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Cinara Stein

EFEITOS DA FOTOTERAPIA NAS DOENÇAS CARDIOVASCULARES

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DEDICATÓRIA

Àqueles que muito amo, em especial aos meus pais,
Noeli e Alcido, que com humildade souberam
me ensinar o verdadeiro valor da educação...

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LISTA DE ABREVIATURAS

BrdU: 5-bromo-2-desoxiuridina

CAT: catalase

CK: creatina quinase

CRM: cirurgia de revascularização do miocárdio

CVM: contração voluntária máxima

DAC: doença arterial coronariana

DCV: doenças cardiovasculares

DMT: dor muscular tardia

EVA: escala visual analógica

IAM: infarto agudo do miocárdio

IC: insuficiência cardíaca

LDH: lactato desidrogenase

LED: *Light Emitting Diode* - Diodos emissor de luz

LLLT: *Low level laser therapy* – Laserterapia de baixa intensidade

MSCs: células mesenquimais

Nm: nanômetro

OMS: Organização Mundial da Saúde

ON: óxido nítrico

SOD: superóxido dismutase

TC6M: teste de caminhada de 6 minutos.

VEGF: *Vascular endothelial growth fator* – Fator de crescimento endotelial vascular

1RM: 1 repatição máxima

ABSTRACT

This study tests the hypothesis that low intensity laser therapy (LLLT) has effects on cardiovascular diseases (CVD) and the acute application of LLLT before an exercise test would increase the distance covered after myocardial revascularization (CABG) surgery. In this context, the first study (article 1) analyzed effects of treatment with phototherapy on CVD through an integrative review. Searched was conducted in MEDLINE (PubMed), EMBASE, Cochrane Library and Physiotherapy Evidence Database (PEDro) from the inception to August 2016. Studies with application of low- level laser therapy (LLLT) and/or light-emitting diode therapy (LEDT) in cardiovascular diseases were included, and the outcomes was functional capacity, infarct size, cytokines, oxidative stress, vascular endothelial growth factor (VEGF), angiogenesis, cardiac damage markers, pain and healing. The selected nineteen full- texts were composed of clinical trials (5), pilot studies (2), single case study (2) and animal models studies (10). Results showed phototherapy can reduces area of infarct, cardiac damage markers and pain; modulates oxidative stress and inflammatory cytokines; and increases healing, angiogenesis and VEGF. Functional capacity was improved only by LEDT. It is concluded that phototherapy is effectiveness to treat CVD. The second study (article 2) aimed to evaluate the acute effects of LLLT on functional capacity after CABG surgery. Fifteen patients were crossed over during the study, in order to compare the outcomes after active LLLT and placebo LLLT treatments. LLLT (850 nm, 200 mW, 30 J to each point,), using a multi-diode cluster (with five spots, 6 J from each spot) at eight points per leg was performed 3 min before the Incremental Shuttle Walking Test. We analyzed distance walked, Borg scale of perceived exertion, heart rate, arterial blood pressure, lactate dehydrogenase, levels of oxidative damage to lipids, activities of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT), and total thiol levels in peripheral blood. Comparison of the distances walked revealed no significant differences between the LLLT and placebo LLLT ($p= 0.779$) groups. Regarding the Borg scale of perceived exertion ($p= 0.567$), heart rate ($P= 0.506$), systolic ($p= 0.164$) and diastolic ($p= 0.140$) blood pressure, no difference was observed between the LLLT and placebo LLLT groups. The application of LLLT was not able to modulate lactate dehydrogenase enzymes ($p= 0.214$), lipids damage ($p= 0.733$), SOD ($p= 0.202$) and CAT ($p= 0.825$) activity, and total thiol levels ($p = 0.925$). Therefore, the use of LLLT acute did not improve neither functional capacity nor the redox status after CABG.

Keywords: Cardiovascular diseases. Low-Level Laser Therapy. Thoracic Surgery. Functional Capacity. Exercise. Systematic review

RESUMO

Este trabalho testa a hipótese que a laserterapia de baixa intensidade (LLLT) possui efeitos nas doenças cardiovasculares (DCV) e a aplicação aguda da LLLT antes de um teste de exercício aumentaria a distância percorrida depois da cirurgia de revascularização do miocárdio (CRM). Neste contexto, o primeiro estudo (artigo 1) objetivou revisar os efeitos da fototerapia nas doenças cardiovasculares por meio de uma revisão integrativa. A pesquisa foi realizada no MEDLINE (PubMed), EMBASE, Cochrane Library e Physiotherapy Evidence Database (PEDro) desde o início das publicações até agosto de 2016. Incluíram-se estudos com aplicação de LLLT e/ou diodos emissor de luz (LED) em doenças cardiovasculares, e os desfechos avaliados foram capacidade funcional, tamanho do infarto, citocinas, estresse oxidativo, fator de crescimento endotelial vascular (VEGF), angiogênese, marcadores de danos cardíacos, dor e cicatrização. Destes, selecionaram-se dezenove estudos compostos por ensaios clínicos (5), estudo piloto (2), estudo de caso (2) e estudos em modelo animal (10). Os resultados mostraram que a fototerapia pode reduzir a área de infarto, marcadores de danos cardíacos e dor; modula o estresse oxidativo e citocinas inflamatórias; aumenta a cicatrização, angiogênese e VEGF. O aumento da capacidade funcional ocorreu somente pelo LED. Conclui-se que a fototerapia é efetiva para tratar as DCV. O segundo estudo (artigo 2) objetivou avaliar os efeitos agudos da LLLT na capacidade funcional após a CRM. Quinze pacientes foram cruzados durante o experimento, a fim de comparar os resultados dos grupos LLLT e LLLT placebo. LLLT (850 nm, 200 mW, 30 J em cada ponto) foi realizada 3 min antes do teste incremental (*Incremental Shuttle Walking)*, em oito pontos da perna. Foram analisados a distância percorrida, escala de Borg de esforço percebido, frequência cardíaca, pressão arterial, lactato desidrogenase, níveis de dano oxidativo aos lipídeos, atividades das enzimas antioxidantes superóxido dismutase (SOD) e catalase (CAT), e os níveis totais de tiol. A comparação das distâncias percorridas não revelou diferenças significativas entre os grupos LLLT e LLLT placebo ($p = 0,779$). Em relação à escala Borg de esforço percebido ($p= 0,567$), pressão cardíaca ($P = 0,506$), pressão arterial sistólica ($p = 0,164$) e diastólica ($p = 0,140$), não houve diferença entre os grupos LLLT e placebo. A aplicação de LLLT não foi capaz de modular as enzimas lactato desidrogenase ($p = 0,214$), danos nos lipídios ($p = 0,733$), SOD ($p = 0,202$) e CAT ($p = 0,825$) e níveis totais de tiol ($p = 0,925$). Assim sendo, o presente estudo mostrou que o efeito agudo da LLLT não melhorou a capacidade funcional e o estado redox após CRM.

Palavras-chave: Doenças cardiovasculares**.** Laserterapia de baixa intensidade. Cirurgia torácica. Capacidade funcional. Exercício. Revisão sistemática.

Capítulo I – Revisão da Literatura

1.1 INTRODUÇÃO

As doenças cardiovasculares (DCV) são um importante problema de saúde pública mundial. Embora a mortalidade por DCV esteja diminuindo gradualmente nas últimas décadas nos países ocidentais, esta condição ainda causa cerca de um terço de todas as mortes em pessoas com mais de 35 anos (SANCHIS-GOMAR et al., 2016). No Brasil essas doenças ocupam a liderança das causas de óbito e internação (RIBEIRO et al., 2016).

A diminuição pronunciada da capacidade funcional, atividades de vida diária, e na qualidade de vida estão piorando as condições de hospitalização e prognóstico em pacientes com DCV (LEE et al., 2012). A cirurgia de revascularização do miocárdio (CRM) é uma das mais frequentes cirurgias realizadas em todo o mundo e a principal indicação está associada à doença arterial coronariana (DAC) (ALEXANDER & SMITH, 2016). De acordo com um relatório recente, a DAC secundária à aterosclerose se destaca como a principal causa de morbidade e mortalidade nas sociedades industrializadas (WHO, 2016). A aterosclerose pode ser definida como um processo dinâmico, evolutivo, a partir de dano endotelial de origem multifatorial, com características de reparação tecidual (THOMAS et al., 2016).

A realização desta cirurgia tem como objetivos aumentar a sobrevida, aliviar os sintomas da isquemia miocárdica, melhorar a função ventricular, prevenir o infarto do miocárdio, recuperar o paciente física, psíquica e socialmente, prolongar e melhorar a qualidade de vida deste (CANTERO et al., 2012). Porém, a incidência de complicações, após as cirurgias cardíacas, decorrentes dos efeitos deletérios da imobilidade, contribui para o declínio funcional, aumento dos custos assistenciais, redução da qualidade de vida e mortalidade pós-alta (FRANÇA et al., 2012).

Desde a primeira cirurgia de coração em campo aberto, bem-sucedida, realizada por John Lewis em seres humanos e que ocorreu em 1953, muitas técnicas têm sido utilizadas para melhorar o prognóstico da cirurgia e reduzir seus efeitos secundários (LEWIS & TAUFIC, 1953). A laserterapia de baixa intensidade (LLLT) tem sido amplamente utilizada na prática clínica para fins de alívio da dor, estímulo à cicatrização tecidual e na atenuação da fadiga muscular e recuperação muscular pós- exercício, apresentando uma resposta dependente da dose (ENWEMEKA, 2004;

BARONI et al., 2010). Nesse sentido, estudos envolvendo a utilização de LLLT para o tratamento de doenças cardiovasculares vêm sendo publicados recentemente.

Não há na literatura atual uma revisão que abranja todos os efeitos da fototerapia nas DCV. Ainda, como a LLLT representa uma importante opção não farmacológica e, embora seja utilizada principalmente para o alívio da dor e na cicatrização, pesquisadores estão analisando a sua atuação frente à atenuação da fadiga muscular e na recuperação muscular pós-exercício em diversas condições.

Recentemente, estudos de revisão sistemática relataram efeitos positivos da fototerapia, na atenuação da fadiga muscular e na recuperação muscular pós-exercício em humanos (LEAL-JUNIOR et al., 2015; NAMPO et al., 2016). A aplicação de LLLT antes ou depois de exercícios físicos apresenta características distintas e mecanismos comuns de ação. A aplicação de LLLT antes do exercício pode ser explicada por um efeito preventivo contra a disfunção muscular e dano mitocondrial mediada pelas espécies reativas de oxigênio e por espécies reativas de nitrogênio, bem como a modulação do metabolismo energético, e após o exercício físico objetiva não só corrigir a disfunção mitocondrial e metabólica, mas também as microlesões produzidas a partir do estresse mecânico e metabólico resultantes da contração muscular (LIU et al., 2009; LEAL JUNIOR et al., 2010a; SUSSAI et al., 2010).

Apesar da existência de várias pesquisas sobre o efeito da LLLT em doenças e condições clínicas, ainda há informações contraditórias e perguntas que permanecem sem respostas concretas nessa área do conhecimento. O efeito da LLLT no desempenho do exercício desperta o interesse de pesquisadores devido ao potencial efeito benéfico que o uso desse recurso possa ter na atenuação da fadiga muscular e na recuperação muscular pós-exercício nas DCV.

Com o objetivo de melhor discutir esses temas nas próximas sessões, realiza-se uma breve revisão da literatura sobre os aspectos históricos da LLLT, suas características gerais e as teorias que fundamentam a sua utilização. Também são abordados os efeitos da LLLT e sua potencial contribuição no tratamento das doenças cardiovasculares e sobre o desempenho no exercício.

1.2 DOENÇAS CARDIOVASCULARES

Globalmente, as DCV, que incluem DAC, insuficiência cardíaca (IC), e outras doenças cardíacas, representam a principal causa de morte do ser humano (SANCHIS-GOMAR et al., 2016). A OMS estima que as doenças cardiovasculares tenham causado 17 milhões de mortes em todo o mundo em 2012, e este número deverá duplicar nos próximos 15 anos (WHO, 2015).

No Brasil, as taxas de mortalidade por DCV apresentaram uma elevação que acompanhou a industrialização no país, a partir da década de 1930, e subsequente queda a partir da década de 80 (MANSUR ADE et al., 2009). Recentemente, os dados de mortalidade por DCV em mulheres e homens nas cinco regiões do país foram atualizados através de uma revisão. O estudo mostrou que as regiões Sudeste e Sul tiveram as maiores reduções na mortalidade por DCV, tendo a região Nordeste apresentado um aumento da mortalidade por essas doenças. Os resultados se registraram variáveis para as regiões Norte e Centro-Oeste (MANSUR ADE & FAVARATO, 2016). Os achados deste estudo revelaram que as regiões Sudeste e Sul tiveram um comportamento semelhante ao dos países mais desenvolvidos, isto é, a persistente tendência de redução da mortalidade por DCV (NICHOLS et al., 2014).

A DAC, também chamada de aterosclerose coronariana, é uma doença complexa que limita a perfusão em todo o corpo e pode afetar significativamente órgãos vitais. A inflamação é o ponto inicial do processo aterosclerótico, com o desenvolvimento e a estimulação do acúmulo contínuo de componentes da placa aterosclerótica, que formam um trombo (BRASHERS, 2015). O resultado da aterosclerose não tratada é a diminuição do fluxo sanguíneo nos sistemas micro e macrovascular, aumentando significativamente o risco de infarto do miocárdio ou acidente vascular cerebral. O infarto do miocárdio pode ser um evento menor em uma doença crônica, e até mesmo não ser detectado, mas também pode ser um evento catastrófico importante, levando o paciente à morte súbita ou à grave deterioração hemodinâmica (THYGESEN et al., 2007).

Apesar de vários avanços no tratamento da DAC, muitos pacientes se beneficiam muito quando submetidos ao tratamento cirúrgico, especialmente aqueles com doença multiarterial e anatomias complexas (MOHR et al., 2011). A CRM é um pilar no tratamento de pacientes sintomáticos ou daqueles com lesões coronárias produtoras de isquemia e a reduz em maior extensão do que o tratamento clínico (PICCOLO et al.,

2015). O objetivo principal da CRM na doença arterial coronariana estável é melhorar os sintomas e prognóstico (WINDECKER et al., 2014).

Os pacientes submetidos à CRM apresentam um risco relativamente elevado de desenvolver complicações pulmonares e funcionais. Há uma série de efeitos que são vistos principalmente após a CRM, tais como: diminuição da capacidade vital, diminuição da capacidade funcional, hipoxemia arterial e redução da complacência pulmonar (KOLLEF et al., 1995). Estas complicações (incluindo a mortalidade) aumentam o tempo de hospitalização e a necessidade de recursos financeiros (PASQUINA et al., 2003).

Outra síndrome clínica que se caracteriza como um importante e crescente problema de saúde pública é a IC (BRAUNWALD, 2008). Ela é caracterizada pela incapacidade da bomba cardíaca em fornecer perfusão adequada para os tecidos, especialmente para os órgãos vitais (MACIVER & DAYER, 2012). Está bem estabelecido que tanto a prevalência como a incidência da IC estão aumentando (GO et al., 2014). Existem dois tipos primários de IC, categorizados por fração de ejeção: fração de ejeção reduzida e fração de ejeção preservada (YANCY et al., 2013). Além disso, a IC é comumente classificada em estágios de leve a grave usando uma escala baseada em sintomas relacionados à limitação funcional (KAMINSKY & TUTTLE, 2015).

Uma das características de destaque da IC é a intolerância ao exercício, que é acompanhada por sintomas de fadiga e falta de ar (WATSON et al., 2000). À medida que a doença progride, os pacientes vivenciam um declínio funcional com redução da atividade física, o que leva à progressiva piora da intolerância ao exercício. Além disso, é bem conhecido que aqueles com IC têm comprometimento na capacidade de realizar atividades de vida diária e sofrem de redução da qualidade de vida (KAMINSKY & TUTTLE, 2015).

A OMS destacou que o estilo de vida inadequado, como o consumo de tabaco, favoráveis na explosão de doenças cardiovasculares no mundo ocidental (WHO, 2002). Mais de três quartos das mortes por DCV poderiam ser prevenidas com mudanças adequadas no estilo de vida (PERK et al., 2012). De fato, Chow e colaboradores (2010) mostraram que os pacientes aderem menos às modificações do estilo de vida do que aos seus regimes medicamentosos, um mês após a síndrome coronariana aguda (CHOW et al., 2010).

A educação dos pacientes no controle dos fatores de risco cardiovasculares, juntamente com o recondicionamento ao esforço, é um aspecto fundamental da

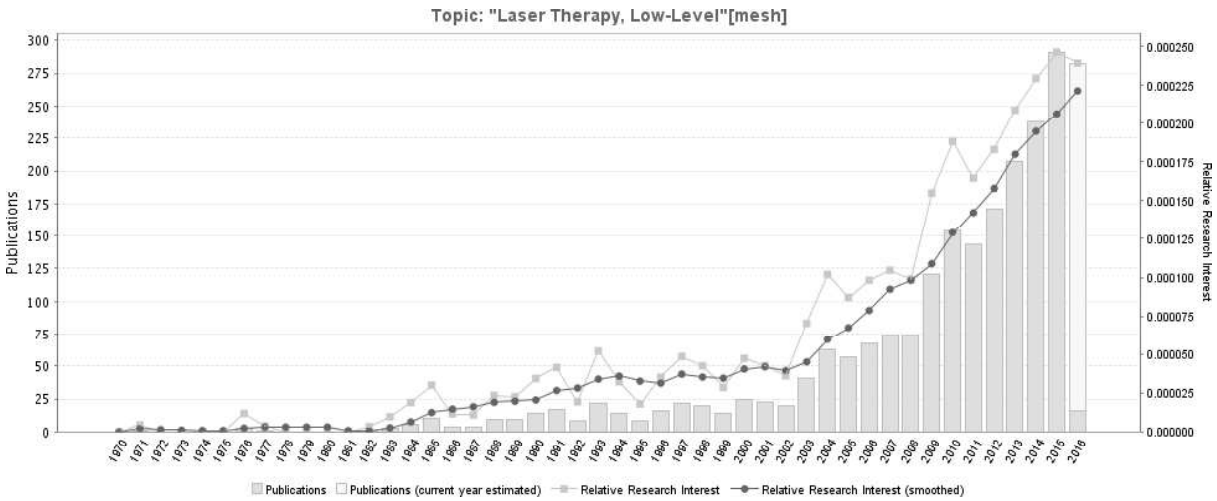
reabilitação cardiovascular. No entanto, apenas uma pequena proporção de pacientes que sofrem de DCV está inserida em programas de reabilitação (ADES et al., 1992; WITT et al., 2004), que são fortemente recomendados e têm provado a sua eficácia (MONPERE, 2002).

1.3 FOTOTERAPIA

Laserterapia de baixa intensidade (LLLT), também conhecida como fotobiomudulação, está se aproximando do seu 50º aniversário. LLLT foi descoberto em 1967 por Endre Mester na Semmelweis Medical University na Hungria (MESTER E, 1967). Ele estava tentando repetir um experimento conduzido primeiramente por Paul McGuff, em Boston, EUA, que tinha usado com sucesso o laser recentemente descoberto do rubi para curar tumores malignos nos ratos. Entretanto, o laser de rubi de Mester, feito sob encomenda, possuía apenas uma fração muito pequena da potência comparado ao laser de McGuff. Apesar de não curar nenhum tumor com seu feixe de laser de baixa intensidade, ele observou um aumento na taxa de crescimento de pelos e melhor cicatrização de feridas nos ratos nos quais havia implantado cirurgicamente os tumores. Esta foi a primeira indicação de que o laser de baixa intensidade poderia ter suas próprias aplicações benéficas na medicina. Mester é creditado como o descobridor de efeitos biológicos positivos de lasers de baixa intensidade (MESTER et al., 1971).

Conforme pode ser visto no gráfico abaixo, da Figura 1, na década de 2000, houve um crescimento na produção de artigos científicos sobre LLLT, e que se manteve constante nos anos seguintes, até a atualidade. Revisões, revisões sistemáticas com metanálise e *overview* de revisões sistemáticas denotam o maior nível de evidência da LLLT em diversas maneiras: para reduzir a inflamação, edema e doenças articulares crônicas (BJORDAL et al., 2003; CHRISTIE et al., 2007; JAMTVEDT et al., 2008); para promover a cicatrização de feridas, tecidos mais profundos, e os nervos (GIGO-BENATO et al., 2005; POSTEN et al., 2005); para o tratamento de distúrbios neurológicos e dor (CHOW et al., 2009; CHANG et al., 2014; HUANG et al., 2015); e, na melhora da performance muscular (LEAL-JUNIOR et al., 2015; NAMPO et al., 2016).

Figura 1- Gráfico: artigos científicos da terapia do LLLT



Fonte: retirada do: **http://www.gopubmed.com/,** acessado em 10 de dezembro de 2016

Laser é um dispositivo que emite luz através de um processo de amplificação óptica baseado na emissão estimulada de fótons. O termo por "laser" se originou como um acrônimo para amplificação de luz por emissão estimulada de radiação (MCGUFF et al., 1963).

A luz laser difere da luz comum devido às suas características específicas, como a monocromaticidade, coerência, colimação. Possui um comprimento de onda bem definido e, portanto, representa uma cor bem delimitada (monocromática), é coerente (ondas sincronizadas) e colimada (radiação paralela) (SCHAWLOW, 1995).

Os lasers podem ser categorizados em dois grupos: lasers de alta potência (superiores a 1 W), os quais são usados para finalidades cirúrgicas como cortes, carbonização ou desnaturação de proteínas através de efeito fototérmico (CHAVANTES, 1990), e lasers de baixa potência (inferiores a 1 W), utilizados para reparação tecidual, alívio de dor e a obtenção de efeitos anti-inflamatórios. Dentre os lasers de baixa potência existem diferentes configurações de comprimento de onda, variando do vermelho (visível) ao infravermelho (invisível). Apesar de a onda vermelha ter sido a mais estudada, a luz infravermelha tem maior poder de penetração nos tecidos (ENWEMEKA, 2001; ENWEMEKA, 2009).

Os métodos mais comuns de administração de radiação da LLLT incluem lasers tais como rubi (694 nm), Argônio (488 e 514 nm), He-Ne (632,8 nm), Krypton (521, 530, 568 e 647 nm), Ga-Al -As (805 ou 650 nm) e Ga-As (904 nm) (LIN et al., 2010). De acordo com Karu (1987), comprimento de onda, densidade de potência

(intensidade), frequência de tratamento e, até mesmo, o tipo de lesão estão relacionados com o aumento da atividade celular. Densidade de energia ou dose também influencia as reações teciduais. A interação da luz com os tecidos é, portanto, baseada em reações fotobiológicas (KARU, 1987).

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

O mecanismo de ação da LLLT foi proposto pela primeira vez por Karu e colaboradores em 1981 (KARU T.I., 1981). Postulou-se que a irradiação laser leva à produção de oxigênio anatômico que, por sua vez, promove a síntese de RNA e DNA. Em 1986, sugeriu-se que a energia do laser pode causar a fotoexcitação do complexo do citocromo c, levando à alteração no estado redox (KARU, 1986). Também tem sido formulada a hipótese de que a irradiação do laser atua sobre as mitocôndrias e reverte a inibição causada pelo óxido nítrico (ON), levando à ativação da cadeia respiratória (KARU, 2000).

Em termos gerais, LLLT é frequentemente utilizado na prática clínica para a promoção da cicatrização de tecidos e controle da dor. As aplicações específicas da LLLT são diversas, em parte porque cada um dos mecanismos listados acima pode ser aplicado a uma série de sistemas corporais. Exemplos incluem tratamento de feridas infectadas, necrose de tecido, lesão de nervo, osteoartrite ou outras dores crônicas, dor miofascial, cicatrização de fratura, lesão tendinosa ou ligamentar e cuidados de incisão pós-cirúrgica.

1.4 LASERTERAPIA DE BAIXA INTENSIDADE NAS DOENÇAS CARDIOVASCULARES

A laserterapia de baixa intensidade nas doenças cardiovasculares vem sendo estudada cada vez mais no intuito de desenvolver novas respostas terapêuticas.

Desfechos como capacidade funcional, tamanho do infarto, estresse oxidativo, fator de crescimento endotelial vascular (VEGF), angiogênese, marcadores de danos cardíacos, dor e cicatrização já apresentam evidências.

Na década de 2000, os primeiros estudos em modelos animais de LLLT nas doenças cardiovasculares começaram a ser publicados. Oron e colaboradores (2001) investigaram a possibilidade da irradiação com LLLT atenuasse o tamanho do infarto, após a indução do IAM em pequenos e grandes animais experimentais. Os autores aplicaram LLLT (810 nm) na área infartada de ratos e cães em várias densidades de potência (2,5 a 20 mW / cm2) após oclusão da artéria coronária. Os resultados indicam que o fornecimento da irradiação com LLLT para miocárdio infartado em ratos e cães tem um grande efeito na redução do tamanho do infarto (ORON et al., 2001).

Com o mesmo objetivo de avaliar o efeito agudo da LLLT no tamanho do infarto, dois estudos subsequentes foram publicados. Tuby e colaboradores (2011) irradiaram LLLT (804 nm) em células mesenquimais (MSCs), e implantaram em corações infartados de ratos. A LLLT aumentou a sobrevivência e/ou proliferação de MSCs pós-implantação em coração isquémico/infartado (TUBY et al., 2011). Ainda, Manchini e colaboradores (2014) testaram a hipótese de que a LLLT reduz a inflamação após IAM aguda em ratas, e melhora a função cardíaca. A LLLT (660 nm) foi colocada em contato com a superfície do miocárdio correspondente à área infartada. Dados sugerem diminuição das citocinas pró-inflamatórias no tamanho do miocárdio e do infarte após a aplicação da LLLT (MANCHINI et al., 2014).

Dois estudos até o momento avaliaram o efeito agudo da LLLT no estresse oxidativo de ratos com IC e IAM. Biasibetti e colaboradores (2014) submeteram ratos com IC ao protocolo LLLT (660 nm) no músculo gastrocnêmio direito durante 10 dias. A LLLT parece reduzir a atividade de superóxido dismutase (SOD) e os níveis de 2'7'diacetato dedicloroflurescеína, alterando o equilíbrio oxidativo no músculo esquelético de ratos com IC (BIASIBETTI et al., 2014). Monich e colaboradores (2011) objetivaram investigar a biomodulação fototerapêutica do músculo cardíaco de ratos após isquemia miocárdica durante todo o período de reperfusão com LLLT (660 nm) e algumas amostras sem modelagem de isquemia com luz vermelha. Os autores descobriram, após a modelagem de isquemia e restauração de perfusão, que a iluminação da luz resulta na aceleração da recuperação da contratilidade miocárdica, aumento da atividade da SOD e redução dos produtos de peroxidação lipídica (MONICH et al., 2011).

O perfil das citocinas após a aplicação da LLLT também foi estudado nas doenças cardiovasculares. Hentschke e colaboradores (2013) avaliaram a influência da LLLT no perfil inflamatório de ratos com IC. Quatro semanas após infarto do miocárdio ou cirurgia simulada, os ratos receberam a aplicação de LLLT (660 nm) no gastrocnêmio direito durante 10 dias consecutivos. LLLT reduziu os níveis de TNF-a e IL-6 e a razão IL-6 / IL-10, bem como o aumento do nível de IL-10 e da razão IL-10 / TNF-α no músculo gastrocnêmio (HENTSCHKE et al., 2013). Yang e colaboradores (2011) testaram a hipótese da LLLT (635 nm) poderia influenciar a expressão de citocinas cardíacas, e contribui para reversão do remodelamento ventricular no modelo de infarto criado por ligadura coronária. A função e a estrutura cardíaca foram avaliadas por ecocardiografia e análise histológica, duas semanas após o IAM. Cinco ratos de cada grupo, sacrificados um dia após a irradiação com laser, tiveram seus tecidos do coração analisados por matrizes de anticorpos de citocinas. Os resultados mostraram a expressão de citocinas regulada pela LLLT no miocárdio lesado no estágio inicial do IAM e, a LLLT reduziu a expansão da área do infarto. No entanto, não se observou melhora da função cardíaca nas 2 semanas após o tratamento a laser (YANG et al., 2011).

Estudos em modelos animais em doenças cardíacas apresentam resultados de VEGF e angiogênese. Tuby e colaboradores (2006) investigaram o efeito da LLLT na expressão de VEGF. O IAM foi induzido por oclusão da artéria descendente esquerda em 87 ratos. Aplicou-se LLLT (804 nm) nos corações intactos e pós-infarto. Ambos os corações, intactos irradiados com laser e pós-infarto, demonstraram aumento significativo na expressão de VEGF em comparação com corações não irradiados com laser. O LLLT causou elevação significativa na angiogênese (TUBY et al., 2006). Tuby e colaboradores (2009) avaliaram possíveis efeitos benéficos do implante de MSCs irradiadas com laser em corações infartados de ratos. Isolaram-se as MSCs a partir da medula óssea de ratos, e observadas em cultura. As células irradiadas com LLLT (810 nm), marcadas com 5-bromo-2-desoxiuridina (BrdU) foram implantadas nos corações. As células não irradiadas, mas marcadas de forma semelhante, foram considerados os controles. Os resultados desse estudo forneceram a primeira evidência de que a LLLT pode aumentar significativamente a sobrevivência e/ou a proliferação de MSCs pós- implantação no coração isquêmico/infartado e aumento da angiogénese (TUBY et al., 2009).

No ano de 2009 começaram a ser publicados os primeiros estudos clínicos sobre LLLT nas doenças cardiovasculares. Um estudo de caso foi publicado por Pinto e colaboradores (2009) com o objetivo de avaliar a resposta da LLLT (685 nm) na deiscência da safenectomia aguda após CRM. No 30º pós-operatório, a LLLT fora aplicada ao redor da borda da ferida. A lesão respondeu com tecido de granulação, diminuição do processo inflamatório e analgesia desde a primeira aplicação (PINTO et al., 2009). Ainda, o mesmo grupo de pesquisadores publicou, em 2014, um estudo piloto com o mesmo objetivo. O estudo incluiu 14 pacientes, divididos em dois grupos. Ambos os grupos receberam o tratamento tradicional, e o grupo intervenção recebeu adicionalmente o tratamento com LLLT (780 nm). As complicações da safenectomaia foram prevenidas no grupo LLLT quando comparado com o grupo controle, que apresentou o dobro de complicações, incluindo taxas críticas de deiscência incisional (PINTO et al., 2014). Recentemente, um ensaio clínico randomizado duplo-cego analisou os efeitos da cicatrização do esterno após aplicação da LLLT em pacientes submetidos a CRM. Os pacientes do grupo LLLT foram submetidos à irradiação nos dias 2, 4, 6 e 8 do pós-operatório e suas incisões de esternotomia fotografadas imediatamente depois da cirurgia e após 8 dias. A esternotomia no grupo LLLT demonstrou menos hiperemia, sangramento incisional e deiscência quando comparada aos grupos controle e plabebo (FERNANDES et al., 2016).

A capacidade funcional de pacientes com IC foi avaliada recentemente por Bublitz et al. (2016). Dois grupos com diagnóstico de IC sistólica foram randomizados para grupo LLLT placebo (n = 10) e grupo LLLT ativo (808 nm; n = 10). A capacidade funcional se analisou pelo teste de caminhada de 6 minutos (TC6M). Os grupos, tanto o placebo quanto o LLLT ativo aumentaram a distância de TC6M em relação aos valores basais ($p = 0,03$ e $p = 0,01$, respectivamente). No entanto, nenhuma diferença foi observada na comparação intergrupos. O grupo LLLT ativo mostrou redução significativa na escala percebida de esforço de Borg em relação ao grupo placebo LLLT ($p = 0,006$) (BUBLITZ et al., 2016).

O efeito agudo da LLLT sobre marcadores de danos cardíacos em pacientes após CRM foi estudado por Khoo et al. (2014). A LLLT (810 nm) foi aplicada durante três dias consecutivos pós-CRM. Realizaram-se medições repetidas de contagem de células sanguíneas e marcadores de danos cardíacos (CPK, CPK-MB, LDH) antes da cirurgia e 5 dias depois da cirurgia. O estudo concluiu que a aplicação da LLLT após a CRM pode

diminuir danos cardíacos celulares, e ajuda a reparação do tecido cardíaco (KAZEMI KHOO et al., 2014).

Dessa maneira, denota-se que a fototerapia vem sendo estudada como forma terapêutica nas doenças cardiovasculares, porém ainda existem respostas contraditórias, com alguns estudos tendo demonstrado efeitos benéficos da fototerapia no tratamento de pacientes com DCV (DE OLIVEIRA et al., 2014; LIMA et al., 2016), outros estudos não encontraram melhora adicional com sua aplicação (STEIN C; BUBLITZ et al., 2016).

1.5 EVIDÊNCIAS DA LASERTERAPIA DE BAIXA INTENSIDADE NO DESEMPENHO DO EXERCÍCIO

Vários estudos clínicos relatam os efeitos positivos da LLLT na atenuação da fadiga muscular e na recuperação muscular pós-exercício em humanos. A LLLT é considerada uma estratégia não invasiva e não farmacológica para alcançar melhorias no desempenho do exercício.

Um dos primeiros estudos realizados para estudar o efeito da LLLT no desempenho muscular foi um estudo piloto. Os autores aplicaram o LLLT (808 nm) no modo pulsado (repetição do pulso de 1 - 10.000 Hz) por 5 min (baixa energia) e 10 min (alta energia) no músculo quadríceps femoral antes da indução à fadiga muscular por estimulação elétrica neuromuscular. O grupo controle recebeu somente estimulação elétrica neuromuscular.Os resultados mostraram que os grupos de LLLT tiveram um menor percentual de fadiga muscular em comparação com o grupo controle (GORGEY et al., 2008).

Vários estudos então vieram dar seguimento a esse achado com diferentes populações, grupos musculares e doses. Leal Junior e colaboradores investigaram os efeitos da LLLT (655 nm) aplicada no bíceps braquial antes do exercício banco Scott. O exercício foi realizado com 75% da carga correspondente à contração voluntária máxima (CVM) até a exaustão. Cinco pontos foram irradiados por 100 s em cada ponto. Os resultados mostraram um aumento significativo no número de repetições do grupo LLLT em relação ao grupo placebo. (LEAL JUNIOR et al., 2008).

Um ensaio clínico duplo-cego e randomizado, controlado por placebo, envolveu também bíceps braquial e fadiga. Este estudo investigou os efeitos da LLLT (cluster com 5 diodos de 810 nm), aplicado antes do exercício banco Scott com 75% da CVM

até a exaustão. Dois pontos do bíceps braquial foram irradiados por 30 s em cada ponto. Houve aumentos em número de repetições e tempo de exercício, diminuição dos níveis de lactato, CK e proteína c-reativa após o exercício (LEAL JUNIOR et al., 2010b).

Os efeitos da LLLT utilizando um *cluster* com 5 diodos de 810 nm sobre o metabolismo energético, dano muscular e dor muscular tardia (DMT) em jovens do sexo masculino foram investigados por Baroni e colaboradores. Foram realizadas cinco séries de 15 contrações excêntricas no quadríceps femoral no dinamômetro isocinético e a aplicação de seis pontos de radiação de LLLT no músculo quadríceps femoral durante 30 s em cada ponto. A LLLT aplicada antes do exercício promoveu uma atenuação do aumento de lactato desidrogenase (LDH) em 48 h após o exercício, uma atenuação do aumento da creatina quinase (CK) no sangue em 24 e 48 horas após o exercício e uma atenuação na perda de CVM imediatamente, 24h e 48h após o exercício. No entanto, a DMT verificada pela escala analógica visual (EVA) foi igual nos dois grupos (BARONI et al., 2010).

Um estudo recente utilizou a LLLT (808 nm) para investigar os efeitos sobre o dano muscular após o exercício. Os autores concluíram que a LLLT atenua a CK 72h após o protocolo de lesão muscular (FELISMINO et al., 2014). Outro ensaio clínico randomizado foi realizado para investigar o efeito agudo da LLLT durante a fadiga em mulheres idosas. A média do número de repetições realizadas durante o protocolo de fadiga foi maior quando os participantes receberam LLLT ativo (808nm) em comparação com a condição de utilização do laser de placebo. Conclui-se que a LLLT antes do exercício pode aumentar o número de repetições durante um exercício de flexão/extensão do joelho, no entanto, não foi eficaz em retardar o início da fadiga eletromiográfica (TOMA et al., 2013).

O uso da LLLT antes de um exercício de corrida foi estudado por De Marchi e colaboradores. O autores aplicaram a LLLT (cluster com 5 diodos de 808 nm) nos membros inferiores de homens não treinados por 5 min antes de um protocolo de corrida progressivo e intenso e concluíram que a LLLT diminui o estresse oxidativo, mostrando um efeito protetor da LLLT no atraso da fadiga muscular (DE MARCHI et al., 2012).

Para analisar o efeito crônico da LLLT Ferraresi e colaboradores aplicaram a LLLT (cluster com 6 diodos de 808 nm) em um programa de treinamento físico com carga correspondente a 80% de uma repetição máxima (1RM). O programa de treinamento se realizou duas vezes por semana, durante 12 semanas com aplicação de

laser imediatamente após cada treino. Sete regiões do quadríceps femoral foram irradiadas por 10s por região. O grupo LLLT teve um maior ganho percentual de 1RM em comparação com o grupo LLLT placebo e do grupo controle após programa de treinamento. Apenas o grupo de LLLT aumentou significativamente a média do pico de torque e pico de torque dos músculos extensores do joelho em dinamômetro isocinético (FERRARESI et al., 2011).

Outro trabalho se realizou, objetivando estudar o efeito crônico da LLLT (cluster com 6 diodos de 808 nm), em um programa de treinamento físico de baixa intensidade (carga relativa ao limiar ventilatório anaeróbio) na bicicleta estacionária, em mulheres jovens. O treinamento físico foi realizado três vezes por semana durante nove semanas consecutivas. Cinco regiões do quadríceps femoral foram irradiadas com LLLT por 10s por região após o treinamento. O grupo que aplicou a LLLT conseguiu diminuir o índice de fadiga dos músculos extensores do joelho em dinamômetro isocinético, não ocorrendo o mesmo nos outros grupos (VIEIRA et al., 2012).

Recentemente, Vanin e colaboradores (2016) realizaram o primeiro estudo para identificar o momento ótimo para fornecer irradiação de fototerapia quando usado em conjunto com programas de treinamento de força. A fototerapia antes e o placebo após as sessões de treinamento mostraram mudanças significativas na contração voluntária máxima e testes de 1RM quando comparados com outros grupos (VANIN et al., 2016).

1.6 Justificativa e objetivos

Apesar da existência de várias pesquisas sobre o efeito da LLLT em doenças cardiovasculares e condições clínicas, ainda há informações contraditórias e perguntas que permanecem sem respostas concretas nessa área do conhecimento. O efeito da LLLT nas doenças cardiovasculares desperta o interesse de pesquisadores devido ao potencial efeito benéfico que o uso desse recurso possa ter no tratamento de doenças que afetam este sistema. Uma revisão integrativa das evidências científicas permite uma análise correta e precisa da eficácia da fototerapia e sua disseminação.

Ainda, o efeito potencial da LLLT sobre as respostas musculares ao exercício tem sido amplamente estudado em diversas situações clínicas, porém em pacientes com doenças cardiovasculares existem poucos estudos. Há um interesse clínico sobre a capacidade funcional para a reabilitação de pacientes após CRM.

Assim, a presente proposta possui dois objetivos principais:

a) revisar os efeitos do tratamento com fototerapia nas doenças cardiovasculares.

b) avaliar o efeito agudo da laserterapia de baixa intensidade na capacidade funcional após a cirurgia de revascularização do miocárdio.

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Capítulo II – Artigo em Inglês

Effects of low-level laser therapy and light-emitting diode therapy on cardiovascular diseases: an integrative review

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EFFECTS OF LOW-LEVEL LASER THERAPY AND LIGHT-EMITTING DIODE THERAPY ON CARDIOVASCULAR DISEASES: AN INTEGRATIVE REVIEW

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Keywords: Heart Diseases, Low-Level Laser Therapy, Review.

ABSTRACT

BACKGROUND AND PURPOSE: Cardiovascular disease (CVD) decreases functional capacity contributing for a worst prognosis of the individuals who suffer of cardiovascular dysfunction. Phototherapy may be a relevant intervention able to improve functional capacity and other related factors associated with CVD. This integrative review investigated the main effects of treatment with phototherapy on cardiovascular dysfunctions. METHODS: Electronic databases were searched to identify studies investigating Low-Level Laser Therapy (LLLT) and/or Light-Emitting Diode Therapy (LEDT) in cardiovascular dysfunctions. The main outcomes observed were functional capacity, myocardial infarcted area, levels of cytokines, oxidative stress biomarkers, vascular endothelial growth factor, angiogenesis, cardiac damage biomarkers, pain and healing. The selection of articles, the assessment of evidence quality and data extraction were made by two independent reviewers. RESULTS: From a total of 1996 articles found in the database, 18 fulfilled the eligibility criteria. Moreover, one study was found from unpublished trials. The selected nineteen full-texts were composed of clinical trials (n = 5), pilot studies (n = 2), single case study (n = 2) and animal models studies (n = 10). The CVD conditions investigated were coronary artery bypass grafting (CABG), heart failure (HF) and myocardial infarction (MI). LLLT, LEDT and association of both were used in two, three, and one study, respectively. In clinical trials, phototherapy intervention was not able to improve functional capacity, but improved healing, decreased cardiac cellular damage biomarkers, and alleviated pain. In animal models studies, intervention with phototherapy reduced infarcted area and concentration of cardiac damage biomarkers, modulated oxidative stress and inflammatory cytokines, and increased levels of VEGF, associated to angiogenesis. Functional capacity was improved only by LEDT in the

animal model study. CONCLUSIONS: This set of data indicates that the use of phototherapy in cardiovascular dysfunction presents a beneficial outcome by the modulation of cytokines, oxidative stress, pain, healing and functional capacity in subjects with cardiovascular diseases.

1. INTRODUCTION

Cardiovascular disease (CVD) remains the leading cause of global morbidity and mortality. Abnormal lipids, smoking, hypertension, diabetes, abdominal obesity, poor diet, and sedentarism account for more than 90 % of the risks to develop CVD as demonstrated in epidemiological studies [1]. The main causes of death by CVD are due to coronary heart disease (CHD), myocardial infarction (MI), stroke, and rheumatic heart disease [2]. The mortality rate for individuals between 35 and 64 years-old are 41% in South Africa, 35% in India, 28% in Brazil, 12% in the USA, and 9% in Portugal [3]. The reasons for high development of CVD, high lethality, and high premature mortality include biological mechanisms, social determinants, and their interactions [4].

Vessels, such as coronary arteries, are affected by CDV. The vessel walls are characterized by lipid accumulation, which increases the risk of blockage by blood clots. This event is called a heart attack whereas blockage of a blood vessel connected to brain causes a stroke [5]. Consequently, patients who suffer from CDV show a pronounced decrease in functional capacity and quality of life, contributing to high hospitalization rate and worse prognosis. [6]. These conditions associated to pain, oxidative stress and imbalance between pro- and anti-inflammatory cytokines are factors which can greatly increase morbidity and mortality. Thus, the development of strategies and new therapeutic modalities could increase the quality of life and lower the mortality rate.

In this context, transcutaneous electrical nerve stimulation [7], neuromuscular electrical stimulation [8], inspiratory muscle training [9], and phototherapy by Low-Level Laser Therapy (LLLT) and Light-Emitting Diodes Therapy (LEDT) [10, 11] are modalities of treatment that have been used in patients with CVD. Phototherapy is widely used in clinical practice in order to relieve pain, reduce inflammation, improve

healing, functional capacity [12, 13] and to modulate inflammatory cytokines and oxidative stress [14, 15].

However, although some studies have shown beneficial effects of phototherapy in treatment of patients with CVD [16, 17], others studies did not find additional improvements with its application [10, 18]. Integrative review of scientific evidence allows correct and accurate analysis of phototherapy's effectiveness and dissemination of its use whether benefits were proven. Therefore, the aim of our study was to review the effects of treatment with phototherapy on cardiovascular diseases.

2. METHODS

2.1 Eligibility criteria

All studies which assessed the effects of LLLT and/or LEDT on cardiovascular diseases were eligible. Exclusion criteria included studies investigating drugs or other electrophysical modalities that were not phototherapy (LLLT and LEDT), commentaries, and editorials.

2.2 Search strategy

Electronic databases used for the present integrative review were accessed in MEDLINE (via PubMed), EMBASE, Cochrane Central Register of Controlled Trials (Cochrane CENTRAL), and Physiotherapy Evidence Database (PEDro) from the inception to August 2016. Individually or combined terms used were "heart diseases", "low-level laser therapy" and "light-emitting diode therapy". Words related to outcomes of interest were not included to enhance sensitivity of search. Language was not a restricted criterion. References in published articles identified through the database search were used as additional sources to identify potential studies. Additionally, thesis

and dissertations produced in Brazil were searched ("bancodeteses.capes.gov.br"), as well as grey literature at Grey Literature Report (http://www.greylit.org). Search terms were adjusted to fit requirements of each electronic database. The complete search strategy used for the PubMed database is shown in Table I.

2.3 Study selection and data extraction

Two reviewers (CS, MH) separately and independently screened titles and abstracts of studies identified in the database. A standard screening checklist based on eligibility criteria was employed for each study, and studies that did not meet all checklist requirements were excluded. Full-text versions of remaining studies, including those potentially eligible and uncertain, were independently retrieved for second review to determine eligibility by two reviewers. Disagreements concerning a study's eligibility were discussed between reviewers, or arbitrated by a third reviewer (CR) when consensus was not reached. The reviewers were not blinded to authors and institutions of studies under review. When the same samples were analyzed in different studies, the study with the largest sample size was selected. Abstracts published in academic conferences were evaluated, and authors were contacted via e-mail to obtain details when necessary.

In the selected studies, data was extracted by the two reviewers (CS, MH), separately and independently, and disagreements in relation to data extraction were resolved by discussion, or arbitrated by the third author (CR). The following data were extracted from the included studies: methodological design, participants, sample and settings, outcomes, intervention and main finding. The outcomes extracted were functional capacity, infarct size, cytokines, oxidative stress, vascular endothelial growth factor (VEGF), angiogenesis, cardiac damage markers, pain and healing.

2.4 Quality of evidence

The quality of evidence across studies followed the principles of GRADE Working Group [19]. The GRADE system is comprised of five items: (1) presence of within-study limitations (risk of bias); (2) inconsistency of results (heterogeneity); (3) indirectness of evidence; (4) imprecision of the effect estimates; and (5) risk of publication bias. Each non-satisfied item downgraded the overall quality. The quality of evidence was classified into four categories: high, moderate, low, and very low. Overall quality was initially considered "low" when the outcome was assessed by only one study. High risk of bias downgraded the quality of evidence to "very low" [19].

3. RESULTS

3.1 Description of selected studies

Eighteen studies [10, 11, 14-17, 20-31] of the 1996 articles found in electronic databases and reference lists met the eligibility criteria. An additional study [18] was found from unpublished clinical trials as well. Therefore, a total of 19 full-text articles were included in this integrative review. A flow diagram of these studies is presented in Figure I and their are summarized in Table II.

Study designs were diversified and comprised of three randomized clinical trials (RCT) [16, 17, 29], one RCT crossover[18], two pilot studies [10, 26] , one clinical trial [21], two single case studies [25, 30] and ten animal models studies [11, 14, 15, 20, 22-24, 27, 28, 31]. Patients who underwent coronary artery bypass grafting (CABG) [16-18, 21, 25, 26, 29, 30] and who had heart failure (HF) [10] participated in the clinical trials . In the animal model studies, rats with HF [11, 14, 20] and myocardial infarction

(MI) were analyzed [15, 22, 23, 27, 28, 31]. One study included rats and dogs with MI [24].

Regarding the phototherapy intervention, LLLT was used in 15 studies [10, 14, 15, 18, 20-29, 31], LEDT was used in three studies [11, 16, 30], and one study evaluated both interventions [17]. Intervention time was acute and ranged from 01 to 10 sessions in 16 studies [10, 14-18, 20-24, 26-29, 31] and three studies intervention time was chronic and ranged from 02 to 08 weeks [11, 25, 30]. Positive and consistent results were obtained using phototherapy (LLLT and LEDT) with 635, 640, 660, 685, 780, 804, 805, 810, 850 and 950 nm wavelengths. Energy density ranged between 0.1 and 22.5 J/cm^2 per point or site, and power output from 0.5 to 400 mW per spot.

3.2 Effects and results of phototherapy interventions in the animal models and clinical trials

3.2.1 Animal model studies

One study evaluated the chronic effects of LEDT (850 nm) on the functional capacity of rats with HF [11]. LEDT was applied directly on the right gastrocnemius muscle, five times per week during eight weeks. Intervention increased functional capacity of rats with HF when compared to control rats in function of distance the covered, the time and the speed of exercise.

Three studies assessed the acute effects of LLLT (810 nm) on infarct size in rats and dogs submitted to MI procedure. Tuby and col. (2011) demonstrated that irradiating LLLT in the tibia of infarcted rats was increased the implantation of mesenchymal stem cells (MSCs) in the heart. This effect improved the scarring process in the infarcted area [31]. Oron and col. (2001) applied LLLT (810 nm) using different power densities (2.5 to 20 mW/cm^2) in the infarcted area after coronary artery occlusion in rats and

dogs. Application of low-energy laser irradiation to infarcted myocardium significantly reduced the infarcted area [24]. Manchini and col. (2014) tested the hypothesis that LLLT could modulate inflammation after acute MI in female rats, and improve cardiac function. Results demonstrated that LLLT diminished pro-inflammatory cytokines (IL- 1β and IL-6) in the heart and infarct size [22].

Two studies evaluated the acute effects of LLLT on oxidative stress markers in rats with HF and MI. Biasibetti and col. (2014) submitted rats with HF to LLLT (660 nm) protocol intervention in the right gastrocnemius muscle during 10 days. LLLT reduced superoxide dismutase (SOD) activity and 2′,7′-Dihydrodichlorofluorescein oxidation levels, indicatinginducing a less oxidative environment in skeletal muscle of HF rats [14]. Monich and col. (2011) aimed to investigate phototherapy biomodulation on the heart muscle submitted to a protocol of ischemia-reperfusion. Two groups of isolated hearts were tested, one being irradiated with LLLT (660 nm) and the non- irradiated heart with red light. Authors found that after ischemia-reperfusion, LLLT improved myocardial contractility post-ischemia, increased SOD activity and reduced of lipid peroxidation [23].

Additionally, two studies evaluated the acute effects of LLLT on inflammatory cytokines in the cardiac tissue of rats. Hentschke and col. (2013) evaluated the influence of LLLT (660 nm on the right gastrocnemius for 10 consecutive days) four weeks after myocardial infarction. LLLT significantly reduced the levels of TNF-α and IL-6 and increased IL-10, followed by decreased of IL-6/IL-10 and increased IL-10/TNF-α ratio in the gastrocnemius muscle [20]. Yang and col. (2011) hypothesized that LLLT (635 nm) could influence the expression of cardiac cytokines and contribute to the reversal of ventricular remodeling in MI models by coronary ligation. Cardiac function and structure were evaluated by echocardiography and histological analysis two weeks after

MI. Five rats from each group were sacrificed one day after laser irradiation, and heart tissues were analyzed by cytokine antibody arrays. Results showed that LLLT regulated cytokine expression in injured myocardium at an early stage of MI, and it reduced the expansion of infarcted area. However, there was no improvement of the heart function 2 weeks following the laser treatment [15].

Levels of VEGF and angiogenesis were investigated in two studies. Tuby and col. (2006) investigated the effects of LLLT on VEGF protein expression in rats submitted to MI. LLLT (804 nm) was applied to intact and post-infarction hearts. Both laser-irradiated intact and post-infarction hearts demonstrated significant increase in VEGF expression compared to laser-unirradiated hearts, followed by enhanced angiogenesis evaluated by 5-bromo-2-deoxyuridine (BrdU), a proliferation marker [27]. In another study, Tuby and col. (2009) evaluated the possible beneficial effects of laser- irradiated mesenchymal stem cells (MSCs) implantation in infarcted hearts of rats. MSCs from rats were isolated from the bone marrow and grown in culture. Cells were irradiated with LLLT (810 nm), labeled with BrdU and were implanted into the infarcted hearts. Non-irradiated cells were similarly labeled and acted as controls. Infarcted hearts were excised three weeks later and cells were stained for BrdU and c-kit immunoreactivity. Laser irradiated cells reduced infarct sized by 53% compared to hearts submitted to non-irradiated cells [28].

3.2.2 Clinical studies

Two studies assessed the acute effects of LLLT on functional capacity in patients with HF and CABG. Recently, Bublitz and col. (2016) randomized in two groups patients diagnosed with systolic HF: placebo LLLT group (n=10) and active LLLT group (808 nm; n=10). A six minute walk test (6MWT) was performed. Both

placebo and active LLLT groups increased 6MWT distance compared to baseline values (P= 0.03 and P= 0.01, respectively). However, no difference was observed at inter- group comparison. The active LLLT group showed significant reduction in perceived exertion Borg scale in relation to the placebo group (P= 0.006) [10]. The unpublished study performed on patients that underwent CABG crossed over fifteen patients during the experimental protocol to compare outcomes after active LLLT and placebo LLLT treatments. Distances walked assessed by Incremental Shuttle Walking Test revealed no significant difference between active or placebo LLLT treatments. Therefore, functional capacity after CABG was not improved by LLLT [18].

Five clinical studies on patients that underwent CABG assessed pain through the visual analogue scale (VAS) and the McGill pain questionnaire [16, 17, 25, 26, 30]. However, one study compared hyperglycemic and normoglycemic CABG patients with internal mammary artery grafts [17]. LLLT intervention was used in two studies [25, 26], LEDT in two studies [16, 30], and one was developed with LLLT and LEDT interventions [17]. The studies designs of these five studies were two RCT [16, 17], one pilot study [26], and two single case studies [25, 30]. Treatment time was acute in three studies ranging between 1-5 sessions [16, 17, 26], and was chronic in two studies [25, 30] with more than two weeks of treatment. All studies demonstrated that LLLT and LEDT were effective in controlling pain.

Healing in CABG patients was assessed by five studies through photographic images and the pressure ulcer scale of healing (PUSH). All the analyses were evaluated by independent researchers [16, 25, 26, 29, 30]. The selected studies were comprised of two RCT [16, 29], one pilot study [26] and two single case studies [25, 30]. LLLT intervention was used in three studies [25, 26, 29] and LEDT in two studies [16, 30]. Treatment time was acute (1-5 sessions) in three studies [16, 26, 29] and chronic (more

than two weeks) in two studies [25, 30]. LLLT and LEDT interventions were both effective ways to in control healing.

One study assessed the effects of LLLT treatment on cardiac damage markers in patients with CABG [21]. LLLT (810 nm) was used during three consecutive days post-CABG in a group of 32 patients, while 28 other patients formed a control group. Complete blood count (CBC) and cardiac damage plasmatic markers (CPK, CPK-MB, LDH) were repeatedly measured before surgery and during 5 days of LLLT application post-CABG (collected one and 12 hours after daily laser irradiation). A significant difference was seen in changes of CPK, CPK-mb and LDH over time $P<0.001$. The study concluded that low-level laser irradiation after CABG surgery can decrease cardiac cellular damage and assist in repairing post-operative cardiac tissue [21].

Quality of evidence

Functional capacity, infarct size, cytokines, oxidative stress, vascular endothelial growth factor (VEGF), angiogenesis and cardiac damage markers outcomes had quality of evidence rated very low-quality. Pain and healing outcomes had quality of evidence rated low-quality. The outcomes were assessed using GRADE scale. The evidence profile is presented in Table III.

4. DISCUSSION

The goal of our integrative review was to join the main findings of the use phototherapy intervention in cardiovascular disease conditions, focusing in the role of LLLT and LEDT effects. These strategies presented effective results as a reduction of infarcted area in animal models of coronary occlusion, modulate positively oxidative stress, inflammatory cytokines, cardiac damage markers, as well as it causes reduction

of pain and improve of healing and angiogenesis processes. However, improvement of functional capacity diverged between LLLT and LEDT.

Employment of phototherapy in patients with CVD has been considered a promising therapy for cardiac rehabilitation, which have been demonstrated by theoretical and experimental analyses of its effectiveness [11, 16]. Mechanism of action of LLLT and LEDT can be associated to beam of light absorption by photoreceptor cells which trigger several chemical responses [13-15]. This photochemical effect is called photobiostimulation [16].

Evaluation of functional capacity after phototherapy intervention was found just in three studies, two clinical and one animal model, despite this outcome be considered an important factor for the functional independence of individuals with CVD. LEDT application in animal model enhanced functional capacity by increase of 32.5% in walking distance after eight weeks of therapy [11]. However, the clinical studies used only a single treatment with LLLT in RCTs, which demonstrated to be ineffective to induce changes after the exercise evaluation test [10, 18]. It may indicate that that the number of phototherapy applications could be an important determinant to reach the threshold of photobiostimulation by accumulation of energy doses to produce a functional improvement. Phototherapy effects in the muscular tissue are related to increase of cytochrome c oxidase activity, an mitochondrial enzyme that contributes for ATP synthesis, augment, and influences peripheral muscle without causes alteration in central hemodynamic control [11]. In this way, to obtain a better translational view from the positive effect observed in the animal model in the clinical field, future trials should be conducted to verify the effects of prolonged phototherapy interventions in subjects with heart disease.

Integrative review results showed that laser therapy reduced infarcted size in animal models of myocardial infarction [22, 24, 31]. Nevertheless, mechanisms associated to cardioprotective effect by LLLT are not clearly understood. Mitochondrial respiration and ATP synthesis augment because of laser therapy may attenuate decrease in ATP in injured cardiomyocytes [24, 32, 33]. Irreversible adverse effects in ischemic zone have slower rate of degeneration due to ATP elevation [24]. Moreover, proper laser power density and timing are necessary to achieve optimal biological effect [24].

Another effect studied were parameters related to oxidative stress in rats with HF and MI [14, 23]. Laser therapy seems to induce increase of antioxidant defenses. Reduction of generation of excessive free radicals because of systemic inflammatory in HF may explain reduction of SOD antioxidant enzyme [14]. Lower need for defense would require a lower SOD antioxidant capacity [34, 35], while in MI the SOD activity is reactivate by LLLT despite of acidosis at ischemic tissue [14, 23].

Moreover the positive effect regarding oxidative stress, phototherapy may be a potential anti-inflammatory therapy without side effect [36]. Important systemic immunomodulatory effects of the laser were cited [15, 20] in this review. It shows a potential use of LLLT employment to contribute in the management of heart diseases [15, 20]. Pro-inflammatory cytokines modulation can influence the prognosis after MI [20] due to its effects in cardiac repair [15]. Laser treatment was associated to increased mobilization from MSCs to regenerate the damaged myocardium, besides of stimulation of collateral vessels formation, infarct healing, and suppression of cardiomyocyte apoptosis by activation the cytoprotective transcription factors as heat shock factor (Hsf)1 and nuclear factor-erythroid 2 p45-related factor (Nrf)2 [15]. At this point, two studies markedly demonstrated an upregulation of VEGF and angiogenesis, which were considered a cardioprotective effects of LLLT [27, 28]. Therefore, the use of LLLT to

attenuate the expression of growth factor or alter in a level of gene expression may offer a new clinical application [27].

Pain was assessed by VAS and McGill questionnaire, and it was reduced in all five studies which evaluate this outcome even they presenting different designs. Patients submitted to CABG surgery usually have chest pain near to sternotomy incision during the first postoperative week, which enhances mainly during chest movements, as to cough or to do deep breath [17]. Whereas good lung ventilation and pervious airway are necessary to prevent respiratory complications, a good analgesic protocol is essential to maintain coughing amongst respiratory exercises in hospitalization period [17]. The process of healing was another important outcome observed in five studies that investigated the use of phototherapy in cardiovascular conditions. LLLT and LEDT accelerate and optimize the tissue repair process [37], which can contribute to prevent CABG's surgical wound dehiscence, a serious complication [38]. Moreover, postoperative complications may occur after procedure of resection of saphenous vein [25].

The major photobiostimulation's mechanism of action is the absorption of photons by mitochondria cytochrome c oxidase. Its results in increase of ATP production which creates large quantities of cell energy [16]. This energy causes normalization of cell's functions as well as pain relief and healing by cellular metabolism augment [16]. Activation of peripheral opioid receptors, and the L- arginine/nitric oxide pathway also may be mechanism whereby phototherapy relieves pain [17, 39]. Mechanisms involved in tissue repair by LLLT include increase in number of dividing cells, and in expression level of inducible nitric oxide synthase, keratinocyte growth factor and keratinocyte growth factor receptor which may facilitate earlier and thicker re-epithelization [40].

Several debates have been proposed about LLLT and LEDT, and in both methods, there is not a proposed irradiation parameters including wavelength and energy density [16]. Our integrative review showed this, and it was possible to observe a great diversity since has not been defined consensus about it. Publications about the use of phototherapy for treatment of cardiac diseases are limited. A problem detected in our integrative review is the variation in methodology, dosimetry and other parameters between studies, and the inclusion criteria and diagnosis of patients. The studies are not standardized, and consequently the results are differing and comparison is difficult. In this way, should be encourage the development of more studies to better understand of physiological mechanisms of action of phototherapy. The standardization of parameters of LLLT and LEDT will be helpful to the observe the best effects in cardiovascular diseases considering its important beneficial.

4.1 Limitations

A limitation of our integrative review is differences in methodology of included studies. Because of it, we cannot carry out a quantitative analysis of studies. The quality of evidence obtained from the included trials was graded as low and very low, which means that there is substantial uncertainty in their results. This might be explained by the high risk of bias inherent to most of the included studies (few patients evaluated and indirectness of outcomes effects).

5. CONCLUSION

Current evidence supports that phototherapy intervention with LLLT and LEDT is effective to contribute in the treatment of cardiovascular diseases. The collected data obtained from this integrative review indicates that laser therapy may represents a

practical tool to contribute as a therapeutic possibility due toimprovement of functional capacity, positive balance between pro- and anti-inflammatory cytokines, modulation of stress oxidative, pain relief, and wound healing. However, new studies, especially clinical trials with larger sample sizes and high methodological quality, are required to establish and prove the beneficial effects of LLLT and LEDT into cardiovascular diseases treatment.

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Table and figures

Table I. Literature search strategy used for the PubMed database

#1 Patient	*"heart diseases" [mesh] OR "heart diseases" OR "Disease, Heart" OR "Diseases, Heart" OR "Heart Disease" OR "Cardiac Diseases" OR "Cardiac Disease" OR "Disease, Cardiac" OR "Diseases, Cardiac" OR "heart failure" [mesh] OR "heart failure" OR "Cardiac Failure" OR "Myocardial Failure" OR "Heart Failure, Left-Sided" OR "Heart Failure, Left Sided" OR "Left-Sided Heart Failure" OR "Left Sided Heart Failure" OR "Heart Failure, Right-Sided" OR "Heart Failure, Right Sided" OR "Right-Sided Heart Failure" OR "Right Sided Heart Failure" OR "Congestive Heart Failure" OR "Heart Failure, Congestive" OR "Heart Decompensation" OR "Decompensation, Heart" OR "thoracic surgery" [mesh] OR "thoracic surgery" OR "Surgery, Thoracic" OR "Surgery, Cardiac" OR "Surgery, Heart" OR "Heart Surgery" OR "Cardiac Surgery" OR "cardiac surgical procedures" [mesh] OR "cardiac surgical procedures" OR "Procedure, Cardiac Surgical" OR "Procedures, Cardiac Surgical" OR "Surgical Procedure, Cardiac" OR "Surgical Procedures, Cardiac" OR "Surgical Procedures, Heart" OR "Cardiac Surgical Procedure" OR "Heart Surgical Procedures" OR "Procedure, Heart Surgical" OR "Procedures, Heart Surgical" OR "Surgical Procedure, Heart" OR "Heart Surgical Procedure" OR "inferior wall myocardial infarction" [mesh] OR "inferior wall myocardial infarction" OR "Myocardial Infarction, Inferior Wall" OR "Diaphragmatic Myocardial Infarction" OR "Diaphragmatic Myocardial Infarctions" OR "Infarction, Diaphragmatic Myocardial" OR "Infarctions, Diaphragmatic Myocardial" OR "Myocardial Infarction, Diaphragmatic" OR "Myocardial Infarctions, Diaphragmatic" OR "Inferior Myocardial Infarction" OR "Infarction, Inferior Myocardial" OR "Infarctions, Inferior Myocardial" OR "Inferior Myocardial Infarctions" OR "Myocardial Infarction, Inferior" OR "Myocardial Infarctions, Inferior" OR "Acute Inferior Myocardial Infarction" OR "anterior wall myocardial infarction" [mesh] OR "anterior wall myocardial infarction" OR "Myocardial Infarction, Anterior Wall" OR "Anterolateral Myocardial Infarction" OR "Anterolateral Myocardial Infarctions" OR "Infarction, Anterolateral Myocardial" OR "Infarctions, Anterolateral Myocardial" OR "Myocardial Infarction, Anterolateral" OR "Myocardial Infarctions, Anterolateral" OR "Anteroseptal Myocardial Infarction" OR "Anteroseptal Myocardial Infarctions" OR "Infarction, Anteroseptal Myocardial" OR "Infarctions, Anteroseptal Myocardial" OR "Myocardial Infarction, Anteroseptal" OR "Myocardial Infarctions, Anteroseptal" OR "Acute Anterior Wall*

Myocardial Infarction" OR "tricuspid valve stenosis" [mesh] OR "tricuspid valve stenosis" OR "Stenoses, Tricuspid Valve" OR "Stenosis, Tricuspid Valve" OR "Tricuspid Valve Stenoses" OR "Valve Stenoses, Tricuspid" OR "Valve Stenosis, Tricuspid" OR "pulmonary valve stenosis" [mesh] OR "pulmonary valve stenosis" OR "Valvular Pulmonic Stenosis" OR "Pulmonic Stenoses, Valvular" OR "Pulmonic Stenosis, Valvular" OR "Valvular Pulmonic Stenoses" OR "Stenosis, Pulmonary Valve" OR "Pulmonary Valve Stenoses" OR "Stenoses, Pulmonary Valve" OR "Pulmonary Stenosis" OR "Pulmonic Stenosis" OR "Pulmonic Stenoses" OR "Stenoses, Pulmonic" OR "Stenosis, Pulmonic" OR "Stenosis, Pulmonary" OR "Pulmonary Stenoses" OR "Pulmonary Stenose" OR "Stenose, Pulmonary" OR "Stenoses, Pulmonary" OR "mitral valve stenosis" [mesh] OR "Mitral Valve Stenoses" OR "Stenoses, Mitral Valve" OR "Stenosis, Mitral Valve" OR "Valve Stenoses, Mitral" OR "Valve Stenosis, Mitral" OR "Mitral Stenosis" OR "Mitral Stenoses" OR "Stenoses, Mitral" OR "Stenosis, Mitral" OR "aortic valve stenosis" [mesh] OR "aortic valve stenosis" OR "Aortic Valve Stenoses" OR "Stenoses, Aortic Valve" OR "Stenosis, Aortic Valve" OR "Valve Stenoses, Aortic" OR "Valve Stenosis, Aortic" OR "Aortic Stenosis" OR "Stenoses, Aortic" OR "Stenosis, Aortic" OR "heart valve diseases" [mesh] OR "heart valve diseases" OR "Disease, Heart Valve" OR "Diseases, Heart Valve" OR "Heart Valve Disease" OR "Valve Disease, Heart" OR "Valve Diseases, Heart" OR "Valvular Heart Diseases" OR "Disease, Valvular Heart" OR "Diseases, Valvular Heart" OR "Heart Disease, Valvular" OR "Heart Diseases, Valvular" OR "Valvular Heart Disease" OR "sternotomy" [mesh] OR "sternotomy" OR "Sternotomies" OR "Median Sternotomy" OR "Median Sternotomies" OR "Sternotomies, Median" OR "Sternotomy, Median" OR "percutaneous coronary intervention" [mesh] OR "percutaneous coronary intervention" OR "Coronary Intervention, Percutaneous" OR "Coronary Interventions, Percutaneous" OR "Intervention, Percutaneous Coronary" OR "Interventions, Percutaneous Coronary" OR "Percutaneous Coronary Interventions" OR "Percutaneous Coronary Revascularization" OR "Coronary Revascularization, Percutaneous" OR "Coronary Revascularizations, Percutaneous" OR "Percutaneous Coronary Revascularizations" OR "Revascularization, Percutaneous Coronary" OR "Revascularizations, Percutaneous Coronary" OR "angioplasty, balloon, coronary" [mesh] OR "angioplasty, balloon, coronary" OR "transluminal Coronary Balloon Dilation" OR "Coronary Angioplasty, Transluminal Balloon" OR "Angioplasty, Coronary Balloon" OR "Angioplasties, Coronary Balloon" OR "Balloon Angioplasties, Coronary" OR "Balloon Angioplasty, Coronary" OR "Coronary Balloon Angioplasties" OR "Coronary Balloon Angioplasty" OR "Balloon Dilation, Coronary Artery" OR "Angioplasty, Transluminal, Percutaneous Coronary" OR

	"Percutaneous Transluminal Coronary Angioplasty" OR "Myocardial ischemia" [mesh] OR "Myocardial ischemia" OR "Ischemia, Myocardial" OR "Ischemias, Myocardial" OR "Myocardial Ischemias" OR "Ischemic Heart Disease" OR "Heart Disease, Ischemic" OR "Disease, Ischemic Heart" OR "Diseases, Ischemic Heart" OR "Heart Diseases, Ischemic" OR "Ischemic Heart Diseases" OR "Rheumatic Heart disease" [mesh] OR "Rheumatic Heart disease" OR "Disease, Rheumatic Heart" OR "Diseases, Rheumatic Heart" OR "Heart Disease, Rheumatic" OR "Heart Diseases, Rheumatic" OR "Rheumatic Heart Diseases" OR "Bouillaud Disease" OR "Disease, Bouillaud" OR "Bouillaud's Disease" OR "Bouillauds Disease" OR "Disease, Bouillaud's" OR "coronary disease" [mesh] OR "coronary disease" OR "Coronary Diseases" OR "Disease, Coronary" OR "Diseases, Coronary" OR "Coronary Heart Disease" OR "Coronary Heart Diseases" OR "Disease, Coronary Heart" OR "Diseases, Coronary Heart" OR "Heart Disease, Coronary" OR "Heart Diseases, Coronary" OR "pulmonary heart disease" [mesh] OR "pulmonary heart disease" OR "Disease, Pulmonary Heart" OR "Pulmonary Heart Diseases" OR "Heart Disease, Pulmonary" OR "Heart Diseases, Pulmonary" OR "Cor Pulmonale" OR "Diseases, Pulmonary Heart" OR "carcinoid heart disease" OR "Carcinoid Heart Diseases" OR "Heart Disease, Carcinoid" OR "Heart Diseases, Carcinoid"
# 2 Intervention	*"Low Level Light Therapy" [Mesh] OR "Low Level Light Therapy" 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 "light-emitting diode therapy" OR "LEDT" OR "light emitting diode"
Search	#1 and #2

Figure 1: The flow diagram of studies included in the review.

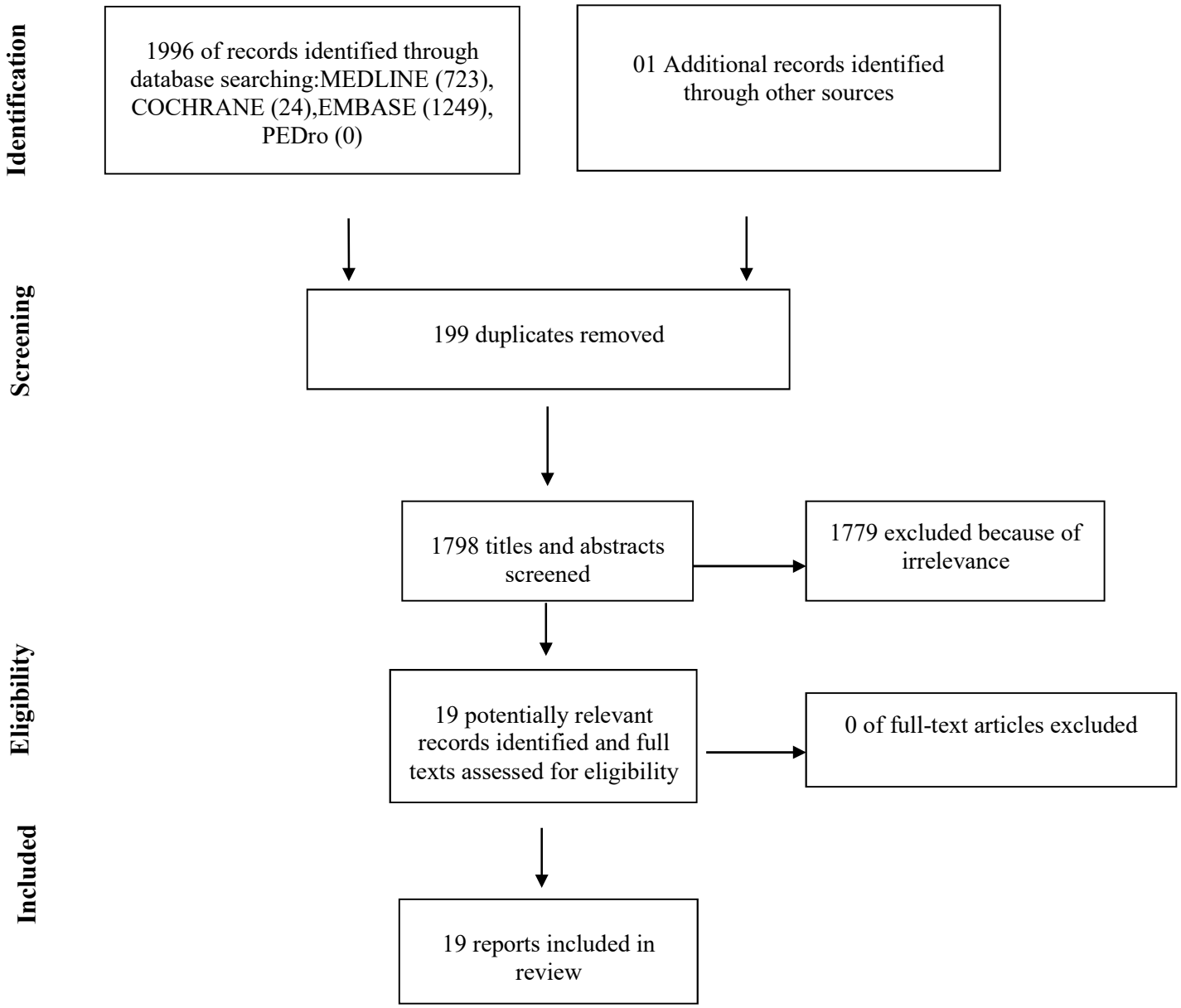

Table II. Characteristics of studies included in integrative review

Author, Year	Method	Participants	Sample &setting	Outcomes	Intervention	Main findings
Animal models studies						
1. Capalonga et al., 2016	Animal model study	Rats with heart failure	n=14 divided into three groups	- Functional capacity	5 sessions/week over 8 weeks: LEDT (850 nm; output power of 50 mW). The energy density was 15 J/cm^2. LEDT was applied directly on the right gastrocnemius muscle	LEDT was able to increase the functional capacity evaluated by distance covered in rats with HF when compared with control rats without intervention
2. Tuby, Maltz and Oron, 2011	Animal model study	Rats with myocardial infarction	n=72 divided into three groups	- Infarct size	1 session: LLLT (804 nm; output power of 400 mW). The energy density was 0.1 J/cm^2. LLLT applied to the bone marrow of the exposed tibia.	Application of LLLT in the exposed tibia was associated to recruitment of the BM-derived MSCs which was associated to better recruitment and, consequently, attenuation of the scaring in the infarcted area.
3. Oron et al., 2001	Animal model study	Rats and dogs with myocardial infarction	n=74 divided into two groups	- Infarct size	Rats: 2 sessions: LLLT (810 nm; output power of 38 mW). The energy density was 0.3 and 0.9 J/cm^2. The laser monitor probe and the infrared sensor card were placed in the chest of the animal at the same distance and angle of the heart. Dogs: 1 session: LLLT (810 nm; output power of 400 mW). The energy density was 1.08 and 2.16 J/cm^2. Laser irradiation was performed directly on the area at risk (anterior and lateral wall) in the myocardium (pericardium was cut and folded laterally) through the "window"' in the chest created during thoracotomy.	LLLT irradiation to infarcted myocardium in rats and dogs reduced significantly the infarcted area
4. Manchini et al., 2014	Animal model study	Rats with myocardial infarction	n=72 divided into three groups	- Infarct size	1 session: LLLT (660 nm; output power of 15 mW). The energy density was	LLLT diminishes the acute inflammation in the myocardium and reduces infarct size

				- Cytokines	22.5 J/cm^2. The laser beam was placed in contact with the myocardium surface corresponding to the infarcted area.	post myocardial infarction
5. Biasibetti et al., 2014	Animal model study	Rats with HF	n=49 divided into six groups	- Oxidative stress	10 daily sessions: LLLT InGaAIP (660 nm; output power of 20 mW). The energy density was 3 and 21 J/cm^2. Placebo LLLT-exposed animals used as controls underwent the same handling procedures, although without laser treatment. The animals received the irradiation medially and laterally to the belly of the gastrocnemius muscle.	LLLT appears to reduce SOD activity and DCFH oxidation levels, changing the oxidative balance in the skeletal muscle of HF rats.
6. Monich et al., 2011	Animal model study	Rats with myocardial infarction	n=280 divided into six groups	- Oxidative stress	1 session: LLLT (660 nm; output power of 0.5 mW). The energy density was 1.03 J/cm^2. In subgroups A, An, C and Cn the right atrium was treated and the light exposure zone spread over the sine atrial node; in subgroups B and D the left ventricle was irradiated.	LLLT results in acceleration of myocardial contractility recovery, rising of the SOD and reduction in the amount of molecular products of lipid peroxidation.
7. Hentschke et al., 2013	Animal model study	Rats with HF	n=49 divided into six groups	- Cytokines	10 daily sessions: LLLT InGaAIP (660 nm; output power of 20 mW). The energy density was 3 and 21 J/cm^2. Placebo LLLT-exposed animals used as controls underwent the same handling procedures, although without laser treatment. The animals received the irradiation medially and laterally to the belly of the gastrocnemius muscle.	LLLT showed systemic and skeletal muscle anti-inflammatory effects in rats with HF.
8. Yang et al., 2011	Animal model study	Rats with myocardial infarction	n=94 divided into three groups	- Cytokines	1 session: LLLT (635 nm; output power of 5 mW). The energy density were 0.1 J/cm^2. LLLT was placed in contact with the myocardium surface	The cytokine expression profile after laser irradiation offers a potential novel mechanism for the use of laser therapy in the treatment of heart disease.

					corresponding to the infarction area.	
9. Tuby, Maltz and Oron, 2006	Animal model study	Rats with myocardial infarction	n=60 divided into four groups	- VEGF - Angiogenesis	7 sessions: LLLT (804 nm; output power of 400 mW). The energy density was 0.6, 1.44 and 2.04 J/cm^2. LLLT was performed by placing the distal tip of the fiber optic directly on the intercostal muscles.	VEGF expression in the infarcted rat heart is markedly upregulated by LLLT and is associated with enhanced angiogenesis and cardioprotection.
10. Tuby, Maltz and Oron, 2009	Animal model study	Rats with myocardial infarction	n=38 divided into three groups	- VEGF - Angiogenesis	1 session: LLLT (804 nm; output power of 400 mW). The energy density was 0.1 J/cm^2. LLLT applied to the tissue cell cultures.	LLLT can significantly increase survival and/or proliferation of MSCs post-implantation into the ischemic/infarcted heart, followed by a marked reduction of scarring and enhanced angiogenesis.
Clinical studies						
11. Bublitz et al., 2016	Pilot study	Patients with HF	n=20 divided into two groups	-Functional capacity	1 session: LLLT cluster probe (808 nm; output power of 100 mW). The energy density was 91 J/cm^2. The active or placebo LLLT application was performed in the middle third of the quadriceps femoris.	Acute effects of LLLT irradiation on skeletal musculature were not able to improve the functional capacity of hospitalized patients with HF.
12. Stein et al.	RCT crossover	Patients who underwent CABG	n=15	- Functional capacity -Oxidative stress	1 session: LLLT (850 nm; output power of 200 mW). The energy density was 85.5 J/cm^2. LLLT at nine points per leg was performed 3 min before the Incremental Shuttle Walking Test.	The use of LLLT did not improve functional capacity nor the redox status after CABG.
13. Lima et al., 2016	RCT	Patients hyperglycemic and normoglycemic patients who underwent CABG	n=120 divided into four groups	- Pain	5 sessions: LLLT (660 nm) and LEDT (640 nm). The energy density was 6 J/cm^2. The irradiation was performed at spots alongside the incision, 2 cm from each other.	LLLT and LED are effective in controlling pain, mainly after postoperative day 6.
14. de Oliveira et al., 2014	RCT	Patients who underwent CABG	N=90 divided into three groups	- Pain	5 sessions: LEDT (640 ± 20 nm; output	The intervention with lighting-emitting diode (LEDT) presented analgesic effect in

				- Healing	power of 70 mW). The energy density per point was 6 J/cm^2. LEDT was applied at spots alongside the incision 2 cm from each other. The placebo group was subjected to the LEDT application process but with the equipment turned off. The control group was only subjected to the assessment protocols and the follow-up.	sternotomy, followed by increase of healing process in surgical incision, preventing dehiscence.
15. Fernandes et al., 2016	RCT	Patients who underwent CABG	n=90 divided into three groups	- Healing	5 sessions: LLLT (660 nm; output power of 40 mW). The energy density per point was 6 J/cm^2. LLLT was applied at spots alongside the incision 2 cm from each other, for a total of eight points. The placebo group was subjected to the LLLT application process but with the equipment turned off. The control group was only subjected to the assessment protocols and the follow-up	LLLT intervention showed a significant capacity to accelerate the healing process after 8 days from the sternotomy procedure compared to control subjects.
16. Pinto et al., 2014	Pilot study	Patients who underwent CABG	n=14 divided into two groups	- Pain - Healing	1 session: LLLT (780 nm; output power of 25 mW). The energy density was 19 J/cm2. LLLT were applied surrounding the entire surgical perimeter wound edge (each 2 cm).	It was observed that treatment with LLLT in the healing process in the area of saphenectomy prevented complication associated the dehiscence, causing a significant improve in the healing compared to group that pass by the traditional process. .
17. Dixit, Maiya and Borkar, 2013	Single case study	Patients who underwent CABG	n=1	-Pain - Healing	5 sessions/week over 2 weeks: LEDT (660 and 950 nm; output power of 10 mW). For the sternal wound, an energy density of 14 J/cm^2 was used and for each shoulder single point was irradiated, anterolaterally with an analgesic dosage of 4 J/cm^2 per day.	LEDT induces biomodulation of dehiscence sternal wound following median sternotomy in CABG. Analgesic effect of laser therapy may be incorporated for early rehabilitation for painful shoulders following mechanical failure of sternum postoperatively in CABG.
18. Pinto et al., 2009	Single case study	Patients who underwent CABG	n=1	-Pain - Healing	2 sessions/week over several weeks: LLLT (685 nm; output power of 20	The use of LLLT in he wound dehiscence in the area from splenectomy for CABG

					mW). The energy density was 4.5 J/cm^{2}. LLLT was punctually applied around the edge of the surgical wound, maintaining an interval of 2 cm from each point, at the whole extent of the dehiscence, maintaining a distance o 0.5 cm away from the skin.	causes a considerable effect in the healing process (anti-inflammatory, analgesic, wound remodeling).
19. Khoo et al., 2014	Clinical trial	Patients who underwent CABG	n=60 divided into two groups	– Cardiac damage biomarkers	3 sessions: LLLT (810 nm; output power of 500 mW). The energy density was 6 J/cm^{2}. The laser was irradiated over the surgery incision (mid sternum) every 1 cm and over the pericardium.	LLLT intervention was associated to decrease levels of cardiac cellular damage biomarkers (CPK, CPK-mb, LDH), which indicates that phototherapy can improve the CABG post-operative prognotic.

Table III. Quality of the evidence.

Quality assessment						Sample	Quality
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision		
Functional capacity							
2	1 RCT crossover, 1 animal model study	serious [1]	---	serious [2]	serious [3]	49	⊕○○○ VERY LOW
Infarct size							
3	Animal models studies	serious[4]	---	serious[5]	serious [3]	218	⊕○○○ VERY LOW
Oxidative stress							
2	Animal models studies	serious[4]	---	serious[6]	serious [3]	329	⊕○○○ VERY LOW
VEGF and Angiogenesis							
2	Animal models studies	serious[4]	---	serious[6]	serious [3]	98	⊕○○○ VERY LOW
VAS							
5	2 RCT, 1 pilot study, 2 single case study	serious[7]	---	serious[8]	---	226	⊕⊕○○ LOW
Healing							
5	2 RCT, 1 pilot study, 2 single case study	serious[7]	---	serious[9]	---	196	⊕⊕○○ LOW
Cardiac damage markers							
1	Clinical trial	serious[10]	---	---	serious [3]	60	⊕○○○ VERY LOW

RCT: randomized controlled trail; HF: heart failure; VEGF: vascular endothelial growth fator; VAS: visual analog scale

1. Studies with different designs; clinical studies evaluating acute effect; one study unpublished
2. Functional capacity was not performed using a gold standard method
3. Few studies
4. Animal models studies
5. One study included rats and dogs with myocardial infarction
6. Studies included rats with HF and myocardial infarction
7. Studies with different designs
8. One study included hyperglycemic and normoglycemic patients who underwent CABG; pain was assessed using an 11 or 10-point visual analog scale
9. Healing was assesses using different methods
10. Study with high risk of bias

Capítulo III – Artigo em Inglês

Acute effects of low-level laser therapy on patients' functional capacity in the postoperative period of coronary artery bypass graft surgery: a randomized, crossover, placebo-controlled trial

(Artigo escrito seguindo o formato da revista Photomedicine and Laser Surgery, fator de impacto: 1.631)

ACUTE EFFECTS OF LOW-LEVEL LASER THERAPY ON PATIENTS' FUNCTIONAL CAPACITY IN THE POSTOPERATIVE PERIOD OF CORONARY ARTERY BYPASS GRAFT SURGERY: A RANDOMIZED, CROSSOVER, PLACEBO-CONTROLLED TRIAL

Running title: LLLT ON PATIENTS' FUNCTIONAL CAPACITY IN THE CABG

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ABSTRACT

***Objective*:** The aim of this study was to evaluate the acute effects of low-level laser therapy (LLLT) on functional capacity after coronary artery bypass graft (CABG) surgery. **Methods:** Fifteen patients were crossed over during the experiment, in order to compare the outcomes after active LLLT and placebo LLLT treatments. LLLT (850 nm, 200 mW, 30 J to each point,), using a multi-diode cluster (with five spots, 6 J from each spot) at eight points per leg was performed 3 min before the Incremental Shuttle Walking Test. We analyzed distance walked, Borg scale of perceived exertion, heart rate, arterial blood pressure, lactate dehydrogenase, levels of oxidative damage to lipids, activities of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT), and total thiol levels in peripheral blood. **Results:** Comparison of the distances walked revealed no significant differences between the LLLT and placebo LLLT ($p= 0.779$) groups. Regarding the Borg scale of perceived exertion ($P = 0.567$), heart rate ($P= 0.506$), systolic ($p= 0.164$) and diastolic ($p= 0.140$) blood pressure, no difference was observed between the LLLT and placebo LLLT groups. The application of LLLT was not able to modulate lactate dehydrogenase enzymes ($p= 0.214$), lipids damage ($p= 0.733$), SOD ($p= 0.202$) and CAT ($p= 0.825$) activity, and total thiol levels ($p = 0.925$). **Conclusions:** The use of LLLT acute did not improve neither functional capacity nor the redox status after CABG. **Trial registration:** Registered as a clinical trial (NCT02688426).

Keywords: Thoracic Surgery; Low-Level Laser Therapy; Functional Capacity; Exercise

INTRODUCTION

Cardiovascular disorders are an important public health problem worldwide [1]. Coronary heart disease (CHD) is a leading cause of mortality and morbidity both in developed and developing countries[2]. A treatment option for patients with advanced atherosclerosis is coronary artery bypass graft (CABG) surgery [3]. This surgery aims to increase survival, relieve symptoms of myocardial ischemia, prevent myocardial infarction, improve ventricular function, and prolong patients' quality of life [4]. However, the incidence of complications after cardiac surgeries contributes to patients' functional decline, reduced quality of life, and post-discharge mortality [5].

Around the world, physical and respiratory therapies are prescribed after cardiac surgeries in order to reduce or prevent postoperative complications [6]. New strategies are currently being studied to maximize the effect of these therapies in a population who has undergone cardiac surgery. In this sense, there is a growing interest in the implementation of other physical therapy interventions, such as breathing exercises, inspiratory muscle training, and transcutaneous electrical nerve stimulation. All these interventions have shown important contributions, leading to a significant impact on functional capacity [7-9]. Furthermore, systematic review studies [10,11] have reported positive effects of phototherapy, using both low-level laser therapy (LLLT) and light emitting diodes therapy (LEDT), on the attenuation of muscle fatigue and on post- exercise muscle recovery in humans. Therefore, phototherapy has been considered a non-invasive and non-pharmacological strategy to achieve improvements in exercise performance.

Positive effects of phototherapy on muscle fatigue markers were firstly evidenced by an animal model study [12]. Thenceforth, human trials have been shown promising findings in untrained healthy young adults submitted to isometric [13] and

dynamic [14] exercise protocols, as well in endurance tests [15]; and these findings are further supported by studies involving from professional athletes [16] to older subjects [17]. However, although patients with cardiovascular disorders could be potentially helped by the phototherapy effects on muscle performance, few studies in this field have been conducted with this population. Experimental studies demonstrated that phototherapy was able to increase inflammatory profile [18], oxidative stress [19] and functional capacity [20] in rats with heart failure (HF). In humans, as far as we know, only a pilot study was conducted and found no influence of LLLT on functional performance in acutely decompensated HF patients [21].

In view of the potential effect of phototherapy on muscle responses to exercise, the clinical interest on functional capacity for the rehabilitation of CABG patients, and the lack of studies involving this population, the aim of this study was to evaluate the effects of LLLT on functional capacity in the rehabilitation period after surgery. Our hypothesis was that acute application of LLLT before an exercise testing would increase the distance walked.

METHODS

Participants/Selection criteria

Patients between 45 and 75 years of age were invited to participate in this study. Sample size was determined based on the fact that most experiments conducted with humans involving light therapy and fatigue have used samples with 10 to 14 participants [21,22]. Therefore, a total of 15 subjects was initially determined. Patients who had undergone CABG were recruited from Instituto de Cardiologia do Rio Grande do Sul (Porto Alegre, Brazil). Patients were invited to participate in the study on the day of hospital discharge, occurred between 6 and 10 days after surgery.

The study's inclusion criteria were: patients in the postoperative period of elective CABG referred to cardiac rehabilitation between 15 and 30 days after surgery, between 45 and 75 years old and who had undergone their first surgery. Exclusion criteria were: patients with decompensated heart failure; presence of any comorbidity such as: unstable angina; neurological disease; severe respiratory disease previously diagnosed by doctor; active infectious disease or fever; disabling peripheral vascular disease; unstable ventricular arrhythmias; diabetes mellitus; use of cardiac pacemaker and musculoskeletal disease that limits physical activity.

Ethical aspects

This study was approved by the Ethics Committee of the Universidade Federal de Ciências da Saúde de Porto Alegre. In accordance with the Declaration of Helsinki, written informed consent was obtained from all patients.

Study design

The study was designed as a randomized, double-blinded, placebo-controlled, crossover trial. Patients performed two visits to the laboratory with one-week interval period between them. Identical data collection procedures were performed in each session, except the LLLT or placebo treatments applied before the exercise testing. The primary outcome of this study was the patients' functional capacity. Additionally, we assessed as secondary outcomes: perceived exertion, heart rate, arterial blood pressure and oxidative stress.

Randomization and blinding procedures

Before data collection, volunteers were randomized by a simple drawing of lots (A and B), which determined whether they would receive active LLLT (A) or placebo LLLT (B) at the functional capacity test. All participants were crossed over during the experiment, in order to compare the outcomes after active LLLT and placebo LLLT treatments. The physical therapy responsible for the treatments performed the code drawn for each patient. Thus, the treatment allocation was concealed from the participants and the assessor who evaluated the functional capacity test. Patients wore opaque goggles during the therapy to protect their eyes from the treatment and help in the study blindness.

Low-level laser therapy

Low-level laser therapy (LLLT) was applied using the Chattanooga® equipment, with an infra-red laser cluster probe of the same manufacturer, consisting of five GaAlAs laser diodes (850 nm), each one with an output power of 200 mW. They were applied on patients immediately before a functional capacity test with the probe held stationary to the skin at a 90° angle with a slight pressure. The location of the application of points was defined by palpation of muscle bellies. Eight points per leg were irradiated to attain a wide muscular area: two points on the vastus medialis; three points on the vastus lateralis; and three points on the rectus femoris. The dose and application location were chosen based on previous studies on LLLT in humans [23,24]. The LLLT treatment was applied for 30 s to each point. Thus, 30 J were applied to each point (6 J per diode), resulting in a total of 240 J for the whole quadriceps muscle (Chart 1). The placebo treatment was performed exactly the same way as the LLLT treatment, but with the device turned off.

Functional Capacity test

The Incremental Shuttle Walking Test (ISWT) required the patient to walk around a 10-m course marked out by cones inset 0.5 m from each end to a symptom- limited maximum. The walking speed was determined by pre-recorded bleeps on an audiotape. The tape also contained instructions, and these were played to the patients in the beginning of the ISWT. After each minute of the test, the walking speed increased. The only verbal intervention given by the operator was to remind patients to increase their pace at the beginning of each minute. Non-invasive blood pressure and heart rate were recorded before and after the ISWT. Breathlessness was measured at rest and immediately upon completion of the ISWT using the Borg scale of perceived exertion (0–10). The following criteria were used to terminate the ISWT: when patient developed dyspnea and/or fatigue to maintain the required speed and/or was at a distance shorter than three meters from the cone when the bleep sounded for the change of directions [25]. All tests were conducted by the same researcher, who was experience with this kind of evaluation and was blinded to patients' allocation.

Blood samples

Blood samples were collected in EDTA vacutainer tubes by a qualified nurse blinded to group allocation. Samples were obtained from the antecubital vein before the application of LLLT or placebo LLLT and exactly 3 min after the end of the functional capacity test. Blood was centrifuged at 1.000 RPM for 10 min at 4°C. Plasma was stored at -80°C until analysis. Erythrocytes were washed in a solution of sodium

chloride (0.9%) and centrifuged at 1.000 RPM for 5 min at room temperature three times. After that, the washed erythrocytes were diluted 1/10 in 1-mmol/L acetic acid and 4-mmol/L magnesium sulphate [26]. The supernatant was used for enzymatic assays.

Biochemical and enzymatic assays

Lactate dehydrogenase (LDH) activity was measured in the plasma by using the LDH Liquiform kit from Labtest® (Labtest Diagnóstica S.A., MG, Brazil), according to the manufacturer's instructions. Lipid peroxidation, measured by the TBARS method, was performed according to Ohkawa et al. (1979) [27]. Briefly, plasma was incubated in a mixed reaction containing 0.8% thiobarbituric acid, 20% acetic acid, and 8.1% sodium dodecyl sulfate, for 1 hour at 100ºC. After the reaction cooled down, tubes were centrifuged at 14,000 RPM for 2 min, and the supernatant was measured spectrophotometrically at 532 nm. Values were calculated based on a standard curve using 1-1-3-3-tetraethoxypropane (Sigma Aldrich, T-9889), and results were expressed in nmol per ml of plasma.

Superoxide dismutase (SOD) enzyme activity was determined based on the inhibition of superoxide radical reaction with pyrogallol [28]. Briefly, the supernatant of washed erythrocytes was mixed with a reaction medium containing 50 mmol/L Tris, 1 mmol/L + 1 mmol/L EDTA buffer (pH 8.2), 24 mmol/L pyrogallol, and 30 mmol/L catalase. Reaction was performed in a spectrophotometer (VersaMax™ ELISA Microplate Reader), and the absorbance changes were measured at 420 nm for 2 minutes. Based on a standard curve using SOD (Sigma Aldrich, S-8160), results were expressed as U SOD per milligram of protein.

Erythrocytes' catalase enzyme activity was determined by measuring the absorption of hydrogen peroxide at 240 nm in a reaction containing 50 mmol/L sodium phosphate buffer (pH 7.4) and 10 mmol/L hydrogen peroxide [29], in a cuvette

spectrophotometer (T60 UV-Visible Spectrophotometer, PG Instruments). Results were expressed as picomoles of hydrogen peroxide reduced per minute per milligram of protein.

In the plasma, total thiol levels were measured by the reaction containing 5-5'-dithiobis-2-nitrobenzoic acid (DTNB) [30]. Briefly, in a mixed reaction of 10 mmol/L DNTB (prepared in a 0.2 mol/L potassium phosphate buffer, pH 8.0) and 143 mmol/L phosphate + 1 mmol/L EDTA buffer (pH 7.4), the reactions were incubated with the samples for 30 min in the dark. Thiol compounds reduced DTNB to TNB, a yellow compound that was measured at 520 nm in spectrophotometer equipment. The results were determined based on a standard curve using reduced glutathione (Sigma Aldrich, G-4251), and expressed as μmol per ml of plasma.

Protein concentration was measured using the total protein kit from Labtest® (Labtest Diagnóstica S.A., MG, Brazil) according to the manufacturer's instructions.

Statistical analysis

The SPSS statistical software package was used to analyze all data. The normal distribution of data was verified by the Shapiro-Wilk test. The parametric data were expressed by mean and standard deviation. The nonparametric data were expressed by median and interquartile intervals. Distance walked, heart rate, arterial blood pressure, CAT and SOD were analyzed by ANOVA for randomized block design and the Borg scale of perceived exertion; LDH, TBARS and thiol were analyzed by **Friedman's test;** post-hoc analysis was conducted by the Wilcoxon test. A P-value < 0.05 was considered statistically significant for all tests.

RESULTS

Baseline characteristics

From March 2015 to December 2015, 35 patients in the postoperative period of CABG were screened for the study. Out of those, 18 patients did not meet the inclusion criteria or met one or more of the exclusion criteria, and therefore 17 patients were randomized. Two of these subjects did not receive the allocated intervention due to withdrawal. A flow diagram of included, excluded and the final number of participants is illustrated in Fig 1. Clinical characteristics of patients are shown in Table 1.

Functional Capacity

No significant difference was observed on distance walked between the LLLT and placebo treatments: 338.00 ± 118.15 vs. 334.67 ± 116.92 m (Fig 2).

Regarding the Borg scale of perceived exertion, no difference was observed between the LLLT and placebo LLLT groups (P = 0.567), as well as when they were compared pre and post-intervention (Table 2). The heart rate did not change between the LLLT and placebo LLLT groups (P = 0.506) (Table 2). Laser therapy did not decrease neither systolic (P = 0.164) nor diastolic blood pressure (P = 0.140) between the LLLT and placebo LLLT groups (Table 2).

Peripheral markers of muscular tissue damage and oxidative stress parameters

Levels of LDH were not different between the LLLT and placebo LLLT groups (P = 0.214) (Table 2).

Regarding the peripheral markers of oxidative stress, no difference was observed in lipid peroxidation between the LLLT and placebo LLLT (P = 0.733) groups, as well as when they were compared pre and post-intervention (Table 2). Laser therapy did not change SOD (P = 0.202) and catalase enzyme activities in the erythrocytes between the LLLT and placebo LLLT groups (P = 0.825) (Table 2). Total thiol levels did not change between the LLLT and placebo LLLT groups (P = 0.925) (Table 2).

DISCUSSION

To the best of our knowledge, this is the first randomized controlled trial evaluating the effects of low-level laser therapy in patients in the postoperative period of CABG. Findings of the present study did not confirm our initial hypothesis, since we demonstrated that LLLT treatment, with the parameters used here, was not able to change the patients' functional capacity.

Randomized clinical trials have shown that a single treatment of phototherapy applied before exercise in healthy young adults [15,23,24], older subjects [17] and athletes [31,32] led to enhanced exercise performance. Since in view of some studies without positive effects of phototherapy before protocols involving electrical stimulation [33], maximal cycling tests [34] or sustained isometric contractions [35], systematic reviews [10,11] have concluded the ergogenic effect of this treatment in healthy subjects. Recently, Vanin et al (2016) conducted the first study to identify the optimal moment to provide phototherapy irradiation when used in conjunction with strength training programs. Phototherapy before and placebo after training sessions showed significant changes in

maximum voluntary contraction and 1RM tests when compared to other groups [36]. However, few studies have focused their attention to the benefits of phototherapy on functional capacity of subjects affected by diseases of the cardiopulmonary system [21,22].

Miranda et al. (2014) conducted the first phototherapy study with cardiopulmonary disease patients in this research field. Isometric exercise tests until exhaustion were performed after a single phototherapy/placebo application, and their findings suggested that 125 J of LEDT on quadriceps muscle was able to improve the tolerance time to exercise [22]. In a recent pilot study, a more functional exercise testing was applied in hospitalized patients with decompensated HF [21]. As a result, 28 J of LLLT in each quadriceps muscle did not change the distance covered at the 6-min walk test compared to placebo. However, improvements were observed on patients' effort sensation and blood lactate levels after LLLT treatment.

Although the sample characteristics and the type of exercise testing used by Bublitz et al. (2016) are similar to those assessed in our study, we expected that using a different laser dosage the benefits on functional performance would be reached [21]. Therefore, a successful dose in previous controlled trials [15,23,24] and recommended by a meta-analysis study [11]. However, no functional enhancement or changes on the secondary outcomes were found in our study. Our results highlight that phototherapy has several parameters, and each combination of them is capable of producing different responses in the human tissues. Moreover, seems reasonable that specific populations (e.g., athletes, healthy subjects and patients) have individual optimal phototherapy treatments to reach the functional improvements. The present study analyzed only one particular wavelength and energy dose of LLLT, and add to the literature that the parameters used here did not cause benefits for patients with CABG. The news evidences show the best results are obtained with the combination of different

wavelengths and different sources of light (super-pulsed-laser and LEDT). Investigators have shown that a combination of different light sources enhance muscular performance [37], increases cytochrome c oxidase activity [38], increased the distance covered, time to exhaustion, and ventilation and decreased the dyspnea score [39].

Therefore, the parameters used, evolving wavelength and dose may have been one of the causes of the results observed in our study. Interestingly, we found no study assessing the acute effects of phototherapy on animals with HF. Experimental studies have verified improvements in inflammatory profile and oxidative stress in rats with HF through LLLT applied by 10 days [18,19], while functional enhances were found after 8 weeks of LEDT in this animal model [20]. Added to the ineffective single session treatments employed in our study and in Bublitz's study, these evidences suggests longer time periods of phototherapy should be used to promote benefits in patients with HF. Considering that a >10% increase in distance walked is considered to indicate a true improvement in functional capacity caused by the therapeutic intervention for patients in the postoperative period of CABG [35], the 32.5% increase in distance covered by rats stimulated with LEDT in Capalonga et al. (2016) [20] is a promising result, and future investigations should address the effects of prolonged phototherapy interventions in humans.

In view of previous studies which phototherapy did not enhance the exercise performance but changed biochemical markers [23,31,34,40], the present study investigated biomarkers of muscle damage and oxidative stress. Changes on these markers could be useful to patients of the present study because could reduce the muscle damage and the generation of excessive free radicals[41]. In this sense, an interesting study used LLLT over the surgery incision for three successive days post-CABG, and found no effect on LDH levels, as well as on muscle damage markers (CPK and CPK-mb) [42].

The biochemical analyses demonstrated that the ISWT does not elicit changes in muscular tissue damage or oxidative damage. In healthy subjects, muscle damage has been mainly assessed by phototherapy studies using high intensity eccentric exercises [23,43], while oxidative stress was already verified with exercise protocols involving progressive-intensity running exercise until exhaustion [15]. Due to the impaired muscle condition of patients from the present study, we expected that the ISWT might cause significant changes on these biomarkers, which not happened. The ISWT is a reproducible measure for patients following CABG, strongly related with performance on a conventional laboratory-based test of cardiorespiratory fitness and considered a useful and safe tool for functional capacity assessment of patients following CABG [25]. However, the present study demonstrated that this test did not change the biochemical markers assessed, independently of the active or placebo treatment. Therefore, we are not able to determine if LLLT treatment may be an efficient tool to counteract the exercise-induced muscle damage and oxidative stress in patients with CABG.

Some limitations may be highlighted. The clinical impact of our findings could be limited by the absence of a group of subjects treated with isolated LLLT (without training). However, in order to bring our promising results to a clinical setting, future studies should be developed with other populations and combines of super pulsed lasers and light-emitting diodes.

CONCLUSION

In conclusion, we found that low-level laser therapy (using the parameters adopted in this study) applied immediately before, in one application, an exercise testing did not increase functional capacity nor improve redox status of patients with CABG. The current study's results showed the importance of continuing research in this

area to better elucidate the effects of LLLT on this and other patient populations and ultimately broaden appropriate clinical application.

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Disclosure Statement

The authors declare that they have no competing interests and no financial competing interest. All authors have read and approved of the manuscript

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Chart 1. Low-level laser therapy protocol characteristics

Model	Laser Ga AIAs (850 nm)
Number of laser diodes	5
Pulse frequency	Continuous output
Optical output	200 mW
Spot diameter	0.7 cm^2
Spot size	0.0376 cm^2
Power density	2.85 W/cm^2
Protocol regime	Immediately before a functional capacity test
Irradiation sites per application	5 (simultaneously)
Irradiation regions per leg	8 (2 points on the vastus medialis; 3 points on the vastus lateralis; and 3 points on the rectus femoris)
Irradiation sites per leg	40 (10 sites on the vastus medialis; 15 sites on the vastus lateralis; and 15 sites on the rectus femoris)
Irradiation sites per patient	16
Dose per point(J/cm2)	85.5
Total energy per point (J)	30
Total energy per leg (J)	240
Total energy per patient (J)	480
Time per point (s)	30
Time per leg (s)	240
Time per patient (s)	480
Total number of treatment	1
Application mode probe	Stationary to the skin

Fig 1 – Consort diagram for study

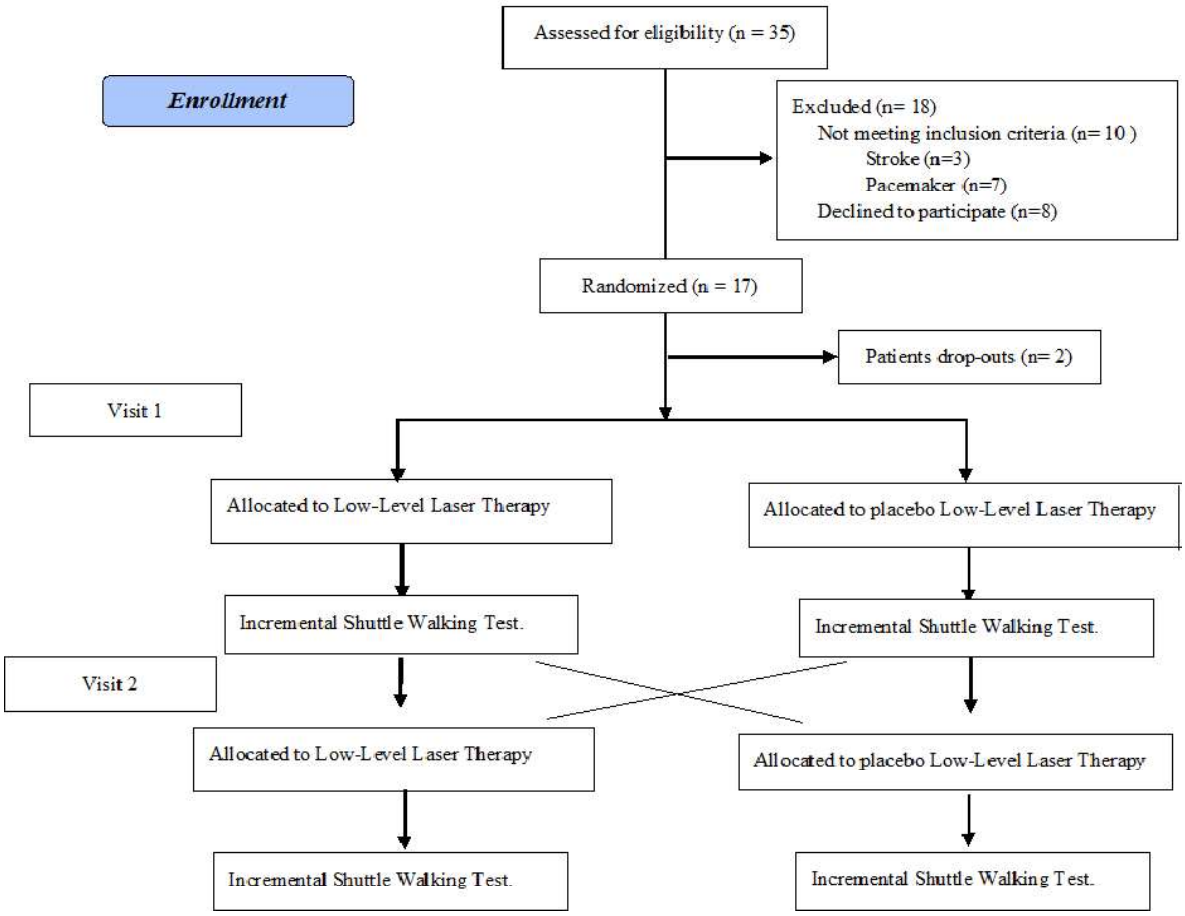
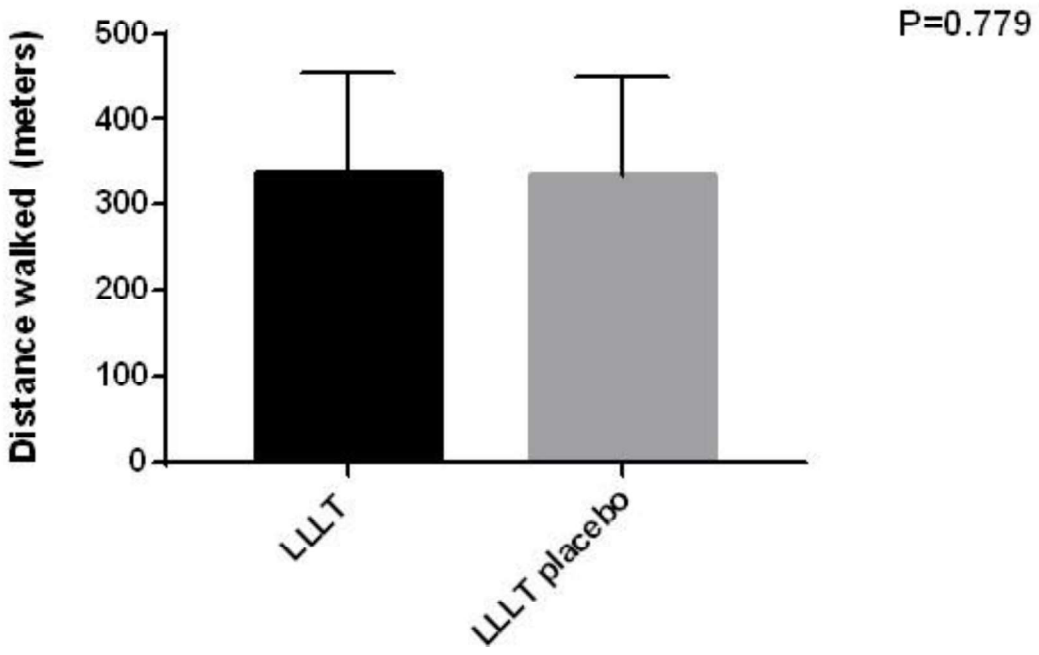


Table 1. Patient characteristics at baseline

Variable	
Age (years)	60.20 ± 9.51
Body mass (kg)	76.79 ± 12.01
Height (m)	1.70 ± 0.05
BMI (kg/m^2)	30.89 ± 4.39
Sex, n (%)	
Male	15 (100%)
Hypertension, n (%)	12
Dyslipidemia, n (%)	10
Left ventricular ejection fraction	69.76 ± 7.27
New York Heart Association, n (%)	
Class I	14 (93%)
Class II	1 (7%)
Drugs, n (%)	
Diuretic	6 (40%)
Spironolactone	1 (7%)
ACEI inhibitors	7 (47%)
Statins	14 (93%)
Beta-blocker	15 (100%)
Antiplatelet	14 (93%)
SBP (mmHg)	111.13 ± 15
DBP (mmHg)	73.80 ± 10.88
Biochemical parameters	
Haemoglobin (g/dL)	10.86 ±1.41
Erythrocytes ($x10^6$/ml)	3.67 ± 0.42
Hematocrits (Packed cell volume)(%)	32.40 ± 3.58
Leukocytes (10^3/ml)	9.42 ± 2.02
Creatinine (ml/dL)	0.85 ± 0.18

Values are expressed as mean ± SD. BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic Blood Pressure;

Fig 2 - Functional capacity assessed by the distance covered during the Sunttle Test



Values are expressed as mean ± SD. LLLT: low-level laser therapy

Table 2 - Functional capacity and peripheral markers of oxidative stress pre- and post-LLLT intervention

	LLLT		LLLT placebo		
	Pre	Post	Pre	Post	p
Heart rate (bpm)	80.93 ± 7.57	96.00 ± 15.37	79.66 ± 9.96	91.40 ± 11.70	0.506
Systolic blood pressure (mmHg)	111.13 ± 14.99	134.60 ± 16.84	124.46 ± 17.39	138.80 ± 20.43	0.164
Diastolic blood pressure (mmHg)	73.80 ± 10.88	83.36 ± 14.59	82.86 ± 18.95	85.20 ± 17.43	0.140
Borg scale of perceived exertion	1 (0 – 2)	4 (3 – 7)	0 (0 – 1)	4 (3 – 5)	0.567
CAT (pmol ml^{-1} of protein)	245.30 ± 83.65	221.95 ± 93.16	234.25 ± 64	226.64 ± 64.34	0.825
SOD (U SOD mg^{-1} of protein)	0.759 ± 0.140	0.613 ± 0.306	0.628 ± 0.149	0.656 ± 0.177	0.202
LDH (IU/l)	60.71 (44.52 – 68.08)	60.71 (40.47 – 89.04)	60.71 (52.62 – 89.04)	64.76 (60.71 – 89.04)	0.214
TBARS (nmol ml^{-1} of plasma)	1.741 (0.620 – 2.671)	1.155 (0.688 – 1.602)	0.846 (0.654 – 1.328)	0.876 (0.507 – 1.463)	0.733
Total thiols (µmol ml^{-1} of plasma)	0.798 (0.617 – 0.973)	0.751 (0.619 – 0.886)	0.743 (0.686 – 0.921)	0.738 (0.576 – 0.920)	0.925

Values are expressed as mean ± SD for heart rate, systolic blood pressure, diastolic blood pressure, SOD and CAT. Values are expressed as median and interquartile intervals for Borg scale of perceived exertion, LDH, TBARS and total thiols. SOD: superoxide dismutase. CAT: catalase. LDH: Lactate Dehydrogenase. TBARS: Thiobarbituric Acid Reactive Substances. LLLT: Low-Level Laser Therapy.

Capítulo IV – Anexos

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: EFEITO AGUDO DA LASERTERAPIA DE BAIXA POTÊNCIA NA CAPACIDADE FUNCIONAL DE PACIENTES NO PÓS - OPERATÓRIO DE CIRURGIA DE REVASCULARIZAÇÃO DO MIOCÁRDIO: ENSAIO CLÍNICO RANDOMIZADO

Pesquisador: Rodrigo
Della Méa Plentz

Área Temática:

Versão: 1

CAAE: 34036614.2.0000.5345
Instituição Proponente: Universidade Federal de Ciências da Saúde de Porto Alegre
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 760.651
Data da Relatoria:
21/08/2014

Apresentação do Projeto:

O presente projeto é parte da tese de doutorado e têm como objetivo verificar o efeito agudo da laserterapia de baixa potência na capacidade funcional de pacientes no pós-operatório de cirurgia revascularização do miocárdio. O mesmo trata-se de um ensaio clínico randomizado cruzado duplo-cego onde os pacientes serão alocados em dois grupos. Os pacientes do grupo A receberão aplicação de LBP no primeiro dia e aplicação placebo no segundo dia de testes, enquanto os pacientes do grupo B receberão placebo no primeiro dia e LBP no segundo dia. A LBP será aplicada de forma bilateral sobre seis pontos no músculo quadríceps femoral dos pacientes com um equipamento com comprimento de onda de 850 nm, composto por 5 diodos de 200 mW e 30 J de energia será administrada sobre cada ponto de aplicação (6 J por diodo). As sessões de testes serão realizadas entre o 15º e o 30º dias de pós-operatório, sendo respeitado um intervalo de 3 dias entre as sessões. Em cada sessão, a aplicação de LBP ou placebo será seguido de avaliações da capacidade funcional, dano muscular e estresse oxidativo.

Objetivo da Pesquisa:

Geral
Verificar o efeito agudo da laserterapia de baixa potência na capacidade funcional de pacientes no pósoperatório de cirurgia revascularização do miocárdio.

Continuação do Parecer: 760.651

Específicos
Verificar o efeito agudo da laserterapia de baixa potência no dano muscular depois de realizado o teste para avaliar a capacidade funcional de pacientes no pós-operatório de cirurgia revascularização do miocárdio. Verificar o efeito agudo da laserterapia de baixa potência no estresse oxidativo depois de realizado o teste para avaliar a capacidade funcional de pacientes no pós-operatório de cirurgia revascularização do miocárdio.

Avaliação dos Riscos e Benefícios:

Os pesquisadores referem que os riscos do procedimento serão mínimos, incluindo um desconforto muscular leve após a realização das avaliações. A aplicação da laserterapia não causa queimaduras e alterações na pele. Todas as avaliações e a aplicação da laserterapia de baixa potência ocorrerão dentro do ambiente hospitalar.
Os benefícios residem no fato do paciente conhecer sua situação de saúde a partir das avaliações, receber um tratamento fisioterapêutico adicional e ainda contar com a possibilidade de melhorar a sua capacidade funcional.

Comentários e Considerações sobre a Pesquisa:

O projeto apresenta metodologia clara e condições de exequibilidade.

Considerações sobre os Termos de apresentação obrigatória:

O TCLE é claro e apresenta informações necessárias. Os documentos necessários, tais como anuência do centro onde será realizado (Instituto de Cardiologia) foram apresentados.

Recomendações:

Conclusões ou Pendências e Lista de Inadequações:

O projeto encontra-se dentro das normas do CEP e encontra-se em condições de aprovação.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

De acordo com o parecer do Relator.

GUIDE FOR AUTHORS

Introduction

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ANNOUNCEMENT

Professor Paolo G. Camici, MD, FESC, FACC, FAHA, FRCP has taken over from Professor Andrew J.S. Coats (Joint Academic Vice-President, Monash University, Australia AND University of Warwick, United Kingdom) as Editor-in-Chief of the International Journal of Cardiology in September 2016.
Having worked for many years in prestigious Universities and hospitals both in the UK and Italy, with a wealth of experience as Consultant, Clinician Scientist, Program Director, Lecturer, Examiner, Society Fellow, Working Group Chairman and member of multiple Editorial Boards, Professor Camici's current position is Professor of Cardiology and Consultant Cardiologist at the Vita-Salute University San Raffaele, in Milan, Italy. He is also Director of the University's Cardiology Training program.

Professor Camici has appointed a prestigious new Editorial Board consisting of five Deputy Editors (Prof. Domenico Cianflone, Prof. Perry Elliott, Prof. Juan Carlos Kaski, Prof. Peter J. Schwartz and Dr. Ornella Rimoldi).
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INTRODUCTION

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Text in these articles should not exceed **3,500** words, **50** references and **4** tables/figures. Additional references and/or methods will be published online only.

This category includes the following types of articles:

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Short communication should contain original data as per the description given under "original articles" but their length should not exceed **1,500** words; **20** references; **2** figures/tables. Case reports are not acceptable under this category.
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3. Huang YY, Chen ACH, Carroll JD, Hamblin MR. Biphasic dose response in low level light therapy. Dose Response 2009;7:358-383.
4. Jenkins PA, Carroll JD. How to report low-level laser therapy (LLLT)/ Photomedicine dose and beam parameters in clinical and laboratory studies. Photomed Laser Surg 2001;29:785-787.
5. WALT. Standards for the design and conduct of systematic reviews with low-level laser therapy for musculoskeletal pain and disorders. Photomed Laser Surg 2006;24:759-760.
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