UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE – UFCSPA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE

Melina Hauck

Efeito do Laser Terapêutico de Baixa Potência na Função Endotelial de Indivíduos Saudáveis e Revisão Sistemática com Meta-análise Sobre Efeitos do Laser Terapêutico de Baixa Potência e de Diodos Emissores de Luz na Dor e Cicatrização Tecidual após Cirurgia de Revascularização do Miocárdio.

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Orientador: Prof. Dr. Rodrigo Della Méa Plentz

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PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE

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De acordo com o estabelecido previamente pela Comissão Coordenadora do Curso de Pós-Graduação em Ciências da Saúde da Universidade Federal de Ciências da Saúde de Porto Alegre, realizou-se aos vinte e sete dias do mês de abril de 2020, às nove horas, na modalidade remota, via Hangout, a defesa da Tese de Doutorado, da aluna **Melina Hauck,** orientada pelo professor **Rodrigo Della Méa Plentz** no Programa de Pós-graduação em Ciências da Saúde da UFCSPA. O trabalho defendido intitula-se de **"Efeitos da fotobiomodulação com laser terapêutico de baixa potência e diodos emissores de luz na função endotelial de indivíduos saudáveis e na cicatrização tecidual e dor após cirurgia de revascularização do miocárdio".** A Banca Examinadora foi composta pelos professores Marcelo Faria Silva (UFCSPA), Bruno Manfredini Baroni (ULBRA), Cinara Stein (HMV) e Ernesto Cesar Pinto Leal Junior (UNINOVE)**.** Após a abertura da sessão a candidata dispôs de 40 minutos para expor seu trabalho. Ao término da apresentação, foi realizada a arguição da candidata pelos membros da banca examinadora. Cada examinador teve até 20 minutos para sua arguição, tendo a candidata igual tempo para resposta. Ao término da Sessão foi anunciada a aprovação da candidata que, após a homologação da Tese de Doutorado, receberá o título de Doutora em Ciências da Saúde: Fisiologia e Patogênese. Nada mais havendo a tratar, foi encerrada a sessão e lavrada a presente ata, que será assinada pelo orientador da aluna e pela Coordenação do Programa.

Porto Alegre, 27 de abril de 2020.

Prof. Pedro Roosevelt Torres Romão
Coordenador do PPGCS

Prof. Rodrigo Della Méa Plentz
Orientador

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Aos meus pais Nelson e Miriam que sempre, nos tempos bons e ruins, optaram por uma educação de qualidade, incentivaram o meu conhecimento, apoiaram minhas decisões e incentivaram minhas ambições.

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Para todos vocês.

"(…) Science and everyday life cannot and should not be separated. Science, for me, gives a partial explanation for life (…)."

(Rosalind Franklin)

RESUMO

Introdução: A fotobiomodulação com Laser terapêutico de baixa potência (LLLT) ou Diodos emissores de luz (LEDT) potencializa os processos energéticos metabólicos, modifica o ambiente intracelular e contribui para a função endotelial pela secreção de óxido nítrico. No manejo de doenças cardiovasculares, pode ser necessário a realização de cirurgia de revascularização do miocárdio que pode desencadear dor pós-operatória e prejuízos na cicatrização tecidual. **Objetivos:** Os objetivos deste estudo foram 1) Avaliar os efeitos da fotobiomodulação com LLLT na função endotelial arterial de indivíduos saudáveis por meio da condução de um ensaio clínico e 2) Revisar sistematicamente os efeitos da fotobiomodulação com LLLT e LEDT após cirurgia de revascularização do miocárdio. **Métodos:** Fizeram parte do ensaio clínico randomizado 22 voluntários (25,45 ± 4,85 anos) que foram submetidos à avaliação da função endotelial pela técnica da dilatação mediada pelo fluxo (n= 22). A função endotelial foi mensurada antes e imediatamente após cada aplicação de fotobiomodulação com LLLT (810 nm, 1000 mW, 0,28 cm^2, 30 J por spot, 107 J/cm^2, 30 s). A revisão sistemática foi conduzida nas bases de dados MEDLINE (PubMed), EMBASE, SCiELO, PEDro e Cochrane Central. De acordo com os critérios de inclusão e exclusão, dois revisores independentes realizaram a seleção dos artigos, extração dos dados e avaliação da qualidade da evidência e metodológica. Os desfechos principais foram dor, cicatrização tecidual e deiscência. **Resultados:** O resultado do ensaio clínico mostrou que a fotobiomodulação com LLLT não aumentou a função endotelial de indivíduos saudáveis. Na condução da revisão sistemática, foram encontrados 1.513 artigos, mas somente oito ensaios clínicos randomizados foram incluídos totalizando dados de 379 pacientes. Os resultados da meta-análise mostram redução da dor após a fotobiomodulação com LLLT e LEDT no 4º 6º e 8º dia de pós-operatório. A cicatrização tecidual e a deiscência melhoraram no 8º dia de pós-operatório. **Conclusões:** A fotobiomodulação com LLLT com dose de 30 J e 60 J não aumentou a função endotelial e doses de energia maiores podem ser necessárias para causar vasodilatação. A fotobiomodulação com LLLT e LEDT parece melhorar a dor pós-operatória, cicatrização tecidual e deiscência após cirurgia de revascularização do miocárdio.

Palavras-chave: Terapia com luz de baixa intensidade; Endotélio; Endotélio vascular; Vasodilatação; Revascularização miocárdica.

ABSTRACT

Introduction: The photobiomodulation with Low-lever laser therapy (LLLT) or Light-emitting diodes therapy (LEDT) enhances the metabolic energy processes, modifies the intracellular environment, and contributes to the endothelial function through secretion of nitric oxide. Cardiovascular diseases may lead to myocardial revascularization surgery which can trigger postoperative pain and damage to tissue healing. **Objectives:** The aims of this study were 1) to evaluate the effects of photobiomodulation with LLLT on the arterial endothelial function of healthy individuals by conducting a clinical trial and 2) to systematically review the effects of photobiomodulation with LLLT and LEDT after myocardial revascularization surgery. **Methods:** Twenty-two volunteers (25.45 ± 4.85 years) composed the randomized clinical trial and underwent endothelial function assessment using the flow-mediated dilation technique (n = 22). Endothelial function was measured before and immediately after each application of photobiomodulation with LLLT (810 nm, 1000 mW, 0.28 cm^2, 30 J per spot, 107 J/cm^2, 30 s). The systematic review was conducted in the MEDLINE (PubMed), EMBASE, SCiELO, PEDro, and Cochrane Central databases. According to the inclusion and exclusion criteria, two independent reviewers performed the selection of articles, data extraction and evaluation of the quality of the evidence and methodological. The main outcomes were pain, tissue healing and dehiscence. **Results:** The result of our clinical trial showed photobiomodulation with LLLT did not improve endothelial function in healthy individuals. In the systematic review, 1,513 articles were found, but only eitgh randomized clinical trials were included totaling data from 379 patients. The results of the meta-analysis show pain reduction after photobiomodulation with LLLT and LEDT on the 4th, 6th and 8th postoperative days. Tissue healing and dehiscence improved on the 8th postoperative day. **Conclusions:** Photobiomodulation with 30 J and 60 J LLLT did not improve endothelial function and higher energy doses may be needed to cause vasodilation. Photobiomodulation with LLLT and LEDT may improve postoperative pain, tissue healing and dehiscence after myocardial revascularization surgery.

Keywords: Low-Level Light Therapy; Phototherapy; Vascular endothelium; Coronary artery bypass; Myocardial revascularization.

SUMÁRIO

1 REVISÃO DA LITERATURA

1.1 FOTOBIOLOGIA

A óptica é um campo da física que estuda a propagação, produção e mecanismos de interação da luz, como por exemplo a fonte de luz laser. Os elétrons que decaem em seus níveis energéticos após serem estimulados produzem um feixe de luz em que todas as pequenas porções chamadas fótons se comportam da mesma maneira (1). A fotobiologia é uma ampla disciplina que inclui os efeitos benéficos e prejudiciais da luz por meio do estudo dos efeitos químicos e biológicos da radiação não ionizante (2). Historicamente, o estudo da fotobiologia é extremamente importante, e dentro da pesquisa da fotobiologia já foram recebidos 12 prêmios Nobel. A radiação não ionizante é capaz de produzir estados excitatórios devido à absorção de fótons pelos fotorreceptores. Essas moléculas receptoras podem reagir com outras moléculas e sofrem alterações fotoquímicas e fotofísicas (2).

Os fótons são os componentes da luz que se propaga na forma de ondas e estes possuem diferentes energias de acordo com seu comprimento de onda. Quando menor o comprimento de onda maior será a energia (2). As radiações não ionizantes são descritas em três grupos: 1 Radiação Ultravioleta (UV) com um curto espectro de onda não visível ao homem e subdivisão em três regiões (UV-C com comprimento de onda de 100-280 nm, UV-B com 280-320 nm e UV-A com 320-400 nm); 2 Radiação Visível com comprimentos de onda de 400-760 nm; e 3 Radiação Infravermelha com comprimento de onda geralmente a partir de 760 nm e não visível ao homem (2).

O feixe de luz possui algumas principais características, como: monocromaticidade, intensidade, direcionalidade e (1). A monocromaticidade refere-se à semelhança da luz carregada pelo fóton estimulante e pelo fóton emitido, pois é composta por apenas um comprimento de onda e se apresenta como um espectro de luz em linha (1). A segunda característica refere-se à intensidade do feixe de luz que pode ser extremamente grande e atingir potências elevadas (1). A direcionalidade refere-se ao caráter direcional do feixe de luz que é composto de ondas caminhando na mesma direção de maneira estreita e com o mínimo de dispersão. A quarta característica refere-se à coerência, que diz que as ondas sucessivas da radiação estão em fase e temporalmente coerente se todo o trem de onda possui a mesma direção e o mesmo comprimento de onda (1). Como essa propriedade define o alinhamento de todas as ondas de luz, a coerência também está relacionada a modificação de um campo elétrico de uma onda de luz durante um período de observação (3). Essa modificação pode

ocorrer por meio da amplitude de fase da onda, e de acordo com variáveis do campo elétrico, pode ser mensurada em uma localização espacial ou temporal (3).

Mudanças na fase do campo de luz estão relacionadas à coerência espacial que são as alterações de um campo de luz em duas localizações espaciais temporalmente correlacionadas. Já as alterações que ocorrem dentro da fase do campo óptico em um determinado local espacial referem-se à coerência temporal (3). A propagação de uma luz coerente através de um tecido biológico pode gerar a perda da sua coerência espacial devido a alterações temporais no meio do tecido (3). Essa propriedade também é responsável pela geração de padrões de interferência chamados “speckle”, pontos granulados formados após interação com algum meio, gerados localmente ao longo do plano perpendicular à direção da propagação (3). Supõe-se que esses padrões podem interferir na irradiância gerada pelo feixe de luz, pois a média da irradiância permanece a mesma, mas a irradiância local pode não ser uniforme (maior dentro e menor ao redor do ponto “speckle”) (3).

Atualmente, sabe-se que as luzes não-coerentes, como a Terapia por Diodos Emissores de Luz (LEDT) são capazes de gerar os mesmos efeitos do Laser Terapêutico de Baixa Potência (LLLT), pois a fotobiomodulação é um fenômeno fotobiológico e a coerência não é essencialmente necessária (4,5). Não há diferenças essenciais nas fontes de luz equivalentes à luz laser que emitem o mesmo comprimento de onda e irradiância (4). A luz laser é uma fonte de luz formada a partir do fenômeno físico de emissão estimulada com criação de feixe de luz monocromática, coerente e de baixa divergência, enquanto os LEDs são fontes de luz baseados no fenômeno da eletroluminescência dos materiais semicondutores (5). Além da coerência, a largura de banda da luz laser é mais estreita (fração de nanômetros) enquanto a luz LEDT possui largura de banda tipicamente de 1-2 nm (5).

A luz deve ser absorvida por qualquer átomo ou molécula antes que as reações fotoquímicas ocorram, de acordo com a primeira lei da fotobiologia, e a sua intensidade ao invés do campo possui o principal papel nesse mecanismo (3). Dessa maneira, a fototerapia envolve a transformação da luz energética em química, cinética ou calórica e nas aplicações terapêuticas a luz será absorvida por um cromóforo específico do tecido biológico (6). A fotobiomodulação com a utilização do LLLT ou LEDTs deve estimular ou inibir a função celular, e dessa maneira, causar efeitos clínicos benéficos (6).

Há diversos parâmetros de irradiação dentro da fotobiomodulação, como comprimento de onda, irradiância, estrutura do pulso, coerência e polarização (6). O comprimento de onda é medido em nanômetros (nm) e caracteriza a propriedade tipo onda da energia eletromagnética (6). A irradiância, também chamada de densidade de potência é calculada

como potência (W) dividido pela área (cm^2), e é medida em W/cm^2 (6). A coerência é responsável pela produção de "speackles" que poderiam influenciar o efeito da fotobiomodulação com os tecidos celulares. Além disso, há um tempo de irradiação que definirá a dose. A energia (J) ou densidade energética (J/cm^2) é geralmente apresentado como o principalmente descritor da dose da fotobiomodulação (6). Mas, torna-se importante ressaltar que a energia possui dois componentes (tempo e potência), porém, não há necessariamente uma reciprocidade entre eles. Ou seja, pode-se dobrar a potência empregada e reduzir o tempo pela metade para entregar uma mesma energia, todavia, provavelmente haverá a geração de diferentes respostas biológica teciduais (6).

1.2 FOTOBIOMODULAÇÃO

A fotobiomodulação (PBM) é uma abordagem terapêutica na qual células e tecidos são expostos ao LLLT e/ou LEDT, com comprimentos de onda visível (400-700 nm) ou próximo ao infravermelho (700-1000 nm) que desencadeia respostas biológicas benéficas (7). O feixe de luz com baixos níveis de energia de fótons aplicado nos tecidos é absorvido por moléculas orgânicas específicas chamados cromóforos (7–9). Após a absorção de energia pelo cromóforo, ocorre a transdução do sinal intracelular e uma foto-resposta celular final (fotobiomodulação e/ou ativação de células-alvo) (8–10).

Os efeitos da PBM estão diretamente ligados à curva dose-resposta bifásica devido aos efeitos inibitórios ou estimulatórios que o mesmo comprimento de onda pode gerar com o uso de densidades energéticas maiores (11). A curva dose-resposta bifásica – também conhecida como lei de Arndt-Schulz – mostra que uma dose muito baixa de energia não causa nenhum efeito celular enquanto qualquer outra dose maior gera um efeito positivo até que um efeito platô seja atingido (11). Porém, se a dose energética é elevada além desse ponto em que os efeitos benéficos começam a diminuir gradativamente nenhum efeito será alcançado e os efeitos inibitórios podem causar danos teciduais (11).

Os fótons de luz devem ser absorvidos nos tecidos por alguma molécula, e o principal fotorreceptor é a enzima terminal citocromo c-oxidase (CCO) da cadeia de transporte de elétrons mitocondrial, e a estimulação dessa enzima ativa múltiplas vias de transdução de sinais (7,8,12,13). A absorção da luz resulta no aumento da síntese de ATP (combustível celular e do metabolismo) e de ROS, as quais também participam do metabolismo celular (8,9,13–15). A PBM influencia a respiração e a sinalização retrógrada mitocondrial (reguladora de diversas atividades celulares em estados patológicos e normais), impactando na regulação do fluxo de canais de íons Ca++, produção de NO via CCO e aumento dos níveis

de ATP (12). A alteração do estado redox por causa do aumento de ROS também propaga sinais mecânicos que podem modificar a matriz celular e as células vizinhas. O aumento intracelular de moléculas de Ca++ também pode induzir ao rearranjo do citoesqueleto (8,9,13–15). Esses estímulos em componentes da mitocôndria e na sinalização mecânica da membrana possivelmente geram um fluxo de elétrons, que modulam a sinalização redox, e consequentemente potencializa (ativa) os processos energéticos metabólicos (16–18). Há aumento do metabolismo aeróbico e da síntese aeróbica de ATP e aprimoramento do consumo de oxigênio (7).

A PBM é capaz de aumentar os níveis do fator de crescimento endotelial vascular (VEGF), aumentando a proliferação celular, a angiogênese e a secreção de NO (15,17,19,20). Sugere-se também, que as células-tronco mesenquimais, precursoras de diversos tipos de células, atuem por meio do estímulo à secreção do VEGF (17,19,20). Ou seja, há uma manutenção da homeostase vascular e modulação da inflamação tecidual, as quais mantém o estado redox e normalizam a função endotelial (17,19,20). A fotobiomodulação não destrói as pontes de hidrogênio nos tecidos e não gera nenhum outro tipo de efeito além dos efeitos fotoquímicos da estimulação celular por meio do aumento do metabolismo celular (10).

Os fatores de crescimento produzidos pelas células endoteliais possuem um papel central na cicatrização e no processo da angiogênese, mas ainda há pouca investigação do papel endotelial no mecanismo e da interação da fotobiomodulação nos tecidos (17). Supõe-se que a PBM com laser pode aumentar o número de capilares e os níveis de fatores angiogênicos, reduzir a fase aguda da inflamação e antecipar a fase de reparação (15). A PBM de células endoteliais umbilicais humanas com laser HeNe (632,5 nm, 5 mW) durante 10, 30, 60 e 90 min (0,09, 0,26, 0,52 e 0,77 J/cm^2) causou um aumento da proliferação das células endoteliais entre o quarto e quinto dia, aumento da secreção de NO, maior expressão de genes e proteínas da eNOS com a dose de 0,26 J/cm^2 e duração de efeito de 72 h (15). Além disso, o laser causou a mesma migração celular que o VEGF, assim como o mesmo padrão de expressão do citoesqueleto, distribuição da vinculina (responsável pela adesão celular e migração), e aumento do comprimento dos tubos capilares (15).

A angiogênese (formação de novos vasos sanguíneos a partir dos vasos pré-existentes) e a cicatrização de feridas é um importante campo de aplicação da PBM. A angiogênese é mediada por diversos fatores de crescimento, principalmente da família do VEGF (21). A partir da secreção desses fatores, as células endoteliais se proliferam e migram para formar novas redes de capilares em locais de dano tecidual (21). Esses novos vasos são super

permeáveis e permitem a liberação de macromoléculas para a degradação da matriz circundante, o que facilita a formação de mais estruturas de redes endoteliais (21). Esse processo de vasculogênese e angiogênese pode ser desencadeado pela estimulação mecânica ou luminosa (21). A luz pode desencadear a liberação do NO de maneira dependente do comprimento da onda (21).

O NO exerce importantes funções na parede vascular além do efeito vasodilatador, como a supressão da resposta inflamatória, inibição da apoptose, regulação da migração celular e angiogênese. Por isso, o estímulo da produção de NO pelas células endoteliais pode gerar um potencial benefício (15). O VEGF é um dos reguladores-chave da angiogênese, e induz seus efeitos biológicos pela ligação com receptores de alta afinidade localizados nas células endoteliais dos vasos sanguíneos (17). Góralczyk e cols. (2015) (17) também irradiaram células endoteliais umbilicais com um laser de semicondutor GaAlAs (635 nm) com 2, 4 e 8 J/cm^2 (duas vezes ao dia em dois dias alternados), e encontraram um aumento do número de células de acordo com a dose de energia (máximo em 4 J/cm^2) e diminuição da concentração solúvel de receptores VEGF. Os receptores em forma solúvel são inibidores fisiológicos da proliferação de células endoteliais. Ou seja, simultaneamente com o aumento da proliferação de células endoteliais, a PBM ocasionou um aumento da ligação de moléculas VEGF com seus receptores, que afetam a proliferação endotelial (17).

Considerando os efeitos da disfunção endotelial no prejuízo da cicatrização de feridas, Amaroli and cols. (2018) (7) isolaram células endoteliais da veia umbilical e aplicaram laser diodo de 808 nm (1 W de potência, 1 W/cm^2 de densidade de potência, dose única de 60 J, tempo de irradiação de 60 s. Os resultados mostraram aumento da lipoperoxidação (dano oxidativo mensurado pelo TBARS: substâncias reativas ao ácido tiobarbitúrico) 1 h após a exposição e a liberação de NO não foi modificada após 30 min. O ensaio de cicatrização de feridas mostrou taxas elevadas no fechamento cicatricial após a PBM. Os resultados mostraram a habilidade da luz laser em estimular a proliferação de células endoteliais e o metabolismo oxidativo, o que resultou em melhora da habilidade de cicatrização de feridas (7). O aumento da produção de ROS foi associado a troca do metabolismo anaeróbico para aeróbico devido à síntese de ATP, necessária para a estimulação da proliferação celular (7).

Em humanos, investigou-se a ação da fotobiomodulação com laser de 632,8 nm com dose de 14,4 J sobre o estresse oxidativo e disfunção mitocondrial em sujeitos com lesão crônica da medula espinhal resultante de trauma (16). Nesses pacientes, houve uma redução do estresse oxidativo e da disfunção mitocondrial, aumento do DNA mitocondrial, da síntese de ATP, da Capacidade Antioxidante Total (CAT) e da Lipoproteína de Alta Densidade

(HDL). Também se observou uma redução do malondialdeído (MDA) e da lipotroteína de baixa intensidade (LDL), o que demonstrou potencial benefício no estado redox desses pacientes (16). Em indivíduos saudáveis, avaliou-se o efeito de três aplicações de PBM de 20 J ao dia (total de 60 J) com o laser transdérmico de 880 nm (20). A fotobiomodulação aumentou os níveis de glutationa, do efeito antioxidante e do potencial angiogênico, além de diminuir a concentração de angiostatina, evidenciando uma mudança na função endotelial vascular (20).

Recente revisão sistemática (22) sumarizou os principais achados experimentais da PBM com o laser terapêutico, e mostrou que a síntese de ATP e a angiogênese podem ser mecanismos cardioprotetores da PBM devido à melhora do suprimento energético, da liberação de fatores de crescimento e, consequentemente, da síntese de NO pelo endotélio (22). Além disso, a PBM parece reduzir as citocinas pró-inflamatórias, aumentar as citocinas anti-inflamatórias e modular o estresse oxidativo (22). Diversos estudos clínicos mostraram melhora da percepção dolorosa e cicatrização tecidual após a utilização da PBM com LLLT (23–26) e LEDT (27). Alguns estudos experimentais demonstraram que ocorre modulação de mediadores químicos inflamatórios, como a bradicinina, assim com a expressão e síntese de beta-endorfinas que poderiam diminuir o limiar excitatório de receptores nervosos da dor (28–30). Além disso, as fibras nervosas de condução do estímulo doloroso são superficiais para a penetração do comprimento de onda da luz da PBM (28).

1.3 DOENÇAS CARDIOVASCULARES

As doenças cardiovasculares (DCVs) são a maior causa de mortalidade mundial (31,32), e possuem vários fatores de risco conhecidos, como a idade avançada, hipertensão arterial, diabetes mellitus e resistência à insulina, dislipidemia, tabagismo, obesidade, doenças inflamatórias e predisposição genética (32,33). Atualmente, cursamos por uma transição epidemiológica, na qual as causas de mortes devido às deficiências nutricionais e doenças infecciosas foram substituídas pelas doenças degenerativas, como as DCVs (34).

Na América Latina e Caribe, essas doenças são responsáveis por um terço total da mortalidade, sendo que aproximadamente um milhão de mortes foram secundárias a elas (34). Além desse total, 42,5% das mortes regionais foram devido à doença arterial coronariana, 28,8% secundárias ao acidente vascular cerebral, e 11,8% atribuídas à hipertensão arterial (34). Dados da Sociedade Brasileira de Cardiologia estimaram em aproximadamente 384 mil o número de óbitos decorrentes de doenças cardiovasculares (35). Além disso, estimou-se que

em uma década (2004-2014), as doenças isquêmicas do coração foram responsáveis por 53,2 mil óbitos a cada 100 mil habitantes no Brasil (35).

De acordo com a Organização Pan-Americana da Saúde (36), as DCVs são um grupo de doenças do coração e dos vasos sanguíneos que inclui a Doença Arterial Coronariana, doença cerebrovascular, Doença Arterial Obstrutiva Periférica, doença cardíaca reumática, cardiopatias congênitas e trombose venosa profunda e embolia pulmonar. A Organização Mundial da Saúde, em 2013, acordou com 194 Estados-Membros, sobre os mecanismos globais para a redução da carga evitável das doenças não transmissíveis, incluindo o "Global action plan for the prevention and control of NCDs 2013-2020". Esse plano incluiu nove metas globais voluntárias, das quais duas metas (pressão arterial e terapia medicamentosa e aconselhamento) atuam diretamente na prevenção e controle de doenças cardiovasculares (36).

A maioria das DCVs é resultado de complicações da aterosclerose, uma patologia multifatorial que causa aumento de fatores inflamatórios, os quais influenciam na formação e ruptura de placas ateroscleróticas (32,33), disfunção endotelial e eventual trombose coronariana (32). Todavia, devido ao aperfeiçoamento da prevenção primária e tratamento farmacológico dos fatores de risco cardiovasculares houve um menor risco para eventos cardiovasculares como também redução do número de casos fatais (31). Dessa maneira, houve um drástico declínio na mortalidade cardiovascular em idades específicas, a idade do primeiro evento cardiovascular tornou-se mais avançada, e os pacientes passaram a ter mais anos de vida após o início da doença cardiovascular (31).

O "Framingham Heart Study" contribuiu para a mudança de foco na atenção à doença cardiovascular, a qual teve passou a ter como estratégia principal a identificação dos pacientes mais suscetíveis a futuros eventos cardiovasculares (37). Essa mudança de estratégia auxiliou na elucidação dos fatores de risco para as doenças cardiovasculares, como a hipertensão, dislipidemia e diabetes mellitus, além disso, o termo fatores de risco foi popularizado no léxico médico (37). Os pesquisadores do estudo Framingham propuseram modelos logísticos multivariáveis com sete fatores de risco: idade, colesterol total, peso, anormalidades no eletrocardiograma, hemoglobina, número de cigarros fumados e pressão arterial sistólica (37). Alguns estudos também propuseram alguns perfis ou scores para risco cardiovascular, todavia o melhor perfil para risco cardiovascular é o "Framingham Risk Score for coronary heart disease", apresentado em 1998 por Wilson e cols., e o risco estimado de 10 anos fornece uma maneira conveniente para classificar os indivíduos como baixo, intermediário ou alto risco de futura doença arterial coronariana (37).

A mudança na atenção às doenças cardiovasculares teve como consequência o aumento da expectativa de vida de pacientes bem como o surgimento de comorbidades, as quais podem levar a um pobre status funcional, baixa qualidade de vida, e aumentar a mortalidade (31). Comorbidade pode ser definida como a presença de uma ou mais doenças crônicas concomitantes a outra determinada doença (31). Um atual estudo holandês demonstrou por meio da análise de uma grande base de dados do país que as principais comorbidades não cardiovasculares em pacientes com doença cardiovascular são baixa visão, problemas de pescoço/coluna, osteoartrite, doença pulmonar obstrutiva crônica, câncer, diabetes e asma (31). A doença arterial coronariana foi a principal comorbidade cardiovascular associada às doenças cardiovasculares (31). A prevenção secundária das DCVs em pacientes com a doença estabelecida inclui o uso de ácido acetilsalicílico, betabloqueadores, inibidores da enzima conversora da angiotensina e estatinas (36). Além disso, quando associado à cessação do tabagismo, essas intervenções secundárias podem prevenir cerca de 75% dos eventos vasculares recorrentes (36). Contudo, intervenções cirúrgicas de alto custo pode ser necessária para o tratamento das DCVs, como a cirurgia de revascularização do miocárdio, angioplastia percutânea, reparação e substituição de válvula cardíaca, transplante de coração e implantação de coração artificial (36).

1.4 CIRURGIA DE REVASCULARIZAÇÃO DO MIOCÁRDIO

A Sociedade Brasileira de Cardiologia (2015) recomenda que a revascularização cirúrgica deva se limitar a pacientes com anatomia favorável, contraindicados ou com falha terapêutica intervencionista, e que estejam nas primeiras horas após o início da alteração isquêmica. A Cirurgia de Revascularização do Miocárdio (CRM) é indicada entre três a sete dias após o episódio do infarto (eletiva) (38). Todavia, esse tempo deve ser determinado individualmente (39). A cirurgia vai ser considerada para grupo restrito de pacientes com anatomia de artérias não favoráveis ao tratamento percutâneo, como a lesão de tronco de coronária esquerda, doença triarterial, doença biarterial com estenose proximal do ramo interventricular anterior; na presença de isquemia recorrente e no comprometimento importante da função ventricular (38). Uma CRM de urgência deve ser considerada se a artéria relacionada ao infarto é importante, a anatomia coronariana é indesejável para a realização de um procedimento percutâneo, se uma grande área do miocárdio está em risco, ou no desenvolvimento de complicações como isquemia recorrente, choque cardiogênico e alterações mecânicas do infarto (38,39).

A esternotomia mediana, parcial ou total, ainda é o acesso cirúrgico mais comumente utilizado na cirurgia cardíaca (40). A cirurgia cardíaca com esternotomia é particularmente agressiva e induz alta carga de estresse com repercussões locais e sistêmicas que alteram a homeostase do organismo, e disparam o gatilho para o desenvolvimento de complicações pós-operatórias (41). Embora raras, complicações na cicatrização esternal podem causar sinais clínicos típicos, como o "click" e/ou evidência de instabilidade no exame físico, tosse ou respiração que podem evoluir para deiscência e mediastinite (40). Imagens de tomografia computadorizada evidenciaram uma redução na vascularização esternal após a cirurgia de revascularização do miocárdio, o que poderia prejudicar a cicatrização tecidual e facilitar o aumento das taxas de infecção (42).

Esse tipo de acesso cirúrgico também pode resultar em profunda e intensa dor pós-operatória que também é exacerbada por movimentos respiratórios, tosse ou durante a fisioterapia respiratória (43). É importante ressaltar que a dor pode causar repercussões importantes nas funções respiratória, cardiovascular e cognitiva dos pacientes, além de aumentar o tempo de internação hospitalar e o risco de morbidade (43). Porém, as complicações irão depender do centro cirúrgico, procedimento, condições gerais do paciente e comorbidades, acometimento de órgãos, aumento do tempo de internação, redução da qualidade de vida e podem aumentar as taxas de mortalidade (41). Dessa maneira, torna-se essencial a atenção primária ao paciente com DCVs por meio de intervenções que reduzam o risco para um evento cardiovascular e preserve sua independência e capacidade funcional.

1.5 PROCESSO ATEROSCLERÓTICO VASCULAR

A aterosclerose, principal causa para o desenvolvimento da doença arterial coronariana, é uma doença inflamatória crônica dos vasos arteriais caracterizada pelo desenvolvimento de complexas placas ateroscleróticas que causam enrijecimento e estreitamento do lúmen arterial, e pela ruptura de placas vulneráveis ou de alto risco (44,45). Desenvolve-se, preferencialmente, em pontos de inclinação e ramificação vascular os quais perturbam o fluxo sanguíneo pela complexidade geométrica vascular (44). A formação da placa aterosclerótica é iniciada e sustentada pela combinação da disfunção endotelial e exposição crônica aos fatores de risco cardiovasculares, como dislipidemia, hipertensão, tabagismo, sexo masculino e diabetes (45). A disfunção da sensibilidade ao fluxo sanguíneo pelo endotélio é o primeiro estágio no processo aterosclerótico (44), e o desenvolvimento da placa é uma resposta mal adaptativa à inflamação não resolvida (45).

A disfunção endotelial permite que a lipoproteína de alta-densidade (LDL) e sua apolipoproteína-B (ApoB) saiam do compartimento sanguíneo e acumulem-se no espaço subendotelial (44). Essa formação lipoproteica estimula a resposta inflamatória devido às modificações pela oxidação, clivagem enzimática e agregação plaquetária (44). Além disso, a oxidação das lipoproteínas (oxLDL) na parede arterial induz a expressão de proteínas quimiotáticas, como a molécula de adesão celular vascular-1 (VCAM-1), selectina-E e selectina-P (45) que geram o recrutamento de monócitos e linfócitos para o endotélio (46).

Com a instalação do processo inflamatório, inicia-se o recrutamento de monócitos que migram através da monocamada endotelial para dentro da camada íntima. Há proliferação e diferenciação de monócitos em macrófagos, que absorvem as lipoproteínas, formando as células de espuma (44). Além disso, as células de espuma também contribuem para o desenvolvimento da placa aterosclerótica, pois apresentam antígenos para monócitos e células-T e secretam mediadores químicos (45). Dessa maneira, as lesões ateroscleróticas permanecem em expansão devido à perpetuação do processo inflamatório, migração e proliferação de células e contínuo acúmulo de lipídeos extracelulares (44).

As células musculares lisas que migram para o espaço subendotelial começam a apresentar elevada taxa de proliferação celular mediada por plaquetas derivadas de fatores de crescimento (45). Também passam a secretar proteínas da matriz extracelular que alteram a produção da elastina para colágeno (44) contribuindo para a formação de uma tampa fibrótica no final do crescimento da placa aterosclerótica (45). Essa cicatriz fibrosa sobreposta à lesão fornece uma barreira protetora entre as plaquetas sanguíneas e o conteúdo plaquetário pró-trombótico da placa aterosclerótica, e influencia a redução da elasticidade e enrijecimento da parede do vaso (44).

O crescimento da placa aterosclerótica gera estreitamento do lúmen do vaso em órgãos específicos, mas após a desestabilização e ruptura de uma placa, ocorre exposição de todo o material pró-trombótico ao fluxo sanguíneo e oclusão local pelo recrutamento e adesão de plaquetas circulantes (44). Além da obstrução ao fluxo sanguíneo, grandes placas que podem estar escondidas na parede do vaso de regiões com remodelação podem gerar trombos devido à perturbação na superfície luminal, assim como fragmentos da placa rompida podem circular (êmbolos) e bloquear outros vasos distantes (44). Ademais, a circulação de monócitos, neutrófilos e plaquetas é um forte preditor e componente essencial para o desenvolvimento das doenças e futuros eventos cardiovasculares (46), assim como o processo de ruptura de uma placa aterosclerótica é a causa mais comum de acidente vascular cerebral e infarto agudo do miocárdio (44).

O enrijecimento arterial e a aterosclerose compartilham de semelhantes mecanismos patofisiológicos e podem ser dois processos sinérgicos que potencializam a ação um do outro no desenvolvimento das alterações vasculares das doenças cardiovasculares (47). A rigidez arterial é caracterizada por deficiência na elastina e colágeno da camada média do vaso sanguíneo, enquanto a aterosclerose envolve a camada íntima e o acúmulo de lipídeos, células inflamatórias, migração de células vasculares e desenvolvimento das células de espuma (47).

O processo de desenvolvimento da aterosclerose também está ligado às citocinas inflamatórias e proteínas de fase aguda (32). O processo inflamatório é um mecanismo de defesa essencial, mas a elevação persistente de marcadores inflamatórios causa um estado crônico de inflamação subclínica (46). Os principais fatores inflamatórios são: interleucina-6, proteína-C-reativa (PCr), fibrinogênio, fator de necrose tumoral alfa (TNF-alfa) e acetilação de glicoproteínas. A interleucina-6 (IL-6) é uma citocina com atividades biológicas de defesa, e a sua produção pelo sistema imune (monócitos e macrófagos), componentes cardiovasculares (células endoteliais), células musculares lisas vasculares e miócitos isquêmicos é precedida pelo efeito de estimuladores, como infecções, proteínas pró-inflamatórias, angiotensina-II, estresse oxidativo e exercício físico (32).

A ativação da sua via de sinalização está relacionada ao aumento do risco para doenças cardiovasculares e isquemia miocárdica pela sua relação com o número e extensão de placas ateroscleróticas (32). A PCr é a primeira linha de defesa contra patógenos, produzida pelos hepatócitos em resposta às citocinas (32), e é considerada um mediador ativo da patogênese da doença vascular como também preditor da disfunção endotelial (46). A PCr aumenta a proliferação e migração das células musculares lisas e o remodelamento vascular via regulação positiva do receptor da angiotensina-1 e geração de ROS (46). Essa citocina também facilita a liberação do TNF-alfa, interleucina 1-beta e IL-6 pelos macrófagos e células espumosas da neointima do vaso sanguíneo (46).

1.6 DISFUNÇÃO VASCULAR

A disfunção endotelial é o evento chave para a iniciação do processo aterosclerótico devido ao desenvolvimento da rigidez funcional das artérias ocasionado pela redução da síntese e liberação de NO pelo endotélio (47). A redução do cofator tetra-hidrobiopterina e/ou aumento dos níveis do dimetilarginina assimétrica (ADMA, inibidor endógeno competitivo para a síntese de óxido nítrico) pode gerar essa redução da síntese de óxido nítrico (33). O ADMA tem sua secreção aumentada pela exposição às partículas de LDL-oxidado e é fator de risco para eventos coronarianos, por isso há associação entre a disfunção endotelial e a

aterosclerose (33). Além disso, os pacientes com aterosclerose e disfunção endotelial também apresentam baixa resposta endotelial quando expostos a fontes exógenas de óxido nítrico, como a nitroglicerina, o que indica uma disfunção concomitante do músculo vascular liso e independente do endotélio (33).

A disfunção das células endoteliais começa em condições de perturbação do fluxo sanguíneo, como as forças mecânicas sobre o endotélio (redução crônica do "pulsatile stretch" e recirculação de fluxo sanguíneo com grande gradiente de "shear stress"), e associada à rigidez arterial estrutural pode causar redução da síntese de NO e do conteúdo e atividade da enzima NOS (47), redistribuição e alteração da comunicação das junções intercelulares e perda da função de barreira (44). Ou seja, o fluxo sanguíneo arterial prejudicado induz disfunção endotelial e predispõe às tendências aterogênicas, enquanto o fluxo sanguíneo arterial uniforme aprimora a integridade endotelial e protege contra a aterosclerose (44).

O dano mediado pelas ROS – estresse oxidativo – também possui um papel importante no desenvolvimento das doenças cardiovasculares. O estresse oxidativo ocorre devido ao desequilíbrio entre a produção oxidante e a defesa antioxidante, e o seu aumento está associado com muitos fatores de risco para o desenvolvimento da aterosclerose, como diabetes, dislipidemia, insuficiência renal, idade avançada, hipertensão e tabagismo (33). A biodisponibilidade do NO é diretamente influenciada pelas ROS, como o radical Ânion Superóxido (O2•-), o H2O2 e o radical Hidroxil (•OH-), os quais são produtos intermediários do metabolismo celular oxidativo (cadeia respiratória mitocondrial), e atuam como sinalizadores moleculares (48). As ROS podem reduzir a biodisponibilidade do NO de maneira direta – destruição – ou de maneira indireta pela formação de lipídios oxidados que destroem o NO ou reduzem a atividade da NOS (33). Além disso, o aumento da produção de ROS mitocondriais bem como a disfunção progressiva da cadeia respiratória mitocondrial (cadeia transportadora de elétrons) presente na doença cardiovascular estão associados à aterosclerose e cardiomiopatia em humanos e modelos animais de estresse oxidativo (33).

1.7 ENDOTÉLIO VASCULAR

As células endoteliais vasculares constituem uma monocamada celular que reveste todo o sistema circulatório e são essenciais para a biologia vascular (33,44). São responsáveis pela interface entre compartimentos sanguíneos e tecido vascular e regulam uma variedade de funções, incluindo o tônus vascular do músculo liso, reações de defesa, angiogênese e homeostase de fluidos teciduais (33,44,49). Por muitos anos, o endotélio foi considerado uma barreira passiva aos elementos contidos no sangue, todavia, atualmente, sabe-se que além

de suas funções de isolamento e transporte, o endotélio possui importante função biológica como o crescimento e tônus vascular e ação anticoagulante (33).

Todas essas funções são realizadas por meio da síntese e secreção de diversas substâncias que agem a nível local e à distância, tornando o endotélio um órgão endócrino, parácrino e autócrino (33). As principais funções endoteliais são sobre a:

1. Permeabilidade vascular: o endotélio regula o transporte de substância através da sua camada (33). A manutenção de uma barreira semipermeável pelo endotélio é importante no controle da passagem de macromoléculas e fluidos entre o sangue e o espaço intersticial (33), e mantém afastadas moléculas e células da parede do vaso vascular (44). Além disso, a comunicação entre as células endoteliais é importante para a troca de íons intercitoplasmáticos, metabólitos e outras pequenas moléculas que participam de muitas funções vasoprotetoras (44). A adesão das interconexões juncionais entre cada célula endotelial faz parte de uma resposta endotelial ao fluxo sanguíneo saudável (44). A permeabilidade pode ser alterada pelo efeito do aumento dos níveis de angiotensina, interleucina-1-beta ou óxido nítrico (NO) (33). Sabe-se que a perda dessa função resulta em inflamação tecidual (44).

2. Tônus vasomotor: as células endoteliais mediam o equilíbrio do tônus dos vasos sanguíneos por meio da síntese e secreção de substâncias vasodilatadoras, como o NO, prostaciclina e fator hiperpolarizante derivado do endotélio (EDHF); e de substâncias vasoconstritoras como angiotensina, endotelina-1, ânion superóxido e tromboxano-A2 (33). A síntese e liberação dessas substâncias vasoativas é uma resposta endotelial ao fluxo sanguíneo. O principal vasodilatador dessa função é o NO que possui efeitos anti-ateroscleróticos no sistema cardiovascular, como a inibição da agregação plaquetária e da proliferação excessiva das células musculares lisas, a prevenção da adesão leucocitária e a capacidade de dilatação vascular (44,49). O EDHF participa de respostas que incluem o aumento da concentração do cálcio intracelular e hiperpolarização dependente do endotélio das células musculares lisas. Essa hiperpolarização evoca o acoplamento elétrico através das junções mioendoteliais e acúmulo de íons potássio no espaço intercelular (44).

3. Homeostase vascular: a regulação do ambiente vascular acontece devido às ações antitrombóticas, por meio da síntese e secreção de substâncias antiaderentes (NO e prostaciclina), anticoagulantes (antitrombina-II, proteína-S, inibitor do fator tissular) e fibrinolíticos (fator tissular plasminogênico) (33,49).

4. Interação com células sanguíneas (leucócitos): o endotélio é capaz de expressar substâncias quimiotáticas e moléculas celulares adesivas (33). As principais substâncias

quimiotáticas produzidas são o fator ativador plaquetário, interleucina-8 e a proteína quimiotática para monócitos (MCP-1), enquanto as principais moléculas vasculares de adesão são as moléculas vasculares de adesão-1 (VCAM-1) e a molécula de adesão intercelular-1 (ICAM-1) (33).

A principal substância responsável pela dilatação vascular dependente do endotélio é o NO (50–52), e com a sua produção as células endoteliais contribuem para que o fluxo sanguíneo mantenha-se de maneira laminar, preservam a fluidez da membrana plasmática, e inibem a proliferação e a migração celular (plaquetas e leucócitos). Esses processos controlam a resposta inflamatória, e impedem a gênese e o desenvolvimento da aterosclerose (50–54). A síntese do NO ocorre por meio da oxidação de um dos dois nitrogênios guanidino da L-arginina, para a geração do NG-hidroxi-L-arginina (NHA), por uma reação catalisada pela enzima óxido nítrico sintase (NOS) com utilização de fosfato de dinucleotídeo de adenina e nicotinamida (NADPH) e oxigênio (O2) (48,52,55). Após esse processo inicial, ocorre a conversão da NHA em NO e L-citrulina com a utilização dos cofatores Flavina Adenina Dinucleotídeo (FAD), Flavina Mononucleotídeo (FMN) e a Tetraidrobiopterina (BH4) (48,52,55). Após sua geração, o NO difunde-se do endotélio para o músculo liso vascular, e estimula a enzima Guanilato Ciclase Solúvel (GCs) a produzir o cGMP (Monofosfato Cíclico de Guanosina). Esse evento causa a redução da concentração de íons cálcio (Ca++) presentes na célula muscular lisa vascular, e consequentemente, o relaxamento vascular dependente do endotélio (48,52,55). A isoforma constitutiva (c-NOS), dependente de Ca++ e de calmodulina, está envolvida na sinalização celular e compreende a NOS endotelial (e-NOS, tipo III) presente nas células endoteliais vasculares e plaquetas (48,52,55).

Somente nas situações em que há uma redução da biodisponibilidade do NO é que se torna mais evidente a participação da Prostaciclina, produto da via das ciclooxigenases (COX), na vasodilatação vascular (53). O EDHF também contribui para a vasodilatação do endotélio na falta do NO, principalmente na microcirculação aonde o shear stress não é elevado, e a sua produção é independente da eNOS e da COX (53). Entre os fatores contráteis, destaca-se o papel da Prostaglandina H2 (PGH2), Tromboxano A2, Angiotensina II (Ang II), Endotelina-1 (ET-1), e das Espécies Reativas de Oxigênio (ROS). A ET-1 promove vasoconstrição e proliferação de células musculares lisas vasculares (53).

Dessa maneira, percebe-se que o endotélio vascular responde por meio da modificação da vasofunção, homeostase, angiogênese e crescimento vascular sendo capaz de perceber alterações de forças hemodinâmicas e na bioquímica sanguínea (44). A disfunção das células endoteliais ocorre em situações patológicas, nas quais as células endoteliais são modificadas,

há prejuízo na secreção das substâncias vasodilatadoras e aumento das moléculas pró-inflamatórias (33). Essas alterações endoteliais induzem a condições pró-coagulantes, antifibrinolíticas, vasoconstritoras e pró-inflamatórias (33), por isso o endotélio é reconhecido como principal contribuidor para a saúde vascular (44) e precursor reversível da aterosclerose (46).

1.8 AVALIAÇÃO DA FUNÇÃO ENDOTELIAL

A dilatação mediada pelo fluxo (FMD) é um estímulo fisiológico responsável pela regulação do tônus e da homeostase vascular da circulação vascular (49). O método de avaliação da função endotelial em humanos mais comumente utilizado é a técnica da FMD na artéria braquial (49), uma mensuração não invasiva que quantifica a função vasodilatadora arterial em resposta ao aumento do fluxo sanguíneo (56). A elevação do fluxo sanguíneo causa a estimulação da liberação de NO pelo endotélio. Dessa maneira, ocorre a vasodilatação que pode ser visualizada e quantificada como um índice da função vasomotora (50). Os "guidelines" para a mensuração da FMD da artéria braquial foram previamente sumarizados (57–59).

Essa avaliação é feita com um aparelho de ultrassom e "probe" linear de alta resolução (> 10 MHz) que é utilizado para a aquisição de imagens longitudinais da artéria braquial em repouso (57–59). Um esfigmomanômetro posicionado ao redor do braço é inflado durante cinco minutos com 50 mmHg acima da pressão arterial sistólica previamente mensurada (57–59). Imediatamente após a desinflação do esfigmomanômetro, são realizadas novas imagens longitudinais da artéria braquial. A artéria dilata em resposta ao fluxo sanguíneo elevado que gera "shear stress" na parede vascular e causa a liberação de NO. Após um período de recuperação de cinco a 10 minutos, pode-se realizar a avaliação da dilatação independente do endotélio com a utilização de nitroglicerina sublingual (57–59).

A função endotelial pode ser rapidamente atenuada em resposta ao estresse oxidativo agudo, como por exemplo devido ao tabagismo, consumo de alto teor de gorduras, e esforço físico intenso (49). Dessa maneira, como a resposta endotelial é dinâmica, intervenções que estão associadas com a diminuição do risco vascular irão melhorar a vasodilatação em algumas semanas, o que permite a mensuração do impacto de novas intervenções em um tempo hábil (49). A liberação de NO em grandes artérias pela FMD é crítica para a prevenção da aterosclerose, ou seja, a redução dessa dilatação está relacionada ao prognóstico de doenças cardiovasculares em humanos (49).

O uso da fotobiomodulação pode gerar efeitos positivos em diversas situações clínicas, como a melhora do suprimento tecidual de oxigênio, do fluxo sanguíneo, da circulação periférica e do metabolismo tecidual. Ao nível endotelial, evidenciou-se aumento do número de capilares e da proliferação de células endoteliais, bem como aumento dos níveis de glutationa, do efeito antioxidante e do potencial angiogênico, sugerindo possíveis alterações na função endotelial vascular. Esses efeitos são demonstrados principalmente em estudos experimentais, todavia, não está esclarecido quais são os efeitos da fotobiomodulação em humanos sobre a responsividade vascular dependente do endotélio, bem como a definição dos melhores parâmetros para a prática baseada em evidências. Para que se obtenha os efeitos necessários, além da escolha adequada do comprimento de onda, também é necessária uma dose de energia suficientemente estimulante. Considerando tais evidências experimentais sobre a ação da fotobiomodulação na manutenção do endotélio saudável, a aplicação vascular da fotobiomodulação pode ser uma ferramenta para melhorar a função endotelial. Dessa maneira, a mensuração da ação vascular possibilitaria uma ampliação do campo de atuação terapêutica da fotobiomodulação por meio da execução com foco em problemas clínicos. Além disso, a investigação da ação da fotobiomodulação em populações específicas ainda necessita de dados científicos que orientem o seu uso assim como investigações que ajudem a esclarecer os potenciais mecanismos envolvidos nesses efeitos.

Como hipóteses deste trabalho, hipotetizamos que: 1) a fotobiomodulação melhora a função endotelial de indivíduos saudáveis; e 2) a fotobiomodulação melhora a dor e cicatrização tecidual de pacientes com doenças cardiovasculares após cirurgia de revascularização do miocárdio. Dessa maneira, investigamos potenciais mecanismos de ação da fotobiomodulação com LLLT na vasodilatação dependente do endotélio de indivíduos saudáveis por meio da condução de um ensaio clínico randomizado e crossover; e revisamos sistematicamente a literatura para analisar os efeitos da fotobiomodulação com LLLT e/ou LEDT na dor, cicatrização tecidual e deiscência em pacientes no pós-operatório de cirurgia de revascularização do miocárdio.

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3 OBJETIVOS

3.1 GERAL

Estudar os efeitos da fotobiomodulação com Laser terapêutico de baixa potência na função endotelial de indivíduos saudáveis, e os efeitos do Laser terapêutico de baixa potência e/ou Terapia por diodos emissores de luz após cirurgia de revascularização do miocárdio.

3.2 ESPECÍFICOS

a. Avaliar os efeitos da fotobiomodulação com Laser terapêutico de baixa potência na função endotelial arterial de indivíduos saudáveis;
b. Revisar sistematicamente os efeitos da fotobiomodulação com Laser terapêutico de baixa potência e/ou Terapia por diodos emissores de luz na dor, cicatrização tecidual e deiscência após cirurgia de revascularização do miocárdio.
c. Analisar quantitativamente (meta-análise) os dados da revisão sistemática.

4 ARTIGOS CIENTÍFICOS

4.1 ARTIGO 1 (EXAME GERAL DE QUALIFICAÇÃO). Submissão para o periódico “European Heart Journal”.

Photobiomodulation therapy on longitudinal sternotomy and saphenectomy after Coronary Artery Bypass Grafting: a systematic review with meta-analysis of randomized clinical trials

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ABSTRACT

Aims. This study aimed to systematically review the effects of photobiomodulation therapy with LLLT and/or LEDT versus placebo and/or control group in sternotomy and saphenectomy after CABG.

Methods and Results. Systematic review and meta-analysis registered in PROSPERO CRD42021155179. Searches were conducted in MEDLINE, EMBASE, SCiELO, PEDro, and Cochrane CENTRAL up to October 2021**.** Randomized clinical trials with patients in CABG postoperative that compared LLLT and/or LED versus placebo and/or control group were included. The main outcome was pain, incision healing, and dehiscence. Two reviewers screened titles and abstracts, selected eligible studies, and extracted data. We assessed the risk of bias using RoB 2.0, and the quality of evidence was assessed using GRADE. We meta-analysis using statistical software R. From 1,513 registers identified, eight randomized clinical trials with 379 patients (60.33± 12.64 years old) were included. Photobiomodulation improves pain from 4^{th} (MD=-0.37; 95%CI=-0.57, -0.17; I^2=21%; n=292), 6^{th} (MD=-1.51; 95%CI=-1.70, -1.33; I^2=22%; n=292) and 8^{th} (MD=-1.59; 95%CI=-1.83, -1.35; I^2=38%; n=292) postoperative day. Also, improves incision healing (MD=-0.90; 95%CI=-1.16, -0.65; I^2=63%; n=225) and dehiscence (MD=-0.51; 95%CI=-0.61, -0.42; I^2=32%; n=305) at 8^{th} postoperative day. The studies present a low risk of bias and moderate quality of evidence.

Conclusion. Photobiomodulation therapy with LLLT and/or LEDT is superior to placebo/control to improve postoperative pain, incision healing, and dehiscence after coronary artery bypass grafting. However, LLLT has the same effect with low energy. Large clinical trials are needed to verify the immediate postoperative effect.

Keywords: Low-Level Light Therapy; Phototherapy; Coronary artery bypass; Myocardial revascularization.

ONE-SENTENCE SUMMARY

This study systematically evaluated the effects of low-level laser therapy and light-emitting diodes in postoperative coronary artery bypass grafting. Meta-analysis of data shown since forty until eighth postoperative day pain may be reduced and incision healing and dehiscence may be improved in eighth postoperative by photobiomodulation with those therapeutic resources.

INTRODUCTION

Cardiovascular diseases (CVDs) are the primary cause of death globally and reached 18.6 million in 2019, besides, the health measures like the sum of years of life lost prematurely and years lived with disability increased over that period (1). People with CVD or who are at high cardiovascular risk need management and costly surgical operations, including coronary artery bypass grafting (CABG) (2). CABG is the gold standard treatment for revascularization of complex multivessel coronary artery disease with medial sternotomy technique, and most of the time, saphenous vein graft (3). However, these incisions may develop healing complications and wound dehiscence, and it's a risk factor for mediastinitis after CABG sternotomy (4).

The vascularity of the sternum may be reduced after CABG which might impair sternal wound healing (5). Besides, inappropriate sternal healing may be asymptomatic due to the high dosage of analgesics in the postoperative period, and this would lead to an increased infection rate (4,5). CABG sternotomy frequently results, also, in postoperative pain which is exacerbated by breathing movements, coughing, and respiratory physiotherapy (6). The great saphenous vein is commonly harvested for CABG and may also be a source of additional postoperative complications like pain, poor wound healing, and infections. Saphenectomy healing is particularly important as the calf muscle pump is an important hemodynamic

component of the venous system as it forces the return of venous blood to the heart from the lower limbs, thereby preventing blood pooling and venous reflux (7). These postoperative complications increase the length of the patient's hospital stay, postoperative costs, and the risk of morbidity (5,6), but also delay the patient's recovery and the return to the job or social activities (8).

Low-level laser therapy (LLLT) and Light-emitting diodes therapy (LEDT) are photobiomodulation therapy (PBM) and have biostimulatory effects that change the intracellular environment. These changes influence the release of growth factors (9), inflammation reduction (10–12), and raise the cell metabolism which can lead to faster healing because of pain relief and better wound healing (9,13–15). CABG guidelines recommend multimodal pain management (16) and PBM may be an effective prophylactic therapy in the early postoperative period for patients who have undergone CABG to prevent sternal and saphenous wound healing complications and, consequently, decrease pain perception.

Clinical studies demonstrated PBM can improve sternotomy pain perception and healing with LLLT (8,10,12,15) and LEDT (11) including in hyperglycemic and normoglycemic patients (14,17). However, Helmy and cols. (8) found no difference between PBM with LLLT and trunk exercises. Saphenectomy healing was also improved by PBM with LEDT (13). Besides positive effects, there is no synthesis of current literature to guide the clinical practice. The aim of this study was systematically reviewing the effects of PBM with LLLT and LEDT versus placebo and/or control group in longitudinal sternotomy and saphenectomy after CABG.

METHODS

Systematic review protocol and Registration

This systematic review was performed following Cochrane Collaboration (18) and The PRISMA Statement of systematic reviews and meta-analysis were used to guide the execution process and reporting (19). The study protocol was registered and can be accessed in PROSPERO CRD42021155179.

Literature search strategy

Search strategy used individually or combined terms “Low-Level Light Therapy”, “Phototherapy”, “Lasers, Semiconductor”, “Coronary Artery Bypass”, “Myocardial Revascularization”, “Sternotomy”, “Saphenous Vein” and a string of words previously proposed which have a high sensibility in search for randomized controlled trials (20). The words related to outcomes of interest were not included to enhance the broadness of the search.

Electronic databases used for literature searches were MEDLINE (accessed by PubMed), EMBASE, SCiELO, Physiotherapy Evidence Database (PEDro), and Cochrane Central Register of Trials (Cochrane CENTRAL) including records available up to October 2021. References list of selected articles was used as an additional source of search to identify potential clinical trials. Search terms were adjusted to fit the requirements of each electronic database, and the complete search strategy used for all databases is available as **supplementary data**. No restriction for date of publication was adopted, and languages were restricted for English, Portuguese, and Spanish at the full-text phase.

Eligibility criteria

Randomized clinical trials (RCT) evaluating the effects of LLLT and/or LEDT versus placebo and/or control group in postoperative CABG were selected for full-text assessment. Studies population with emergency or urgent CABG, morbid obesity, previous thoracic

surgery, respiratory or renal insufficiency after surgery, clinical and/or post-surgery complications, changes in routine analgesic protocols, and morphine sulfate use in postoperative were not included. Full-text articles should have protocol intervention characteristics defined, such as wavelength, power output, spot size, power density, number of spots, energy density, and treatment time per point. The placebo and/or control group should not be exposed to active LLLT and/or LEDT, but conventional physiotherapy or standard care could be ordered as part of usual care to the intervention group. The exclusion criteria were one application of LLLT and/or LEDT, protocol intervention time longer than the hospital postoperative period, and experimental and pilot studies.

Study selection, data extraction, and analysis

Titles and abstracts of papers identified at database searches were screened separately and independently by two reviewers (MH, IS) to determine eligibility following the mentioned eligibility criteria. Full-text versions of selected RCT (potentially eligible and uncertain) were independently retrieved for a complete review to determine eligibility by these two reviewers. Disagreements were discussed between reviewers or arbitrated by a third review (MM) when a consensus was not reached. The reviewers were not blinded to authors and institutions of full-text papers. The data was separately and independently extracted by two reviewers (MH, IS), and disagreements were resolved by discussion or third review (MM). The methodological design, number of subjects, intervention protocol, comparison groups, and outcomes were extracted from selected full-text papers. The data underlying this article are available in the article and its online supplementary material.

The primary outcome extracted was pain assessed by the Visual Analogue Scale (VAS) and incision healing assesses by Score of healing from the photographic analysis. Secondary outcomes extracted were dehiscence assessed by the Score of closure. The number

of participants in each group, mean (or median), standard deviations (SD) (or standard errors or interquartile range), and p-value was extracted for each outcome. Studies with standard errors and p-value were transformed in SD according to chapter 7 of Cochrane Handbook (18). Method of Wan and cols. (21) was used to estimate SD from the interquartile range. Outcome measurements could not be estimated with the methods described above were not included at meta-analysis and was performed a descriptive synthesis. Data presented in graphs were extracted using the WebPlotDigitizer (version 4.5) software.

Data analysis

The estimated pooled-effect was obtained by comparison of the end of intervention mean and standard deviation (SD) of each group included for metanalysis. Calculation of a change score requires measurement of the outcome twice and in practice may be less efficient for outcomes that are unstable or difficult to measure precisely, where the measurement error may be larger than true between-person baseline variability (18). Besides, data from only one comparator group were included in the meta-analysis. Studies with multiple intervention groups (experimental versus placebo versus control) were combined to create a single pair-wise comparison (experimental versus placebo and control) following Cochrane Handbook (18).

Results are presented as mean difference (MD) with 95% confidential interval (CI). Analyses were performed using the inverse of variance method with a random-effects model. The p-value <0.05 was considered statistically significant. Statistical heterogeneity between studies was assessed by the chi-squared test (Chi^2) and inconsistency test (I^2). I^2 interpretation follows the thresholds described in Cochrane's Handbook (0% to 40%: heterogeneity might not be important; 30% to 60%: may represent moderate heterogeneity; 50% to 90%: may represent substantial heterogeneity; 75% to 100%: considerable heterogeneity. I^2 values in

intersections of these thresholds were judged following the strength of evidence for heterogeneity (p-value from the Chi^2 test) (18). Subgroup analysis was performed considering LLLT and LEDT applications and Q-test for subgroup differences was performed. Meta-analysis was made by statistical software R (version 3.6.1), using the statistical package 'meta'(22).

Risk of bias and quality of evidence assessment

The risk of bias was measured using the Excel tool implementation of Revised Cochrane risk-of-bias tool for randomized trials (RoB 2)(23). ROB 2 included studies independently by review authors (MH, IS). RoB 2 is structured into a fixed set of five domains of bias, including randomization process, deviations from intended interventions, missing outcome data, measurement of the outcome, and selection of the reported results. Overall bias was generated, and judgment obtained can be 'Low' or 'High' risk of bias or can express 'Some concerns'. The Grading of Recommendations Assessment, Development and Evaluation (GRADE) was used to the quality of evidence assessment by the GRADEpro GDT app (24). GRADE is a common, sensible, and transparent approach to grading the quality (or certainty) of evidence. GRADEpro GDT app was used to summarizing the evidence and dissemination, and seamlessly making sure it adheres to the GRADE methodology.

RESULTS

Description of studies

Eight RCT (10–15,17,25) from 1,513 potentially relevant articles found in electronic databases and reference list searches fulfilled eligibility criteria and were included in the systematic review. **Figure 1** shows the PRISMA flow diagram of included studies. Clinical trials provided data from 379 individuals with a mean age of 61.07±7.63 years old in LLLT

and/or LEDT groups, and 59.60±10.03 in comparison groups. The gender was balanced between groups, male gender was prevalent with 88 (50.3%) individuals in the experimental group (LLLT or LEDT) and the female gender with 151 (50.1%) in comparison groups. Lima and cols. (14) and Lima and cols. (17) not reported genders. Besides, these studies (14,17) divided groups into normoglycemic and hyperglycemic, but in our study, we included only normoglycemic individuals. Also, studies with the same sample in any of the groups were not duplicated (11,12,14,15,17).

Six studies (10,12,14,15,17,25) applied LLLT in sternotomy (12,14,15,17) or in saphenectomy (10,25). LEDT was the experimental group in four studies (11,13,14,17), but only one applied it in saphenectomy (13). Two studies presented the mean length of sternotomy (11) or saphenectomy (13) which was 19.76±0.93cm and 21±1.11cm, respectively. The saphenous vein was removed mainly of the right leg and utilized in one graft vessel in the study of Araújo Júnior and cols. (13). All patients made internal mammary artery grafting in studies of Lima and cols. (14,17). Antihypertensive drugs were used by all patients of Fernandes and cols. (12,15) and Lima and cols. (14,17) studies. In the study of Pinto and cols. (25), all the laser group was hypertensive, 71.4% had dyslipidemia, and diabetes versus 71.4% with hypertension, dyslipidemia, and diabetes in the control group (25). Araújo Júnior and cols. (13), de Oliveira and cols. (11) and Gonzaga and cols. (10) not reported comorbidities or medicines.

Experimental protocol had five (11,12,14,15,17), three (10,13), or two (25) sessions. LLLT or LEDT was initiated immediately after surgery in six studies (11,12,14,15,17), and continued during the 2nd (10–12,14,15,17), 4th (10–12,14,15,17), 6th (11,12,14,15,17), and 8th (11,12,14,15,17) postoperative day. Araújo Júnior and cols. (13) applied LEDT in the 2nd, 3^{rd,} and 4th postoperative days, while Pinto and cols. (25) applied LLLT only in the 2nd and 4th days. Probes of PBM were applied parallel to the incision with 2cm between each in all

studies (10–15,17,25). Six studies applied eight spots (11–15,17), and one 20 spots (10) alongside incision. Pinto and cols. (25) do not present spots number because the probe was applied according to the wound's size surrounding the entire surgical perimeter. Probe application was in contact with the skin in seven studies (10–15,17) and was protected by a translucent film in six studies (10–12,14,15,17). Pinto and cols. (25) do not describe probe contact with skin or translucent film for protection. The main wavelength and energy were 660nm and 2.4J with LLLT (12,14,15,17) and 640±20nm and 10.6J with LEDT (11,13,14,17) application per point. The total energy by session was 19.2J with LLLT and 84.8J with LEDT.

The comparison protocol was a placebo and/or control group. The placebo group was the comparison group of two studies (10,13) and the control group was the comparison of one (25). Most of the studies had both placebo and control groups (11,12,14,15,17). Placebo groups were LLLT (10,12,15) and LEDT (11,13,14,17) applications with equipment turned off according to the process of the experimental protocol. Control groups received no type of intervention (11,12,14,15,17), except for Pinto and cols. (25) control group which received conventional therapy that was not described. **Table 2** summarizes the characteristics of randomized clinical trials included in this systematic review.

Outcomes

Pain was assessed with VAS in the 2nd, 4th, 6^{th,} and 8th postoperative days in three studies with LLLT (12,14,25), and two with LEDT (11,14). Three studies (11,12,14) were included in meta-analysis of pain totaling 292 individuals. Pinto and cols. (25) reported only the percentage of pain in initial evaluation versus final evaluation. Pain percentage was 0% in all evaluated times (LLLT and control group) (25). In the 2nd postoperative day, there was no difference between experimental and placebo/control group with 0% heterogeneity

(MD=0.18, 95%CI=-0.37 to 0.73, p=0.51; **Figure 2A**). However, LEDT seems superior to LLLT with 0% heterogeneity (MD=-0.27, 95%CI=-0.61 to 0.07 versus 0.69, IC95%=0.29 to 1.08, p=0.0003; **Figure 2A**). In the 4th postoperative day experimental group was superior to placebo/control group with 21% heterogeneity (MD=-0.37, 95%CI=-0.57 to -0.17, p=0.0003; **Figure 2B**), but with no difference between LLLT and LEDT (MD=-0.48, 95%CI=-0.70 to -0.25 versus -0.22, IC95%=-0.50 to 0.07, p=0.16; **Figure 2B**). This result remained in 6th postoperative day with experimental superior to placebo/control group with 22% heterogeneity (MD=-1.51, 95%CI=-1.70 to -1.33, p<0.0001; **Figure 2C**), and no difference between LLLT and LEDT (MD=-1.37, 95%CI=-1.61 to -1.14 versus 95%CI=-1.65 -1.88 to -1.42, p=0.09; **Figure 2C**). In the 8th postoperative day, 38% heterogeneity was found with difference between experimental and placebo/control group heterogeneity (MD=-1.59, 95%CI=-1.83 to -1.35, p<0.0001; **Figure 2D**). There was no difference between LLLT and LED (MD=-1.51, 95%CI=-1.84 to -1.19 versus -1.65, IC95%=-2.08 to -1.22, p=0.61; **Figure 2D**).

Incision healing was assessed by the score of healing from photographic analysis, and measured in six studies (10,11,13,15,17,25). LLLT was the experimental group in four studies (10,15,17,25), but only two studies (15,17) were included in quantitative analysis. Gonzaga and cols. (10) presented data like area reduction percentage, and in the LLLT group, this area was reduced concerning the control group. LLLT improved 70% of erythema, and 26% of edema and hematoma in the 7th postoperative day whereas the control group had a worsening in the inflammatory process in the study of Pinto and cols. (25). Three studies used an LED group (11,13,17), but only two studies (11,17) were included in the meta-analysis. Araújo Júnior and cols. (13) also presented data like area reduction percentage, and a significant reduction was observed in the LED group in the 5th postoperative day compared to the placebo group. Subgroup meta-analysis with 225 individuals shown difference between

experimental and placebo/control group with 63% heterogeneity (MD=0.90, 95%CI=-1.16 to -0.65, p<0.0001; **Figure 3A**). There was no difference between LLLT and LED (MD=-0.96, 95%CI=-1.16 to -0.65 versus -0.78, IC95%=-1.01 to -0.55, p=0.49; **Figure 3A**).

Dehiscence assessed by the score of incision closure was evaluated in six studies (10,11,13,15,17,25), and LLLT like the experimental group was applied in four (10,15,17,25) of these studies. A meta-analysis of dehiscence included three studies with LLLT (10,15,17) and also with LED (11,13,17) because of Pinto and cols. (25) data cannot be extracted. This study related two individuals of the LLLT group who presented dehiscence versus four of the control group (25). Meta-analysis totaling 305 individuals showed difference between experimental and placebo/control group with 32% heterogeneity (MD=-0.51, 95%CI=-0.61 to -0.42, p<0.0001; **Figure 3B**). There was no difference between LLLT and LED (MD=-0.49, 95%CI=-0.60 to -0.38 versus -0.52, IC95%=-0.69 to -0.35, p=0.78; **Figure 3B**).

Risk of bias and quality of evidence

The assessment of the risk of bias evidenced most information is from trials with a low risk of bias. One of five RCT with pain outcomes presented a high risk of bias for two domains and overall bias. Three of seven RCT with incision healing presented a high risk of bias in one domain and overall bias. Dehiscence outcome also with seven studies evidenced only one study with two domains for high risk of bias and overall bias. Biases' risks were high mainly because of the measurement of the outcome and selection of the reported results. **Figure 4** presents the detailed risk of bias considering each outcome of interest. GRADE quality of evidence with RCT included in meta-analysis demonstrate moderate certainty in the pain, incision healing, and dehiscence outcomes in LLLT and LED compared to placebo/control groups after CABG surgery. The summarizes of the evidence are presented in **supplementary data**.

DISCUSSION

This systematic review and meta-analysis about effects of PBM therapy versus placebo and/or control group in longitudinal sternotomy and saphenectomy after CABG showed LLLT (660nm and 2.4J) and/or LEDT (640±20nm and 10.6J) may reduce pain as from 4th postoperative day, and incision healing and dehiscence until 8th postoperative day in the discharge of hospital. To the best of our knowledge, this is the first systematic review of RCT about this topic. Despite a broad literature search, we found only eight RCT (10–15,17,25) but could perform a quantitative approach of data with three studies in pain, two in incision healing, and three in dehiscence with LLLT and LEDT because of differences in the outcome reporting.

The spectrum of visible light is generally employed because these wavelengths can penetrate superficial tissue and ranging from 600 to 700nm may have a good effect on pain, inflammation, and tissue repair (26–28). WALT Guidelines (29) had no recommendations about this wavelength, but the most of studies included in this systematic review used 660nm (LLLT) (10,12,14,15,17) and 640± 20nm (LEDT) (11,13,14,17). The positive results were with probe applied stationary for at least 60s with LLLT (10,12,14,15,17) and 152s with LEDT (11,13,14,17) per point of treatment, and with a fluence of 6J/cm^2 per spot (11–15,17). The use of adequate doses is necessary for PBM therapy effects. Low irradiance and prolonging of radiation time to achieve the ideal fluence or dose will not give an adequate result because of the biphasic dose-response curve (27,30,31). Low-energy PBM therapy may be stimulating mitochondria and increases metabolism while higher intensity may be producing the opposite effect (27,30,31).

PBM with LLLT and LEDT both promote cell biostimulation by primary photochemical response called photoactivated cells (26). Mitochondrial intracellular

chromophores like cytochrome C oxidase (CCO) absorb very low levels of light which influence the cell response because of cellular metabolism stimulation (31). The proposed mechanism is PBM may cause nitric oxide (NO) and CCO photo-dissociation and reverses the mitochondrial respiration inhibition (27,30). This mitochondrial stimulation produces chemical signaling molecules like ATP, cyclic adenosine monophosphate, NO, and oxygen reactive species which active cell proliferation signaling pathways' transcription factors (27,30,31). The activity of these pathways causes growth factors to increase, and consequently, increasing angiogenesis, neovascularization, and collagen synthesis (27,30,31).

All included studies at quantitative analysis showed PBM with LLLT and/or LEDT can improve pain assessed with VAS (11,12,14) from the middle of the postoperative period (eight days). In accordance, Sanchez and cols. (32) demonstrated LLLT (808nm, 30mW, 30J/cm^2, 28s) reduces thermal and mechanical hyperalgesia in an experimental model of neuropathic pain. The previous meta-analysis of 21 head-to-head comparisons totaling 1,462 participants showed a difference between LLLT and control groups in pain of patients with musculoskeletal disorders (33). A systematic review of de Andrade and cols. (34) showed moderate evidence for PBM with LLLT in the control of neuropathic pain, but without a standard of parameters used. However, in our systematic review, the study of Pinto and cols. (25) was the only study that related no pain in saphenectomy of patients after CABG surgery. Pain relief can reduce the risk of postoperative morbidities which could increase patients' length of hospital stays like pulmonary complications, higher myocardial oxygen consumption, and rehabilitation delay (35). It is known that pain is both the transduction of noxious environmental stimulus of actual or potential tissue injury and a complex sensory or emotional experience that depends on cognitive and emotional processing (36). Thus, multiple techniques such as pharmacologic and non-pharmacologic interventions like PBM to the management of pain after cardiac surgery are considered a preferred strategy (35). The

reduction of one point in VAS was considered the minimal clinically important difference for pain in the postoperative period (MYLES ET AL 2017), and in our results, we found reducing of 1.59 points in VAS after PBM therapy.

The mechanisms for PBM-mediated pain relief are not fully understood, but are related to inflammatory process modulation, stimulation and increasing of endorphins synthesis, and excitation and conduction of peripheral nerves (34). In experimental models, modulation of the chemical mediators of inflammation (pro-inflammatory peptides like bradykinin) and beta-endorphin synthesis and expression by PBM therapy could limit the reduction of excitability threshold in painful receptors (27,32,34). The noxious stimuli of pain are transduced by myelinated A-gama and unmyelinated C fibers of nociceptors' nerve endings which are very superficial for penetration depths of wavelengths used in PBM (27). PBM could also decrease mitochondrial membrane potentials of nerve endings nociceptors by the formation of reversible varicosities along the axons which disrupted the nerve's cytoskeleton and block anterograde transport of ATP-rich mitochondria in dorsal root ganglion neurons (27,37). These mechanisms probably normalize the speeds of transmission of nerve impulses and lead to neural inhibition (27,32). The analgesic effect is initially at the epidermal neural network, but elongated cytoplasm of neurons also endings on the surface of the skin, and these effects move to nerves in subcutaneous tissues, sympathetic ganglia, and neuromuscular junctions within muscles (27).

In this meta-analysis, incision healing and dehiscence were improved after PBM with LLLT and LEDT application assesses by the score of healing and incision closure from photographic analysis (10,11,13,15,17) until hospital discharge. Another meta-analysis of four RCT (38) demonstrated PBM with LLLT (660 and 850 nm; 60 mW/cm^2; 2 to $4J/cm^2$) in diabetic foot ulcer reduced the ulcer area after 15 days of treatment in 32 patients. Also, besides no difference in pain relief of LLLT and trunk exercises, Helmy and cols. (8) shows

PBM therapy was more efficacious in decreasing sternal separation after CABG surgery which led to faster healing and rapidly return to activities of daily life (8). Several mechanisms are believed to support beneficial wound healing responses. Inflammatory cells influence the wound healing process and supposedly PBM application may suppress inflammatory cells causing cellular changes like the synthesis of collagen and extracellular matrix, recruitment of cytokines and growth factors, migration, proliferation, and differentiation of different cell types (28,38). Light irradiation recruits important cytokines and growth factors leading to cellular functions improvement due to increasing in CCO of the mitochondrial respiratory chain, and ATP production which stimulates wound healing (8,9,28,38). Besides of high heterogeneity in incision healing outcomes with LLLT, we cannot explore this because of the articles number included in the meta-analysis. Moderate heterogeneity in dehiscence with LEDT could be explained due to a wide SD in one study, however, this result did not influence the overall prism.

RoB 2 evidenced a low risk of bias in the overall bias of included articles, but a high risk of bias of Pinto and cols. (25) and Gonzaga and cols. (10) may be affecting our result in the meta-analysis because of reporting of selective bias. Nevertheless, besides meta-analysis with a small number of studies, the quality of evidence was moderate according to GRADE assessment in all outcomes because of a low number of patients. These results qualify our systematic review and meta-analysis and should collaborate with clinical practice due to its importance. Based upon a small number of RCT, PBM therapy with LLLT and LEDT after CABG had been described with good results, and demonstrated improvement in pain, incision healing, and dehiscence outcomes with no adverse events using this physical therapy intervention. Control of postoperative pain is especially important because it can prevent hemodynamic changes that may increase postoperative morbidity. However, in the postoperative period in the intensive care unit, CABG patients receive routinely analgesics

drugs and are very manipulated by the medical team what possibly explains the no effect of PBM therapy in this meta-analysis.

Limitations

The limitations of this systematic review are the small number of RCT with a moderate sample size which could affect the result. Data presentation and selective reporting of bias also were important limitations because of outcome extracting from graphics and data transformation which may be underestimated or overestimated meta-analysis results. Despite similar parameters in experimental protocols were still used different parameters that can interfere in clinical practice. But this study also has strengths, including the analysis of critically relevant CABG postoperative outcomes, a broad search in the principal health database, following the gold standard methods for systematic reviews of interventions.

Conclusion

This systematic review and meta-analysis of randomized clinical trials showed PBM therapy with LLLT (660nm and 2.4J) and/or LEDT (640±20nm and 10.6J) is superior to placebo and/or control to improve pain, incision healing, and dehiscence in patients who underwent CABG surgery. However, besides the positive effects of both LLLT and LEDT, low energy was used in LLLT. This study reports an important therapeutic with significant potential, minimally invasive, and with moderate quality of evidence assessed by GRADE. Besides good results, we suggest conducting new large clinical trials with interventions after CABG surgery to verify the immediate postoperative effect.

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CONFLICT OF INTEREST (COI) STATEMENT

The authors declare no conflicts of interest.

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FIGURE LEGENDS

Figure 1. PRISMA 2020 flow diagram of the selection and inclusion process of the studies.

Figure 2. The meta-analysis of postoperative pain with LLLT and/or LEDT versus placebo/control group. **(A)** Pain measured on the second postoperative day. **(B)** Pain measured on the fourth postoperative day. **(C)** Pain measured on the sixth postoperative day. **(D)** Pain measured on the eighth postoperative day.

Figure 3. The meta-analysis of **(A)** incision healing and **(B)** dehiscence with LLLT and/or LEDT versus placebo/control group measured on the eighth postoperative day.

Figure 4. Risk of bias of included studies at systematic review considering the outcome of interest evaluated with RoB 2.

Figure 5. GRADE quality of evidence in randomized clinical trials with experimental groups of LLLT and LEDT measuring pain, incision healing, and dehiscence.

Table 1. Characteristics of randomized clinical trials included in systematic review.

Author, Year	Public trial registry	Region of treatment	Eligibility criteria	Intervention	N	Comparator	N	Age± SD	Male gender	Protocol
1. Araújo Júnior et al., 2018	https://ensaiosclinicos.gov.br/rg/RBR-38wgx6	Saphenectomy	**Inclusion:** surgery with extracorporeal circulation; longitudinal sternotomy; saphenectomy by; hemodynamic stability; skin phototype I to IV, according to Fitzpatrick's classification; BMI <29.9 kg/m^2; agreement to participate in the study by signing the free and informed consent form. **Exclusion:** altered protocol of analgesia; syndrome of low cardiac output; coagulation disorders; renal insufficiency with creatinine> 1.8 mg/dl; respiratory system infections requiring mechanical ventilation; and/or stroke.	LEDT	20	Placebo group	20	60.2± 11.56	**IG:** 13 **PG:** 13	**IG:** 3 sessions of LEDT irradiation daily between 2 and 4 days after surgery (640±20nm wavelength, 10.6J per point, 152s of treatment time per point, 1.77cm^2 spot size): 8 spots (2cm between each) parallel to incision and in contact with skin. **PG:** LEDT application process with equipment turned off. **Outcomes:** Incision healing and Dehiscence.
2. de Oliveira et	https://ensaiosclinicos.gov.br	Sternotomy	**Inclusion:** elective CABG; longitudinal sternotomy incision; ages	LEDT	30	Placebo and	30/30	59.9± 9.16	**IG:** 16	**IG:** 5 sessions of LEDT irradiation immediately after surgery; and 2, 4,

al., 2014	/rg/RBR-38wgx6		between 18 and 75 years. **Exclusion:** morbid obesity; previous thoracic surgery; emergency or urgent CABG; respiratory or renal insufficiency; low cardiac output syndrome; changes in analgesic protocols; any other complication			Control group			**PG:** 20 **CG:** 19	6 and 8 days after surgery (640±20nm wavelength, 10.6J per point, 152s of treatment time per point, 1.77cm^2 spot size): 8 spots (2cm between each) alongside and parallel to incision and in contact with skin; probe protected by translucent film. **PG:** LEDT application process with equipment turned off. **CG:** No intervention. **Outcomes:** Pain, Incision healing and Dehiscence.
3. Fernandes et al., 2016	https://ensaiosclinicos.gov.br/rg/RBR-	Sternotomy	**Inclusion:** age between 18 and 75 years; both genders; hemodynamic stability; BMI <29.9 kg/m^2.	LLLT	30	Placebo and Control	30/30	59.6± 9	**IG:** 19 **PG:** 19	**IG:** 5 sessions of LLLT irradiation immediately after surgery; and 2, 4, 6 and 8 days after surgery (660nm

	38wgx6		**Exclusion:** previous thoracic surgery; emergency or urgent CABG; respiratory insufficiency; renal insufficiency with serum creatinine≥1.8 mg/dL; low cardiac output syndrome; changes in analgesic protocols.			group			**CG:** 20	wavelength, 2.4J per point, 60s of treatment time per point, 0.4cm^2 spot size): 8 spots (2cm between each) alongside and parallel to incision and in contact with skin; probe protected by translucent film. **PG:** LLLT application process with equipment turned off. **CG:** No intervention. **Outcomes:** Incision healing and Dehiscence.
4. Fernandes et al., 2017	https://ensaiosclinicos.gov.br/rg/RBR-38wgx6	Sternotomy	**Inclusion:** elective CABG; longitudinal sternotomy; extracorporeal circulation; ages between 18 and 75 years-old; both genders; hemodynamically stable;	LLLT	30	Placebo and Control group	30/ 30	59.7± 8.93	**IG:** 19 **PG:** 20 **CG:** 19	**IG:** 5 sessions of LLLT irradiation immediately after surgery; and 2, 4, 6 and 8 days after surgery (660nm wavelength, 2.4J per point, 60s of treatment time per point, 0.4cm^2

			BMI <29.9 kg/m2. **Exclusion:** previous thoracic surgery; emergency or urgent CABG; respiratory or renal insufficiency; low cardiac output syndrome; changes in analgesic protocols; other postsurgery complications.							spot size): 8 spots (2cm between each) alongside and parallel to incision and in contact with skin; probe protected by translucent film. **PG:** LLLT application process with equipment turned off. **CG:** No intervention. **Outcome:** Pain.
5. Gonzaga et al., 2018	https://ensaiosclinicos.gov.br/rg/RBR-38wgx6	Saphenectomy	**Inclusion:** age between 45 and 75 years; skin phototype I to IV (Fitzpatrick classification); CABG with a longitudinal sternotomy; saphenectomy; extracorporeal circulation; hemodynamic stability; BMI <29.9 Kg/m^2. **Exclusion:** low cardiac output	LLLT	20	Placebo group	20	61.5 ± 11.42	**IG:** 16 **PG:** 13	**IG:** 3 sessions of LLLT irradiation immediately after surgery; and 2 and 4 days after surgery (660nm wavelength, 0.22J per point, 5.7s of treatment time per point; spot size of 0.04cm^2): 20 spots (2cm between each) alongside and parallel to incision and in contact with skin;

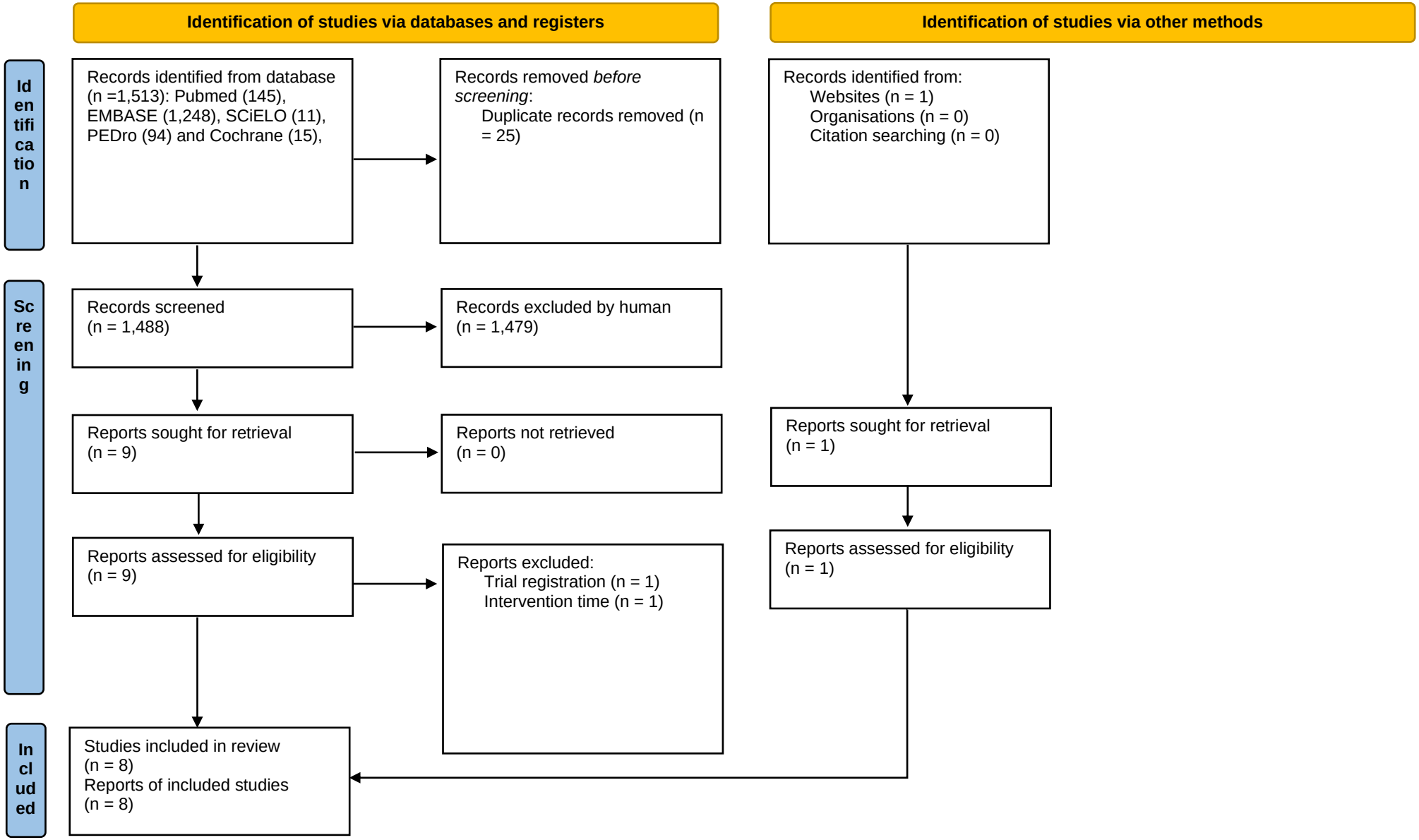
			syndrome; postoperative coagulation disorder; renal insufficiency with serum creatinine‡1.8 mg/dL; respiratory insufficiency; stroke; alteration of the consciousness level; changes in analgesic protocols.						probe protected by translucent film. **PG:** LLLT application process with equipment turned off. **Outcome:** Incision healing and Dehiscence.
6. Lima et al., 2016	https://ensaiosclinicos.gov.br/rg/RBR-38wgx6	Sternotomy		LLLT and LEDT	19/19	Placebo and Control group	19/18	58.9± 9.15	Not reported	**IG:** 5 sessions of LLLT or LEDT irradiation immediately after surgery; and 2, 4, 6 and 8 days after surgery (LLLT= 660nm wavelength, 2.4J per point, 60s of treatment time per point; spot size of 0.4cm^2; LEDT= 640±20nm wavelength, 10.6J per point, 152s of treatment time per point; spot size of 1.77cm^2): 8 spots (2cm between each) alongside and

									parallel to incision and in contact with skin; probe protected by translucent film. **PG:** LEDT application process with equipment turned off. **CG:** No intervention. **Outcome:** Pain.
7. Lima et al., 2017	https://ensaiosclinicos.gov.br/rg/RBR-38wgx6	Sternotomy	**Inclusion:** elective CABG; longitudinal sternotomy; extracorporeal circulation; anastomosing left internal mammary artery; saphenous vein grafts; age between 18 and 75 years-old; both genders; hemodynamic stability; BMI <29.9 kg/m^2.	LLLT and LEDT	19/19	Placebo and Control Group	19/18	58.9± 9.15	Not reported.	**IG:** 5 sessions of LLLT or LEDT irradiation immediately after surgery; and 2, 4, 6 and 8 days after surgery (LLLT= 660nm wavelength, 2.4J per point, 60s of treatment time per point; spot size of 0.4cm^2; LEDT= 640±20nm wavelength, 10.6J per point, 152s of treatment time per point; spot

			Exclusion: type I and II diabetes mellitus; previous thoracic surgery, or emergency or urgent CABG; respiratory or renal insufficiency; low cardiac output syndrome; changes in analgesic protocols; other postoperative complications.						size of 1.77cm2: 8 spots (2cm between each) alongside and parallel to incision and in contact with skin; probe protected by translucent film. **PG:** LEDT application process with equipment turned off. **CG:** No intervention. **Outcome:** Incision healing and Dehiscence.
8. Pinto et al., 2014	Not available	Saphenectomy	**Inclusion:** prodromal signals on the saphenous incision after CABG. **Exclusion:** immunosuppression; infection; respiratory, kidney, or liver failure; class III or IV	LLLT + Conventional therapy	7	Control group	7	63 ± 3.33	**IG:** 5 **CG:** 7	**IG:** 2 sessions of LLLT irradiation at 2 and 4 days after surgery (780nm wavelength, 0.75J per point, 30s of treatment time per point; spot size of 0.04cm^2): spots (2cm between each) surrounding

	obesity.	entire surgical perimeter; nº spots according to wound's size. **CG:** Conventional therapy. **Conventional therapy:** not reported. **Outcomes:** Pain, Incision healing and Dehiscence.

IG= Intervention group; PG= Placebo Group; CG= Control Group; LLLT= Low-Level Laser Therapy; LEDT= Light-Emitting Diode Therapy; CABG= Coronary Artery Bypass Grafting; BMI= Body Max Index. Data presented like mean ± standard deviation.


Identification of studies via databases and registers
Identification of studies via other methods
Identification
Screening
Included
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Records removed *before screening*:
Duplicate records removed (n = 25)
Records identified from:
Websites (n = 1)
Organisations (n = 0)
Citation searching (n = 0)
Records screened (n = 1,488)
Records excluded by human (n = 1,479)
Reports sought for retrieval (n = 9)
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Reports assessed for eligibility (n = 9)
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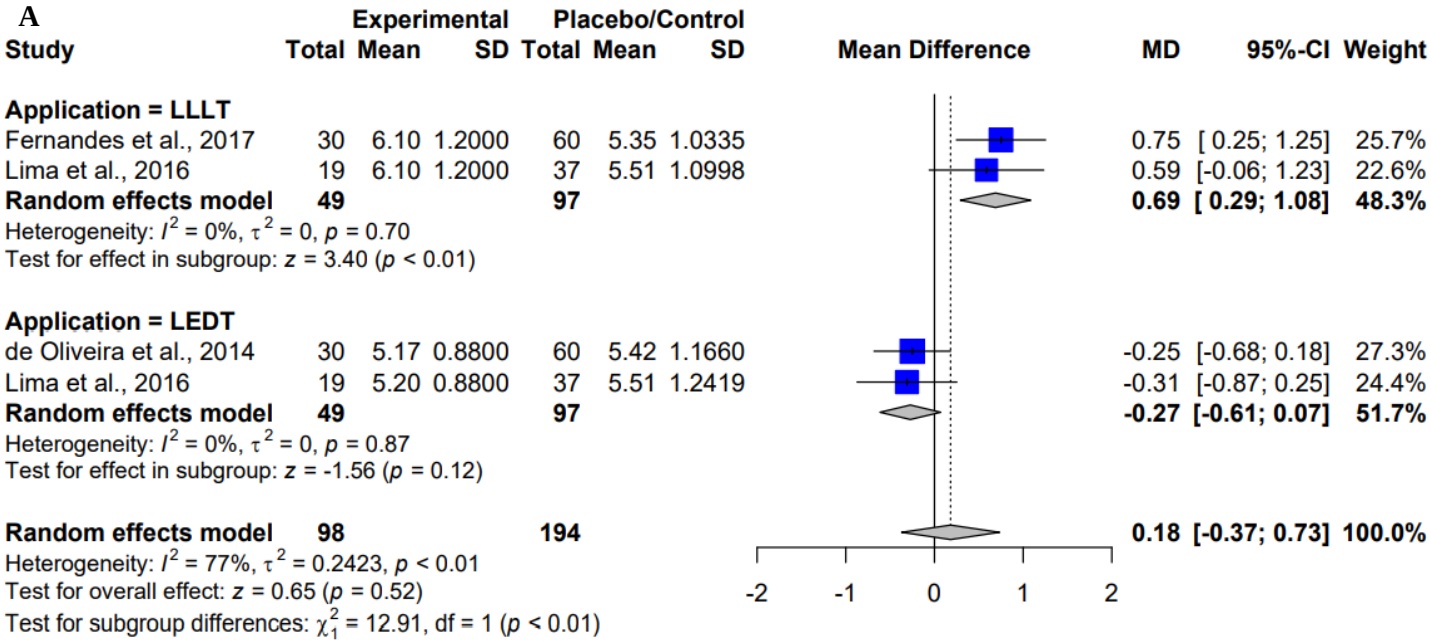
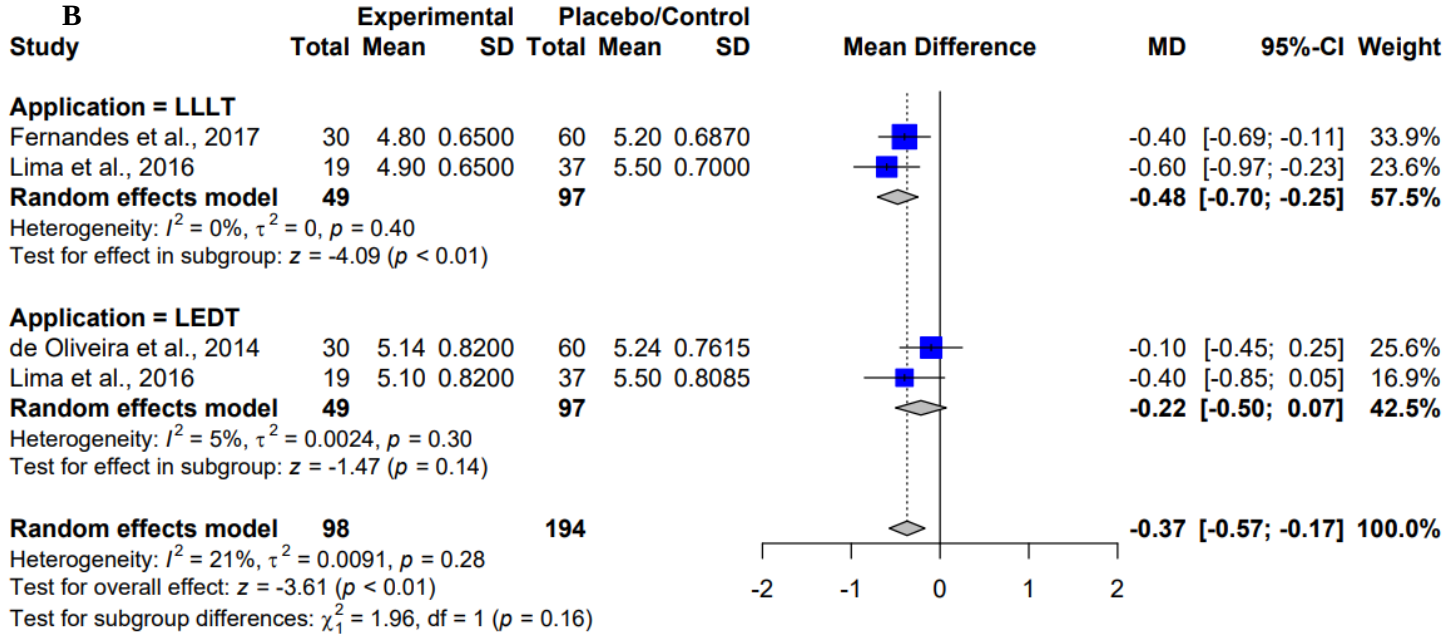
Trial registration (n = 1)
Intervention time (n = 1)
Reports assessed for eligibility (n = 1)
Studies included in review (n = 8)
Reports of included studies (n = 8)

A


Experimental
Placebo/Control
Study Total Mean SD Total Mean SD
Mean Difference
MD 95%-CI Weight
Application = LLLT
Fernandes et al., 2017 30 6.10 1.2000 60 5.35 1.0335 0.75 [0.25; 1.25] 25.7%
Lima et al., 2016 19 6.10 1.2000 37 5.51 1.0998 0.59 [-0.06; 1.23] 22.6%
Random effects model 49 97 0.69 [0.29; 1.08] 48.3%
Heterogeneity: I^2 = 0%, τ^2 = 0, p = 0.70
Test for effect in subgroup: z = 3.40 (p < 0.01)
Application = LEDT
de Oliveira et al., 2014 30 5.17 0.8800 60 5.42 1.1660 -0.25 [-0.68; 0.18] 27.3%
Lima et al., 2016 19 5.20 0.8800 37 5.51 1.2419 -0.31 [-0.87; 0.25] 24.4%
Random effects model 49 97 -0.27 [-0.61; 0.07] 51.7%
Heterogeneity: I^2 = 0%, τ^2 = 0, p = 0.87
Test for effect in subgroup: z = -1.56 (p = 0.12)
Random effects model 98 194 0.18 [-0.37; 0.73] 100.0%
Heterogeneity: I^2 = 77%, τ^2 = 0.2423, p < 0.01
Test for overall effect: z = 0.65 (p = 0.52)
Test for subgroup differences: χ^2_1 = 12.91, df = 1 (p < 0.01)
-2 -1 0 1 2

B


Experimental
Placebo/Control
Study Total Mean SD Total Mean SD
Mean Difference
MD 95%-CI Weight
Application = LLLT
Fernandes et al., 2017 30 4.80 0.6500 60 5.20 0.6870 -0.40 [-0.69; -0.11] 33.9%
Lima et al., 2016 19 4.90 0.6500 37 5.50 0.7000 -0.60 [-0.97; -0.23] 23.6%
Random effects model 49 97 -0.48 [-0.70; -0.25] 57.5%
Heterogeneity: I^2 = 0%, τ^2 = 0, p = 0.40
Test for effect in subgroup: z = -4.09 (p < 0.01)
Application = LEDT
de Oliveira et al., 2014 30 5.14 0.8200 60 5.24 0.7615 -0.10 [-0.45; 0.25] 25.6%
Lima et al., 2016 19 5.10 0.8200 37 5.50 0.8085 -0.40 [-0.85; 0.05] 16.9%
Random effects model 49 97 -0.22 [-0.50; 0.07] 42.5%
Heterogeneity: I^2 = 5%, τ^2 = 0.0024, p = 0.30
Test for effect in subgroup: z = -1.47 (p = 0.14)
Random effects model 98 194 -0.37 [-0.57; -0.17] 100.0%
Heterogeneity: I^2 = 21%, τ^2 = 0.0091, p = 0.28
Test for overall effect: z = -3.61 (p < 0.01)
Test for subgroup differences: χ^2_1 = 1.96, df = 1 (p = 0.16)
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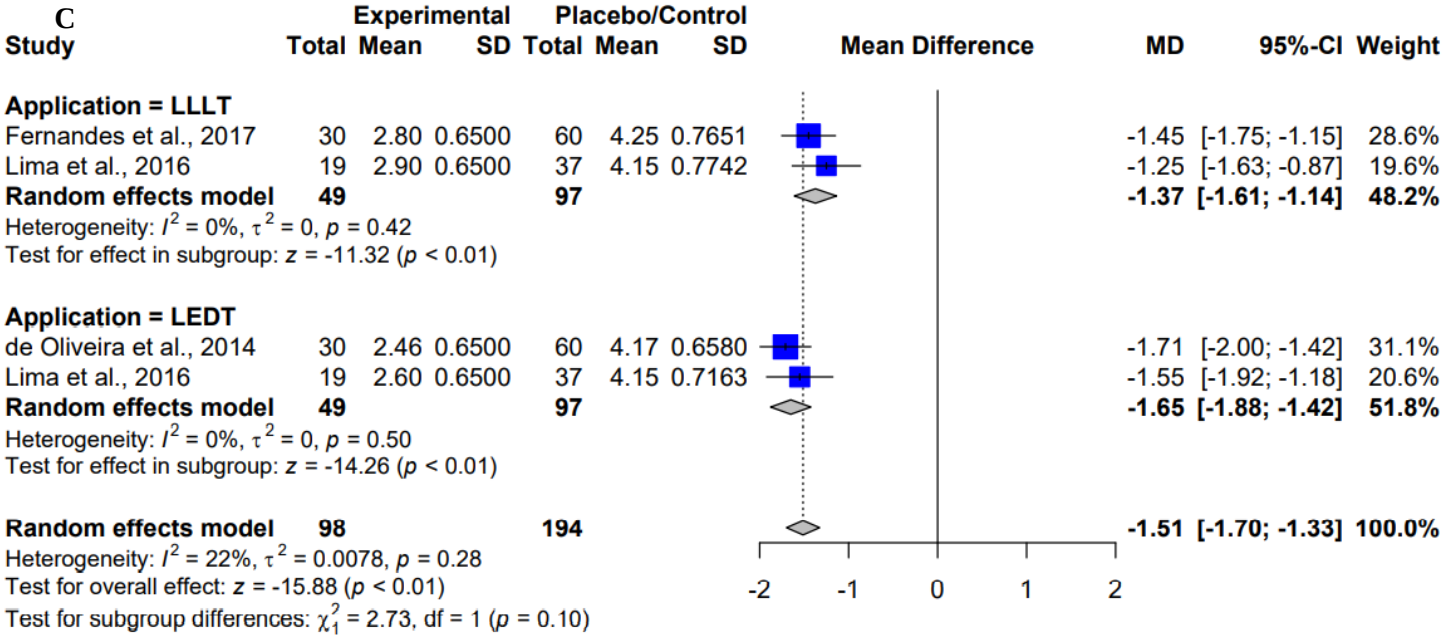
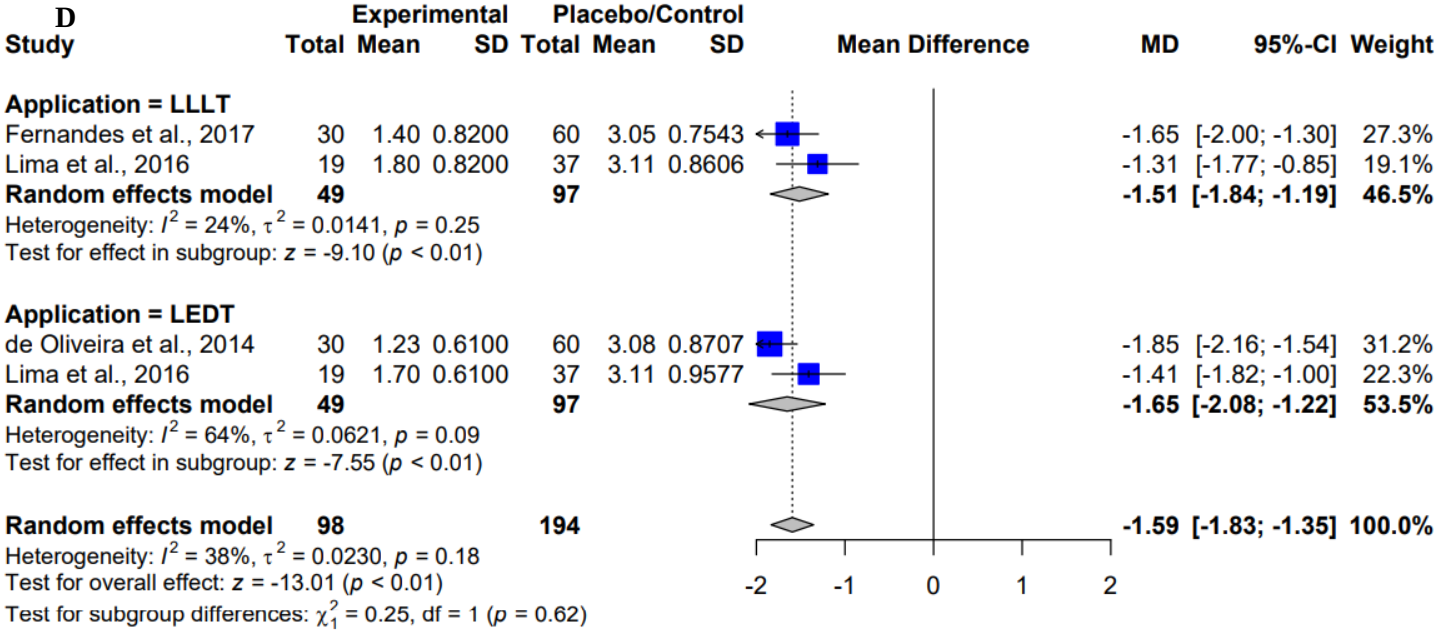
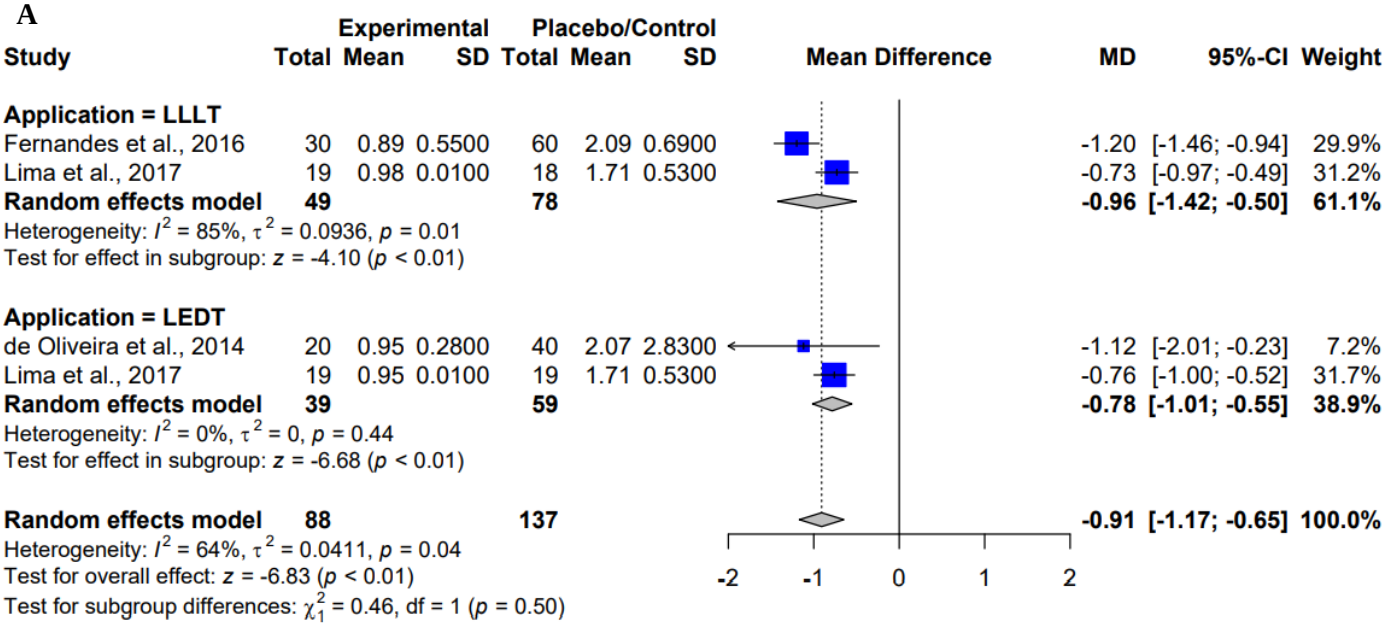
C


Study | Experimental Total Mean SD | Placebo/Control Total Mean SD | Mean Difference | MD 95%-CI Weight
Application = LLLT
Fernandes et al., 2017 30 2.80 0.6500 60 4.25 0.7651 -1.45 [-1.75; -1.15] 28.6%
Lima et al., 2016 19 2.90 0.6500 37 4.15 0.7742 -1.25 [-1.63; -0.87] 19.6%
Random effects model 49 97 -1.37 [-1.61; -1.14] 48.2%
Heterogeneity: I^2 = 0%, τ^2 = 0, p = 0.42
Test for effect in subgroup: z = -11.32 (p < 0.01)
Application = LEDT
de Oliveira et al., 2014 30 2.46 0.6500 60 4.17 0.6580 -1.71 [-2.00; -1.42] 31.1%
Lima et al., 2016 19 2.60 0.6500 37 4.15 0.7163 -1.55 [-1.92; -1.18] 20.6%
Random effects model 49 97 -1.65 [-1.88; -1.42] 51.8%
Heterogeneity: I^2 = 0%, τ^2 = 0, p = 0.50
Test for effect in subgroup: z = -14.26 (p < 0.01)
Random effects model 98 194 -1.51 [-1.70; -1.33] 100.0%
Heterogeneity: I^2 = 22%, τ^2 = 0.0078, p = 0.28
Test for overall effect: z = -15.88 (p < 0.01)
Test for subgroup differences: χ^2_1 = 2.73, df = 1 (p = 0.10)
-2 -1 0 1 2

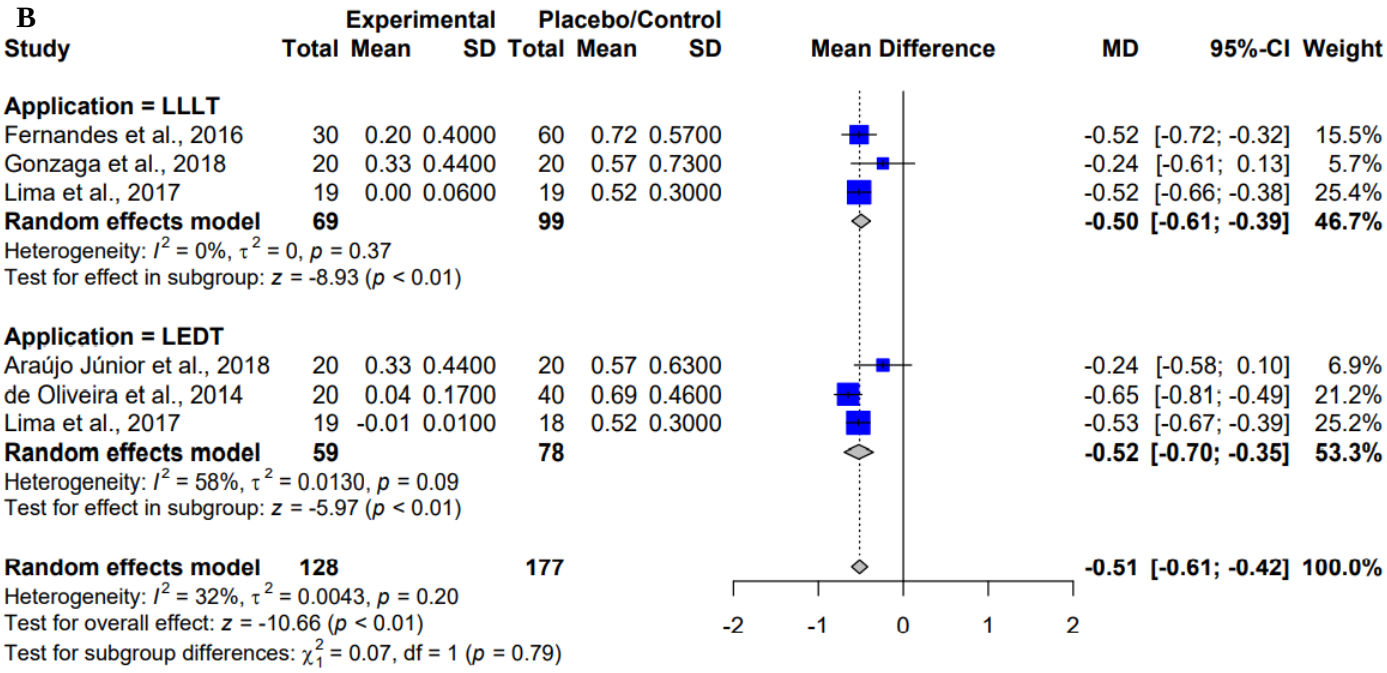
D

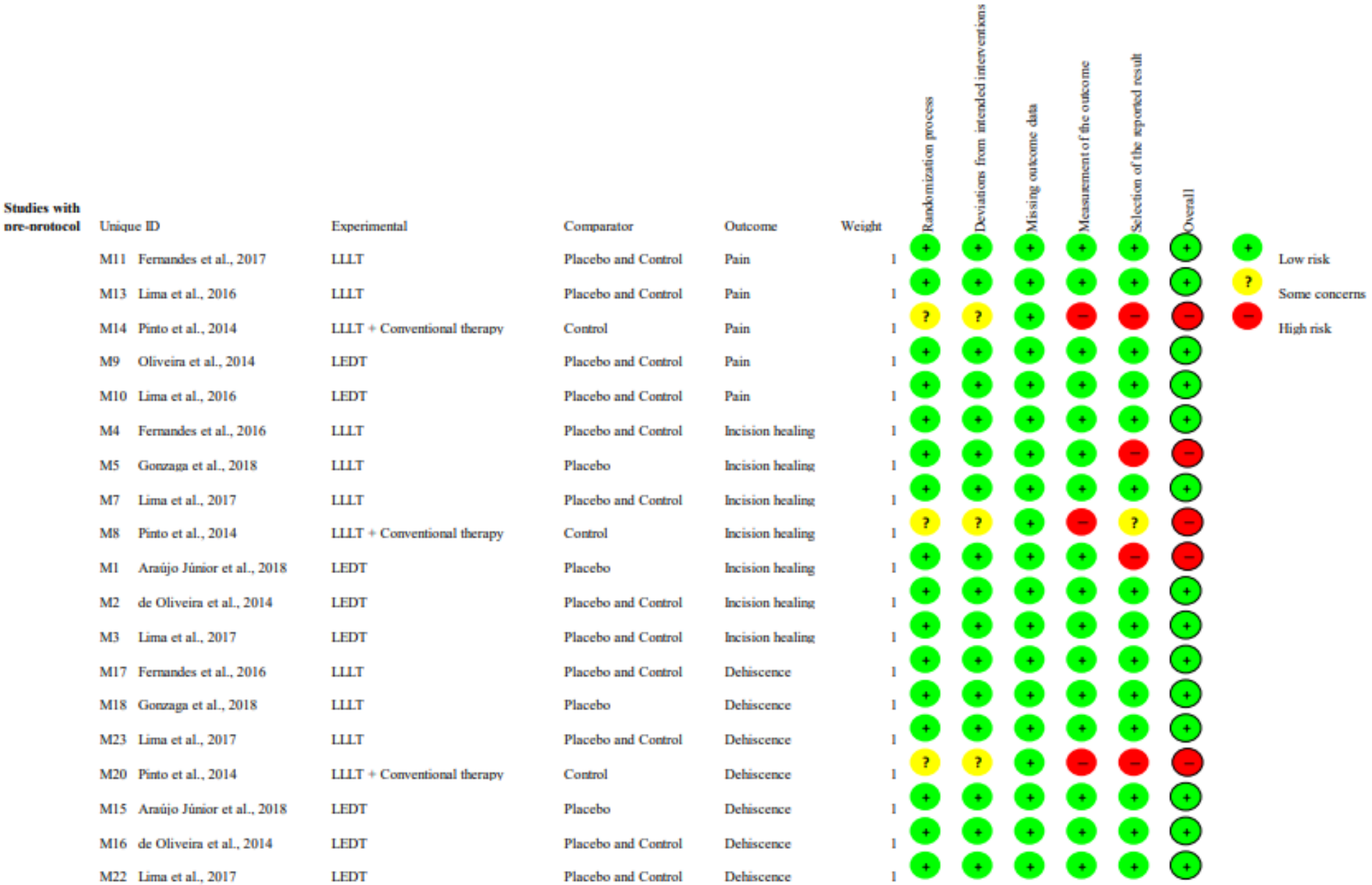

Study | Experimental Total Mean SD | Placebo/Control Total Mean SD | Mean Difference | MD 95%-CI Weight
Application = LLLT
Fernandes et al., 2017 30 1.40 0.8200 60 3.05 0.7543 -1.65 [-2.00; -1.30] 27.3%
Lima et al., 2016 19 1.80 0.8200 37 3.11 0.8606 -1.31 [-1.77; -0.85] 19.1%
Random effects model 49 97 -1.51 [-1.84; -1.19] 46.5%
Heterogeneity: I^2 = 24%, τ^2 = 0.0141, p = 0.25
Test for effect in subgroup: z = -9.10 (p < 0.01)
Application = LEDT
de Oliveira et al., 2014 30 1.23 0.6100 60 3.08 0.8707 -1.85 [-2.16; -1.54] 31.2%
Lima et al., 2016 19 1.70 0.6100 37 3.11 0.9577 -1.41 [-1.82; -1.00] 22.3%
Random effects model 49 97 -1.65 [-2.08; -1.22] 53.5%
Heterogeneity: I^2 = 64%, τ^2 = 0.0621, p = 0.09
Test for effect in subgroup: z = -7.55 (p < 0.01)
Random effects model 98 194 -1.59 [-1.83; -1.35] 100.0%
Heterogeneity: I^2 = 38%, τ^2 = 0.0230, p = 0.18
Test for overall effect: z = -13.01 (p < 0.01)
Test for subgroup differences: χ^2_1 = 0.25, df = 1 (p = 0.62)
-2 -1 0 1 2

A


Study | Experimental Total Mean SD | Placebo/Control Total Mean SD | Mean Difference | MD 95%-CI Weight
Application = LLLT
Fernandes et al., 2016 30 0.89 0.5500 60 2.09 0.6900 -1.20 [-1.46; -0.94] 29.9%
Lima et al., 2017 19 0.98 0.0100 18 1.71 0.5300 -0.73 [-0.97; -0.49] 31.2%
Random effects model 49 78 -0.96 [-1.42; -0.50] 61.1%
Heterogeneity: I^2 = 85%, τ^2 = 0.0936, p = 0.01
Test for effect in subgroup: z = -4.10 (p < 0.01)
Application = LEDT
de Oliveira et al., 2014 20 0.95 0.2800 40 2.07 2.8300 -1.12 [-2.01; -0.23] 7.2%
Lima et al., 2017 19 0.95 0.0100 19 1.71 0.5300 -0.76 [-1.00; -0.52] 31.7%
Random effects model 39 59 -0.78 [-1.01; -0.55] 38.9%
Heterogeneity: I^2 = 0%, τ^2 = 0, p = 0.44
Test for effect in subgroup: z = -6.68 (p < 0.01)
Random effects model 88 137 -0.91 [-1.17; -0.65] 100.0%
Heterogeneity: I^2 = 64%, τ^2 = 0.0411, p = 0.04
Test for overall effect: z = -6.83 (p < 0.01)
Test for subgroup differences: χ^2_1 = 0.46, df = 1 (p = 0.50)
-2 -1 0 1 2

B


Study | Experimental Total Mean SD | Placebo/Control Total Mean SD | Mean Difference | MD 95%-CI Weight
Application = LLLT
Fernandes et al., 2016 30 0.20 0.4000 60 0.72 0.5700 -0.52 [-0.72; -0.32] 15.5%
Gonzaga et al., 2018 20 0.33 0.4400 20 0.57 0.7300 -0.24 [-0.61; 0.13] 5.7%
Lima et al., 2017 19 0.00 0.0600 19 0.52 0.3000 -0.52 [-0.66; -0.38] 25.4%
Random effects model 69 99 -0.50 [-0.61; -0.39] 46.7%
Heterogeneity: I^2 = 0%, τ^2 = 0, p = 0.37
Test for effect in subgroup: z = -8.93 (p < 0.01)
Application = LEDT
Araújo Júnior et al., 2018 20 0.33 0.4400 20 0.57 0.6300 -0.24 [-0.58; 0.10] 6.9%
de Oliveira et al., 2014 20 0.04 0.1700 40 0.69 0.4600 -0.65 [-0.81; -0.49] 21.2%
Lima et al., 2017 19 -0.01 0.0100 18 0.52 0.3000 -0.53 [-0.67; -0.39] 25.2%
Random effects model 59 78 -0.52 [-0.70; -0.35] 53.3%
Heterogeneity: I^2 = 58%, τ^2 = 0.0130, p = 0.09
Test for effect in subgroup: z = -5.97 (p < 0.01)
Random effects model 128 177 -0.51 [-0.61; -0.42] 100.0%
Heterogeneity: I^2 = 32%, τ^2 = 0.0043, p = 0.20
Test for overall effect: z = -10.66 (p < 0.01)
Test for subgroup differences: χ^2_1 = 0.07, df = 1 (p = 0.79)
-2 -1 0 1 2


Studies with pre-protocol
Unique ID
Experimental
Comparator
Outcome
Weight
Randomization process
Deviations from intended interventions
Missing outcome data
Measurement of the outcome
Selection of the reported result
Overall
M11 Fernandes et al., 2017 LLLT Placebo and Control Pain 1
M13 Lima et al., 2016 LLLT Placebo and Control Pain 1
M14 Pinto et al., 2014 LLLT + Conventional therapy Control Pain 1
M9 Oliveira et al., 2014 LEDT Placebo and Control Pain 1
M10 Lima et al., 2016 LEDT Placebo and Control Pain 1
M4 Fernandes et al., 2016 LLLT Placebo and Control Incision healing 1
M5 Gonzaga et al., 2018 LLLT Placebo Incision healing 1
M7 Lima et al., 2017 LLLT Placebo and Control Incision healing 1
M8 Pinto et al., 2014 LLLT + Conventional therapy Control Incision healing 1
M1 Araújo Júnior et al., 2018 LEDT Placebo Incision healing 1
M2 de Oliveira et al., 2014 LEDT Placebo and Control Incision healing 1
M3 Lima et al., 2017 LEDT Placebo and Control Incision healing 1
M17 Fernandes et al., 2016 LLLT Placebo and Control Dehiscence 1
M18 Gonzaga et al., 2018 LLLT Placebo Dehiscence 1
M23 Lima et al., 2017 LLLT Placebo and Control Dehiscence 1
M20 Pinto et al., 2014 LLLT + Conventional therapy Control Dehiscence 1
M15 Araújo Júnior et al., 2018 LEDT Placebo Dehiscence 1
M16 de Oliveira et al., 2014 LEDT Placebo and Control Dehiscence 1
M22 Lima et al., 2017 LEDT Placebo and Control Dehiscence 1
Low risk
Some concerns
High risk

Supplementary Table 1. The literature search strategy used for the PubMed database.

Id.	Search strategy
# 1	(Low-Level Light Therapy)[Mesh] OR (Light Therapies, Low-Level) OR (Light Therapy, Low-Level) OR (Low Level Light Therapy) OR (Low-Level Light Therapies) OR (Therapies, Low-Level Light) OR (Therapy, Low-Level Light) OR (Photobiomodulation Therapy) OR (Photobiomodulation Therapies) OR (Therapies, Photobiomodulation) OR (Therapy, Photobiomodulation) OR (LLLT) OR (Laser Therapy, Low-Level) OR (Laser Therapies, Low-Level) OR (Laser Therapy, Low Level) OR (Low-Level Laser Therapies) OR (Laser Irradiation, Low-Power) OR (Irradiation, Low-Power Laser) OR (Laser Irradiation, Low Power) OR (Low-Power Laser Therapy) OR (Low Power Laser Therapy) OR (Laser Therapy, Low-Power) OR (Laser Therapies, Low-Power) OR (Laser Therapy, Low Power) OR (Low-Power Laser Therapies) OR (Low-Level Laser Therapy) OR (Low Level Laser Therapy) OR (Low-Power Laser Irradiation) OR (Low Power Laser Irradiation) OR (Laser Biostimulation) OR (Biostimulation, Laser) OR (Laser Phototherapy) OR (Phototherapy, Laser) OR (Phototherapy)[Mesh] OR (Phototherapy) OR (Phototherapies) OR (Therapy, Photoradiation) OR (Photoradiation Therapies) OR (Therapies, Photoradiation) OR (Light Therapy) OR (Light Therapies) OR (Therapies,

	Light) OR (Therapy, Light) OR (Photoradiation Therapy) OR (Lasers, Semiconductor)[Mesh] OR (Laser, Semiconductor) OR (Semiconductor Laser) OR (Semiconductor Lasers) OR (Semiconductor Diode Lasers) OR (Diode Laser, Semiconductor) OR (Diode Lasers, Semiconductor) OR (Laser, Semiconductor Diode) OR (Lasers, Semiconductor Diode) OR (Semiconductor Diode Laser) OR (Diode Lasers) OR (Diode Laser) OR (Laser, Diode) OR (Lasers, Diode) OR (light-emitting diode) OR (LED)
#2	(Coronary Artery Bypass)[Mesh] OR (Artery Bypass, Coronary) OR (Artery Bypasses, Coronary) OR "Bypasses, Coronary Artery) OR (Coronary Artery Bypasses) OR (Coronary Artery Bypass Surgery) OR (Bypass, Coronary Artery) OR (Aortocoronary Bypass) OR (Aortocoronary Bypasses) OR (Bypass, Aortocoronary) OR (Bypasses, Aortocoronary) OR (Bypass Surgery, Coronary Artery) OR (Coronary Artery Bypass Grafting) OR (CABG) OR (Myocardial Revascularization)[Mesh] OR (Myocardial Revascularizations) OR (Revascularization, Myocardial) OR (Revascularizations, Myocardial) OR (Internal Mammary Artery Implantation) (AND (Sternotomy)[Mesh] OR (Sternotomies" OR (Median Sternotomy) OR (Median Sternotomies) OR (Sternotomies, Median) OR (Sternotomy, Median) OR (Saphenous Vein)[Mesh] OR (Saphenous Veins) OR (Vein, Saphenous) OR (Veins, Saphenous) OR

	(Saphenectomy))
#3 *	(randomized controlled trial [pt] OR controlled clinical trial [pt] OR randomized controlled trials [mh] OR random allocation [mh] OR double-blind method [mh] OR single-blind method [mh] OR clinical trial [pt] OR clinical trials [mh] OR ("clinical trial"[tw]) OR ((singl*[tw] OR doubl*[tw] OR trebl*[tw] OR tripl*[tw]) AND (mask*[tw] OR blind*[tw])) OR ("latin square"[tw]) OR placebos [mh] OR placebo*[tw] OR random*[tw] OR research design [mh: noexp] OR comparative study [mh] OR evaluation studies [mh] OR follow-up studies [mh] OR prospective studies [mh] OR crossover studies [mh] OR control*[tw] OR prospectiv*[tw] OR volunteer*[tw]) NOT (animal [mh] NOT human [mh])
Search	#1 AND #2 AND #3

* Robinson KA, Dickersin K. Development of a highly sensitive search strategy for the retrieval of reports of controlled trials using PubMed. Int J Epidemiol. 2002; 31: 150–153.

Supplementary Table 2. The literature search strategy used for the EMBASE database.

Id.	**Search strategy**
#1	('low level laser therapy'/exp OR 'low level laser therapy' OR 'light emitting diode'/exp OR 'light emitting diode')
#2	('coronary artery bypass graft'/exp OR 'coronary artery bypass graft') OR 'heart muscle revascularization'/exp OR 'heart muscle revascularization' OR 'sternotomy'/exp OR 'sternotomy')
#3	('saphenous vein graft'/exp OR 'saphenous vein graft')

Search	#1 AND #2 AND #3 AND [humans]/lim AND [embase]/lim

Supplementary Table 3. The literature search strategy used for the SCiELO database.

Id.	**Search terms**
#1	Low level laser therapy
#2	Light emitting diode
#3	Coronary artery bypass
#4	Myocardial revascularization
#5	Sternotomy
#6	Saphenous vein graft
#7	#1 OR #2
#8	#3 OR #4 OR #5 OR #6
Search	*#7 AND #8*

Supplementary Table 4. The literature search strategy used for the PEDro database.

Advanced Search	***Search 1***	***Search 2***
Therapy	Electrotherapies, heat, cold	Electrotherapies, heat, cold
Problem	Pain	Skin lesions, wound, burn
Subdiscipline	Cardiothoracis	Cardiothoracis
Method	Clinical trial	Clinical trial

Supplementary Table 5. The literature search strategy used for the COCHRANE Central database.

Id.	**Search strategy**
#1	(“Low-level light therapy”) OR (“Phototherapy”) OR (“Lasers, Semiconductor”)
#2	(“coronary artery bypass”) OR (“myocardial revascularization”) OR (“sternotomy”) OR (“saphenous vein graft”)
#3	#1 AND #2
Limits	Trials

Author(s): Melina Hauck, Isadora R. Sisto, Miriam A. Zago Marcolino, Cinara Stein and Rodrigo D. M. Plentz.
Question: Should Light-Emitting Diodes Therapy (LEDT) vs. Placebo be used after Coronary Artery Bypass Grafting surgery (CABG)?
Setting: LEDT compared to Placebo.
Bibliography:
(Pain and Incision healing) De Oliveira RA, Fernandes GA, Lima ACG, et al (2014) The effects of LED emissions on sternotomy incision repair after myocardial revascularization: A randomized double-blind study with follow-up. Lasers Med Sci 29:1195–1202 . doi: 10.1007/s10103-013-1503-2
(Pain and Dehiscence) Lima ACG, Fernandes GA, Gonzaga IC, et al (2016) Low-Level Laser and Light-Emitting Diode Therapy for Pain Control in Hyperglycemic and Normoglycemic Patients Who Underwent Coronary Bypass Surgery with Internal Mammary Artery Grafts: A Randomized, Double-Blind Study with Follow-Up. Photomed Laser Surg 34:244–251 . doi: 10.1089/pho.2015.4049
(Incision Healing and Dehiscence) Lima ACG, Fernandes GA, De Barros Araújo R, et al (2017) Photobiomodulation (Laser and LED) on Sternotomy Healing in Hyperglycemic and Normoglycemic Patients Who Underwent Coronary Bypass Surgery with Internal Mammary Artery Grafts: A Randomized, Double-Blind Study with Follow-Up. Photomed Laser Surg 35:24–31 . doi: 10.1089/pho.2016.4143

Certainty assessment							№ of patients		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	LEDT	Placebo	Relative (95% CI)	Absolute (95% CI)		
Pain (follow-up: mean 8 days; assessed with: Visual Analogic Scale; Scale from: 0 to 11)												
3	randomised trials	not serious	not serious	not serious	serious[a,b]	none	49	97	-	MD **1.65 lower** (2.08 lower to 1.22 lower)	⨁⨁⨁◯ Moderate	IMPORTANT
Incision healing (follow-up: mean 8 days; assessed with: Photographic analysis; Scale from: 0 to 3)												
2	randomised trials	not serious	not serious	not serious	serious[a,b]	none	39	77	-	MD **0.77 lower** (0.94 lower to 0.6 lower)	⨁⨁⨁◯ Moderate	IMPORTANT
Dehiscence (follow-up: mean 8 days; assessed with: Score of incision closure; Scale from: 0 to 3)												
2	randomised trials	not serious	not serious	not serious	serious[a,b]	none	39	77	-	MD **0.57 lower** (0.67 lower to 0.46 lower)	⨁⨁⨁◯ Moderate	IMPORTANT

CI: confidence interval; **MD:** mean difference

Explanations

a. Confidence Interval 95%.
b. Low number of patients.

Author(s): Melina Hauck, Isadora R. Sisto, Miriam A. Zago Marcolino, Cinara Stein and Rodrigo D. M. Plentz.
Question: Should Low-Level Laser Therapy (LLLT) vs. Placebo be used after Coronary Artery Bypass Grafting surgery (CABG)?
Setting: LLLT compared to Placebo.
Bibliography:
(Pain, Incision healing and Dehiscente) Fernandes G, Araújo Júnior R, Lima A, et al (2017) Low-intensity laser (660 NM) has analgesic effects on sternotomy of patients who underwent coronary artery bypass grafts. Ann Card Anaesth 20:52–56 . doi: 10.4103/0971-9784.197836
(Pain) Lima ACG, Fernandes GA, Gonzaga IC, et al (2016) Low-Level Laser and Light-Emitting Diode Therapy for Pain Control in Hyperglycemic and Normoglycemic Patients Who Underwent Coronary Bypass Surgery with Internal Mammary Artery Grafts: A Randomized, Double-Blind Study with Follow-Up. Photomed Laser Surg 34:244–251 . doi: 10.1089/pho.2015.4049
(Incision Healing and Dehiscence) Lima ACG, Fernandes GA, De Barros Araújo R, et al (2017) Photobiomodulation (Laser and LED) on Sternotomy Healing in Hyperglycemic and Normoglycemic Patients Who Underwent Coronary Bypass Surgery with Internal Mammary Artery Grafts: A Randomized, Double-Blind Study with Follow-Up. Photomed Laser Surg 35:24–31 . doi: 10.1089/pho.2016.4143
(Dehiscente) Gonzaga ICA, De Barros Araújo R, Lima ACG, et al (2018) Effectiveness of Low-Intensity Laser Therapy on Tissue Repair Following Saphenectomy in Patients Who Underwent Coronary Artery Bypass Graft: A Randomized, Double-Blind Study. Photomed Laser Surg 36:18–23 . doi: 10.1089/pho.2017.4329

Certainty assessment							№ of patients		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	LLLT	Placebo	Relative (95% CI)	Absolute (95% CI)		
Pain (follow-up: mean 8 days; assessed with: Visual Analogic Scale; Scale from: 0 to 11)												
2	randomised trials	not serious	not serious	not serious	serious[a,b]	none	49	97	-	MD **1.51 fewer** (1.84 fewer to 1.19 fewer)	⨁⨁⨁◯ Moderate	IMPORTANT
Incision healing (follow-up: mean 8 days; assessed with: Photographic analysis; Scale from: 0 to 3)												
2	randomised trials	not serious	not serious	not serious	serious[a,b]	none	49	97	-	MD **0.95 lower** (1.4 lower to 0.5 lower)	⨁⨁⨁◯ Moderate	IMPORTANT
Dehiscence (follow-up: mean 8 days; assessed with: Score of incision closure; Scale from: 0 to 3)												
3	randomised trials	not serious	not serious	not serious	serious[a,b]	none	69	117	-	MD **0.5 lower** (0.59 lower to 0.41 lower)	⨁⨁⨁◯ Moderate	IMPORTANT

CI: confidence interval; **MD:** mean difference

Explanations

a. Confidence Interval 95% = −0.82; 0.00.
b. Low number of patients.

2 ARTIGO 2 (DEFESA DA TESE DE DOUTORADO)**.** Submissão para o periódico “Lasers in Medical Sciences”.

ENDOTHELIAL FUNCTION IN HEALTHY INDIVIDUALS AFTER PHOTOBIOMODULATION THERAPY WITH LOW-LEVEL LASER THERAPY (LLLT): CROSSOVER RANDOMIZED CONTROLED TRIAL

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DECLARATIONS

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Conflicts of interest. The authors declare that they have no conflict of interest.

Ethics approval. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Human Research Ethics Committee of UFCSPA (No 3.531.840).

Consent to participate. Informed consent was obtained from all individual participants included in the study.

Consent for publication. Not applicable.

Availability of data and material. Data are available with corresponding author.

Code availability. Not applicable.

Authors' contributions. All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Melina Hauck, Jociane Schardong, Gabriela Donini, Tatiana Coser Normann and Rodrigo Della Méa Plentz. The first draft of the manuscript was written by Melina Hauck and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

ABSTRACT

Purpose. Photobiomodulation therapy (PBM) causes stimulatory effects that raise cell metabolism and can be applied by low-level laser therapy (LLLT). The aim of the study was to evaluate the effects of LLLT on endothelial function of healthy individuals.

Methods. Triple-blinded crossover randomized controlled trial with 22 healthy volunteers aged 25.45 ± 4.85 years-old. The volunteers were randomized for application order of PBM with LLLT (810 nm, 1000 mW, 0.28 cm^2, 30 s): 1) 30 J (107 J/cm^2) per spot, 2) 60 J (214 J/cm^2) per spot or 3) placebo (equipment power off). The LLLT application was over the radial and ulnar artery region with a total of two parallel spots. Before and immediately after PBM therapy, the endothelial function (%FMD) was measured by the flow-mediated dilation technique by the high-resolution ultrasound.

Results. The %FMD increases after all PBM therapy interventions (pre *versus* post; p=0.016).

Conclusion. PBM with LLLT dose of 30 J and 60 J did not improve endothelial function. Higher energy densities may be necessary to causes vasodilation and a time-dependent dilation could exist.

Trial Registration Number: NCT03252184 (01/09/2017).

Keywords: Low-Level Light Therapy; Phototherapy; Endothelial function; Endothelium, vascular; Nitric oxide.

INTRODUCTION

Photobiomodulation (PBM) is a light therapy generated from a low-level laser therapy (LLLT) or light-emitting diode (LEDT) which causes stimulatory effects by the primary photochemical response with changes in the intracellular environment, and it's called photoactivated cells [1]. PBM has as main applications to promote cell growth and recovery from injury because of inflammatory signals' reduction and pain relief [2, 3]. Low levels of light are absorbed by chromophores like cytochrome C oxidase (CCO) causing nitric oxide (NO) and CCO photo-dissociation that reverses the mitochondrial respiration inhibition [4, 5]. The proposed mechanism is PBM may influence the cellular respiratory chain and the cell response because of stimulation of cellular metabolism [2, 3, 6, 7].

The LLLT is composed of coherent beams of a single wavelength in the visible to near-infrared spectrum [1, 7], not ablative or thermal mechanism [3], and is a noninvasive and inexpensive treatment [8]. Properties of light like tissue penetration and specific wavelength will influence the PBM effects and are sources of research [9]. Fluence (energy density) is referred to as dose, however, the right combination of individual values is more important than energy density and time [3]. It is assumed that increasing energy density could lead to vasodilation representing a dose-dependent photo dilation effect until the limit of biphasic dose-response [3, 10]. The PBM may increase NO bioavailability and the raising levels of NO could mediate light-induced vasodilation by nitric oxide synthase (NOS) during the conversion of L-arginine to L-citrulline in the oxygen-dependent pathway [10].

The NO, prostaglandin I2 and endothelium-derived hyperpolarizing factor contribute to vascular health, but NO plays a key role in vasodilation due to maintenance of basal vasodilator tone of blood vessels in response

to increased shear stress and certain pharmacologic stimuli contributing to endothelial function [11, 12]. Endothelial cells are endocrine organs with complex functions that line the entire circulatory system from the heart to the smallest capillaries [13–16]. These cells react sensibly to changes in the hemodynamic flow because mechanosensors on and in these cells, which leads to intracellular signaling cascades and/or structural proteins [16, 17].

Thus, endothelial function plays a role in a wide range of homeostatic functions by vasoactive factors that affect vasomotion, thrombosis, platelet aggregation, and inflammation [11, 14, 16]. Physiological stimulus like flow-mediated dilation (FMD) regulating vascular tone and homeostasis of the peripheral circulation mainly through NO release [11, 12], and the endothelial function evaluation by noninvasive FMD technique is considered a predictor of a cardiovascular event [12, 13, 18, 19] and all-cause mortality [19]. FMD could be the gold standard to understand the effects of treatments on peripheral artery conduit vessel function [11, 15, 18].

Previous experimental studies showed that LLLT caused endothelial cell proliferation [8], stimulated oxidative metabolism [8], dilation of coronary artery segments [20], and hypotension in spontaneously hypertensive rats [21]. Another clinical trial showed change in vascular endothelial function measured by glutathione levels, total antioxidant effect and angiogenic potential in healthy individuals after transdermal LLLT [24]. The largest number of studies is experimental, however, to the authors' knowledge, no study evaluated the endothelial function in response to PBM therapy with LLLT in humans. We hypothesized that different doses of LLLT may promote endothelium-dependent vasodilation. Thus, the aims of our study were to evaluate the effects of LLLT on the arterial endothelial function in healthy individuals.

METHODS

Design overview and compliance with ethical standards

This randomized placebo-controlled, triple-blinded, crossover clinical trial followed recommendations of the Consolidated Standards Of Reporting Trials (CONSORT) [25], was approved by the Human Research Ethics Committee (nº 3.531.840) of Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), and was registered in the ClinicalTrials.gov (NCT03252184). The evaluations were carried out at the Physiotherapy Laboratory of UFCSPA. All volunteers that fulfilled the eligibility criteria were informed of the study protocol and provided written free and informed consent.

Participants

All volunteers age between 18 to 33-years-old, body mass index (BMI) <30 kg/m2; non-smokers; without cutaneous/subcutaneous lesions in the left arm; and without skeletal muscle, rheumatic, cardiovascular, metabolic, neurological, oncological, immunological, hematological, psychiatric and cognitive disorders. The enrolled participants were not taking any type of medication. Before and during the interventions, the volunteers were requested to not drink alcohol, coffee or citrus juice, and do not perform intense physical activity 72 h before the evaluations. The exclusion criteria in the intervention's day were arterial diameter < 0.25 mm or > 0.50 mm and endothelial dysfunction assessed by FMD (%FMD < 8 %) [18, 26].

Outcomes

The outcome of this study was the endothelial function (%FMD) measured by FMD technique with high-resolution ultrasound and transducer, and the evaluation of endothelium-independent vasodilation by nitroglycerin.

Design, randomization, and follow-up

The sample of 24 healthy volunteers that fulfilled the eligibility criteria was organized into three blocks to perform a crossover study, and the randomized volunteers were submitted to the following treatments to evaluated endothelial function: 1) 30 J, 2) 60 J and 3) placebo. However, two volunteers were not included based on the exclusion criteria. **Figure 1** shows the CONSORT 2010 Flow diagram.

The randomization was performed using the website www.randomization.com by researcher (JS) and the information about this was sealed in a brown envelope. The brown envelope was opened immediately before the intervention by a trained team (GD, TN) to ensure concealment of allocation. The volunteers, and the outcomes assessor (MH) were blinded to the type of intervention. The volunteers used protective goggles and headphones with music for blinding and outcomes assessor was not in the room during interventions.

Intervention

The PBM therapy was performed with a cluster probe gallium-aluminum-arsenide (GaAlAs) diode laser Thor DD2 (Thor® Photomedicine Ltd, London, UK) emitting a wavelength of 810 nm that was calibrated by the biomedical sector of Hospital de Clínicas de Porto Alegre (Porto Alegre, RS, BR) before and after study. The energy of 30 J was delivered per cluster probe applied and the cluster probe was placed perpendicular to and in contact with the skin for a total of two parallel points. The LLLT parameters and local of points are in **Figure 2**. The points were applied over the radial and ulnar artery region (medial face) of the left arm located by palpation: 1) 30 J was applied by one active cluster probe and one placebo cluster probe; 2) 60 J was applied by two active cluster probe; and 3) placebo was applied by two inactive cluster probe. The treatments were performed on three days with 48 h intervals. The placebo treatment was made with the same LLLT and cluster probe equipment; however, the device was turned off and did not transmit any type of light therapy. The cluster probe was cleaned with 70 % alcohol between each volunteer.

Evaluation of endothelial function

All the evaluations were made with high-resolution ultrasound and 3-12 MHz linear transducer (Vivid-i GE, GE General Electric Company, USA). The endothelium-dependent vasodilation was evaluated by FMD technique with the arm cuff placed above transducer at the brachial artery in according to the American Heart Association Guidelines and adjustments [18, 26]. Ultrasound images were acquired before (baseline evaluation) and after the cuff disinflation around the upper arm (reactive hyperemia evaluation; until 120 seconds) like in **Figure 3**. The cuff was inflated to 50 mmHg above systolic blood pressure for five minutes. The cardiac cycle was monitoring by electrocardiographic and the onset of the R-wave was used to identify the diastole end, which corresponds to the minimum diameter of the artery. Thus, the brachial artery diameter was measured at the same time in the cardiac cycle. Vessel diameter measurements were made from an average of three consecutive heart cycles (three measurements per cycle) at baseline and reactive hyperemia evaluation. The average data of reactive hyperemia evaluation were compared to baseline evaluation and are expressed as percentage changes in

(%FMD). Mid-artery pulsed doppler velocity signals were used to evaluate basal blood flow and blood flow immediately after the cuff release (obtained no later than 15 seconds after cuff deflation). This evaluation was performed before and immediately after PBM therapy in the three days of treatment.

The endothelium-independent vasodilation was measured by sublingual nitroglycerin spray (NTG; 0.4 mg) and it is expressed like change in the vessel dilation before and after NTG. This evaluation was realized after all interventions in subjects with blood pressure within regular parameters (systolic blood pressure: 120 mmHg, diastolic blood pressure: 80 mmHg). The evaluations were carried out by a trained researcher in handling and image acquisition with ultrasound.

Data analysis

The sample size was estimated in 16 volunteers in each study group (30 J, 60 J, and placebo) considering 20 % losses while conducting the study based on a previous study [28]. Sample calculation considered an alpha error (5%) and beta error (95%) rates, 3.4 % of the difference between means and vasodilation (placebo versus pulsed waveform of therapeutic ultrasound), and 2.5 % of standard deviation. Sample characteristics are presented like mean and standard deviation. In the statistical analysis (version 23 software SPSS), the distribution of variables was tested by the Shapiro-Wilk normality test and was applied ANOVA for repeated measures over percent delta analysis of studies groups. Effect size was measured by Cohen's d. Results are presented as mean and standard error, or mean difference with 95% confidence intervals (95% CI). Probability of less than 5 % (p-value < 0.05) was considered to indicate statistical significance.

RESULTS

Participants

Twenty-four healthy individuals who fulfilled the eligibility criteria initially composed the sample size, however, two volunteers were excluded due to endothelial dysfunction (%FMD > 8 %). The clinical characteristics of 22 volunteers are presented in **Table 1**. The body max index and the waist-hip ratio of female and male were according to the World Health Organization [29].

Endothelium-dependent vasodilation and measurements of the brachial artery

The delta %FMD measurements (n= 22) in response to energy of 60 J, Placebo and 30 J PBM therapy are shown in **figure 4**. Statistical difference was not founded between interventions with small effect size (p=0.702; d de Cohen= 0.24). The mean of delta %FMD was 10.4% with 60 J, 7.3% with 30 J and 4.7% with placebo. Measurements of the brachial artery before and after PBM therapy in millimeters are presented ate **table 2**. Hyperemic arterial diameter increases after PBM with 30 J (mean difference= 0.518 mm, 95% CI= 0.44 to 0.59, p<0.001), 60J (mean difference= 0.496 mm, 95% CI= 0.42 to 0.57, p<0.001), but also with placebo (mean difference= 0.560 mm, 95% CI= 0.48 to 0.63, p<0.001) when compared to the baseline arterial diameter. The groups were not different from each other (p=0.856). There was no difference in blood flow (p=0.208) and hyperemic blood flow (p=0.704).

Endothelium-independent vasodilation

The endothelial integrity was evaluated by endothelium-independent vasodilation mediated by the smooth muscle of the vessels' relaxation. However, only 11 subjects could perform this measurement and the nitroglycerin spray increased the vessel diameter (pre nitroglycerin= 3.28 ± 0.47 mm versus post-nitroglycerin= 4.23 ± 0.35 mm, $p<0.0001$).

DISCUSSION

This randomized placebo-controlled, triple-blinded, crossover clinical trial shown that both 30 J (107 J/cm^2) and 60 J (214 J/cm^2) energies of PBM therapy LLLT (810 nm, 1000 mW, 0.28 cm^2, 30 s) not improved the endothelium-dependent vasodilator function in healthy volunteers. In this study about feasibility mechanisms over endothelium, the parameters were manipulated by the wavelength and energy density, and it can easily penetrate the tissues resulting in stimulatory effects on cells and tissues. However, depending on the area irradiated by this beam of photons, the power density and the cellular effects produced will be very different [3, 7].

It has been suggested that the light energy is absorbed by intracellular chromophores. The mitochondrial electron transport stimulation as shown by electrical charge across the mitochondrial and adenosine triphosphate (ATP) synthesis has been considered as the primary mechanism of PBM because of a shift in overall cell redox potential [3–5, 7]. This mitochondrial stimulation also produces chemical signaling molecules like cyclic adenosine monophosphate, NO and oxygen reactive species which active cell proliferation signaling pathways' transcription factors [4, 5, 7]. The greater oxidation could induce transcriptional changes like the activation of transcription factors such as nuclear factor kappa B (NF-κB) and growth factor production [3, 7]. In this mechanism, the NO also is a secondary messenger produced in a small amount from mitochondria by dissociation from intracellular stores [30], increasing the nitrite reductase activity of CCO [31], or by the greater activity of an isoform of the NOS [20].

Previous experimental studies showed endothelial cells exposed to diode laser (808 nm, 100 J/cm^2, and 150 J/cm^2) applied with handpiece device caused endothelial cell proliferation and stimulated oxidative metabolism [8]. Lower energy densities of 0.26 J/cm^2 with 632.5 nm LLLT also caused a proliferation of cells and increased NO [32], and a dose-dependent effect over cell proliferation with 2 and 4 J/cm^2 (635 nm LLLT) [33]. PBM applied to the rat's abdominal region with diode laser (660nm, 96 J/cm^2, 6 points) caused a reduction above 50% in systolic and diastolic arterial pressure and in the mean arterial pressure in a spontaneous hypertensive experimental model [21]. Besides these previous positive effects, the expected dose-response over endothelial function was not found in our results. It's important discuss about our sample size that was not achieved and may have influenced our results. Besides, the ambiental factors like temperature, silence and luminosity are controlled in part because of characteristic of the building and living room were conducted the treatments. The respect with requests by volunteers it's not guaranteed either.

The shorter wavelengths (600 to 700 nm) may be considered the best choice to treat the superficial tissue, whereas longer wavelengths (780 to 950 nm) are preferred to treat deeper tissues [7]. However, the biphasic dose-response curve of PBM may be observed in which low or high-power density and/or energy density may have excitatory or inhibitory effects [3, 9, 10]. Lower thresholds could lead to no different illumination from daylight and the upper thresholds (~750 mW/cm^2 at 800 to 900 nm) could be fixed by photothermal effect [7]. These concepts which are relevant to the successful PBM therapy application may have

affected our results because of the choice of the parameters. Another important factor is the number of photons that penetrate the tissue to arrive at target tissue which can be very low to cause the mitochondrial stimulation effect because of the optical properties of the tissue and the linear reciprocity [7]. Besides, the spot diameter at the surface of the target tissue could affect the distribution of energy across the tissue surface. The irradiation over the exact center of the beam will receive the indicated power output or more while tissue at the periphery of the irradiation will only receive about 13% of that power [7]. This way, the application of two parallel spots rather than two overlapping spots at tissue surface over the radial and ulnar artery region may not have generated the desired power density to causes the increase of endothelial function.

The time of brachial artery vasodilation in a time-dependent manner was found in previous experimental studies. Keszler and cols. (2017) [22] found a response to light in a time-dependent manner in blood vessel diameter exposed to an LEDT source (670 nm, 0, 0.75, 1.5, 3, 6, and 12 J/cm^2). The dilation of cultured endothelial cells increases over baseline after five minutes of exposition to PBM [22]. Besides, under pathological conditions of NO depletion, these cells produce NO independently from NOS enzyme activity [22]. However, in this study, only the chromaticity was explored, and possible results associated with energy density were not related. This time-dependent dilation may affect our result of endothelial function because result suggests that just four minutes after LLLT intervention is that the endothelial function must be measured. The only study that evaluated endothelial function in humans after exposition to PBM therapy also found results that seem support it [24]. The energy dose of 20 J/day totaling a total dose of 60 J (energy density of 1.6 mW/cm^2) applied with semiconductor laser (808 nm) modulates the endothelial function by increasing its antioxidative and angiogenic potential measured by an increase in reduced glutathione and a reduction in the angiostatin levels. However, only after 24 h after the last irradiation that blood samples were collected [24].

The effect of PBM therapy with LLLT over the endothelial function of healthy individuals there was not yet been tested using a direct measurement like FMD. Nitric oxide has important effects like suppression of the inflammatory response, inhibition of apoptosis, regulation of cell migration and angiogenesis [11, 13, 14, 26]. Nevertheless, endothelial dysfunction is characterized by reduced vasodilation, a proinflammatory state, and prothrombic properties due to free radicals which can disrupt the balance of NO decreasing its bioavailable, damage the endothelium and leave it overly permeable [11, 14]. It appears long before the formation of structural atherosclerotic changes [12] and reduced NO bioavailability for the microcirculation contributes to vasoconstriction [16]. This way, a possible mechanism of PBM therapy with LLLT over vascular cells with time-dependent dilation could be a therapeutic strategy for clinical problems like tissue healing which requires a dynamic and complex process of angiogenic response [8].

LIMITATIONS

As limitations of this study we can mention: 1) acute effects of PBM therapy with LLLT over endothelial function because of it seems that effects could be measured from four minutes after interventions; 2) the use of another wavelength and energy density; 3) absence of biochemical analysis that could adequately characterize our sample and evidence changes causing endothelial dysfunction; 4) thermography before and immediately after PBM interventions to explore if there are thermal effects; and 5) the sample that was composed mainly by women, but besides our attempts to recruit men, two of the men included were excluded due to endothelial dysfunction. However, besides limitations, we found good results that indicated its necessary a

higher energy density to enhance endothelial function and possible time-dependent dilation that could support future clinical trials in this and other populations.

CONCLUSION

This randomized placebo-controlled, triple-blinded, crossover clinical trial showed both 30 J (107 J/cm^2) and 60 J (214 J/cm^2) energies of PBM therapy with LLLT (810 nm, 1000 mW, 0.28 cm^2, 30 s) did not improve endothelial function. However, higher energy densities may be necessary to causes vasodilation and a time-dependent dilation could exist.

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Table 1. Clinical characteristics of the studied individuals.

Characteristic	**PBM therapy with LLLT (n= 22)**
Gender (men/%)	5/22,7%
Age (years)	25.45 ± 4.85
Systolic blood pressure (mmHg)	103.4 ± 11.59
Diastolic blood pressure (mmHg)	72.72 ± 8.11
Heart rate (bpm)	68.40 ± 5.22
Body max index (kg/m^2)	22.97 ± 2.88
Waist-hip ratio of female (cm)	0.74 ± 0.06
Waist-hip ratio of male (cm)	0.77 ± 0.15

PBM= Photobiomodulation; LLLT= Low-level laser therapy.

Table 2. Measurements of the brachial artery before and after laser interventions.

Endothelial function	**30J (n= 22)**	**60J (n= 22)**	**Placebo (n= 22)**
Baseline diameter (mm)	0.30 ± 0.36	0.29 ± 0.31	0.29 ± 0.32
Hyperemic diameter (mm)	0.35 ± 0.37*	0.34 ± 0.34*	0.35 ± 0.33*
p value	**0.001**	**0.001**	**0.001**

* hyperemic diameter versus baseline diameter.

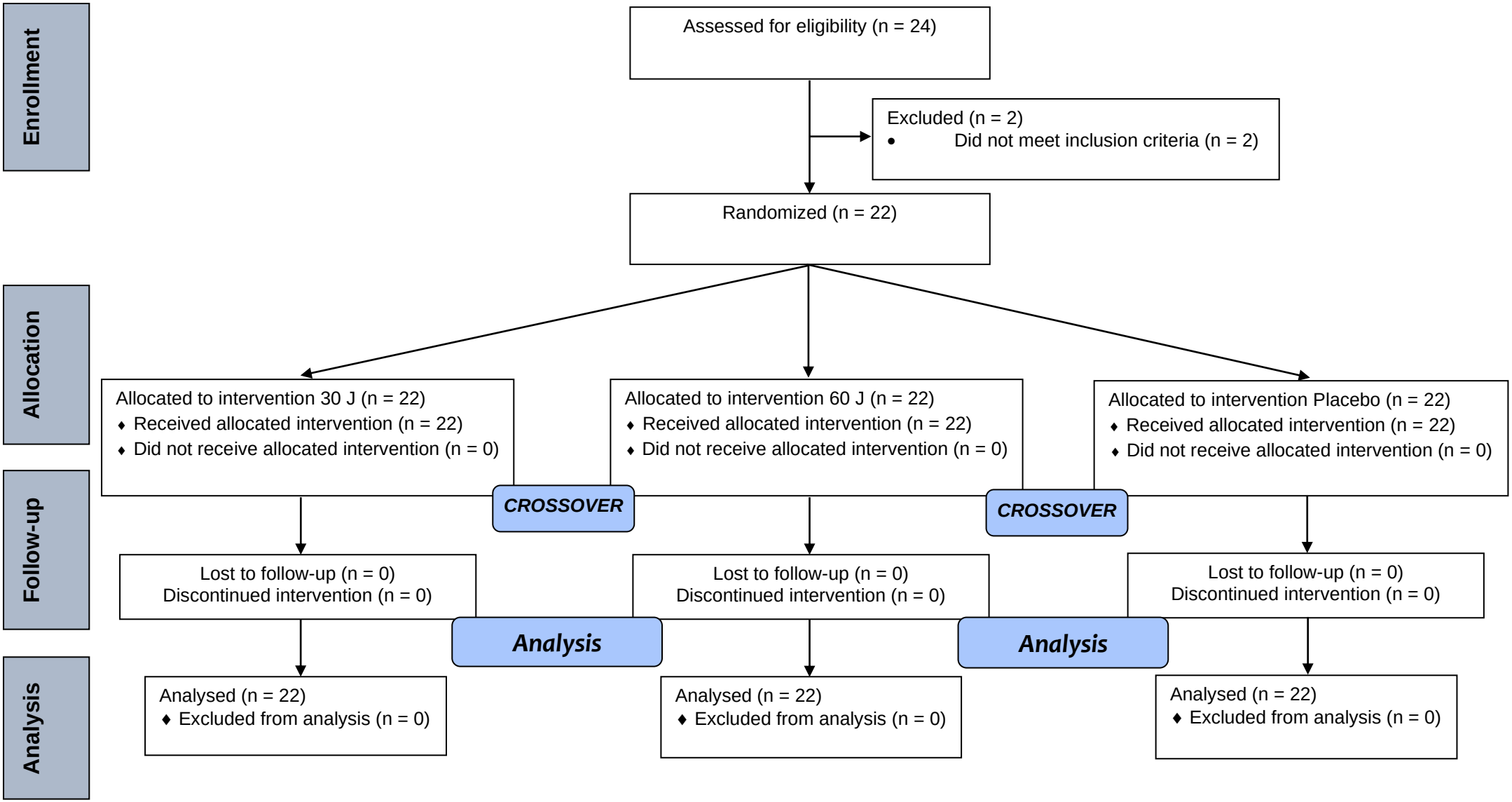
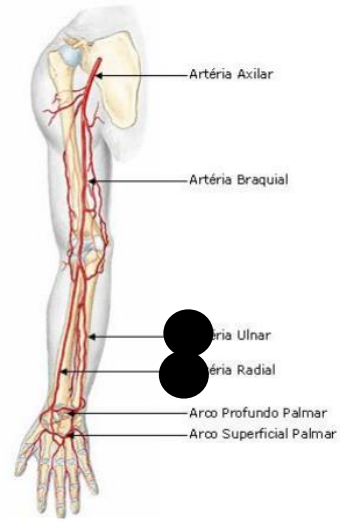


Figure 1. CONSORT 2010 Flow diagram of participants.



Parameters

Equipment: Thor DD2 control unit

Energy per diode (J): 6

Energy per cluster probe (J): 30

Treatment time per cluster probe (s): 30

Number of parallel cluster probe: 2

Figure 2. The parameters used by photobiomodulatio therapy with low-level laser therapy and the local of the cluster probe were Applied.

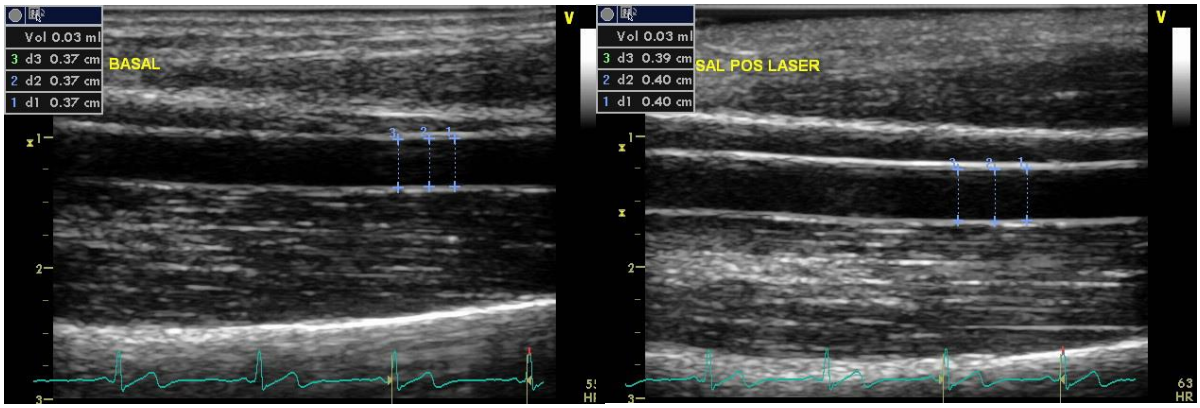


Figure 3. The measurement of endothelial function (%FMD) by ultrasound images that were acquired before (baseline evaluation: first image) and immediately after the cuff disinflation (reactive hyperemia evaluation: second image).

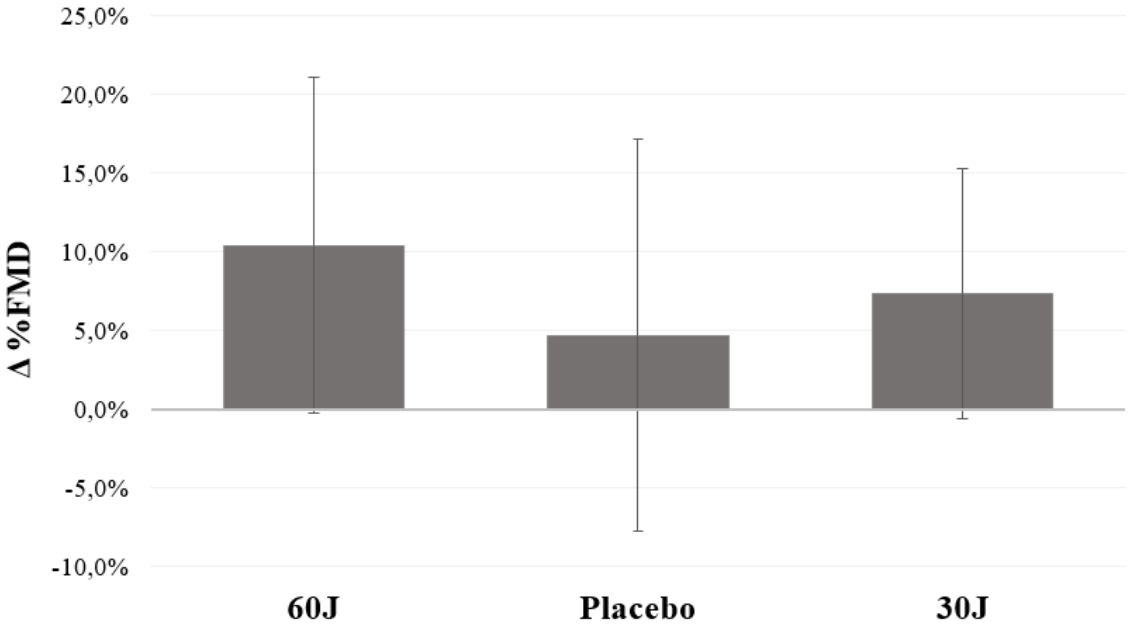


Figure 4. The results in percentage change (delta) of %FMD in all PBM therapy interventions.

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	2
	2b	Specific objectives or hypotheses	3
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	3
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	-
Participants	4a	Eligibility criteria for participants	3
	4b	Settings and locations where the data were collected	3
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	4
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	4
	6b	Any changes to trial outcomes after the trial commenced, with reasons	-
Sample size	7a	How sample size was determined	4
	7b	When applicable, explanation of any interim analyses and stopping guidelines	-
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	4
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	4
Allocation concealment	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	4

mechanism			
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	4
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	4
	11b	If relevant, description of the similarity of interventions	-
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	5
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	-
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	6
	13b	For each group, losses and exclusions after randomisation, together with reasons	5
Recruitment	14a	Dates defining the periods of recruitment and follow-up	5
	14b	Why the trial ended or was stopped	-
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	5
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	5
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	5
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	5
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	5
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	5
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	6
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	6
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	6
Other information			

Registration	23	Registration number and name of trial registry	4
Protocol	24	Where the full trial protocol can be accessed, if available	4
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	2

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

5 CONCLUSÃO

Os resultados desse estudo mostraram que: 1) a fotobiomodulação com LLLT (810 nm, 1000 mW, 0,28 cm^2, 30 s) de 30 J (107 J/cm^2) e 60 J (214 J/cm^2) não aumentaram a vasodilatação dependente do endotélio mensurada com a técnica FMD; e 2) a fotobiomodulação com LLLT (660 nm, 2,4 J por ponto, 19,2 J por sessão) e/ou LEDT (640 ± 20 nm, 10,6 J por ponto, 84,8 J por sessão) pode melhorar a dor, cicatrização tecidual e deiscência em pacientes no pós-operatório de cirurgia de revascularização do miocárdio.

As células endoteliais regulam diversas funções por meio da permeabilidade vascular, tônus vasomotor, homeostase vascular e da interação com as células sanguíneas. Dessa maneira, o endotélio controla a passagem de macromoléculas e fluidos e uma comunicação fluida entre suas células; equilibram o tônus dos vasos sanguíneos principalmente em resposta ao fluxo sanguíneo; produzem e secretam substâncias que controlam a atividade trombótica, de coagulação e de fibrinólise dentro dos vasos sanguíneos; mas também expressa substâncias e moléculas que auxiliam na angiogênese e vasculogênese. Ou seja, possui um papel central nos eventos cicatriciais. Também, pacientes com DCVs estabelecidas em potencial risco para evento cardiovascular, ou após evento cardiovascular, possuem alteração da função endotelial, processos ateroscleróticos e cascatas inflamatórias em decorrência da progressão da doença. Em adequadas condições fisiológicas, o endotélio contribui para a homeostase vascular, e por consequência, pode postergar a gênese e a progressão da aterosclerose.

Nossos resultados parecem sugerir que existe intervalo de tempo necessário para que ocorra um efeito vasodilatador após a aplicação da fotobiomodulação com LLLT, assim como a utilização de uma dose energética maior pode ser necessária para gerar uma resposta vascular; a manipulação de outros parâmetros como a aplicação duplicada de spots poderia influenciar essa resposta. Além disso, nossa revisão sistemática de ensaios clínicos randomizados mostrou que os sintomas álgicos podem ser reduzidos em 1,59 pontos e o processo cicatricial pode ser potencializado após cirurgia cardíaca com a utilização da fotobiomodulação com LLLT e/ou LEDT e sumarizou pontualmente os principais parâmetros utilizados para a obtenção desses resultados. Essas implicações clínicas poderão guiar a prática clínica baseada em evidências, e a fotobiomodulação pode ser uma possibilidade de intervenção terapêutica em DCVs, especialmente na reabilitação cardíaca Fase I (intra-hospitalar). Além disso, sabe-se que a disfunção endotelial é fator de risco para eventos adversos em pacientes com DCVs, e a possibilidade de melhorar a resposta vascular dependente do endotélio também pode vir a ser uma terapêutica importante. Assim,

recomenda-se a realização ensaios clínicos amplos com a utilização de outros parâmetros de irradiação com o LLLT de acordo com nossos resultados, bem como e em outras populações.

6 ANEXO

ANEXO A – Parecer do Comitê de Ética em Pesquisa com Seres Humanos - UFCSPA

UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE

Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Efeitos da Fotobiomodulação com Laser Terapêutico de Baixa Potência na Função Endotelial e nos Marcadores Sanguíneos e Endoteliais de Indivíduos Saudáveis.

Pesquisador: Rodrigo Della Méa Plentz
Área Temática:
Versão: 2
CAAE: 14167019.0.0000.5345
Instituição Proponente: Universidade Federal de Ciências da Saúde de Porto Alegre
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 3.531.840

Apresentação do Projeto:
Trata-se de um ensaio clínico randomizado cruzado (crossover), controlado e duplo-cego, que pretende aplicar o protocolo de fotobiomodulação com laser terapêutico de baixa potência em pessoas saudáveis com a finalidade de avaliar a função endotelial, da vasodilatação e dos marcadores sanguíneos de função endotelial, e temperatura tecidual. A amostra será composta por 24 sujeitos saudáveis selecionados pelos critérios de elegibilidade, e aleatoriamente randomizados quanto à ordem de intervenção dos grupos em cada uma das partes da pesquisa. Os indivíduos selecionados serão aleatoriamente distribuídos em uma ordem de quatro grupos homogêneos: grupo 1 (dose de energia de 2J), grupo 2 (dose de energia de 4J), grupo 3(dose de energia de 6J) e grupo 4 (placebo). Após serem submetidos a fotobiomodulação (laserterapia) serão realizadas avaliações, antes e imediatamente após a intervenção. Será realizada: avaliação da função endotelial arterial por meio da técnica da dilatação mediada pelo fluxo; avaliação da temperatura corporal pela termografia com Termovisor; avaliação do tempo de vasodilatação imediatamente após e por até 20min após a fotobiomodulação pelo ecógrafo; e análise de exames laboratoriais. Para a mensuração desses desfechos, a pesquisa será dividida em duas partes: 1- avaliação do efeito da fotobiomoduçação sobre a função endotelial; e 2- avaliação do efeito da vasodilatação imediatamente após e por até 20min após a fotobiomodulação. Todas as intervenções com

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fotobiomodulação serão realizadas com laser diodo cluster probe composto por cinco diodos devidamente calibrado (Thor® Photomedicine, DD2, Londres, Inglaterra) com os seguintes parâmetros de aplicação: comprimento de onda de 810nm com uma potência de saída de 1000mW (200mW por diodo); área do feixe de 0,029cm2; densidade de potência de 34,5Wcm2 (6,9J/cm2 por diodo); dose de 30J por cluster probe (6J em cada diodo); densidade de energia de 1.034J/cm2 (206,9J/cm2 por diodo); tempo de aplicação de 30s por cada aplicação do cluster probe; modo de emissão contínuo. Na primeira parte da pesquisa, para as intervenções nos grupos 1 e 2, serão utilizadas, respectivamente, as doses de energia de 30J e 60J. No grupo 3, será realizada a intervenção placebo com o equipamento desligado. Na segunda parte da pesquisa, o grupo 1 e 2 receberão as doses de energia de 30J e 60J.

Objetivo da Pesquisa:

GERAL: Avaliar os efeitos do fotobiomodulação laser terapêutico de baixa potência na função endotelial arterial de indivíduos saudáveis.

ESPECÍFICOS:1) Avaliar o tempo de vasodilatação da artéria braquial imediatamente após e por até 20 min após aplicação da fotobiomodulação.2) Avaliar os efeitos da fotobiomodulação nos marcadores sanguíneos (hemograma) e endoteliais (cGMP, L-arginina, nitrito e nitrato).3) Analisar associações entre os marcadores e os metabólitos com a função endotelial devido aos efeitos da fotobiomodulação.4) Analisar a temperatura local antes e após a fotobiomodulação.5)Analisar a temperatura local antes e após a mensuração da função endotelial arterial.6) Avaliar possíveis

associações entre os marcadores sanguíneos e endoteliais e função endotelial com a temperatura local.7) Comparar os efeitos das doses de energia da fotobiomodulação sobre a função endotelial, marcadores sanguíneos e endoteliais.

Avaliação dos Riscos e Benefícios:

Riscos: A intervenção realizada apresenta um risco mínimo ao sujeito. A fotobiomodulação é um procedimento não-invasivo e transcutâneo, realizado por meio da aplicação de feixes de luz sobre o braço na região da artéria radial e ulnar. Durante a sua aplicação, não há percepção de nenhuma sensação, e os indivíduos receberão um óculos adequado para a aplicação do laser diodo. Essa técnica é utilizada como recurso terapêutico principalmente por profissionais fisioterapeutas. O equipamento é um recurso seguro (pois mostra pouca capacidade de incidir danos) e de baixo custo na sua aplicação. As pesquisas científicas feitas para investigação dos efeitos desse tipo de energia de fótons perpetuam desde 1988, com um expressivo crescimento na última década no Brasil, todavia, ressalta-se a necessidade da condução de ensaios clínicos para o esclarecimento de mecanismos

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de ação e geração de efeitos visando o direcionamento da prática clínica.

Benefícios: Os benefícios associados à participação do indivíduo consistem em realizações de avaliações das condições: a) bioquímicas, b) vasculares e (c)hemodinâmicas. Todos os resultados de exames bioquímicos serão entregues aos participantes da pesquisa, além da interpretação dos resultados por meio do relato verbal em relação à responsividade vascular.

Comentários e Considerações sobre a Pesquisa:

Os pesquisadores esclareceram como os indivíduos serão selecionados, anexando o seguinte texto ao projeto na página 19 "Os participantes serão
estudantes dos cursos da UFCSPA ou universidades próximas, os quais serão
convidados a participar por meio de convite divulgado em redes sociais, ou
pessoalmente (Anexo 2). Caso o participante não esteja na universidade no dia das
avaliações, lhe forneceremos passagem de ônibus para custear sua ida e volta."

Os pesquisadores também esclareceram que os participantes serão convidados nas redes sociais ou pessoalmente; apresentaram o texto convite que será divulgado nas redes sociais; e informaram o meio de divulgação para seleção dos participantes. O TCLE foi ajustado conforme sugestões do parecer fornecido pelo CEP.

Considerações sobre os Termos de apresentação obrigatória:

Apresentam: Termo de Anuência do responsável pelo laboratório do Departamento de Fisioterapia; Termo de Compromisso da entrega de relatório; e TCLE modificado.Incluiram cartaz convite e carta resposta ao CEP.

Recomendações:

1) Incluir na PB o Projeto,em formato word, com as correções realizadas.

2) Levar ao CEP ou incluir na PB o TCLE sem marcações em amarelo, para que possa ser carimbado pelo CEP e retirado pelos pesquisadores para ser fornecido aos participantes.

Conclusões ou Pendências e Lista de Inadequações:

Atender aos dois itens descritos nas Recomendações deste Parecer, item anterior.

Considerações Finais a critério do CEP:

De acordo com o parecer do Relator.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação

Endereço: Rua Sarmento Leite ,245
Bairro: Sarmento **CEP:** 90.050-170
UF: RS **Município:** PORTO ALEGRE
Telefone: (51)3303-8804 **E-mail:** cep@ufcspa.edu.br

UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE

Continuação do Parecer: 3.531.840

Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1323708.pdf	03/07/2019 23:02:38		Aceito
Outros	CartazConvite.pdf	03/07/2019 23:02:03	Rodrigo Della Méa Plentz	Aceito
Outros	CartaCEP.pdf	03/07/2019 23:01:34	Rodrigo Della Méa Plentz	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.pdf	03/07/2019 23:01:01	Rodrigo Della Méa Plentz	Aceito
Outros	Relatorio.pdf	20/05/2019 22:28:51	Rodrigo Della Méa Plentz	Aceito
Folha de Rosto	FolhadeRosto.pdf	23/04/2019 13:11:25	Rodrigo Della Méa Plentz	Aceito
Outros	Anuencia.pdf	23/04/2019 13:09:56	Rodrigo Della Méa Plentz	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TermoConsentimentoLivreEsclarecido.pdf	28/03/2019 20:31:16	Rodrigo Della Méa Plentz	Aceito
Projeto Detalhado / Brochura Investigador	ProjetoDetalhado.pdf	28/03/2019 20:29:40	Rodrigo Della Méa Plentz	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

PORTO ALEGRE, 26 de Agosto de 2019

Assinado por:
Luciane Dalcanale Moussalle
(Coordenador(a))

Endereço: Rua Sarmento Leite ,245
Bairro: Sarmento **CEP:** 90.050-170
UF: RS **Município:** PORTO ALEGRE
Telefone: (51)3303-8804 **E-mail:** cep@ufcspa.edu.br

ANEXO B – *"Submission guidelines" da revista "European Heart Journal"*

Instructions to authors

1. Scope of the Journal

The *European Heart Journal* (EHJ) is an international, peer-reviewed journal, engaged in publishing the highest quality material, both clinical and scientific, on all aspects of Cardiovascular Medicine. It is an official Journal of the European Society of Cardiology (ESC) and is published 48 times a year. It includes articles related to research findings, technical evaluations, and clinical reviews. It also provides a forum for the exchange of information on all aspects of Cardiovascular Medicine, including educational issues.

1.1 Publication Ethics and Malpractice Statement

European Heart Journal and Oxford University Press are members of the Committee on Publication Ethics (COPE). This journal follows the guidance provided in COPE's Core Practices. The journal also subscribes to the International Committee of Medical Journal Editors (ICJME) Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly work in Medical Journals. The journal expects all parties involved in the publication of content in *European Heart Journal* (the publisher, editors, authors, and reviewers) to follow these guidelines on best practice and publication ethics. The Editors are further supported by the ESC Journal Family Ethics Committee.

2. Preparation of manuscripts

2.1 Article categories

Manuscript Type	General Information	Parameters	Figures	Tables	Misc.
Clinical Research Article	Innovative game-changing original clinical studies that significantly advance the field in the prevention, diagnosis and treatment of cardiovascular diseases. (case studies and reports not accepted).	<5000 words (excl. references, figure legends and tables) Up to 40 authors Structured Graphical abstract *	✔	✔	Only up to 30 references will be published; if more references are submitted, the reference list will be published online only.
Meta-analysis	Innovative meta-analyses, in particular in controversial	<5000 words (excl. referenc	✔	✔	Only up to 30 references will be published; if more references are submitted, the

Manuscript Type	General Information	Parameters	Figures	Tables	Misc.
	areas, which significantly advance the field in the prevention, diagnosis and treatment of cardiovascular diseases	es, figure legends and tables) Up to 40 authors			reference list will be published online only.
Translational Research article	Preclinical studies with relevant clinical implications, that are translatable to human disease. A Translational Perspective (up to 100 words) describing the value of the work in clinical operations is also required.	<5000 words (excl. references, figure legends and tables) Up to 40 authors Structured Graphical abstract *	✔	✔	Only up to 30 references will be published; if more references are submitted, the reference list will be published online only.
State of the Art Review	Scholarly, comprehensive clinical and translational reviews of a timely topic of high	<5000 words (excl. references, figure legends	✔	✔	Only up to 50 references will be published; if more references are submitted, the reference list will be published online only.

Manuscript Type	General Information	Parameters	Figures	Tables	Misc.
	relevance to the cardiovascular community, aiming to summarize and critically evaluate research in the field. Typically by invitation only, but proposals from known authorities will be considered.	and tables) Up to 40 authors Graphical abstract **			
		<1500 words max 15 references one figure or table Graphical abstract **			
Editorial	By invitation only.	Up to 3 authors	✔	✔	-
Viewpoint	Brief opinion pieces	<1500 words	✔	✔	-

Manuscript Type	General Information	Parameters	Figures	Tables	Misc.
	authored by leading experts in the field, covering a wide range of provocative topics, typically challenging current dogma or discussing a controversial issue, thus stimulating discussion. These articles reproduce the format utilized in the ESC annual meeting. One statement is proposed on a hot and controversial topic and the authors write in favour (pro) or against (contra) the proposed statement. The debates are preceded	max 15 references one figure or table Graphical abstract ** Up to 3 authors <5000 words (excl. references, figure legends and tables)			
Debate Article			✔	✔	Only up to 50 references will be published; if more references are submitted, the reference list will be published online only.

Manuscript Type	General Information	Parameters	Figures	Tables	Misc.
	by an introduction written by a member of the Editorial Board.				
Special article	Typically, these include position and consensus documents proposed by working groups, associations and task forces, and other papers not covered by the other article categories.	<5000 words (excl. references, figure legends and tables) Graphical abstract **	✔	✔	Only up to 50 references will be published; if more references are submitted, the reference list will be published online only.
Discussion Forum	An opportunity for readers to submit in-depth letters on Clinical Research articles published in EHJ in the last 6 months. We encourage our readers to discuss all other articles	<500 words (excl. references) One figure or table (if informative) Up to 5 references (incl. the	✔	✔	Authors of the discussed paper will be invited to reply to the discussion forum article if it is accepted for publication.

Manuscript Type	General Information	Parameters	Figures	Tables	Misc.
	via Twitter (or other social media platforms). Contributions should strike a constructive, professional and polite tone. No original data should be presented.	reference of the discussed paper). Up to 3 authors			
Cardiovascular Flashlight	Striking and illustrative clinical images depicted by electrocardiograms, echocardiograms, tomography images, X-rays, or pathology specimens. See 'Further guidance' below.	<250 words (excl. references) Up to 3 authors	✔	✗	Images in this category may be published on the cover of the Journal.
Case Reports	The Journal does not accept case reports. However, case reports can be	N/A	N/A	N/A	N/A

Manuscript Type	General Information	Parameters	Figures	Tables	Misc.
	submitted to European Heart Journal - Case Reports – an Open Access journal that publishes high quality, educationally valuable case reports, images, and quality improvement projects in all aspects of cardiology. See the Instructions to Authors of this journal for more information.				
CardioPulse	By invitation only	<1000 Max 10 references Up to 4 authors Up to 1 figure, image or video	✔	✗	These papers are invite only. EHJ welcomes suggestions for CardioPulse content. Please email cardiopulse.ehj@unicatt.it

*** Structured Graphical Abstracts:**

Authors of Clinical Research and Translational Research articles are advised to provide a Structured Graphical Abstract. This includes the following parts:

- Flowchart. Headings: Key question(s), key findings(s), take-home message(s) – up to 40 words under each heading
- Graphical Abstract

Please use this document to help you prepare your Structured Graphical Abstract. The Flowchart and the Graphical Abstract should be uploaded as separate files in Editorial Manager using the appropriate file name. Please provide both elements of the Structured Graphical Abstract at revise stage.

The Graphical Abstract element should clearly summarize the focus and findings of the article. This can be one of the key images/figures/graphs of the article. Please see more information about the preparation of figures.

**** Graphical Abstracts:**

Authors of State of the Art Reviews, Editorials, Viewpoints, and Special Articles are advised to provide a Graphical Abstract. The Graphical Abstract should clearly summarize the focus and findings of the article and will be published at the beginning of the contribution. This can be one of the key images/figures/graphs of the article.

The Graphical Abstract should be submitted for peer review at initial submission stage as a separate file (as a .tif, .tiff, .eps, .ai, or .pdf file type) using the appropriate file name in Editorial Manager. Please see more information about the preparation of figures.

Further guidance for Cardiovascular Flashlights:

- Images should be submitted as one figure, with separate designated panels, as required
- While the image may consist of individual panels, its outer perimeter should exhibit a 1:1 aspect ratio
- The clinical message contained in the picture should be amplified in a 250-word description (no references), which will be included with the image
- The image should be submitted with the names of no more than four authors
- With your submission, authors are required to provide a short abstract (ca 30 words) for administrative reasons and a short title
- Authors are encouraged to include videos (formats: avi or mp4) with the submission, which can be published in the article. If a video is submitted, a still image must also be provided for the printed article

- It is the Journal's editorial policy not to accept case studies or reports

2.2 Authorship

All individuals listed as authors should qualify for authorship and should have participated sufficiently in the work to take public responsibility for appropriate portions of the content. Authors included in the manuscript should meet all of the following conditions: 1) substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to be published. Any other contributors to the work who do not qualify for authorship should be listed in an acknowledgement section. For further information about authorship, please refer to the ICMJE guidelines.

Conflict of interest: All authors must declare any potential conflicts of interest. The Journal requires;

1. An International Committee of Medical Journal Editors (ICJME) disclosure of potential conflicts of interest (COI) form to be submitted for each author. All completed ICMJE forms must be received when a revised manuscript is submitted. Each form must have the author's surname in the document name. Forms must be submitted even if there is no conflict of interest. It is the responsibility of the corresponding author to ensure that all authors adhere to this policy.
2. A Conflict of Interest statement to be included under the "Disclosure" header in the submitted manuscript. This paragraph should contain all information in the summary section of the completed ICJME form (this can be copy and pasted directly), plus any information from the 'Comments' section of the form that is not incorporated into the summary. If no conflict exists, please state that 'The Author(s) declare(s) that there is no conflict of interest'.

A detailed definition of conflicts of interests can be found here.

Author Responsibility Information: The corresponding author is responsible for completing the Author Responsibility Information which can be found in the "Additional Information" section of the submission process in the EHJ Editorial Manager online submission system. This information must be completed by the corresponding author and it stipulates the roles and responsibilities of each individual author who contributes to the submission. This information is required for submission.

Registration of clinical trials: All clinical trials, in particular those involving pharmaceuticals, devices, or aspects relating to nutrition, should be registered prospectively in publicly accessible databases (Clinical Trials and Clinical Trials Register), and the paper should include registration numbers and the name of the register. EHJ requires clinical trials to be reported according to CONSORT guidelines.

Animal experimentation: EHJ aims at detailed and high quality reporting of animal experiments and suggests authors follow the ARRIVE guidelines when preparing their manuscript. Authors may be required to provide evidence that they obtained ethical and /or legal approval prior to conducting the research.

Author Queries: Authors may send queries concerning the submission process, review process, and journal procedures to ehj@unicatt.it. After the manuscript has been prepared in accordance with the Instructions to Authors, please go to the online submission system. First-time users must click "Register" on the navigation menu at the top of the screen. The system will send an automatic e-mail with the user name and password. Detailed guidelines for authors and reviewers are available at the submission site.

2.3 Manuscript Preparation

Word Count: All submitted manuscripts must not exceed 5000 words (or for Viewpoints 1500 words, Editorials 1500 words and Discussion Forum contributions 500 words), excluding tables, figure legends, and references. The number of tables and figures should be appropriate to the manuscript content and should not be excessive in number.

Style and spelling: Oxford English spelling should be used. Authors whose first language is not English are requested to have their manuscripts checked carefully before submission. This will greatly help expedite the review process by helping to ensure that the academic content of the paper is fully understood by journal editors and reviewers. There are many specialist language editing companies that offer editing services and you can use any of these. Authors are liable for all costs associated with such services.

Abbreviations: Standard SI units of measurement should only be used.

Sections of the manuscript: Clinical and Basic Science papers should be divided into the following sections: (1) Title page, (2) Abstract and Keywords, (3) Translational Perspective (translational aspects; applicable only for Basic Science papers), (4) Introduction, (5) Methods, (6) Results, (7) Discussion, (8) Acknowledgements, (9) References, (10) Figure legends, (11) Appendices, (12) Text tables, (13) Figures, and (14) Supplementary files (if any).

General format: Prepare the manuscript text using a Word processing package (save in .doc format). Submission of PDF text files is not permitted. Manuscripts should be double-spaced, including text, tables, legends, and references. Each page should be consecutively numbered and all pages must contain line numbers that restart at each page . Please avoid footnotes; use instead, and as sparingly as possible, parentheses within brackets. Enter text in the style and order of the journal. Type references in the correct order and style of the journal (see Reference Format below). Type unjustified, without hyphenation, except for compound words, and type headings in the style of the journal. Use the TAB key once for paragraph indents. Where possible, use Times New Roman for the

text font and Symbol for the Greek and special characters. Use the word processing formatting features to indicate Bold, Italic, Greek, Maths, Superscript, and Subscript characters. Clearly identify unusual symbols and Greek letters. Differentiate between the letter "O" and zero, and the letter "I" and the number 1. Mark the approximate position of each figure and table. Check the final copy of your paper carefully since any spelling errors may be retained in a typeset version.

Title page: The title page should include the following: (1) the title, (2) the name(s) of authors, (3) the institution(s) where the work was performed, (4) the position, institution, and location of all authors, (5) the telephone number, fax number, and e-mail address of the corresponding author, (6) the institutional affiliations of the authors (including corporate appointments) should be acknowledged in a footnote.

Abstract: All abstracts must be restricted in length to 250 words and should also be submitted as a separate file (for administrative purposes only). The abstract should be formatted with the following headings: (1) Aims, (2) Methods and Results, (3) Conclusion, (4) Keywords. A maximum of six keywords may be submitted.

Translational Perspective (for Basic Science papers only): A clinical summary of ca 100 words to provide the reader with a brief take-home message on relevant translational aspects for clinical applications. In the event of publication, this summary will appear below the abstract in both the online and print versions of the journal. It will also be included in the electronic Table of Contents sent to readers.

Tables: Tables should be typed with double spacing, but minimizing redundant space, and each table should be uploaded as a separate file. Wherever possible, tables should be submitted in portrait - as opposed to landscape - layout. Each table should be numbered in sequence using Arabic numerals. Tables should also have a title above and an explanatory footnote below.

Figures: Figures should be limited to the number necessary for clarity and must not duplicate data given in tables or in the text. Standard submissions should have no more than 8 total figures and tables. Any number exceeding this should be designated as supplementary online-only material. They must be suitable for high quality reproduction and should be submitted in the desired final printed size so that reduction can be avoided. Figures should be no larger than 125 (height) x 180 (width) mm (5 x 7 inches) and should be submitted under the respective header ("Figure") and in files separates from that of the main manuscript.

'One-sentence Summary': short non-technical summary stating the novelty of the article in simple language. Please use the third person, not first person (i.e. do not use 'I' or 'we').

Electronic submission of figures: Figures should be saved in TIFF format at a resolution of at least 300 pixels per inch at the final printed size for colour figures and photographs, and 1200 pixels per inch for black and white line drawings. While some formats can be converted into TIFFs by the publisher, conversion may alter the tones, resolution and contrast of the image. Digital colour art should be submitted in CMYK rather than RGB format, since the printing process requires colours to be separated into CMYK, and this conversion can alter the colour intensity and brightness. Please keep in mind that colours can appear differently on different screens and printers.

Photographs: Photographs should be of sufficiently high quality, i.e. JPG or TIFF formats with a minimum file size of 1 MB, and 300 dpi. Please ensure that the photographs are of high quality with respect to detail, contrast, and low noise, to enable them to withstand loss of contrast and detail inherent in the printing process.

EHJ does not charge for colour figures

Line drawings: Please provide these as clear, sharp illustrations, suitable for reproduction as submitted. All labeling should be on the original. Faint and grey shading or stippling will be lost upon reproduction and should be avoided. If a figure has various shadings, please ensure that it is easy to differentiate between them, using standard shadings (see the hard copy of the journal for examples). There should be sufficient white space between lines and dots to ensure the areas will not fill in and look grey. If stippling is used, this should be made up of clear black dots with visible white space between them. Ensure that the size of the lettering is in proportion with the overall dimensions of the drawing. Ideally, the drawings should be submitted in the desired final printed size to avoid reduction. If submitting line drawings, which require reduction, please check that the lettering will be clearly legible after the drawing has been reduced to the size at which it will be printed. After reduction, letters should not be smaller than 2 mm in height.

Videos: Videos can now be published in the online article with a still image of the video appearing in the print version. Authors should submit videos in mp4 or avi format. Still images to be used in the article must be provided and should represent as best as possible the main subject of the video. Video files should be clearly named as video 1, video 2 etc, and still images should be named ‘video 1 still image’. Any supplementary videos not published in the article should be uploaded as supplementary data (see Supplementary Data).

Figure and video legends: These should be on a separate, numbered manuscript sheet grouped under the heading "Legends" on a separate sheet of the manuscript after the References. Define all symbols and abbreviations used in the figure. Common abbreviations and others in the preceding text should not be redefined in the legend.

Acknowledgements: Substantive contributions of individuals should be noted in an Acknowledgements section and entered before the Conflict of Interest (COI) statement.

Declaration of Helsinki: The authors should state that their study complies with the Declaration of Helsinki, that the locally appointed ethics committee has approved the research protocol and that informed consent has been obtained from the subjects (or their legally authorized representative).

Meta-Analysis/Systematic Review - Additional Instructions: If your research is a systematic review or meta-analysis, the Editors recommend that it be registered on the PROSPERO platform.
For meta-analyses, please provide in a separate table - either in the manuscript or supplementary appendix - a summary of the included studies with the following information:

- Study Name (with reference to bibliography)
- Publication Year
- Study Design
- Sample Size
- Inclusion Criteria
- Exclusion Criteria
- Follow-up period
- Primary Outcomes
- Secondary Outcomes
- The link to the public trial registry, if applicable.

2.4 Reference format

References should be identified in the text by Arabic numerals and numbered in the order cited. All references should be compiled at the end of the article in a Vancouver-like style (i.e. author-number system), although complete information should be given for each reference, including the title of the article, abbreviated journal title, and page numbers. If there are six or more authors, then use the first three followed by 'et al.'

Personal communications, manuscripts in preparation, and other unpublished data should not be cited in the reference list but may be mentioned in parentheses in the text. Authors should obtain permission from the source to cite unpublished data. Titles of journals should be abbreviated in accordance with Medline. If a journal is not listed in Medline, its name should be written out in full.

Article citation example:

1. Schroeder S, Baumbach A, Mahrholdt H, *et al*. The impact of untreated coronary dissections on the acute and long-term outcome after intravascular ultrasound guided PTCA. Eur Heart J 2000;21:137-145. doi: 10.1053/euhj.1999.1754

Book citation example:

2. Nichols WW, Rourke MF. *Aging, High Blood Pressure and Disease in Human*. 3rd ed. London/Melbourne: Lea and Febiger; 1990. p91–101.

Chapter citation example:

3. Nichols WW, O'Rourke MF. Aging, high blood pressure and disease in humans. In: Arnold E, ed. McDonald's Blood Flow in Arteries: Theoretical, Experimental and Clinical Principles. 3rd ed. London/Melbourne/Auckland: Lea and Febiger; 1990. p398-420.

Webpage citation example:

4. Panteghini M. Recommendations on use of biochemical markers in acute coronary syndrome: IFCC proposals. eJIFCC 14. http://www.ifcc.org/ejifcc/vol14no2/1402062003014n.htm (28 May 2004)

Where the date in parenthesis refers to the access date.

Conference proceedings citation example:

5. Koza JR 23-O derivatives of OMT: highly active against H. *influenzae*. In: *Programs and Abstracts of the Forty-third Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL, 2003*. Abstract F-1187, p.242. American Society for Microbiology, Washington, DC, USA.

Lecture series citation example:

6. Koza JR The craft and career of writing (lecture, Northwestern University, Evanston, IL, April 26, 2000).

Unpublished material citation example:

7. Citation: Personal communication: (J. Bloggs, personal communication).

Electronic article citation example:

8. Oyama K, Raz I, Cahn A, et al. Obesity and effects of dapagliflozin on cardiovascular and renal outcomes in patients with type 2 diabetes mellitus in the DECLARE-TIMI 58 trial. *Eur Heart J*, doi: 10.1093/eurheartj/ehab530. Published online ahead of print 24/08/21

If using EndNote to facilitate referencing citations (not required for submission), this journal's style is available for use.

2.5 Statistics

The application of adequate statistical methods is a prerequisite for publication in the EHJ (for a basic statement see 'Uniform Requirements for Manuscripts Submitted to Biomedical Journals', Ann Intern Med 1997 126: 36-47). The rationale of the EHJ regarding the statistical methods applied is 'Be as simple as possible, but as sophisticated as needed'. For example, clinical trials with their formalized framework must meet more specific statistical standards than pathophysiological studies. Please follow this link for a summary of relevant points (and pitfalls) regarding study design, analysis and reporting. For studies with a sophisticated design, the collaboration of a professional statistician is recommended.

2.6 Permissions information

If illustrations or figures are to be duplicated from previously published work, written permission must be obtained from both the publisher and the author, and a credit line indicating the source must be added in the relevant Figure Legend. If text material (250 to 300 words) is to be reproduced from published sources, written permission is required from both publisher and author. For shorter quotations, it is sufficient to add a bibliographic credit. Letters containing reprint permission for the reproduction of either text or illustrations must be included in the manuscript upload. Please indicate if it was not possible to obtain permissions.

If all illustrations and figures in the manuscript are original, have not been previously published and therefore do not require permission to reproduce, please include the following statement in the file uploaded for Permissions Information: "The authors do hereby declare that all illustrations and figures in the manuscript are entirely original and do not require reprint permission."

2.7 Supplementary data

Supporting material that is not essential for inclusion in the main text of the manuscript, but would benefit the reader, can be made available as online-only content. The material should not be essential to understanding the conclusions of the paper, but should contain data that is additional or complementary and directly relevant to the article content. Such information might include more detailed methods, extended data sets/data analysis, list of investigators, or additional figures.

All supplementary data must be provided in suitable electronic formats (more information). All material to be considered as Supplementary data must be submitted at the same time as the main manuscript for peer review. It cannot be altered or replaced after the paper has been accepted for publication. Please

ensure that the material intended as Supplementary data is clearly indicated as such upon submission and is referred to in the main manuscript, where necessary.

2.8 Sources of funding

Details of all funding sources for the work in question should be given in a separate section entitled 'Funding'. This should appear before the 'Acknowledgements' section.

The following rules should be followed:

a. The sentence should begin: ‘This work was supported by …’
b. The full official funding agency name should be given, i.e. ‘the National Cancer Institute at the National Institutes of Health’ or simply 'National Institutes of Health' not ‘NCI' (one of the 27 sub-institutions) or 'NCI at NIH’ (full RIN-approved list of UK funding agencies)
c. Grant numbers should be complete and accurate and provided in brackets as follows: ‘[grant number ABX CDXXXXXX]’
d. Multiple grant numbers should be separated by a comma as follows: ‘[grant numbers ABX CDXXXXXX, EFX GHXXXXXX]’
e. Agencies should be separated by a semi-colon (plus ‘and’ before the last funding agency)
f. Where individuals must be specified for certain sources of funding, the following text should be added after the relevant agency or grant number 'to [author initials]'.

An example is given here: ‘This work was supported by the National Institutes of Health [P50 CA098252 and CA118790 to R.B.S.R.] and the Alcohol & Education Research Council [HFY GR667789].

Oxford Journals will deposit all NIH-funded articles in PubMed Central. Authors must ensure that manuscripts are clearly indicated as NIH-funded using the guidelines above.

Crossref Funding Data Registry: In order to meet your funding requirements, authors are required to name their funding sources, or state if there are none, during the submission process. For further information on this process or to find out more about CHORUS, visit the CHORUS initiative.

2.9 Availability of Data and Materials

Where ethically feasible, the *European Heart Journal* strongly encourages authors to make all data and software code on which the conclusions of the paper rely available to readers. Authors are required to include a Data Availability Statement in their article.

We suggest that data be presented in the main manuscript or additional supporting files, or deposited in a public repository whenever possible. Information on general repositories for all data types, and a list of recommended repositories by subject area, is available on the Research Data Policy page.

Data Availability Statement

The inclusion of a Data Availability Statement is a requirement for articles published in the *European Heart Journal*. Data Availability Statements provide a standardised format for readers to understand the availability of data underlying the research results described in the article. The statement may refer to original data generated in the course of the study or to third-party data analysed in the article. The statement should describe and provide means of access, where possible, by linking to the data or providing the required unique identifier.

More information and example Data Availability statements.

Data Citation

The *European Heart Journal* supports the Force 11 Data Citation Principles and requires that all publicly available datasets be fully referenced in the reference list with an accession number or unique identifier such as a digital object identifier (DOI). Data citations should include the minimum information recommended by DataCite:

- [dataset]* Authors, Year, Title, Publisher (repository or archive name), Identifier

*The inclusion of the [dataset] tag at the beginning of the citation helps us to correctly identify and tag the citation. This tag will be removed from the citation published in the reference list.

2.10 Reporting Demographic Information for Study Participants

The *EHJ* adopts the definitions of sex and gender proposed by the *Institute of Medicine Report*, the *United States National Institutes of Health* and the *Canadian Institutes of Heart Research*.

De-identified information on demographics (e.g. age, sex, race/ethnicity, and/or socioeconomic indicators) should be described where available. This information should be placed in the methods and/or results section of the main article and/or supplement. Authors are required to explain demographic variables that have been collected and are not included.

Parameter	Recommendations

Parameter	Recommendations
Age	Study inclusion and exclusion according to age should be mentioned in the methods section as should be stratification by age group. In the results section median values and range should be given.
Sex	This term refers to biological factors and information to this issue should be given in the methods section and data given in the results section for both females and males. If only one sex is investigated, the reason for that should be given.
Gender	This term refers to the cultural and psychosocial aspects. If appropriate this should be defined in the methods section and results given separately in the results section.
Ethnicity	If multi-ethnic populations are

Parameter	Recommendations
	studied, the classification should be defined in the methods section and the results given separately in the results section.

Lack of compliance will not prevent the publication of the manuscript, but it will be disclosed similarly to conflict of interest disclosures.

Sex stratified data should be uniformly provided (in the work when relevant, in the Appendix or Cloud when not), and codified in the International Committee of Medical Journal Editors (ICMJE) disclosure form.

Further information can be found in the *Sex and Gender Equity in Research* (SAGER) guidelines.

3. Review of manuscripts

3.1 Standard Review Process

All manuscripts submitted to the EHJ will be assessed by the Editorial Board. Some manuscripts will be returned to authors at this stage if they are deemed more appropriate for another journal, if the paper fails to meet submission requirements, or if they are deemed to have insufficient priority. Submissions that advance in the publication process will undergo appropriate peer review, and all papers provisionally accepted for publication will undergo a detailed statistical review.

3.2 Fast Track Review Process

Please note: Fast Track petitions are only considered for original research contributions.

To petition for fast track review status, corresponding authors must send their manuscript by e-mail to: fasttrack.eurheartj@zhh.ch, ensuring that the manuscript adheres to the EHJ's Instructions to Authors. An accompanying cover letter should detail why the authors deem the manuscript suitable for fast track review. All files accompanying the petition should be attached individually (no ZIP file). The Editorial Board will decide as to whether the manuscript is suitable for fast track or regular review. When petitioning for fast track review,

corresponding authors *should not* enter their manuscripts simultaneously as regular submissions

The Editorial Office will communicate within 48 hours whether or not the fast track review process has been approved. Alternately, the submission may be considered in a standard review process. Please note that fast track review does not in any way guarantee acceptance of the manuscript.

The EHJ Editorial Office will notify the corresponding authors if their manuscript has been selected for Fast Track review. At this time they must then submit the manuscript in its entirety in the Editorial Manager system and notify the EHJ Editorial Office immediately after submission (ehj@unicatt.it). The article type will then be converted into a Fast Track and reviewers will be invited.

For manuscripts entering fast track review, the initial Editorial decision will be made within 5 consecutive days. If provisionally accepted, a revised manuscript must be returned to the EHJ Editorial Office as stipulated in the relevant decision letter. If the manuscript is accepted for publication, it should be published online 10 days after acceptance and in print as soon as possible, provided galley proofs are returned to the publisher within 48 hours. The corresponding authors will receive a 'Welcome to Oxford Journals' email, which will notify them of the DOI of their paper and contain a link to the online license to publish, which must be completed before the paper can be published.

3.3 Appealing a Decision

If you have reason to believe that the review process or final decision has not been fair or well-informed, you may submit an appeal via email to the Editorial Office (ehj@unicatt.it). The appeal should be provided in a word document as an attachment to the email and should not exceed 2 pages.

Appeals can be submitted within 1 month of the final decision on the manuscript. Appeals received after this date will not be considered. Please note that the Journal does not consider appeals for manuscripts that were rejected without peer review.

The appeal should include:

- Author name
- Manuscript title
- Manuscript ID
- An explanation regarding why you feel that the decision was unfair or not merited.
- Specific comments in relation to the reviewer reports
- Email and contact details

The appeal will be considered carefully by the Editor-in-Chief and Editorial Board at *European Heart Journal*. The Journal will endeavour to respond as quickly as possible. Please note that we will consider one appeal per manuscript.

4. Other manuscript processing

4.1 Manuscript transfer within the ESC Journal Family

Oxford University Press (OUP) and the European Society of Cardiology (ESC) have introduced a means for transfer of manuscripts among the ESC family journals. Authors submitting to the EHJ will be given the opportunity to indicate whether or not their manuscript could be considered for transfer to a specialty journal if the EHJ is unable to consider their manuscript further.

If authors agree during the upload process to have their manuscript transferred, and the manuscript is henceforth approved for transfer, there will be no need for re-submission and any reviewer comments will be transferred, resulting in a reduced time to a decision. Please follow this link for more information.

5. Manuscript Acceptance

5.1 Copyright Information

It is a condition of publication in the EHJ that authors grant an exclusive licence to the ESC. This ensures that requests from third parties to reproduce articles are handled efficiently and consistently and thus ensures that the article is disseminated as widely as possible. As part of the licence agreement, authors may use their own material in other publications, provided the EHJ is acknowledged as the original place of publication, and Oxford University Press (OUP) is notified in writing in advance. Upon receipt of accepted manuscripts at OUP, authors will be invited to complete an online licence to publish form.

Please note that by submitting an article for publication, OUP will retain the email address of the corresponding author for the purpose of further communication regarding the article. In the event of a change of personal information, OUP must be informed immediately. Upon acceptance for publication, OUP will contact the corresponding author directly. Please note that OUP does not retain copies of rejected articles. Please add eurheartj.oup@kwglobal.com to your safe senders list to avoid key emails about the publication of your article potentially going into your spam folder.

5.2 Proofs

Page proofs will be submitted to the corresponding author electronically. These should be checked thoroughly for any changes or typographic errors.

It is the publisher's intent to review and correct the proofs and publish the accepted work as soon as possible. To achieve this, it is mandatory that all corrections are returned to OUP within 3 days. Subsequent additional corrections will not be possible, hence please ensure that all amendments are marked up comprehensively in the proofs.

5.3 Publication embargos

If authors have embargos on papers, for example, if they are presenting their research results at a subsequent conference, publication can be delayed accordingly. Authors should include a note in the cover letter at submission, and also when returning proofs, about the embargo and the exact date and time the paper can be published.

The European Society of Cardiology may promote and make available to certain parties the finalised version of an article shortly prior to publication in the journal.

5.4 Media Activity

In the event of manuscript acceptance, the authors may elect to issue a press release. In this case, please contact the publisher, OUP (wordmason@mac.com) or the ESC (press@escardio.org), copying the EHJ Editorial Office (ehj@unicatt.it) in all correspondence.

5.5 Open access option for authors

EHJ offers the option of publishing under either a standard licence or an open access licence. Please note that some funders require open access publication as a condition of funding. If you are unsure whether you are required to publish open access, please do clarify any such requirements with your funder or institution.

Should you wish to publish your article open access, you should select your choice of open access licence in our online system after your article has been accepted for publication. You will need to pay an open access charge to publish under an open access licence.

Details of the open access licences and open access charges.

OUP has a growing number of Read and Publish agreements with institutions and consortia which provide funding for open access publishing. This means authors from participating institutions can publish open access, and the institution may pay the charge. Find out if your institution is participating.

Third-Party Content in Open Access papers

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5.6 Preprint policy

Authors of original research and review articles, excluding ESC Guidelines, retain the right to make an Author's Original Version (pre-print) available through various channels, and this does not prevent submission to the Journal provided that the following conditions are met:

1. During submission, authors must acknowledge pre-print server deposition and provide any associated accession numbers or DOIs;
2. Versions of a manuscript that have been altered as a result of the peer review process may not be deposited;
3. The pre-print version cannot itself have been indexed in MEDLINE or PubMed;
4. Upon publication, authors are responsible for updating the archived pre-print with a DOI and link to the published version of the article.

Should the paper be accepted and published in the Journal, the authors are required to update the status of any preprint, including your published paper's DOI, as described on our Author Self-Archiving policy page. The Journal DOI should be considered as the one representing this published work in all credits, citation, and attribution. Sharing of data from manuscripts that are under review or accepted but not yet published is expressly forbidden, unless permission is received from the Journal Editorial Office.

For further information see our Online Licensing, Copyright and Permissions policies.

5.7 Self-archiving and post-print policy

Authors of all article types, excluding ESC Guidelines, may enter their Accepted Manuscript (post-print) in PubMedCentral, other subject repositories, or institutional repositories so long as it is clearly stipulated that public availability be delayed by 12 months after the first online publication. For further details on this policy, please visit: Author Self-archiving Policy.

5.8 Online access and offprints

Details of free online access will be sent to the corresponding author, who may then circulate them to co-authors. Offprints can be claimed using the Oxford Journals Author Services site, and the corresponding author will receive a link when the paper enters production. Late orders submitted after the journal is in press are subject to increased prices.

ANEXO C – *"Submission guidelines" da revista "Lasers in Medical Science"*

Instructions for Authors

Types of papers

- Original Article – limited to 4000 words, 45 references, no more than 5 figures
- Review Article – limited to 5000 words, 50 references, no more than 5 figures
- Brief Report - limited to 2000 words, 25 references, no more than 4 figures -

Case Reports will not be accepted!

- Letter to the Editor – up to 600 words

Manuscript Submission

Manuscript Submission

Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication anywhere else; that its publication has been approved by all co-authors, if any, as well as by the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out. The publisher will not be held legally responsible should there be any claims for compensation.

Permissions

Authors wishing to include figures, tables, or text passages that have already been published elsewhere are required to obtain permission from the copyright owner(s) for both the print and online format and to include evidence that such permission has been granted when submitting their papers. Any material received without such evidence will be assumed to originate from the authors.

Online Submission

Please follow the hyperlink "Submit online" on the right and upload all of your manuscript files following the instructions given on the screen.

Please ensure you provide all relevant editable source files. Failing to submit these source files might cause unnecessary delays in the review and production process.

Editorial Procedure

Double-blind peer review

This journal follows a double-blind reviewing procedure. Authors are therefore requested to submit:

A blinded manuscript without any author names and affiliations in the text or on the title page. Self-identifying citations and references in the article text should be avoided.

A separate title page, containing title, all author names, affiliations, and the contact information of the corresponding author. Any acknowledgements, disclosures, or funding information should also be included on this page.

Title page

Title Page

Please use this **template title page** for providing the following information.

The title page should include:

- The name(s) of the author(s)
- A concise and informative title

- The affiliation(s) of the author(s), i.e. institution, (department), city, (state), country
- A clear indication and an active e-mail address of the corresponding author
- If available, the 16-digit ORCID of the author(s)

If address information is provided with the affiliation(s) it will also be published. For authors that are (temporarily) unaffiliated we will only capture their city and country of residence, not their e-mail address unless specifically requested.

Abstract

Please provide a structured abstract of 150 to 250 words which should be divided into the following sections:

- Purpose (stating the main purposes and research question)
- Methods
- Results
- Conclusion

For life science journals only (when applicable)

Trial registration number and date of registration

Trial registration number, date of registration followed by “retrospectively registered”

Keywords

Please provide 4 to 6 keywords which can be used for indexing purposes.

Declarations

All manuscripts must contain the following sections under the heading 'Declarations'. If any of the sections are not relevant to your manuscript, please include the heading and write 'Not applicable' for that section.

To be used for non-life science journals

Funding (information that explains whether and by whom the research was supported)

Conflicts of interest/Competing interests (include appropriate disclosures)

Availability of data and material (data transparency)

Code availability (software application or custom code)

Authors' contributions (optional: please review the submission guidelines from the journal whether statements are mandatory)

To be used for life science journals + articles with biological applications

Funding (information that explains whether and by whom the research was supported)

Conflicts of interest/Competing interests (include appropriate disclosures)

Ethics approval (include appropriate approvals or waivers)

Consent to participate (include appropriate statements)

Consent for publication (include appropriate statements)

Availability of data and material (data transparency)

Code availability (software application or custom code)

Authors' contributions (optional: please review the submission guidelines from the journal whether statements are mandatory)

Please see the relevant sections in the submission guidelines for further information as well as various examples of wording. Please revise/customize the sample statements according to your own needs.

Text

Text Formatting

Manuscripts should be submitted in Word.

- Use a normal, plain font (e.g., 10-point Times Roman) for text.
- Use italics for emphasis.
- Use the automatic page numbering function to number the pages.
- Do not use field functions.
- Use tab stops or other commands for indents, not the space bar.
- Use the table function, not spreadsheets, to make tables.
- Use the equation editor or MathType for equations.
- Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).

Manuscripts with mathematical content can also be submitted in LaTeX.

Headings

Please use no more than three levels of displayed headings.

Abbreviations

Abbreviations should be defined at first mention and used consistently thereafter.

Footnotes

Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.

Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols.

Always use footnotes instead of endnotes.

Acknowledgments

Acknowledgments of people, grants, funds, etc. should be placed in a separate section on the title page. The names of funding organizations should be written in full.

Scientific style

Generic names of drugs and pesticides are preferred; if trade names are used, the generic name should be given at first mention.

Units and abbreviations

- Please adhere to internationally agreed standards such as those adopted by the commission of the International Union of Pure and Applied Physics (IUPAP) or defined by the International Organization of Standardization (ISO). Metric SI units should be used throughout except where non-SI units are more common [e.g. litre (l) for volume].

- Abbreviations (not standardized) should be defined at first mention in the abstract and again in the main body of the text and used consistently thereafter.

Drugs

- When drugs are mentioned, the international (generic) name should be used. The proprietary name, chemical composition, and manufacturer should be stated in full in Materials and methods.

References

Citation

Reference citations in the text should be identified by numbers in square brackets. Some examples:

1. Negotiation research spans many disciplines [3].
2. This result was later contradicted by Becker and Seligman [5].
3. This effect has been widely studied [1-3, 7].

Reference list

The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text. Do not use footnotes or endnotes as a substitute for a reference list.

The entries in the list should be numbered consecutively.

- Journal article

Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. https://doi.org/10.1007/s00421-008-0955-8

Ideally, the names of all authors should be provided, but the usage of “et al” in long author lists will also be accepted:

Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. N Engl J Med 965:325–329

- Article by DOI

Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. J Mol Med. https://doi.org/10.1007/s001090000086

- Book

South J, Blass B (2001) The future of modern genomics. Blackwell, London

- Book chapter

Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257

- Online document

Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

- Dissertation

Trent JW (1975) Experimental acute renal failure. Dissertation, University of California

Always use the standard abbreviation of a journal’s name according to the ISSN List of Title Word Abbreviations, see

ISSN.org LTWA

If you are unsure, please use the full journal title.

For authors using EndNote, Springer provides an output style that supports the formatting of in-text citations and reference list.

EndNote style (Download zip, 4 kB)

Authors preparing their manuscript in LaTeX can use the bibtex file spbasic.bst which is included in Springer's LaTeX macro package.

Tables

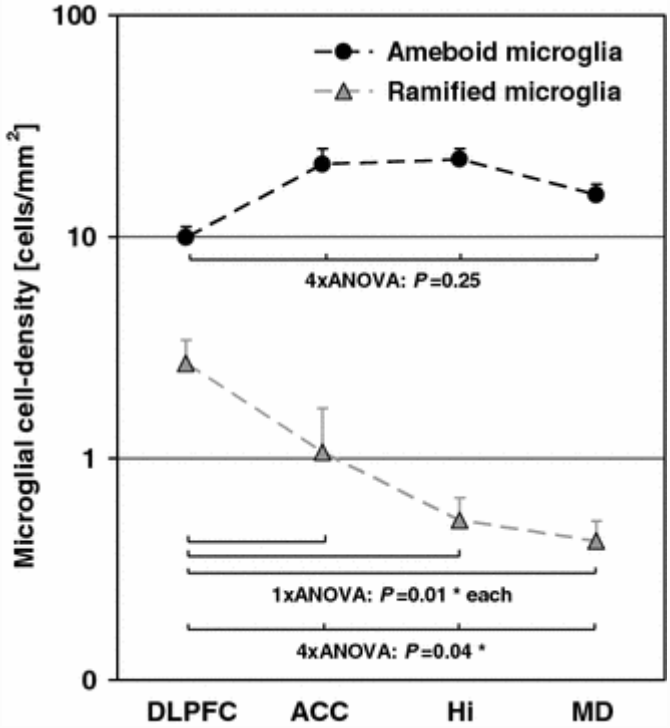
- All tables are to be numbered using Arabic numerals.
- Tables should always be cited in text in consecutive numerical order.
- For each table, please supply a table caption (title) explaining the components of the table.
- Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.
- Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

Artwork and Illustrations Guidelines

Electronic Figure Submission

- Supply all figures electronically.
- Indicate what graphics program was used to create the artwork.
- For vector graphics, the preferred format is EPS; for halftones, please use TIFF format. MSOffice files are also acceptable.
- Vector graphics containing fonts must have the fonts embedded in the files.
- Name your figure files with "Fig" and the figure number, e.g., Fig1.eps.

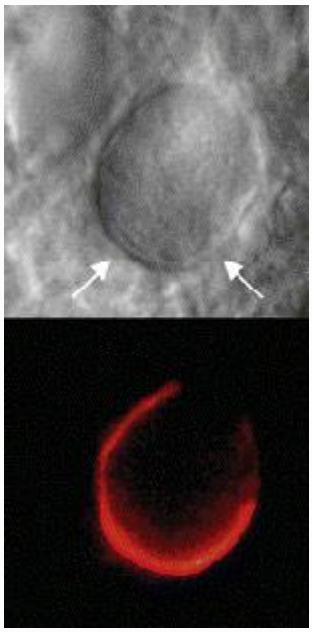
Line Art



- Definition: Black and white graphic with no shading.
- Do not use faint lines and/or lettering and check that all lines and lettering within the figures are legible at final size.

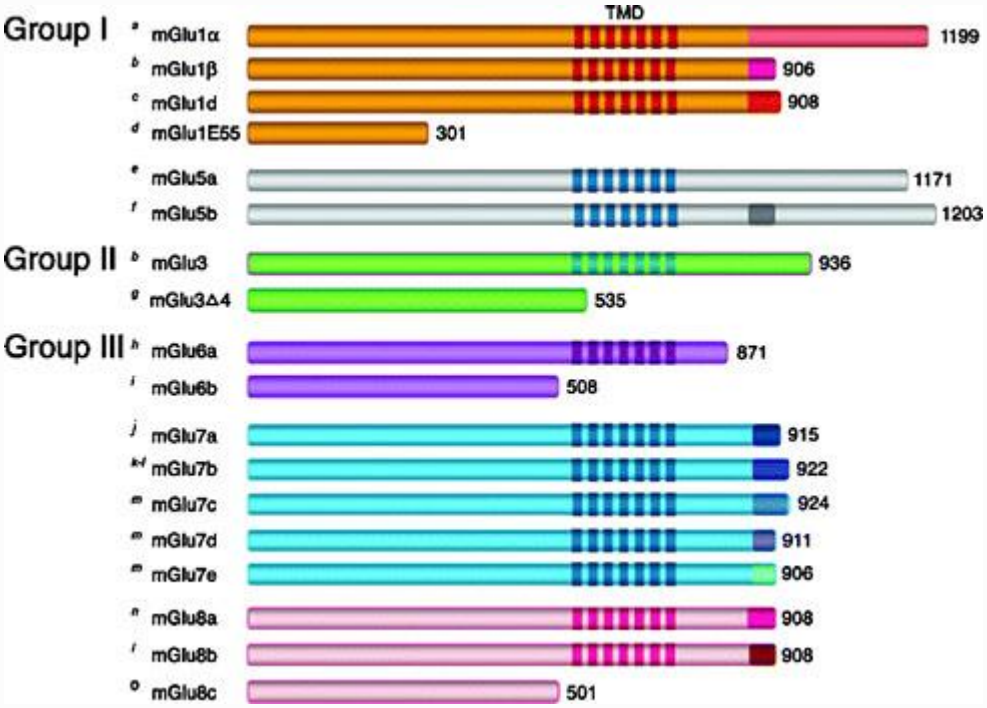
- All lines should be at least 0.1 mm (0.3 pt) wide.
- Scanned line drawings and line drawings in bitmap format should have a minimum resolution of 1200 dpi.
- Vector graphics containing fonts must have the fonts embedded in the files.

Halftone Art



- Definition: Photographs, drawings, or paintings with fine shading, etc.
- If any magnification is used in the photographs, indicate this by using scale bars within the figures themselves.
- Halftones should have a minimum resolution of 300 dpi.

Combination Art



- Definition: a combination of halftone and line art, e.g., halftones containing line drawing, extensive lettering, color diagrams, etc.
- Combination artwork should have a minimum resolution of 600 dpi.

Color Art

- Color art is free of charge for online publication.
- If black and white will be shown in the print version, make sure that the main information will still be visible. Many colors are not distinguishable from one another when converted to black and white. A simple way to check this is to make a xerographic copy to see if the necessary distinctions between the different colors are still apparent.
- If the figures will be printed in black and white, do not refer to color in the captions.
- Color illustrations should be submitted as RGB (8 bits per channel).

Figure Lettering

- To add lettering, it is best to use Helvetica or Arial (sans serif fonts).
- Keep lettering consistently sized throughout your final-sized artwork, usually about 2–3 mm (8–12 pt).
- Variance of type size within an illustration should be minimal, e.g., do not use 8-pt type on an axis and 20-pt type for the axis label.
- Avoid effects such as shading, outline letters, etc.
- Do not include titles or captions within your illustrations.

Figure Numbering

- All figures are to be numbered using Arabic numerals.
- Figures should always be cited in text in consecutive numerical order.
- Figure parts should be denoted by lowercase letters (a, b, c, etc.).
- If an appendix appears in your article and it contains one or more figures, continue the consecutive numbering of the main text. Do not number the appendix figures,"A1, A2, A3, etc." Figures in online appendices (Electronic Supplementary Material) should, however, be numbered separately.

Figure Captions

- Each figure should have a concise caption describing accurately what the figure depicts. Include the captions in the text file of the manuscript, not in the figure file.
- Figure captions begin with the term Fig. in bold type, followed by the figure number, also in bold type.
- No punctuation is to be included after the number, nor is any punctuation to be placed at the end of the caption.
- Identify all elements found in the figure in the figure caption; and use boxes, circles, etc., as coordinate points in graphs.
- Identify previously published material by giving the original source in the form of a reference citation at the end of the figure caption.

Figure Placement and Size

- Figures should be submitted separately from the text, if possible.
- When preparing your figures, size figures to fit in the column width.
- For large-sized journals the figures should be 84 mm (for double-column text areas), or 174 mm (for single-column text areas) wide and not higher than 234 mm.

- For small-sized journals, the figures should be 119 mm wide and not higher than 195 mm.

Permissions

If you include figures that have already been published elsewhere, you must obtain permission from the copyright owner(s) for both the print and online format. Please be aware that some publishers do not grant electronic rights for free and that Springer will not be able to refund any costs that may have occurred to receive these permissions. In such cases, material from other sources should be used.

Accessibility

In order to give people of all abilities and disabilities access to the content of your figures, please make sure that

- All figures have descriptive captions (blind users could then use a text-to-speech software or a text-to-Braille hardware)
- Patterns are used instead of or in addition to colors for conveying information (colorblind users would then be able to distinguish the visual elements)
- Any figure lettering has a contrast ratio of at least 4.5:1

Electronic Supplementary Material

Springer accepts electronic multimedia files (animations, movies, audio, etc.) and other supplementary files to be published online along with an article or a book chapter. This feature can add dimension to the author's article, as certain information cannot be printed or is more convenient in electronic form.

Before submitting research datasets as electronic supplementary material, authors should read the journal's Research data policy. We encourage research data to be archived in data repositories wherever possible.

Submission

- Supply all supplementary material in standard file formats.
- Please include in each file the following information: article title, journal name, author names; affiliation and e-mail address of the corresponding author.
- To accommodate user downloads, please keep in mind that larger-sized files may require very long download times and that some users may experience other problems during downloading.

Audio, Video, and Animations

- Aspect ratio: 16:9 or 4:3
- Maximum file size: 25 GB
- Minimum video duration: 1 sec
- Supported file formats: avi, wmv, mp4, mov, m2p, mp2, mpg, mpeg, flv, mxf, mts, m4v, 3gp

Text and Presentations

- Submit your material in PDF format; .doc or .ppt files are not suitable for long-term viability.
- A collection of figures may also be combined in a PDF file.

Spreadsheets

- Spreadsheets should be submitted as .csv or .xlsx files (MS Excel).

Specialized Formats

- Specialized format such as .pdb (chemical), .wrl (VRML), .nb (Mathematica notebook), and .tex can also be supplied.

Collecting Multiple Files

- It is possible to collect multiple files in a .zip or .gz file.

Numbering

- If supplying any supplementary material, the text must make specific mention of the material as a citation, similar to that of figures and tables.
- Refer to the supplementary files as “Online Resource”, e.g., "... as shown in the animation (Online Resource 3)", “... additional data are given in Online Resource 4”.
- Name the files consecutively, e.g. “ESM_3.mpg”, “ESM_4.pdf”.

Captions

- For each supplementary material, please supply a concise caption describing the content of the file.

Processing of supplementary files

- Electronic supplementary material will be published as received from the author without any conversion, editing, or reformatting.

Accessibility

In order to give people of all abilities and disabilities access to the content of your supplementary files, please make sure that

- The manuscript contains a descriptive caption for each supplementary material
- Video files do not contain anything that flashes more than three times per second (so that users prone to seizures caused by such effects are not put at risk)

Ethical Responsibilities of Authors

This journal is committed to upholding the integrity of the scientific record. As a member of the Committee on Publication Ethics (COPE) the journal will follow the COPE guidelines on how to deal with potential acts of misconduct.

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- The manuscript should not be submitted to more than one journal for simultaneous consideration.
- The submitted work should be original and should not have been published elsewhere in any form or language (partially or in full), unless the new work concerns an expansion of previous work. (Please provide transparency on the re-use of material to avoid the concerns about text-recycling (‘self-plagiarism’).
- A single study should not be split up into several parts to increase the quantity of submissions and submitted to various journals or to one journal over time (i.e. ‘salami-slicing/publishing’).
- Concurrent or secondary publication is sometimes justifiable, provided certain conditions are met. Examples include: translations or a manuscript that is intended for a different group of readers.

- Results should be presented clearly, honestly, and without fabrication, falsification or inappropriate data manipulation (including image based manipulation). Authors should adhere to discipline-specific rules for acquiring, selecting and processing data.
- No data, text, or theories by others are presented as if they were the author's own ('plagiarism'). Proper acknowledgements to other works must be given (this includes material that is closely copied (near verbatim), summarized and/or paraphrased), quotation marks (to indicate words taken from another source) are used for verbatim copying of material, and permissions secured for material that is copyrighted.

Important note: the journal may use software to screen for plagiarism.

- Authors should make sure they have permissions for the use of software, questionnaires/(web) surveys and scales in their studies (if appropriate).
- Authors should avoid untrue statements about an entity (who can be an individual person or a company) or descriptions of their behavior or actions that could potentially be seen as personal attacks or allegations about that person.
- Research that may be misapplied to pose a threat to public health or national security should be clearly identified in the manuscript (e.g. dual use of research). Examples include creation of harmful consequences of biological agents or toxins, disruption of immunity of vaccines, unusual hazards in the use of chemicals, weaponization of research/technology (amongst others).
- Authors are strongly advised to ensure the author group, the Corresponding Author, and the order of authors are all correct at submission. Adding and/or deleting authors during the revision stages is generally not permitted, but in some cases may be warranted. Reasons for changes in authorship should be explained in detail. Please note that changes to authorship cannot be made after acceptance of a manuscript.

*All of the above are guidelines and authors need to make sure to respect third parties rights such as copyright and/or moral rights.

Upon request authors should be prepared to send relevant documentation or data in order to verify the validity of the results presented. This could be in the form of raw data, samples, records, etc. Sensitive information in the form of confidential or proprietary data is excluded.

If there is suspicion of misbehavior or alleged fraud the Journal and/or Publisher will carry out an investigation following COPE guidelines. If, after investigation, there are valid concerns, the author(s) concerned will be contacted under their given e-mail address and given an opportunity to address the issue. Depending on the situation, this may result in the Journal's and/or Publisher's implementation of the following measures, including, but not limited to:

- If the manuscript is still under consideration, it may be rejected and returned to the author.
- If the article has already been published online, depending on the nature and severity of the infraction:

- an erratum/correction may be placed with the article

- an expression of concern may be placed with the article
- or in severe cases retraction of the article may occur.

The reason will be given in the published erratum/correction, expression of concern or retraction note. Please note that retraction means that the article is **maintained on the platform**, watermarked "retracted" and the explanation for the retraction is provided in a note linked to the watermarked article.

- The author's institution may be informed
- A notice of suspected transgression of ethical standards in the peer review system may be included as part of the author's and article's bibliographic record.

Fundamental errors

Authors have an obligation to correct mistakes once they discover a significant error or inaccuracy in their published article. The author(s) is/are requested to contact the journal and explain in what sense the error is impacting the article. A decision on how to correct the literature will depend on the nature of the error. This may be a correction or retraction. The retraction note should provide transparency which parts of the article are impacted by the error.

Suggesting / excluding reviewers

Authors are welcome to suggest suitable reviewers and/or request the exclusion of certain individuals when they submit their manuscripts. When suggesting reviewers, authors should make sure they are totally independent and not connected to the work in any way. It is strongly recommended to suggest a mix of reviewers from different countries and different institutions. When suggesting reviewers, the Corresponding Author must provide an institutional email address for each suggested reviewer, or, if this is not possible to include other means of verifying the identity such as a link to a personal homepage, a link to the publication record or a researcher or author ID in the submission letter. Please note that the Journal may not use the suggestions, but suggestions are appreciated and may help facilitate the peer review process.

Authorship principles

These guidelines describe authorship principles and good authorship practices to which prospective authors should adhere to.

Authorship clarified

The Journal and Publisher assume all authors agreed with the content and that all gave explicit consent to submit and that they obtained consent from the responsible authorities at the institute/organization where the work has been carried out, **before** the work is submitted.

The Publisher does not prescribe the kinds of contributions that warrant authorship. It is recommended that authors adhere to the guidelines for authorship that are applicable in their specific research field. In absence of specific guidelines it is recommended to adhere to the following guidelines*:

All authors whose names appear on the submission

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