterogeneity of the included studies, represented by the inconsistency (I^2). However, it is emphasized that this limitation may be a result of the low number of participants who composed the included studies. Other point that would be addressed is the relationship of CK with muscle damage. Despite the widespread use of the CK level as an indirect indicator, caution should be used in its application in clinical practice. Some studies also report that the CK levels may not be a reliable marker of muscle damage and could represent only a membrane disruption or increase in permeability.[39,43]

The strengths are related to a comprehensive search strategy with no language restriction, the assessment of methodological quality by the PEDro Scale, the use of the GRADE system to rate the quality of evidence, and a preregister protocol[19,21] (see details in **Appendix 4, Supplemental Digital Content 6**, http://links.lww.com/JSM/A175). The rationale used to perform a stratified analysis should also be considered as a strength because this is a field that few have investigated in the current scenario. In addition, the findings have important implications for clinical practice, optimizing the use of phototherapy based on the exercise protocols used.

Thus, in summary, the hypothesis that the magnitude of the effects can be influenced by magnitude and type of damage caused by exercise, presenting a relationship, was confirmed. Phototherapy, when applied directly to muscle tissue damaged by exercise that provokes higher levels of CK (localized exercise), provides the best results.

CONCLUSIONS

The findings of this study suggest that phototherapy has beneficial effects on the management of CK activity. The results also demonstrate a possible relationship based on damage caused by exercise, providing a greater effect with moderate-quality evidence in studies that used protocols with localized exercise.

The findings of the study allow clinicians and researchers a more effective implementation of phototherapy in post-exercise recovery, considering the CK level. However, for a better accurate of muscle damage, more invasive investigations, such as muscle biopsies, should be considered because the CK level may not be a reliable marker.

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***Appendix* Table 1. Search strategy**

MEDLINE, EMBASE, SPORTDiscus and CENTRAL (Cochrane Central Register of Controlled Trials)

- **Up to 03 October, 2016**

1. (biostimulation) OR (laser biostimulation) OR (laser therapies) OR (laser therapy) OR (laser phototherapy) OR (LEDT) OR (light therapies) OR (light therapy) OR (light-emitting diode therapy) OR (LLLT) OR (low level laser therapies) OR (low level laser therapy) OR (low level laser therapies) OR (low level light therapies) OR (low level light therapy) OR (low power laser irradiation) OR (low power laser therapies) OR (low power laser therapy) OR (low-level laser therapies) OR (low-level laser therapy) OR (low-level light therapies) OR (low-level light therapy) OR (low-power laser irradiation) OR (low-power laser therapies) OR (low-power laser therapy) OR (photobiomodulation) OR (photobiomodulation therapies) OR (photobiomodulation therapy) OR (photobiostimulation) OR (photoradiation therapies) OR (photoradiation therapy) OR (phototherapies) OR (phototherapy)
2. (exercise) OR (exercises) OR (physical exercise) OR (physical exercises)
3. 1 AND 2

PEDro (Physiotherapy Evidence Database)

- **Up to 03 October, 2016**

Abstract and title:

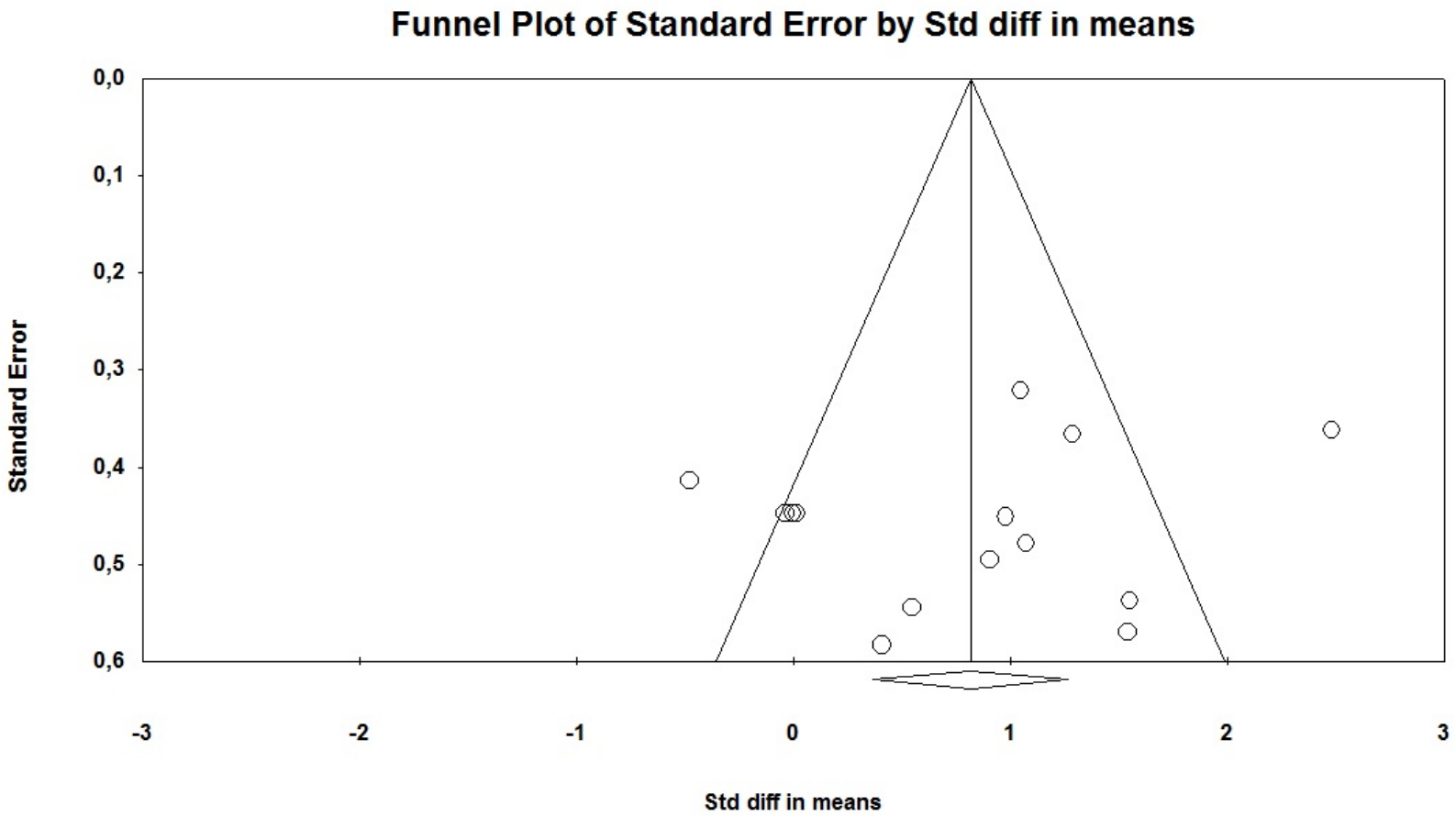
1. Biostimulation
2. Laser Biostimulation
3. Laser Therapies
4. Laser Therapy
5. Laser Phototherapy
6. LEDT
7. Light Therapies
8. Light Therapy
9. Light-emitting diode therapy
10. LLLT
11. Low Level Laser Therapies
12. Low Level Laser Therapy
13. Low Level Light Therapies
14. Low Level Light Therapy
15. Low Power Laser Irradiation
16. Low Power Laser Therapies
17. Low Power Laser Therapy
18. Low-Level Laser Therapies
19. Low-Level Laser Therapy
20. Low-Level Light Therapies
21. Low-Level Light Therapy
22. Low-Power Laser Irradiation
23. Low-Power Laser Therapies
24. Low-Power Laser Therapy
25. Photobiomodulation
26. Photobiomodulation Therapies
27. Photobiomodulation Therapy
28. Photobiostimulation
29. Photoradiation Therapies
30. Photoradiation Therapy
31. Phototherapies
32. Phototherapy

Method: clinical trial

***Appendix* Table 2**. Information of data extraction

Study	Time point	Value extracted	*Control condition*			*Phototherapy*		
			Mean or change	**SD**	**Sample size**	**Mean or change**	**SD**	**Sample size**
Antonialli et al., 2014[31]	96h post-exercise (group 3)	Mean and SD	1077.81	372.23	10	1078.5	41.25	10
Baroni et al., 2010[5]	48h post-exercise	Mean and SD	1327.58	949.82	18	435.95	238.04	18
De Marchi et al., 2012[30]	Post-exercise	Mean and SD	290.42	127.11	22	178.26	82.36	22
de Paiva et al., 2016[33]	1 hour post-exercise	Mean and SD	56.92	16.86	10	56.69	16.03	10
Felismino et al., 2014[4]	72h post-exercise	Change and SD SD obtained from SE	841	623.5	11	357	318.4	11
Ferraresi et al., 2015[9]	Post-exercise (group 1)	Mean and SD	406.1	150.5	12	499.6	232	12
Leal et al., 2009[29]	Post-exercise	Change and SD	26.7	15.3	8	-20	40	8
Leal et al., 2009[6]	Post-exercise	Change and SD	28.49	22.62	9	2.52	7.04	9
Leal et al., 2009[28]	Post-exercise	Change and SD	4.33	8.65	10	-3.04	4.47	10
Leal et al., 2010[27]	Post-exercise	Mean and SD	525.7	386.5	9	263.6	134.2	9
Leal et al., 2011[26]	Post-exercise	Change and SD	-1.72	21.57	6	-8.55	9.56	6
Reis et al., 2014[25]	Post-exercise	Mean and SD	412.5	107.5	27	215	33	27
Vanin et al., 2016[20]	48 hours post-exercise (group 2)	Mean and SD SD obtained from SE	745.6	59.26	7	712	63.49	7
Zagatto et al., 2016[32]	Post-exercise	Mean and SD SD obtained from SE	107.66	161.97	10	114.06	178.44	10

SD: standard deviation; SE: standard error


Funnel Plot of Standard Error by Std diff in means
Standard Error
0,0
0,1
0,2
0,3
0,4
0,5
0,6
-3
-2
-1
0
1
2
3
Std diff in means

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	2
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	2
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	3
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	3
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	3
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Appendix
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	3
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	3
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	3
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	4
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	4
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	4

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	4
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	4
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	5
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	5
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	6
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	6
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	6
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Appendix
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	6
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	6
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	8, 9
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	9
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	10

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: **www.prisma-statement.org**.

***Appendix* Table 3. Physiotherapy Evidence Database Scores of Included Studies**

Study	Random allocation	Concealed allocation	Baseline comparability	Blinding subjects	Blinding therapists	Blinding assessors	Adequate follow-up	Intention-to-treat analysis	Between-group comparisons	Point estimates / variability	Score (0-10)
Antonialli et al., 2014[31]	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes	8/10†
Baroni et al., 2010[5]	Yes	No	Yes	Yes	No	Yes	No	No	Yes	Yes	6/10*
De Marchi et al., 2012[30]	Yes	Yes	Yes	Yes	Yes	No	No	No	No	Yes	6/10*
de Paiva et al., 2016[33]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	10/10†
Felismino et al., 2014[4]	Yes	No	Yes	Yes	No	Yes	No	No	Yes	Yes	6/10*
Ferraresi et al., 2015[9]	Yes	Yes	No	Yes	Yes	Yes	Yes	No	No	Yes	7/10†
Leal et al., 2009[29]	Yes	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	7/10*
Leal et al., 2009[6]	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes	8/10*
Leal et al., 2009[28]	Yes	No	No	Yes	Yes	Yes	Yes	No	Yes	Yes	8/10*
Leal et al., 2010[27]	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Yes	Yes	8/10*
Leal et al., 2011[26]	Yes	Yes	No	Yes	Yes	Yes	No	No	No	Yes	6/10*
Reis et al., 2014[25]	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes	8/10†
Vanin et al., 2016[20]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	10/10†
Zagatto et al., 2016[32]	Yes	No	Yes	Yes	No	Yes	No	No	Yes	Yes	6/10*

** Trials assessed and listed on the PEDro Database*

† Trial assessed by the 2 independent raters

***Appendix* Table 3. Quality of evidence (GRADE system)**

Pooled estimate (Outcome: CK)	**Quality assessment**				**Participants (n)**		**Effect**	**Quality**
	Risk of bias*	Inconsistent†	Imprecision‡	Reporting Bias§	Control Condition	Phototherapy	Std. Mean Difference (95% IC)	
Overall pooled	Limitation	No limitation	No limitation	Undetected	169	169	0.77 [0.32, 1.22]	⊕⊖⊖⊖ Moderate
General exercise	Limitation	Limitation	Limitation	n/a	67	67	0.61 [-0.05, 1.26]	⊕⊕⊕⊖ Very Low
Localized exercise	No limitation	No limitation	Limitation	n/a	102	102	0.89 [0.26, 1.51]	⊖⊖⊕⊖ Moderate

CK: creatine kinase, GRADE: Grades of Recommendation, Assessment, Development and Evaluation
**More than 25% of participants from studies with low methodological quality (Physiotherapy Evidence Database score 7 points).*
† 75% of participants or less from studies with findings in the same direction.
‡ Fewer than 300 participants for each outcome.
§ Inspection of funnel plot asymmetry and the Egger test (Overall analysis; P-value = 0.426).
n/a: not applicable; was not performed due to the insufficient number of studies (<10 studies).

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ORIGINAL RESEARCH

Effect of low-level laser therapy (LLLT) and light-emitting diodes (LEDT) applied during combined training on performance and post-exercise recovery: protocol for a randomized placebo-controlled trial

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KEYWORDS
Phototherapy;
Recovery of function;
Athletic performance

Abstract
Background: Previous studies have shown positive results of phototherapy for improving performance and accelerating recovery; however, the effects of phototherapy during training and after a primary adaptation remain unclear. The aim of this randomized controlled trial is to analyze the effects of phototherapy and combined training on clinical, functional, and psychological outcomes and on vascular endothelial growth factor.
Methods: This randomized placebo-controlled trial by stratified sample will involve 45 healthy male participants. In phase 1, the participants will undergo six weeks of combined training (sprints and squats). In phase 2, participants will be allocated through stratified randomization (based on adaptation capacity) into three groups: active phototherapy group (AG), placebo group (PG), and non-treatment control group (CG). A new six-week training program will then start and the participants will receive the recovery strategy between sprints and squats. The primary outcome will be maximal isometric contraction. The secondary outcomes include strength and power testing, maximal incremental test, squat jump, sprint test, muscle soreness, pain

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threshold, perceptions of exertion and recovery, psychological questionnaire, and vascular endothelial growth factor.
Conclusions: This will be the first trial to include phototherapy during training. We believe that this strategy will combine the ergogenic and prophylactic effects in the same session. Furthermore, an application protocol performed after primary adaptation may reflect the real effect of the technique.

Introduction

Phototherapy has been the focus of important research in sports science in recent years.[1] Also known as the phenomenon of photobiomodulation or photobiostimulation,[2] this technique is the application of monochromatic light that can influence cellular activity through inhibition or stimulation of chemical and biological functions.[3] Phototherapy has two main light sources, i.e., laser and LED (light emitting diode), that are applied separately and, in recent years, in combination, and has demonstrated a significant therapeutic advance with subsequent clinical improvement.[4]

Experimental studies and clinical trials have identified the possible physiological effects of this technique on muscle tissue undergoing structural or metabolic stress.[5] The main findings showed the formation of giant mitochondria able to provide high respiratory rates, a higher volume of energy, and greater mitochondrial density in the tissues[6] with consequent synthesis of adenosine triphosphate (ATP),[7] modulation of gene expression with regeneration of musculoskeletal tissue,[5] increases in local microcirculation[8] and modulation of endothelial function with angiogenesis upregulated by the technique with vascular endothelial growth factor (VEGF) as mediator.[9,10] These interactions have repercussions in exercise and its mechanisms of action are related to two main effects, i.e., ergogenic and protective or prophylactic.[11] Borsa et al.[11] relate the ergogenic effects of changes in microcirculation, reduction in lactic acid production,[12–14] improvement in mitochondrial function, and increase in antioxidant capacity.[15–17] The protective effect is closely related to muscle cell protection against muscle damage,[17] producing anti-inflammatory and antioxidant effects.[11]

From these mechanisms, positive results can be observed in a range of outcomes, as discussed in recent systematic reviews[3,11] and clinical trials.[4,12,14–23] The magnitude of the effects of phototherapy can be influenced by the different application techniques, such as wavelength, energy density, and power, as well as the type of damage and time of application.[3,24] Recent studies[3,4] have aimed to eliminate these potential biases by identifying the best management application. Regarding the time of application, Leal-Junior et al.[3] emphasize that 92% of the studies included in a systematic review opted to apply the technique before exercise. However, studies have shown positive effects on different markers from application both before and after exercise, although no justification for such a choice is given other than author preference.

Thus, investigation into the management of phototherapy adjusted to a model of training (between structural and metabolic exercise), could combine the ergogenic and protective effects in the same session. In addition, phototherapy applied after control of the primary adaptation to exercise may reflect the real effect of the technique. Thus, the objective of the study is to analyze the effect of phototherapy with different light sources (low-level laser therapy and light-emitting diodes) and combined training on clinical, functional, and psychological outcomes and on vascular endothelial growth factor. Accordingly, the study hypothesis is that the use of a phototherapy protocol, adjusted to a specific time of physical stress, will lead to significant improvements in the recovery process and in damage prevention.

Methods

Study design and settings

We will conduct a randomized, placebo-controlled clinical trial with parallel groups at Universidade Estadual Paulista (UNESP), Presidente Prudente, SP, Brazil. The trial has been prospectively registered at ClinicalTrials.gov (NCT02918916) and approved by Human Research Ethics Committee of UNESP, Presidente Prudente, SP, Brazil (1.389.046/2016). The study follows the SPIRIT 2013 checklist (Standard Protocol Items: Recommendations for International Trials)[25] and TIDieR (Template for Intervention Description and Replication)[26] to improve the information and quality of intervention reporting.[27] Prior to the procedures, the participants will receive oral and written instructions and sign a consent form agreeing to participate in the study. All personal data will be confidential.

Participants will undergo a 12-week training program divided into two phases. In phase 1 (six weeks), participants will undergo training consisting of sprints and squats in the same session. After this period, the participants will undergo new tests and be randomly allocated into the groups that comprise the study. In phase 2, a new six-week training period will begin. In this phase, the participants will train normally with the adjusted loads and between sprints and squats will receive the recovery strategy related to the group to which they belong.

A flowchart of the design, composition of the groups, and phases is presented in Fig. 1.

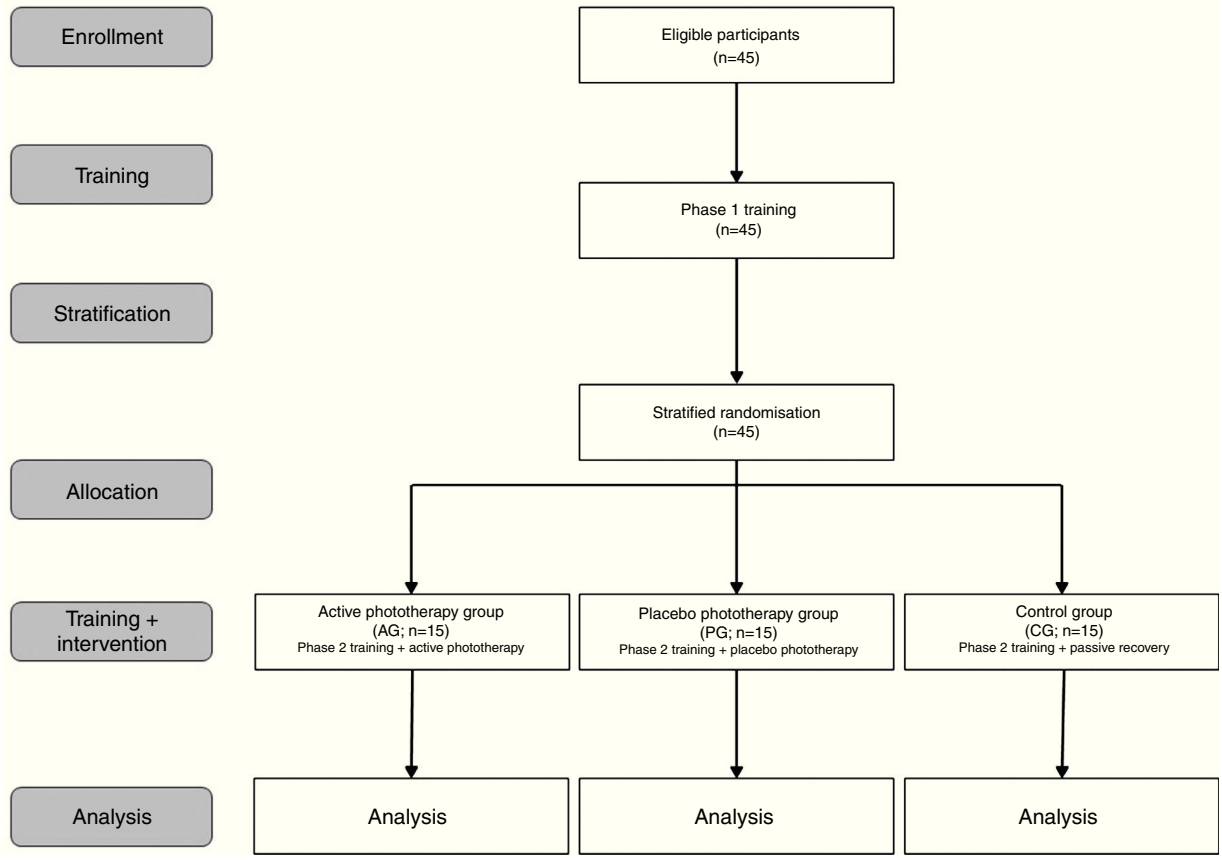

Figure 1 Study design.

Participants

A total of 45 healthy male volunteers will be recruited from the local university (UNESP) via online media advertisement, personal invitation, telephone, and SMS and registered in the database of the Sports Physical Therapy Laboratory. These procedures are recommended by Treweek et al.[28] as a strategy to improve participant recruitment.

Initial contact with all participants will be made by telephone to establish their willingness to participate and a meeting will be scheduled to evaluate the eligibility criteria.

The following inclusion criteria will be employed:

- Healthy patient (self-report);
- Male gender;
- Age between 18 and 30 years.

Participants with the following will be excluded from the study:

- History of cancer;
- Presence of anemia, inflammation, or diabetes;
- History of muscle injury in the lower limbs or spine in the previous six months.
- Occurrence of musculoskeletal injury during the study.

Participants will be instructed to maintain their daily diet routine and abstain from anti-inflammatory and analgesic drugs, as well as any exercise not presented during the study. This information will be reinforced during data collection and monitored by self-report to further analysis.[29]

Randomization procedures

Participants will be randomly allocated to the groups after phase 1. The stratification process will be carried out based on the concept of responders and non-responders to adaptation capacity,[30] defined in this study as functional and clinical responses. Each response will be recorded before and after phase 1.

Adaptation capacity (AC) will be extracted using the following equation:

$$AC = 0.5\,FP + 0.5\,CP$$

Functional parameter (FP)

A priori knowledge will be used based on the study of Kotzamanidis et al.,[31] who observed average gains of 8.5% after combined training. This value is set as the ceiling of strength gain capacity. Therefore, gains $\geq$8.5% in strength test will

correspond to 100% of FP and gains <8.5% will be inserted into the following equation:

$$FP = \frac{(\text{final test value} - \text{baseline test value}) * 100}{8.5}$$

Clinical parameter (CP)

The perception of recovery will be assessed using a 10-point Likert scale.[32] Each point on the scale is considered as 10% of CP. Participants will be evaluated at the end of session 10.

Participants will be stratified into three strata based on their adaptation capacity:

- Participants with AC values below the median;
- Participants with AC values between the median and the 3rd quartile;
- Participants with AC values above the 3rd quartile.

A block randomization[33] with blocks of three will be used to allocate participants of each stratum. Participants will be allocated to groups in a 1:1:1 ratio into the following groups: active phototherapy group (AG), placebo phototherapy group (PG), and control group (CG).

To ensure allocation concealment, randomization to groups will be undertaken by an independent researcher who will be instructed not to inform the participants or other researchers.[34]

Details of procedures

Training program

The training program will be based on combined training described by Marques et al.[35] The program is considered individualized and based on a load moved in 1 m s^{-1} in a strength and power test.

The training consists of a combination of maximal sprints and explosive squats. Sessions will be held twice a week (48-hour interval) for six weeks, followed by a second six-week program. Phase 1 of the training program (six weeks) aims to induce adaptation and making the participants' level of physical activity more uniform. Phase 2 corresponds to the experimental phase, in which the recovery strategies will be given following stratified randomization.

The reference study[35] and a pilot study did not show any adverse effects from training. However, the adherence or adverse events will be recorded for further analysis. To improve adherence to the protocol, the participants will receive telephone reminders or SMS messages.[28]

A detailed description of the training program is presented in Table 1.

Recovery strategies

Recovery strategies will be applied between sprint and squat training. We chose this moment of application because of the possibility of associating ergogenic and protective effects of phototherapy in the same session.

A trained therapist will apply active phototherapy bilaterally using the MR4 LaserShower 50 4D emitter (Multi Radiance Medical, USA) to six sites on the quadriceps (two centrally – rectus femoris and vastus intermedius; two laterally – vastus lateralis; two medially – vastus medialis) in direct contact with the skin. The option to apply phototherapy at these sites was chosen due to this muscle group being more directly involved in squats and the most worked during sprints. To receive intervention, the participant will be placed in the supine position. Phototherapy parameters are described in Table 2.

The optical power will be calibrated before irradiation in each participant using a Thorlabs thermal power meter (Model S322C, Thorlabs, Newton, NJ, USA) and the dosage applied will be 30J per site (180J per muscle). This dosage is considered the optimal dose to improve performance, muscle soreness, and biochemical markers related to skeletal muscle damage.[4]

The same procedures as in the active phototherapy group will be applied to the placebo group; however, the emitter will be disabled. The device will emit the same sounds regardless of the programmed mode (active phototherapy).

The control group participants will remain seated for passive recovery supervised by an independent therapist[36] during the period when the other groups are receiving recovery strategies.

Primary and secondary outcome measures and assessment points

The primary outcome will be the peak torque obtained in the maximal voluntary isometric contraction. Secondary outcomes are other functional, clinical, and psychological outcomes and VEGF, described below. Data will be collected in a specific time point (Table 3). Each assessor will be responsible for the same outcome throughout the study. Performance tests will be completed in two sessions separated by a day of rest and will be performed in the order described below.

In the first session, the sprint test, squat jump, and maximal incremental test will be completed. In the second session, the strength and power tests and maximal voluntary isometric contraction (MVIC) will be performed. Additionally, care will be taken to allow sufficient rest (15 min) between all tests on the same day to limit the effects of fatigue on the following test. This rest is based on time to recovery of each test.[37–41]

Primary outcome

Maximal voluntary isometric contraction (MVIC)

Participants will be positioned with the dominant leg on the isokinetic dynamometer (Biodex System 4 Pro, New York, USA). Prior to the MVIC, they will do a warm up consisting of 10 repetitions of knee flexion–extension at 180° s^{-1} throughout the range of motion. The MVIC will be determined by the highest maximal isometric torque over three contractions of five seconds at 60° of knee flexion. The repetitions will be separated by a 2-min rest interval.[17]

Table 1 Training program.

Phase	Week	Sessions	Sprint training					Squat training			
			Warm up*	Intensity†	Sets × Repetitions	Rest between sets		Warm up*	Intensity†	Sets × Repetitions	Rest between sets
Phase 1	1	1	3 × 20 m	Maximum	2 × 15 m	120 s	10 min between training	1 × 8 + 1 × 8	80%	1 × 8	120 s
		2	3 × 20 m		2 × 15 m	120 s		1 × 8 + 1 × 8	80%	2 × 8	120 s
	2	3	3 × 25 m		2 × 20 m	120 s		1 × 8 + 1 × 8	85%	1 × 8	120 s
		4	3 × 25 m		2 × 20 m	120 s		1 × 8 + 1 × 8	85%	2 × 6	120 s
	3	5	3 × 30 m		2 × 25 m	150 s		1 × 8 + 1 × 8	90%	1 × 6	120 s
		6	3 × 30 m		2 × 25 m	150 s		1 × 8 + 1 × 8	90%	2 × 8	120 s
	4	7	3 × 35 m		2 × 30 m	180 s		1 × 8 + 1 × 6	95%	1 × 6	180 s
		8	3 × 35 m		2 × 30 m	180 s		1 × 8 + 1 × 6	95%	2 × 5	180 s
	5	9	3 × 25 m		2 × 20 m	150 s		1 × 8 + 1 × 6	100%	2 × 6	180 s
		10	3 × 25 m		2 × 20 m	150 s		1 × 8 + 1 × 6	100%	2 × 5	180 s
	6	11	3 × 30 m		1 × 25 m	180 s		1 × 8 + 1 × 6	90%	2 × 6	120 s
		12	3 × 30 m		1 × 25 m	180 s		1 × 8 + 1 × 6	80%	1 × 8	120 s
	7						Interval between phases△				
Phase 2	8	13	3 × 20 m	Maximum	2 × 15 m	120 s	10 min between training	1 × 8 + 1 × 8	80%	1 × 8	120 s
		14	3 × 20 m		2 × 15 m	120 s		1 × 8 + 1 × 8	80%	2 × 8	120 s
	9	15	3 × 25 m		2 × 20 m	120 s		1 × 8 + 1 × 8	85%	1 × 8	120 s
		16	3 × 25 m		2 × 20 m	120 s		1 × 8 + 1 × 8	85%	2 × 6	120 s
	10	17	3 × 30 m		2 × 25 m	150 s		1 × 8 + 1 × 8	90%	1 × 6	120 s
		18	3 × 30 m		2 × 25 m	150 s		1 × 8 + 1 × 8	90%	2 × 8	120 s
	11	19	3 × 35 m		2 × 30 m	180 s		1 × 8 + 1 × 6	95%	1 × 6	180 s
		20	3 × 35 m		2 × 30 m	180 s		1 × 8 + 1 × 6	95%	2 × 5	180 s
	12	21	3 × 25 m		2 × 20 m	150 s		1 × 8 + 1 × 6	100%	2 × 6	180 s
		22	3 × 25 m		2 × 20 m	150 s		1 × 8 + 1 × 6	100%	2 × 5	180 s
	13	23	3 × 30 m		1 × 25 m	180 s		1 × 8 + 1 × 6	90%	2 × 6	120 s
		24	3 × 30 m		1 × 25 m	180 s		1 × 8 + 1 × 6	80%	1 × 8	120 s

* Warm up: sprints in which gradually increasing speed until they reach the maximum speed; squats with lighter loads to those in training.
† Sprints at full speed; Squat load defined by percentage of the load that a subject is able to displace at an average speed of 1 m s^{-1}.
△ Adjust load.

Table 2 Phototherapy parameters.

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	0.07125
Spot size (cm^2) – each	0.44
Number of red LEDs	4 red
Wavelength (nm)	640
Frequency (Hz)	2
Average mean optical output (mW) – each	15
Power density (mW/cm^2) – each	16.66
Dose (J) – each	3.42
Spot size (cm^2) – each	0.9
Number of infrared LEDs	4 infrared
Wavelength (nm)	875
Frequency (Hz)	16
Average mean optical output (mW) – each	17.5
Power density (mW/cm^2) – each	19.44
Dose (J) – each	3.99
Spot size (cm^2) – each	0.9
Magnetic field (mT)	35
Irradiation time per site (s)	228
Total dose applied (J)	180
Aperture of device (cm^2)	20

Secondary outcomes

There will be 12 secondary outcomes to understand the comprehensive process of post-exercise recovery and performance.

- *Strength and power testing*: these tests will be carried out with a bar.[35] Participants starting in the upright position with the bar in contact with the shoulders and will be required to perform the squat with continuous movement until their thighs reach the horizontal plane, then immediately perform the reversed motion at maximum speed. Information about the eccentric distance reached and concentric speed of each repeat will be recorded by a linear velocity transducer (T-Force System Ergotech, Murcia, Spain), which will also provide visual and auditory feedback.

  For the strength test, the warm up will consist of 10 repetitions at 40–60% of maximum perception. The last acceptable repetition with the highest possible load will be considered 1RM (repetition maximum). The rest period between sets is two minutes.

  To determine the velocity of displacement during the concentric phase of full squat, the load willbe adjusted until the highest velocity is obtained. The velocity index is calculated by the average value of peak velocity obtained with each load. The tests will be carried out gradually and with increments of 10 kg for each set with two trials executed with each load.

  For the power test, the warm up will consist of five repetitions with the bar. Bar displacement and peak and mean velocity will be recorded by an encoder. A computer program (Isocontrol Dinámico, 3.6, Spain) will be used to calculate the velocity of displacement for each repetition throughout the range of motion. An interval of 2–3 min will be given between trials. The best trial with each load will be recorded.
- *Maximal incremental test*: the maximum oxygen consumption (VO2max) and maximum aerobic speed (MAS) will be determined on a treadmill (Inbramed, Inbrasport, Brazil) using an incremental protocol.[42] Participants will do a five-minute warm up at 7 km h^{-1}. After five minutes of rest, the participants will perform the incremental protocol consisting of running at a starting velocity of 10 km h^{-1}, with increments of 1 km h^{-1} per minute until exhaustion and a fixed inclination of 1%. The test will finish through voluntary exhaustion or the presence of clinical alterations. Gas analysis will be measured continuously and the average value of every 30 s will be recorded (VO2000, MedGraphics, Minnesota, USA). The MAS will be recorded as the speed corresponding to the final complete step in the incremental test.
- *Squat jump*: participants will remain with the sole of their feet in contact with the jump platform (Multisprint, Hidrofit, Brazil), legs at 90° of flexion, hands on hips, trunk upright, and without previous movements. The participant will perform a jump keeping their legs straight (180°) and touch the platform again. Each participant will perform three complete tests with a 30-second rest interval.[43] The best measure of the tests will be adopted.
- *Sprint test*: participants will perform three maximum sprints exceeding 30 m with 3-min rest intervals. Performance times at 10, 20, and 30 m will be recorded (Multisprint, Hidrofit, Brazil).[35] The average of the two best tests will be adopted.
- *Muscle soreness*: values of muscle soreness in lower limbs will be obtained through an 11-point visual analog scale (VAS),[17] with 'zero' corresponding to absence of soreness and '10′ to the maximum level supported by the participant.[44]
- *Pain threshold*: pressure algometry will be applied at five specific sites of the quadriceps in the dominant leg.[45] Participants will be instructed to signal when the pressure sensation becomes discomfort. The pressure will not exceed 2.55 kgf.[46]
- *Perception of exertion*: the level of exertion reported by participants will be assessed using the Borg Scale (6–20 points), with 'six' corresponding to 'very easy' and '20′ to 'exhaustive'.[47]
- *Perception of recovery*: the level of perception of recovery will be assessed by a 10-point Likert scale, with 'one' corresponding to 'not recovered' and '10′ to 'fully recovery'.[29,32]
- *Psychological questionnaire*: participants will be instructed to mark an ''X'' on a 10-cm visual analog scale, with 'zero' corresponding to 'least possible'

Table 3 Time point of outcomes.

Outcomes		Time point											
		SW* (phase 1) Stratification analysis Before adaptation						SW* (phase 2) Recovery effect After adaptation					
		M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12
Performance tests	Strength and power tests	√					√						√
	Maximal voluntary isometric contraction	√					√						√
	Maximal incremental test	√					√						√
	Squat jump	√					√						√
	Sprint test	√					√						√
	Muscle soreness	√	√	√	√	√	√	√	√	√	√	√	√
	Pain threshold	√	√	√	√	√	√	√	√	√	√	√	√
	Perception of exertion			√	√				√		√		
	Perception of recovery			√	√				√	√	√		
	Psychological questionnaire	√	√					√					
	Belief questionnaire						√						√
	Vascular endothelial growth factor	√					√						√

SW, specific week (phase 1: session 9 and 10; phase 2: session 21 and 22).
M1: baseline; SW (phase 1) = M2: before training; M3: after sprint training; M4: after complete training; M5: 60 min after complete training; M6: after phase 1. SW (phase 2) = M7: before training; M8: after sprint training; M9: after recovery strategy; M10: after complete training; M11: 60 min after complete training; M12: after phase 2.
* Specific week: training sessions with higher energetic spending leaving for the last two weeks, composed of higher volume and intensity.

and '10′ 'most possible' for each rating (readiness for exercise, fatigue, vigor, sleepiness, and pain).[48]

- *Belief questionnaire*: after phase 1, participants will answer a belief questionnaire to measure their 'belief' in the effectiveness of the technique. Participants will be instructed to mark an ''X'' on a 5-point Likert scale, with 'zero' corresponding to 'not effective' and '10′ to 'extremely effective'. After application of the recovery techniques, the participants will complete a similar questionnaire to measure their perceived effectiveness of the technique.[48]
- *Vascular endothelial growth factor (VEGF)*: 10 ml of blood will be collected and plasma from this sample will be stored at −80 °C for later analysis. The plasma level of VEGF will be analyzed using the ELISA method (enzyme-linked immunosorbent assays) following the manufacturer's instructions (RayBio, Norcross, GA, USA).[49]

Masking/blinding

This study will ensure that assessors will be blinded. Although the therapist is not blinded, he will not have access to which recovery strategy is being applied (phototherapy or placebo). One participating researcher will be responsible for programming the phototherapy device based on random allocation. In this study design, it will not be possible to blind the participants and therapists due to the control condition.

Sample size calculations

Based on data from a previous study[4] that used the same light source of phototherapy, for a three-arm parallel study (with SD = 38.86 Nm on MVIC with $\alpha = 0.05$ and $\beta = 0.8$, to detect a difference of 10%) a total sample of 45 will be required, allowing for sample loss.

Statistical analysis

The statistical analysis will be conducted in SPSS (version 18; SPSS Inc., Chicago, IL, USA). After the adaptation process, the data will be analyzed as described below.

For outcomes with two time points: analysis of covariance (ANCOVA) with general linear models will be used to test between-group differences, with the difference/delta value as a dependent factor, group variable as fixed factor, and baseline values as covariate. (Outcomes: performance tests, VEGF, belief questionnaire, perception of exertion.)

For outcomes with three or more time points: initially, sphericity of the data will be tested by Mauchly's test. In case of violation of the sphericity assumption, the Greenhouse–Geisser corrections will be used. Analysis of variance (ANOVA) with repeated measurements will be used to test between-group differences followed by Bonferroni or Dunn post hoc test. (Outcomes: perception of recovery, psychological questionnaire, muscle soreness, and pain threshold.)

The data will be double entered. All participants will be included in the analyses following an intention-to-treat approach and the significance level will be set at $p < 0.05$.

Discussion

Phototherapy is a strategy commonly used by physical therapists to minimize the changes caused by exercise, promote post-exercise recovery, and improve performance. However, different application times have been used. A recent review[3] showed that 92% of included studies chose phototherapy before exercise, but it did not justify the choice. However, evidence has shown positive effects in the application before and after exercise in the ability to prevent injuries related to muscle fatigue and also in the improvement of the recovery process.[3,4,50] Thus, the building of a rationale based on adjustment of phototherapy to training seems relevant. We believe that this strategy could combine the ergogenic and prophylactic effects of phototherapy in the same session. Furthermore, an application protocol performed after control of the primary adaptation process resulting from the exercise may be able to reflect the real effect of the technique.

This trial will contribute to evidence-based use of phototherapy in training programs, providing a better understanding of the technique and improved logistics for the recovery process. It contemplates the items of checklists for protocol studies to minimize bias, and it was prospectively registered. We chose to perform randomization with stratification by adaptation process to improve the methodological quality of the study. To achieve this, we will use measures of performance and perception of recovery. As this strategy has not yet been conducted, it is not possible to infer about its full efficiency, and such condition may represent a potential limitation. Data will be published after the study is completed.

Conflicts 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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Artigo 3

Study title: Phototherapy applied during combined training does not promote additional effects on performance and post-exercise recovery: a randomized controlled trial, placebo-controlled

Short title: Phototherapy applied during a combined training

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Keywords: phototherapy; recovery of function; athletic performance

ABSTRACT

Background: Previous studies have shown positive results of the phototherapy on the improvement of performance and post-exercise recovery acceleration. However, the effects of phototherapy adjusted to a model of training remain unclear.

Purpose: The aim of this study was to analyze the effects of phototherapy applied during combined training on functional, clinical and psychological outcomes and vascular endothelial growth factor.

Methods: Thirty-nine healthy male participants performed a 12-week (24 sessions) training program divided in two phases. Phase 1: six weeks of combined training with sprints and squats. After a stratified randomization (based on adaptation capacity), participants were allocated into three groups: phototherapy, placebo, and non-treatment control. Phase 2: new six-week training (load adjusted) with recovery strategy applied between sprints and squats. Phototherapy (30J per site) was applied on six sites of quadriceps bilaterally. Functional performance, clinical and psychological outcomes and vascular endothelial growth factor (VEGF) were assessed.

Results: Clinical and psychological outcomes demonstrated no significant differences. There was a significant effect for time with large and medium effect size for some functional outcomes [$P<0.05$; Squat jump (ES=0.187); Sprint 10m (ES=0.210); Sprint 20m (ES=0.210); Sprint 30m (ES=0.251); maximal voluntary isometric contraction (ES=0.091)]. The VEGF analyses also demonstrated a significant effect for time with large effect size [$P<0.001$; VEGF (ES=0.559)]. There were no significant group and interaction effects.

Conclusions: Twelve weeks of combined training of sprints and squat improve some functional outcomes and increase of vascular endothelial growth factor concentration; however, no evidence was found that phototherapy applied during training promotes additional effects.

INTRODUCTION

Phototherapy has been the focus of different researches, currently. Its effects have been related to the interaction of light with comophores, influencing the cellular activity through the inhibition or stimulation of chemical and biological functions[1]. Among its attributions, studies have shown that phototherapy is able to improve performance and accelerate the recovery process[2].

The physiological effects of phototherapy were associated with two main effects, ergogenic and protective or prophylactic[3]. Positive results of these mechanisms can be observed in a range of outcomes as discussed in recent systematic reviews[4, 5]. The magnitude of the effects can be influenced by the different application techniques, such as wavelength, energy density and power, as well as type of damage and time of application[6].

A systematic review published by our research group suggests that phototherapy can have a different repercussion on general and localized exercises[4]. A recent guideline provides clinical recommendations regarding methods of applying phototherapy[2]. The authors highlight in 'when to irradiate' a differentiation for acute and chronic effects[2]. This study also mention that, according to the International Olympics Committee, phototherapy may be a beneficial therapeutic agent in promoting acute muscle recovery[2, 7].

The phototherapy application aimed at evaluating chronic effects, as in exercise training, began to be investigated recently in humans[8-12]. Divergences are identified to types of training and times of application. Regarding the type of training, studies have investigated the effects of phototherapy mainly on strength training[8, 12] (predominant structural exercise) and on endurance training[9, 11] (predominant metabolic exercise). Studies have also shown positive effects on different markers its application both before and after exercise[13].

Thus, investigating the management of phototherapy adjusted to a combined training (between structural and metabolic exercise) may be an effective way to associate its ergogenic and

protective effects in the same session[13]. In addition, phototherapy applied after control of the primary adaptation process resulting from exercise may be able to reflect the real effect of the technique.

Thus, the objective of this study was to analyze the effect of phototherapy interacting with combined training on clinical and functional outcomes and vascular endothelial growth factor. The study hypothesis was that the use of phototherapy adjusted to a training program would provide significant results, reflecting in an improvement of the recovery process and performance.

METHODS

Participants

Thirty-nine healthy, male volunteers (aged 18-30 years; height 176.95 ± 6.16 cm; weight 75.27 ± 12.03 kg; body mass index 23.96 ± 3.00 kg/m²) participated in this study. Based on data from a previous study[14] that used the same light source of phototherapy for a three-arm parallel study (with SD=28.71 Nm on MVIC with α=0.05 and β=0.8, to detect a difference of 10%) a total sample of 11 per group was required.

Participants were instructed to maintain their daily diet routine and abstain from anti-inflammatory and analgesic drugs, as well as the execution of any exercise not proposed during the study. Twenty minutes before each session (tests or training) participants ingested 30g of carbohydrate-based gel supplement followed by 180 ml of water to standardize the energy reserve.

The participants were recruited from the local university (Univ Estadual Paulista) via online media advertisement and approach of participants, via telephone and SMS, registered in the database of the Laboratory of Physiotherapy in Sports.

Inclusion criteria were participants who did not present the following characteristics: history of cancer, presence of anemia, inflammation, or diabetes and history of muscle injury in the lower limbs or spine in the previous six months. Exclusion criteria were the occurrence of musculoskeletal injury during the study.

Study design and settings

All procedures were approved by the Human Research Ethics Committee of the São Paulo State University (Protocol number: 1.389.046/2016) and registered at ClinicalTrials.gov (ID: NCT02918916). Prior to the procedures, the participants received oral and written instructions regarding the procedures and objectives of the study and signed a consent form agreeing to participate.

The study was carried out at São Paulo State University. Participants performed a 12-week training program, divided into phases 1 and 2. In phase 1 participants performed a sex weeks training consisting of sprints and squats. After this period, the participants performed new performance tests and were randomly allocated, based on adaptation capacity, into the groups that comprise the study: active phototherapy group (AG), placebo phototherapy group (PG) and control group (CG). In Phase 2 the participants performed the same training program with the adjusted loads and received the recovery strategy between sprint and squats.

The flowchart of participants is presented in Figure 1.

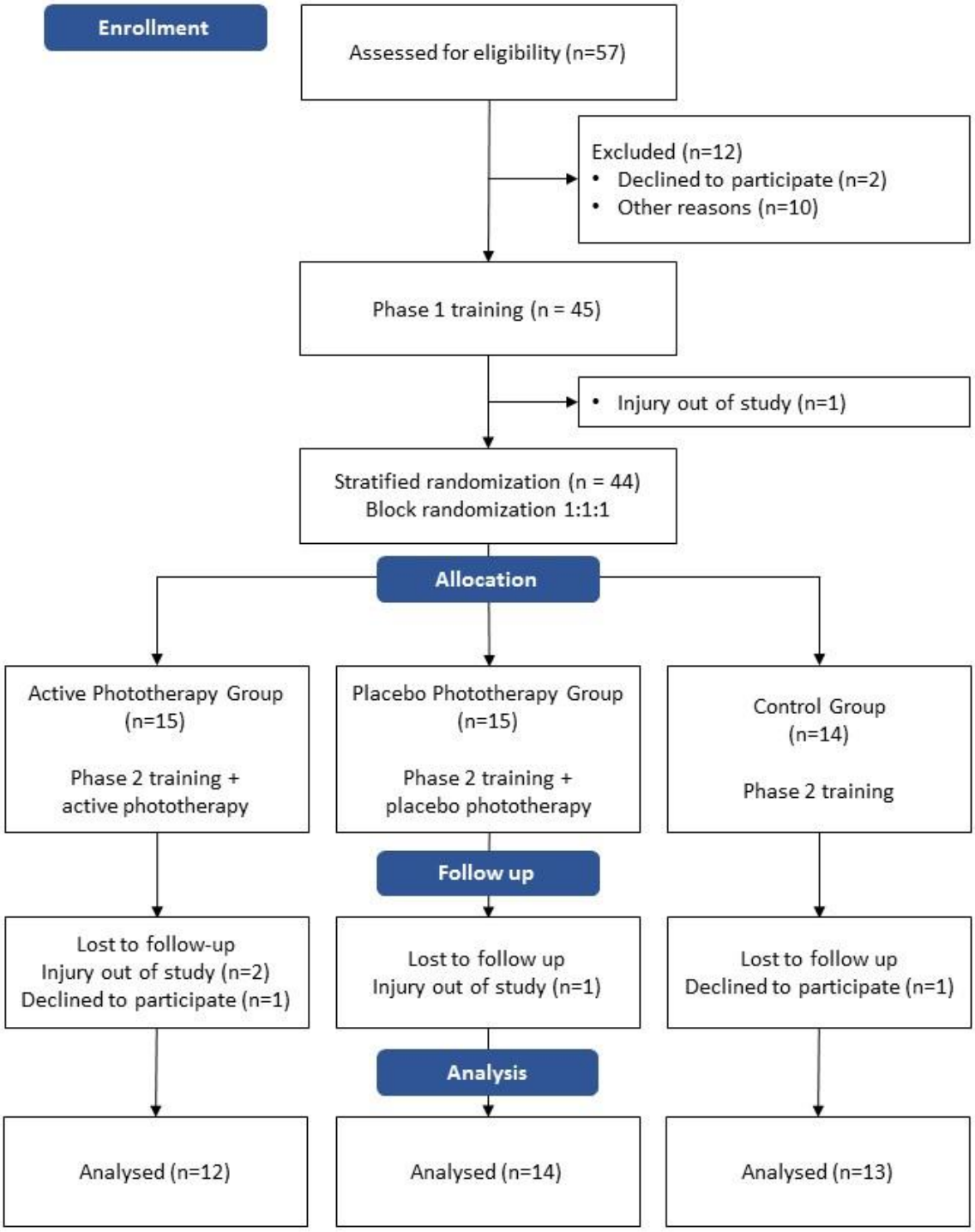

Figure 1. Flowchart of participants.

Randomization procedures

A randomized controlled trial by a stratified sample was performed. The stratification process was conducted based on the concept of responders and non-responders to adaptation capacity[15]. Each response was recorded before and after phase 1.

The adaptation capacity (AC) was extracted using the equation $AC = 0.5\,FP + 0.5\,CP$, where FP is the functional parameter (strength test) and CP is the clinical parameter (perception of recovery). Participants were stratified into three stratum (quartiles) based on their adaptation capacity and allocated into groups by a block randomization. The complete randomization process was described by Machado et al.[13].

Details of procedures

Training program

The training program was adapted from an individualized combined training described by Marques et al.[16]. The training consists of a combination of sprints and explosive squats performed during six-weeks twice a week (48 hours of interval), which was duplicated into two phases for this study.

Phase 1 of the training program had the objective of inducing adaptation and equating the participants level of physical activity. Phase 2 corresponded to the experimental phase, in which, after the stratified randomization, the recovery strategies were applied. A detailed description of the training is presented in Table 1.

Table 1. Training program

Phase	Week	Sessions	*Sprint Training*				*Squat Training*			
			Warm up*	Intensity†	Sets x Repetitions	Rest between sets	Warm up*	Intensity†	Sets x Repetitions	Rest between sets
Phase 1	1	1	3x20m	Maximum	2x15m	120s	1x8+1x8	80%	1x8	120s
		2	3x20m		2x15m	120s	1x8+1x8	80%	2x8	120s
	2	3	3x25m		2x20m	120s	1x8+1x8	85%	1x8	120s
		4	3x25m		2x20m	120s	1x8+1x8	85%	2x6	120s
	3	5	3x30m		2x25m	150s	1x8+1x8	90%	1x6	120s
		6	3x30m		2x25m	150s	1x8+1x8	90%	2x8	120s
	4	7	3x35m		2x30m	180s	1x8+1x6	95%	1x6	180s
		8	3x35m		2x30m	180s	1x8+1x6	95%	2x5	180s
	5	9	3x25m		2x20m	150s	1x8+1x6	100%	2x6	180s
		10	3x25m		2x20m	150s	1x8+1x6	100%	2x5	180s
	6	11	3x30m		1x25m	180s	1x8+1x6	90%	2x6	120s
		12	3x30m		1x25m	180s	1x8+1x6	80%	1x8	120s
	7		INTERVAL BETWEEN PHASES Δ							
Phase 2	8	13	3x20m	Maximum	2x15m	120s	1x8+1x8	80%	1x8	120s
		14	3x20m		2x15m	120s	1x8+1x8	80%	2x8	120s
	9	15	3x25m		2x20m	120s	1x8+1x8	85%	1x8	120s
		16	3x25m		2x20m	120s	1x8+1x8	85%	2x6	120s
	10	17	3x30m		2x25m	150s	1x8+1x8	90%	1x6	120s
		18	3x30m		2x25m	150s	1x8+1x8	90%	2x8	120s
	11	19	3x35m		2x30m	180s	1x8+1x6	95%	1x6	180s
		20	3x35m		2x30m	180s	1x8+1x6	95%	2x5	180s
	12	21	3x25m		2x20m	150s	1x8+1x6	100%	2x6	180s
		22	3x25m		2x20m	150s	1x8+1x6	100%	2x5	180s
	13	23	3x30m		1x25m	180s	1x8+1x6	90%	2x6	120s
		24	3x30m		1x25m	180s	1x8+1x6	80%	1x8	120s

10 minutes between training

* Warm up: sprints in which gradually increasing speed until they reach the maximum speed; squats with lighter loads to those in training

† Sprints at full speed; Squat the load is defined based on the percentage of the load that a subject is able to displace the propulsive an average speed of 1 m. s^{-1}

Δ Adjust load

Recovery strategies

Recovery strategies were applied between sprint and squat training due to the preliminary hypothesis of possible association of ergogenic and protective effects of phototherapy in the same session.

Active phototherapy was applied using a MR4 LaserShower 50 4D emitter (Multi Radiance Medical, USA), bilaterally to six sites of the quadriceps (two centrally – rectus femoris and vastus intermedius; two laterally – vastus lateralis; two medially – vastus medially) in direct contact with the skin[14]. The option to apply phototherapy at these sites was made because this muscle group is more directly involved during squats and the most worked during sprints. The dosage (30J per site; 180J per muscle) was based on findings of Antonialli et al.[14]. More parameters are described in Table 2.

The same procedures were applied to the placebo group; however, the emitter was disabled. An independent researcher was responsible for programming the device based on random allocation.

The control group participants performed passive recovery (remained seated) supervised by an independent researcher while the other groups were receiving the recovery strategies. Participants from all groups remained seated to receive the recovery strategies.

Table 2. Phototherapy parameters

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/cm²) – each	0.07
Dose (J) – each	0.07125
Spot size (cm²) – each	0.44
Number of red LEDs	4 red
Wavelength (nm)	640
Frequency (Hz)	2

Average mean optical output (mW) - each	15
Power density (mW/cm²) – each	16.66
Dose (J) – each	3.42
Spot size (cm²) – each	0.9
Number of infrared LEDs	4 infrared
Wavelength (nm)	875
Frequency (Hz)	16
Average mean optical output (mW) - each	17.5
Power density (mW/cm²) – each	19.44
Dose (J) – each	3.99
Spot size (cm²) – each	0.9
Magnetic field (mT)	35
Irradiation time per site (s)	228
Total dose applied (J)	180
Aperture of device (cm²)	20

Primary and secondary outcome measures and assessment points

Maximal voluntary isometric contraction (MVIC) was considered the primary outcome. Secondaries are other functional and clinical outcomes and VEGF. The same assessor was responsible for the same test throughout study.

Performance tests were completed in two sessions separated by 24 hours. The first session consisted of sprint test and squat jump. In the second session, strength and power test and MVIC were performed. An interval of 15 minutes between tests was administered to limit effects of fatigue on subsequent test. This rest is based on time to recovery of each test described in literature[17-21].

Primary outcome

Maximal voluntary isometric contraction (MVIC): Participants were positioned with the dominant leg on the isokinetic dynamometer (Biodex System 4 Pro, New York, USA). Prior to the MVIC, a warm up consisting of 10 repetitions of knee flexion-extension at 180°.s^{-1} throughout the

range of motion was performed. The MVIC was determined by the highest maximal isometric torque over three contractions of five seconds at 60° of knee flexion. The repetitions were separated by a 2-minute rest interval[22].

Secondary outcomes

Strength and power test: Tests were carried out using a guided bar[16]. Participants were required to perform the squat with continuous movement until their thighs reached the horizontal plane, and immediately to perform the opposite movement at maximum speed without losing the contact of the feet with the ground.

For the strength test, the warm up consisted of 10 repetitions at 40-60% of maximum perception. The last acceptable repetition with the highest possible load was considered as 1RM (repetition maximum). A progression from warm up load was performed with increment of 10 kg. 2 minutes rest interval between sets were considered.

For the power test, the warm up consisted of five repetitions with the bar. To determine the load moved at $1 m.s^{-1}$ the tests were carried out gradually with increments of 10 kg for each set and two attempts for each load. An interval of 3 minutes was given between attempts. The best measure with each load was recorded. Information about the distance held eccentrically and concentric speed of each repetition were recorded by a linear velocity transducer (T-Force System Ergotech, Murcia, Spain) and analyzed by its specific software (Isocontrol Dinámico, 3.6, Spain).

Squat jump: Participants performed three squat jumps on a platform without previous movements (Multisprint, Hidrofit, Brazil). A rest interval of 30 seconds was considered between jumps. The best measure of the tests was adopted[23].

Sprint test: Participants performed three maximum sprints exceeding 30 meters with rest intervals of 3 minutes. Performance times at 10, 20 and 30 meters were recorded by phothocells (Multisprint, Hidrofit, Brazil). The average of the two best measures was adopted[16].

Muscle soreness: Values of muscle soreness in the lower limbs were obtained by a visual analog scale (VAS 0-10)[22], with 'zero' corresponding to absence of soreness and '10' to the maximum level supported by the participant[24].

Pain threshold: Pressure algometry was applied at five specific sites of the quadriceps of the dominant leg[25]. Participants were instructed to signalize when the pressure sensation became discomfort. The pressure did not exceed 2.55 Kgf[26].

Perception of exertion: The level of exertion reported by participants was assessed using the Borg Scale (6-20 points), with 'six' corresponding to 'very easy' and '20' to 'exhaustive'[27].

Perception of recovery: The level of perception of recovery was assessed by a 10-point Likert Scale, with 'one' corresponding to 'not recovered' and '10' to 'fully recovered'[28, 29].

Psychological questionnaire: Participants filled out a questionnaire about readiness for exercise, fatigue, vigor and sleepiness. The information about pain was excluded due to application of VAS[30].

Vascular endothelial growth factor (VEGF): 10 ml of blood was collected and the plasma from this sample was stored at -80°C for later analysis. The plasma concentration of VEGF was analyzed using the ELISA method (enzyme-linked immunosorbent assays) following the manufacturer's instructions (R&D Systems, Minneapolis, USA)[31].

Statistical analysis

The statistical analysis was conducted using the software SPSS (version 18; SPSS Inc, Chicago, Illinois, USA).

Initially, sphericity of the data was tested by Mauchly's test. In case of violation of the sphericity assumption, the Greenhouse-Geisser corrections were used. Analysis of variance (ANOVA) with repeated measurements was used to test between-group differences followed by Bonferroni post hoc test. Effect size was calculated using partial eta-squared and interpreted as small (≥0.01), medium (≥0.06), or large (≥0.14)[32]. All statistical analysis assumed a significance level of 5%.

RESULTS

Clinical and psychological outcomes demonstrated no significant differences at any time or group (see Supplementary material 1). Table 3 shows the results for functional outcomes. There was a significant effect for time with large effect size for squat jump, sprint 10, 20 and 30 meters [P<0.05; Squat jump (ES=0.187); Sprint 10 meters (ES=0.210); Sprint 20 meters (ES=0.210); Sprint 30 meters (ES=0.251)]; and medium effect size for MVIC [P<0.05; MVIC (ES=0.091)]. There were no significant group and interaction effect for functional outcomes.

The VEGF analyses also demonstrated a significant effect for time with large effect size [P<0.001; VEGF (ES=0.559)] (Figure 2). No significant effect for group or interaction was found.

Table 3. Functional outcomes (1 RM, load lifted at 1m/s, squat jump, MVIC and sprint) on baseline, six-weeks and 12-weeks

		Baseline	After 6-weeks	After 12-weeks	Summary of effects
1 RM (kg)	Active Group	67.66 ± 15.89	69.00 ± 14.08	71.91 ± 17.36	Time (P=0.331; ES=0.030); Group (P=0.266; ES=0.071); Time x Group (P=0.888; ES=0.014)
	Placebo Group	61.92 ± 14.68	64.28 ± 17.04	63.50 ± 16.41	
	Control Group	71.23 ± 14.12	71.76 ± 13.33	73.00 ± 15.61	
Load lifted at 1m/s	Active Group	29.82 ± 9.99	32.20 ± 13.48	29.07 ± 11.92	Time (P=0.250; ES=0.038); Group (P=0.900; ES=0.006); Time x Group (P=0.454; ES=0.049)
	Placebo Group	27.04 ± 13.67	30.28 ± 13.46	29.27 ± 16.31	
	Control Group	27.53 ± 11.71	30.16 ± 13.66	34.71 ± 14.98	
Squat jump (cm)	Active Group	31.10 ± 3.9	32.04 ± 6.10	32.15 ± 4.80	Time (P=0.001; ES=0.187); Group (P=0.758; ES=0.015); Time x Group (P=0.356; ES=0.058)
	Placebo Group	29.06 ± 6.93	29.50 ± 5.59	32.29 ± 5.89	
	Control Group	30.40 ± 6.09	30.80 ± 4.77	32.71 ± 5.13	
MVIC (peak torque/body weight)	Active Group	339.04 ± 60.71	318.02 ± 56.33	333.02 ± 65.52	Time (P=0.032; ES=0.091); Group (P=0.932; ES=0.004); Time x Group (P=0.582; ES=0.038)
	Placebo Group	315.15 ± 70.63	316.75 ± 65.84	334.25 ± 76.47	
	Control Group	330.86 ± 84.25	312.53 ± 53.86	346.06 ± 81.37	
Sprint 10 m (s)	Active Group	1.779 ± 0.12	1.734 ± 0.11	1.728 ± 0.10	Time (P=0.001; ES=0.210); Group (P=0.841; ES=0.010); Time x Group (P=0.774; ES=0.019)
	Placebo Group	1.823 ± 0.14	1.749 ± 0.12	1.746 ± 0.12	
	Control Group	1.829 ± 0.19	1.732 ± 0.10	1.732 ± 0.13	
Sprint 20 m (s)	Active Group	3.172 ± 0.22	3.116 ± 0.25	3.120 ± 0.19	Time (P=0.001; ES=0.210); Group (P=0.841; ES=0.010); Time x Group (P=0.774; ES=0.019)
	Placebo Group	3.249 ± 0.24	3.166 ± 0.22	3.176 ± 0.23	
	Control Group	3.227 ± 0.26	3.128 ± 0.18	3.127 ± 0.21	
Sprint 30 m (s)	Active Group	4.548 ± 0.38	4.450 ± 0.30	4.412 ± 0.27	Time (P<0.001; ES=0.251); Group (P=0.752; ES=0.016); Time x Group (P=0.938; ES=0.008)
	Placebo Group	4.632 ± 0.40	4.500 ± 0.35	4.515 ± 0.35	
	Control Group	4.544 ± 0.36	4.429 ± 0.28	4.416 ± 0.33	

Values are presented as mean ± SD

1 RM: one repetition maximum; MVIC: maximal voluntary isometric contraction.

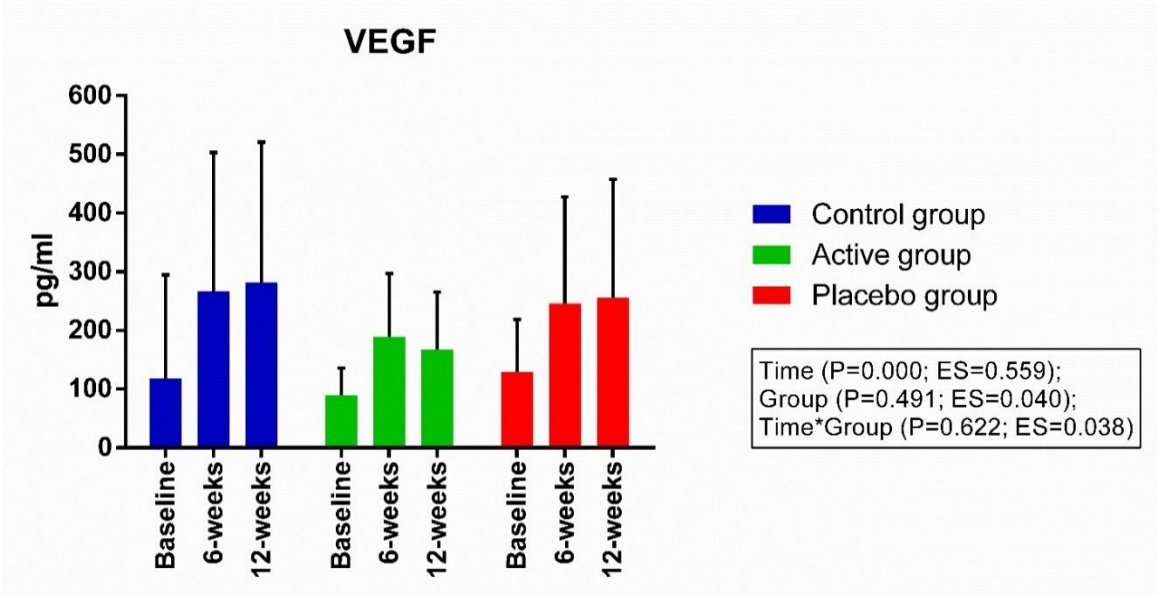

Figure 2. VEGF analysis (pg/ml; mean and SD).

DISCUSSION

This study aimed to assess the effects of phototherapy during a combined training on clinical and functional outcomes and vascular endothelial growth factor. The main findings of this study were that combined training improve some functional outcomes and increase of vascular endothelial growth factor concentration, however, the phototherapy acted in similar way compared to control and placebo group.

Combined training has been proposed with the aim of optimizing performance adaptations, suggesting higher gains than single-element training programs. Favorable results on functional outcomes are identified in studies combining strength and sprint training, strength and plyometric training, strength and flexibility training and strength and power training. The 12 weeks of combined training promoted gains in squat jump, maximal voluntary isometric contraction and sprint for all groups with a clinically relevant effect. These gains represent the best results of functional performance for this study, as the squat jump was found to be a good predictor of athletic performance, demonstrating an important relationship with sprint

performance[33, 34]. The sprint test (10, 20 and 30 meters) results are in accordance with Marques et al.[16] which provide evidence that the duplication of the training with load readjustment was successful.

Another fact that may have contributed to the improvement of functional performance is the similar movement pattern applied during the training and the tests as suggested by Villareal et al.[20]. The authors highlight the findings regarding the speed of execution of the squat, a variable closely related to the specificity of the proposed training[20]. The similar movement patterns reproduced in the present study refer to the tests performed using the guided bar and the sprint test. This is partially confirmed by the present study, which found improvements for the sprint test. However, no evidence was found regarding the load lifted at 1m/s and 1 RM tests. These findings may be justified by their different measurement methods. Although biomechanical factors such as range of motion, movement pattern and execution technique were respected, the functional performance tests considered the amount of load moved (kg) and not the specificity and the individuality of the training, which was, in this case, the speed of execution of the movement (m/s).

As previously shown, no additional phototherapy effects on performance were identified. Systematic reviews have shown positive effects for the application of this technique[3, 6], however, it should be noted that most of the included clinical trials evaluate the acute effects of phototherapy, considering only a single stress. Studies involving training and phototherapy present unclear results, and usually only demonstrate superiority of the technique in relation to the control group (without training).

No change was identified in clinical and psychological outcomes. Considering these results, it is observed that the proposed 12-week training program was able to promote positive adaptations in participants without causing major clinical changes. This information also seems noteworthy when considering the results of the phototherapy group for functional performance.

Our research laboratory in a recent meta-analysis[4] have identified that the effects of phototherapy on the concentration of creatine kinase depend on the type of stress applied, showing greater effects after localized exercises. Machado et al.[4] justify that this finding may be due to the magnitude of the damage caused by the exercise, presenting better results for the application of phototherapy when exercise causes greater damages (higher concentrations of creatine kinase).

Effects related to the training alone were also observed in the VEGF variable. The combined training of 12 weeks was able to increase the concentration of VEGF for all groups, presenting clinical relevance. Studies have shown that this cytokine regulates the formation of new blood vessels. Skriver et al.[31] point out that changes in the concentration of this marker in humans after exercise are not yet fully elucidated, with divergent results. In a systematic review involving the effects of exercise on VEGF, Vital et al.[35] discusses that training frequencies less than three times a week may not be enough to promote increased VEGF production in the elderly. However, as observed in the present study, regarding a healthy young population, the frequency of twice a week seems to be enough. The VEGF is considered a systemic angiogenic cytokine and its production can occur in peripheral and central areas[36]. Its release mechanisms are mainly related to the hemodynamic stimulus and can be influenced by the exercise intensity[37, 38]. However, the findings suggest that phototherapy was not able to promote additional effects. In contrast, Vieira et al.[39] identified a 31% increase in VEGF concentration in the group that performed strength training associated with phototherapy. However, the authors reinforce that these effects come from preliminary results, which does not allow to identify definitive conclusions[39]. Thus, from the findings of this study, it is suggested that phototherapy applied bilaterally twice a week for six weeks does not cause any systemic or cumulative effects, identified by both VEGF concentration and functional outcomes.

The study presents high methodological quality with a noteworthy design. The stratified randomization process allowed a better distribution of responders and non-responders into groups. This type of control has been considered important and innovative when considering inter and intra-variability responses. The application protocol performed after control of the primary adaptation process resulting from the exercise may be able to reflect the real effect of the technique, which can be considered a strength of the study. We also highlight that this is the first study to investigate the effect of phototherapy during a combined training. Therefore, the phototherapy was applied on strategic sites according to greater muscle demand and no superior effects to the control or placebo condition were found. Thus, we suggest that future investigations consider the application of phototherapy to other muscle groups used during this type of training, in addition to those applied in this study (i.e. hamstring, gastrocnemius), as well as the use of an equipment that involves all the musculature. Such options should be investigated to verify whether the absence of systemic results and muscle function are confirmed even with the use of other methods of application.

CONCLUSION

Twelve weeks of combined training of sprints and squat improve some functional outcomes and increase vascular endothelial growth factor concentration; however, no evidence was found that phototherapy applied during training promote any additional effects.

The findings contribute to the practice based on evidence to the use of phototherapy in training programs, suggesting that the positive effects commonly observed in acute exercises cannot be extrapolated to training programs, and that different training protocols require different irradiation protocols/moments.

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CONFLICTS OF INTEREST

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Supplementary Table 1. Clinical outcomes on specific training* before session training

		Phase 1 - ST	Phase 2 - ST	Summary of effects
Muscle soreness	Active group	0.16 ± 0.38	0.08 ± 0.28	P=0.5863
	Placebo group	0.21 ± 0.80	0.14 ± 0.53	P=0.7929
	Control group	0.15 ± 0.37	0.08 ± 0.28	P=0.5863
Perception of recovery	Active group	9.50 ± 0.79	9.66 ± 0.65	P=0.4832
	Placebo group	9.28 ± 1.43	8.92 ± 1.54	P=0.5205
	Control group	8.38 ± 1.93	8.53 ± 2.43	P=0.7938
Perception of exertion	Active group	7.66 ± 1.37	8.50 ± 1.83	P=0.2335
	Placebo group	8.71 ± 3.29	8.78 ± 2.51	P=0.9250
	Control group	8.15 ± 2.47	9.46 ± 2.57	P=0.2453

Values are presented as mean ± SD

** ST: Specific training (data collected on session 10 and 21)*

Supplementary Table 2. Clinical outcomes on specific training* after session training

		Phase 1 - ST	Phase 2 - ST	Summary of effects
Muscle soreness	Active group	0.25 ± 0.62	0.16 ± 0.57	P=0.7545
	Placebo group	0.85 ± 1.23	0.57 ± 0.85	P=0.2631
	Control group	0.38 ± 0.65	0.30 ± 0.48	P=0.7533
Perception of recovery	Active group	6.91 ± 2.15	7.16 ± 1.74	P=0.6671
	Placebo group	6.57 ± 2.02	5.64 ± 2.46	P=0.2596
	Control group	6.53 ± 2.57	5.84 ± 2.23	P=0.3308
Perception of exertion	Active group	11.83 ± 2.58	11.66 ± 1.61	P=0.7723
	Placebo group	11.85 ± 2.98	10.64 ± 2.73	P=0.2515
	Control group	11.84 ± 3.41	12.30 ± 1.70	P=0.6539

Values are presented as mean ± SD

** ST: Specific training (data collected on session 10 and 21*

Supplementary Table 3. Clinical and psychological outcomes on test day

		Baseline	After six-weeks	After 12-weeks	Summary of effects
Muscle soreness	Active Group	0.83 ± 1.33	0.25 ± 0.86	0.41 ± 1.44	Time (P=0.087; ES=0.068);
	Placebo Group	0.42 ± 0.93	0.07 ± 0.26	0.14 ± 0.36	Group (P=0.392; ES=0.051);
	Control Group	0.53 ± 0.87	0.15 ± 0.55	0.38 ± 0.65	Time*Group (P=0.967; ES=0.006)
Pain threshold*	Active group	2.16 ± 0.58	2.38 ± 0.38	2.38 ± 0.38	Time (P=0.106; ES=0.071);
	Placebo group	2.26 ± 0.67	2.33 ± 0.59	2.33 ± 0.59	Group (P=0.995; ES=0.000);
	Control group	2.14 ± 0.70	2.41 ± 0.29	2.41 ± 0.2	Time*Group (P=0.744; ES=0.016)
Physically ready*	Active group	7.08 ± 3.14	8.54 ± 1.56	8.64 ± 1.77	Time (P=0.114; ES=0.063);
	Placebo group	7.63 ± 2.14	7.85 ± 1.88	8.08 ± 1.36	Group (P=0.912; ES=0.005);
	Control group	7.79 ± 1.82	8.40 ± 1.65	7.46 ± 1.79	Time*Group (P=0.242; ES=0.074)
Mentally ready*	Active group	6.96 ± 3.72	9.07 ± 2.17	8.17 ± 2.62	Time (P=0.265; ES=0.036);
	Placebo group	8.31 ± 1.85	7.71 ± 2.45	7.93 ± 1.87	Group (P=0.987; ES=0.001);
	Control group	7.72 ± 2.30	8.38 ± 1.81	8.15 ± 1.81	Time*Group (P=0.188; ES=0.081)
Fatigue*	Active group	1.40 ± 1.97	0.75 ± 0.96	1.52 ± 1.90	Time (P=0.193; ES=0.045);
	Placebo group	2.82 ± 2.58	2.27 ± 2.03	1.57 ± 1.76	Group (P=0.180; ES=0.091);
	Control group	2.59 ± 2.49	1.96 ± 1.65	2.66 ± 2.51	Time*Group (P=0.262; ES=0.070)
Vigorous*	Active group	7.60 ± 2.59	8.51 ± 1.42	8.20 ± 1.90	Time (P=0.438 ES=0.021);
	Placebo group	7.43 ± 1.98	7.08 ± 1.93	7.83 ± 1.76	Group (P=0.316; ES=0.062);
	Control group	7.16 ± 2.11	7.74 ± 1.71	6.66 ± 1.07	Time*Group (P=0.122; ES=0.100)
Sleepy*	Active group	3.47 ± 3.67	2.04 ± 2.49	2.70 ± 2.63	Time (P=0.436; ES=0.021);
	Placebo group	2.75 ± 3.04	1.92 ± 1.73	2.82 ± 3.23	Group (P=0.937; ES=0.004);
	Control group	2.22 ± 2.50	2.60 ± 2.42	2.72 ± 2.54	Time*Group (P=0.690; ES=0.027)

Values are presented as mean ± SD

** Domains of psychological questionnaire*

Conclusão

A partir da pesquisa realizada pode-se observar que a fototerapia apresenta uma relação de dose-resposta, demonstrando que a magnitude dos efeitos da técnica pode ser influenciada por diferentes fatores.

Por meio da revisão sistemática com meta-análise verifica-se que a fototerapia apresenta efeitos favoráveis no controle da atividade da creatina quinase. Destaca-se que os efeitos podem ser influenciados pelo tipo de estresse, apresentando efeito significante e de moderada qualidade apenas para estudos que aplicaram a fototerapia no processo de recuperação após exercício localizado.

Ainda com relação a magnitude dos efeitos, observa-se por meio do ensaio clínico randomizado que a fototerapia não promove efeitos adicionais ao treinamento combinado de sprints e agachamentos em desfechos funcionais, clínicos, psicológicos e no fator de crescimento endotelial vascular. Ainda que a aplicação da técnica tenha sido ajustada ao momento e tipo de estresse, destaca-se que as doses utilizadas para efeitos agudos podem não apresentar a mesma efetividade para efeitos crônicos, como para o treinamento.

Dessa forma, sugere-se que pesquisas científicas sigam a mesma direção, investigando os melhores métodos de aplicação da fototerapia para cada momento e tipo de estresse específico.

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