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Capacidade funcional em ratos com insuficiência cardíaca: efeitos da terapia laser de baixa intensidade associada ao treinamento de força.

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RESUMO

A intolerância ao exercício permanece como um das características predominantes na insuficiência cardíaca (IC). A terapia laser de baixa intensidade (TLBI) emerge como uma ferramenta capaz de melhorar o desempenho ao exercício, porém pouco se sabe sobre seus efeitos na capacidade funcional na IC. O Treinamento de Força (TF) é parte integrante de um programa de reabilitação cardíaca, porém seu efeito no Consumo Máximo de Oxigênio (VO_{2max}) na IC ainda não está estabelecido. O modelo animal de IC induzida por Infarto Agudo do Miocárdio (IAM) é usado extensamente para avaliar potenciais terapias nessa síndrome. Nesse contexto, é essencial definir se os ratos submetidos à cirurgia de IAM desenvolverão primariamente uma redução da capacidade funcional. A conclusão de que o modelo animal (ratos) de IC induzida por IAM é capaz de reduzir a capacidade funcional é dificultada por metodologias e resultados discrepantes. Portanto, os objetivos dessa tese são: 1) avaliar a influência da TLBI associada ao TF na força máxima da musculatura esquelética, VO_{2max}, distância percorrida e tempo de permanência em esteira em ratos com IC induzida por IAM; 2) caracterizar esse modelo animal quanto a capacidade funcional e; 3) revisar sistematicamente o impacto desse modelo animal no VO_{2max}, na distância percorrida e no tempo de permanência em esteira. Por meio de três estudos experimentais inéditos e uma revisão sistemática com meta-análise em ratos, conclui-se que: 1) a TLBI associada ao TF aumenta o ganho de força da musculatura esquelética máximo, o VO_{2max} e a tolerância ao exercício comparado ao TF isolado em ratos com IC induzida por IAM; 2) o VO_{2max} e a tolerância ao exercício são dependentes do tamanho do infarto do miocárdio e; 3) ratos submetidos ao IAM reduzem o VO_{2max}, o tempo de permanência em esteira e a distância percorrida quando comparado a ratos controle. Nossos resultados contribuem de forma significativa para o entendimento dos efeitos da TLBI associada ao TF nos parâmetros de capacidade funcional na IC. Adicionalmente, nossos resultados mostram que animais com grandes áreas de infarto (>40%) são um bom modelo para testar novas terapias que possam promover alterações em variáveis de capacidade funcional.

Palavras-chave: Fototerapia. Exercício físico. Força máxima. Consumo máximo de oxigênio. Tolerância ao exercício. Ratos. Infarto agudo do miocárdio.

ABSTRACT

The exercise intolerance remains as a markedly manifestation in heart failure (HF). Low-level Laser Therapy (LLLT) emerges as a tool capable to improve the exercise performance, however little is known about the effects of LLLT in the functional capacity in HF. Resistance Training (RT) is a part of a cardiac rehabilitation program, however their effects in the Maximal Oxygen Uptake (VO_{2max}) still not established. The animal model of HF following myocardial infarction (MI) is widely used to evaluate potential therapies in this syndrome. In this context, its essential to define if rats underwent to MI develops primarily a reduction in functional capacity. The conclusion that the rat model of IC following MI is capable to reduce the functional capacity is hampered by discrepant methodologies and results. Therefore, the aims of this thesis are: 1) assess the influence of the LLLT associate with RT in the maximal strength, VO_{2max}, distance to run and time to exhaustion in rats with HF following MI; 2) characterize this animal model as the functional capacity e; 3) systematically review the impact of this animal model in VO_{2max}, distance run and time to exhaustion. Through by three inedited animal studies and one systematic review with meta-analysis in rats, we conclude: 1) LLLT associated with RT improves maximal strength gain, VO_{2max} and exercise tolerance compared to RT alone in HF-MI rats; 2) VO_{2max} and exercise tolerance area MI dependents and; 3) rats underwent to MI systematically reduce VO_{2max}, time to exhaustion and run distance compared to control rats. Our results contribute to the understanding about the effects of LLLT associated with HF in functional capacity parameters in HF. Additionally, our results show that animals with large MI (>40%) are a suitable model to test novel therapies that can change functional capacity variables.

Keywords: Phototherapy. Physical exercise. Maximal strength. Maximal oxygen uptake. Exercise tolerance. Rats. Myocardial infarction.

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1 CONTEXTUALIZAÇÃO

Durante a realização do meu mestrado no Programa de Pós-Graduação em Ciências da Saúde da Universidade Federal de Ciências da Saúde de Porto Alegre, nosso grupo de pesquisa (Grupo de Pesquisa em Interação Cardiopulmonar – GPIC), coordenado pelo Prof. Pedro Dal Lago, iniciou um linha de pesquisa sobre a aplicação da Terapia Laser de Baixa Intensidade (TLBI) na Insuficiência Cardíaca (IC). O primeiro trabalho realizado nessa linha de pesquisa e tema da minha dissertação de mestrado evidenciou que a TLBI é capaz de melhorar o perfil inflamatório (redução da concentração de TNF- α e IL-6 e aumento da concentração de IL-10) na musculatura esquelética de ratos com IC induzida por infarto agudo do miocárdio (IAM) (HENTSCHKE *et al.*, 2013). Observando o ciclo vicioso entre inflamação/estresse oxidativo na IC (KHAPER *et al.*, 2010), delineamos um segundo estudo que evidenciou que a TLBI é capaz de reduzir o estresse oxidativo na musculatura esquelética de ratos com IC (BIASIBETTI *et al.*, 2014). Porém, até a presente data, nenhum estudo foi realizado com o objetivo de observar o impacto da TLBI na capacidade funcional no modelo animal de IC.

Apesar do notável progresso na abordagem terapêutica de pacientes com IC, a intolerância ao exercício permanece como uma das características predominantes dessa síndrome (CONRAADS *et al.*, 2013). Sintomas que caracterizam a IC, incluindo a fadiga, estão frequentemente relacionados a anormalidades na musculatura esquelética (MIDDLEKAUFF, 2010). Nesse contexto, a inflamação (CICOIRA *et al.*, 2001; GIELEN *et al.*, 2005) e o estresse oxidativo (COIRAULT *et al.*, 2007) parecem desempenhar um importante papel na miopatia periférica na IC.

A reabilitação cardíaca pode ser útil em pacientes com IC clinicamente estáveis com o objetivo de melhorar a capacidade funcional, a duração do exercício, a qualidade de vida relacionada à saúde e a mortalidade (YANCY *et al.*, 2013). Evidências mostram que o treinamento de força (TF) aumenta a força muscular, e isso está associado à melhora do *status* clínico, à qualidade de vida e à capacidade ao exercício (JANKOWSKA *et al.*, 2008). Entretanto, o efeito do TF no consumo máximo de oxigênio (VO_{2max}) em pacientes com IC ainda não está estabelecido, com alguns estudos descrevendo nenhum efeito (MAGNUSSON *et al.*, 1996; HARE *et al.*, 1999; PU *et al.*, 2001; JANKOWSKA *et al.*, 2008).

Paralelamente, um crescente número de evidências apresenta a TLBI como uma emergente ferramenta capaz de melhorar o desempenho ao exercício em humanos (FERRARESI *et al.*, 2011; LEAL-JUNIOR *et al.*, 2015). Nesse contexto, estudos clínicos mostram que a TLBI associada ao TF aumenta o ganho de força máxima comparada ao TF isolado em indivíduos saudáveis (FERRARESI *et al.*, 2011). Estudos em animais mostram que a fototerapia aumenta o desempenho muscular em camundongos saudáveis submetidos ao TF (FERRARESI *et al.*, 2014).

Ainda, evidencia-se que a TLBI aumenta o desempenho ao exercício (VO_{2max} - e tempo em esteira) em um protocolo de corrida com intensidade progressiva até a exaustão em humanos (DE MARCHI *et al.*, 2012). Recentemente, observou-se que a TLBI associada ao treinamento aeróbico aumenta o VO_{2max} devido à redução de marcadores inflamatórios (IL-6 e TNF-α) em ratos idosos (AMADIO *et al.*, 2015). Esse montante de evidências sugere, em último plano, a aplicabilidade da TLBI associada à programas de treinamento físico.

Entretanto, até o momento, não conhecemos nenhum estudo que avaliasse a influência da TLBI associada ao TF nos parâmetros de capacidade funcional (força máxima, VO_{2max}, distância percorrida e tempo de permanência em esteira) em ratos com IC. Apresentamos aqui a hipótese de que o TF associado à TLBI aumenta os parâmetros de capacidade funcional em ratos com IC induzida por IAM quando comparado ao TF isolado.

Nesta tese foram desenvolvidos quatro artigos. O primeiro artigo se intitula *"Low-level Laser Therapy Enhances the Skeletal Muscle Strength Gain Promoted by Resistance Training in Rats with Heart Failure after Large Myocardial Infarction".* Os resultados inéditos desse estudo demonstram que a TLBI (aplicada na musculatura esquelética) associada ao TF aumenta o ganho de força máxima comparada ao TF isolado em ratos com IC e grande área de infarto. Paralelamente a esse trabalho, nosso laboratório adquiriu uma esteira para pequenos animais equipada com uma caixa metabólica com análises de trocas gasosas (AVS Projetos, São Carlos, SP, Brasil). Dois trabalhos iniciais de que participei como colaborador foram realizados por nosso grupo de pesquisa e evidenciaram que a fototerapia aplicada na musculatura esquelética aumenta o VO_{2max} e a tolerância ao exercício em ratos saudáveis (PERINI *et al.*, 2015, aceito para publicação) e com IC (CAPALONGA *et al.*, 2015, submetido). Os resultados inéditos desse primeiro artigo e dos dois trabalhos anteriormente descritos, bem como o fato de os mecanismos de ação

propostos na TLBI incluírem a modulação do metabolismo energético e o aumento da capacidade oxidativa de células musculares (FERRARESI *et al.*, 2012) nos instigaram a observar os efeitos da TLBI associada ao TF no VO_{2max} e na tolerância ao exercício em ratos com IC.

Dessa forma, o segundo artigo elaborado para esta tese se intitula *"Maximal oxygen uptake and exercise tolerance are improved in rats with heart failure subjected to low-level laser therapy associated with resistance training".* Os resultados inéditos desse estudo demonstram que a TLBI associada ao TF aumenta o VO_{2max} e a tolerância ao exercício (distância percorrida e tempo de permanência em esteira) comparada ao TF isolado em ratos com IC após indução do IAM. Entretanto, durante a realização desse estudo, concluiu-se que é essencial definir se os ratos submetidos à cirurgia de indução do IAM desenvolveram ou não a síndrome da IC. Especialmente neste caso, é essencial definir se o IAM causará primariamente uma redução do VO_{2max} e da tolerância ao exercício para, assim, testar novas terapias com potencial de melhorar essas variáveis de capacidade funcional. Em nosso conhecimento, nenhum estudo avaliou o consumo de oxigênio e a produção de CO_2 (VO_2 e VCO_2) bem como a tolerância ao exercício em ratos submetidos à cirurgia de indução do IAM classificados segundo o tamanho da área de infarto. Dessa forma, fez-se necessária a realização de um trabalho que objetivasse avaliar o VO_2 e o VCO_2 e a tolerância ao exercício em ratos submetidos à cirurgia de IAM e classificados segundo a área de infarto do miocárdio.

Assim, o terceiro artigo elaborado para esta tese se intitula *"Functional capacity in a rat model of heart failure: impact of myocardial infarction size".* Nesse estudo, propusemos uma abordagem integrada da avaliação do tamanho da área de infarto do miocárdio, da função ventricular (invasiva e não invasiva), do VO_2 do VCO_2 e da tolerância ao exercício em ratos controle (*sham*), ratos com pequenos tamanhos de IAM (<40%) e ratos com grandes tamanhos de IAM (>40%). Os resultados inéditos desse trabalho sugerem que o VO_{2max} e a tolerância ao exercício (tempo de permanência em esteira até exaustão) estão prejudicadas apenas em ratos com grandes tamanhos de IAM. Interessantemente, observamos na construção desse estudo dois pontos principais em estudos que avaliam a capacidade funcional (VO_{2max} e tolerância ao exercício) em ratos submetidos à cirurgia de IAM: 1) apresentam uma grande variedade de metodologias/protocolos; 2) apresentam resultados discrepantes. Assim, a conclusão de que o modelo animal (ratos) de IC

induzida por IAM é capaz de reduzir a capacidade funcional quando comparada a ratos controle é dificultada. Ao nosso conhecimento, até o momento não existem revisões sistemáticas na literatura sobre o impacto desse modelo animal nas variáveis de capacidade funcional.

Dessa forma, o quarto artigo elaborado para esta tese se intitula *"Impact of a rat model of heart failure on functional capacity: A systematic review and meta-analysis"*. Os resultados dessa revisão sistemática mostram que o modelo animal (ratos) de IC induzida por IAM reduz o VO_{2max}, o tempo de permanência em esteira até a exaustão e a distância percorrida quando comparado a ratos controle, apesar de: 1) grande variedade de espécies, sexo e idade dos animais incluídos; 2) diversas áreas de IAM; 3) diferentes períodos de avaliação após a indução do IAM; 4) diferentes características dos protocolos utilizados; 5) falhas metodológicas (ausência de cegamento e cálculo amostral/poder do estudo; 6) alta heterogeneidade observada na meta-análise.

Apesar de estar a cronologia de elaboração dos artigos descrita acima, optamos por apresentá-los de forma que seja possível construir um conhecimento sobre o tema proposto nesta tese. Assim, primeiramente, apresentamos o artigo *"Impact of a rat model of heart failure on functional capacity: A systematic review and meta-analysis"*. Em seguida, o artigo *"Functional capacity in a rat model of heart failure: impact of myocardial infarction size".* Os resultados inéditos desses trabalhos sugerem que o modelo animal (ratos) de IC induzida por IAM, em especial animais com grandes áreas de IAM, causa intolerância ao exercício e, em último plano, é um bom modelo animal para testar novas terapias com potencial biológico de alterar variáveis de capacidade funcional na IC. Assim, apresentamos o artigo *"Maximal oxygen uptake and exercise tolerance are improved in rats with heart failure subjected to low-level laser therapy associated with resistance training"* e, por fim, o artigo *"Low-level Laser Therapy Enhances the Skeletal Muscle Strength Gain Promoted by Resistance Training in Rats with Heart Failure after Large Myocardial Infarction"*.

Por fim, posso comentar que os processos de realização do doutorado e dos artigos que compõem essa tese exigiram-me a aquisição de novas habilidades prático/experimentais e teóricas, além das adquiridas durante o mestrado. Além dos procedimentos realizados há bastante tempo no nosso laboratório (ex: indução do IAM através da ligadura da artéria coronária descendente esquerda em ratos e

avaliação invasiva da função cardíaca), nosso laboratório adquiriu duas novas ferramentas de avaliação que exigiram-me aprendizado e treinamento para sua correta execução. Juntamente com o Prof. Marlus Karsten e a colega Júlia Perini implementamos em nosso laboratório as avaliações de VO_2 e VCO_2 e de tolerância ao exercício em ratos e, juntamente com o colega Lucas Capalonga, a avaliação não-invasiva da função cardíaca por ecocardiografia. Em especial, essa ultima técnica exigiu muito estudo sobre sua estrutura conceitual e muitas horas de prática para sua correta execução e exposição dos resultados. Além disso, pude participar intensamente da co-orientação da aluna de iniciação científica Júlia Perini (PERINI *et al.*, 2015, aceito para publicação) e como colaborador dos mestrados de Micheli Biasibetti (BIASIBETTI *et al.*, 2014), Douglas Rossato (ROSSATO *et al.*, 2015), Jéssica Lima (LIMA *et al.*, 2015) e Lucas Capalonga (CAPALONGA *et al.*, 2015, submetido) realizados no laboratório.

Os coautores dos manuscritos apresentados nesta tese participaram das diferentes etapas dos estudos: elaboração do planejamento experimental, realização de experimentos, análise de resultados e/ou elaboração do corpo do manuscrito. As versões finais dos artigos foram aprovadas por todos os coautores.

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3 OBJETIVOS

3.1 Objetivo geral:

Avaliar a influência da terapia laser de baixa intensidade (TLBI) associada ao treinamento de força (TF) nos parâmetros de capacidade funcional em ratos com insuficiência cardíaca (IC) induzida por infarto agudo do miocárdio (IAM).

3.2 Objetivos específicos:

- revisar sistematicamente o impacto do modelo animal (ratos) de IAM no VO_{2max}, na distância percorrida e no tempo de permanência em esteira;
- avaliar o VO_2 e o VCO_2 e a tolerância ao exercício em ratos submetidos à cirurgia de IAM e classificados segundo a área de infarto do miocárdio;
- avaliar a influência da TLBI associada ao TF no VO_{2max}, na distância percorrida e no tempo de permanência em esteira em ratos com IC induzida por IAM;
- avaliar a influência da TLBI associada ao TF no ganho de força máxima em ratos com IC induzida por IAM.

4 ARTIGO 1

Impact of a rat model of heart failure on functional capacity: A systematic review and meta-analysis.

(Artigo formatação *Heart Failure Reviews*; Fator de impacto:3.787)

Impact of a rat model of heart failure on functional capacity: A systematic review and meta-analysis.

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ABSTRACT

Studies that have evaluated functional capacity in myocardial infarction (MI) rats demonstrate a wide range of methodologies and discrepant results, making it difficult to conclude whether the rat model of heart failure (HF) following MI can actually induce a reduction in functional capacity relative to controls. There are no literature reviews on the repercussions of the rat model of HF following MI on functional capacity, aside from a methodological quality assessment. The aim of the present report was to evaluate the impact of a rat model of HF after MI following left coronary artery (LCA) ligation in maximal oxygen uptake (VO_{2max}), run time to fatigue and distance to run. As a secondary aim, we assessed the methodological quality of the studies. Thirty-one controlled studies were identified in MEDLINE (Pubmed), Web of Science and Scielo (Scientific Electronic Library Online). A meta-analysis showed that the rat model of HF after MI following LCA ligation reduced VO_{2max} (-13.58 ml· kg^{1}·min^{-1}; 95% CI, -16.96 to -10.20; $p < 0.00001$; I^2:92%), run time to fatigue (-9.60 minutes; 95% CI, -11.02 to -8.17; $p < 0.00001$; I^2:24%) and distance run (-122.53 meters; 95% CI, -200.80 to -44.25; $p < 0.002$; I^2:86%) relative to control rats. These studies exemplify methodological limitations such as the absence of blinding and sample size estimation/power calculations. Rat models of HF after MI following LCA ligation are systematically associated with VO_{2max}, run time to fatigue and distance run reduction compared with controls. Methodological improvements in protocols for functional capacity assessment in this widely used animal model of HF are necessary.

Keywords: Myocardial infarction. Rats. Maximal oxygen uptake. Run time to fatigue. Distance run.

Introduction

Reduction of functional capacity is an important feature of heart failure (HF) and it is used as a parameter for the severity of the disease [1]. Cardiopulmonary exercise testing is the gold standard method for the assessment of functional ability in HF. Oxygen uptake (VO_2) rises during incremental exercise and peak VO_2 (VO_{2peak}) represents the highest rate of oxygen uptake achieved [2,3]. This measurement is used as a good short-term predictor of mortality in HF patients [4].

Animal models of HF are essential to the understanding of the fundamental aspects of HF pathophysiology and to the development of novel preventive and reparative therapies for this prevalent and fatal disease [5-7]. Particularly, myocardial infarction (MI) in rodents is the animal model most frequently used to experimentally reproduce human HF [8]. MI following coronary artery ligation in rats is an extensively used animal model in pathophysiological [9-12] and therapeutic studies of HF [13-16], with advantages and disadvantages including high variability in the resulting size of the MI, cardiac remodelling and left ventricular dysfunction [8,5,17,18].

Our research group and others have noted that not all postinfarction rats develop features of HF and some only present moderate HF [11,17,19,20]. This classification is based on different variables, such as the presence of pleural effusion [20], a combination of clinical and pathologic HF features and some newly proposed factors, such as echocardiography classification [17], lung congestion and right ventricular hypertrophy [21], infarcted size [11] and (in

particular) left ventricular end-diastolic pressure after hemodynamic evaluation [19,22], with little or no classification based on functional capacity parameters.

Therefore, in animal studies on HF treatment, it is essential to define whether MI rats present HF or not in order to accurately assess the effects of treatments [17]. Exercise intolerance is the cardinal clinical manifestation of HF [23] and, in this context, it is important to determine the functional capacity repercussions of the rat model of HF following MI essentially for two aims: to characterize HF syndrome in general and to test novel therapies that could potentially modify the variables of functional capacity in particular.

The general observations of studies that have evaluated the functional capacity parameters ($VO_{2max,}$ run time to fatigue and distance run) in postinfarction rats demonstrate a wide range of methodologies and discrepant results, making it difficult to conclude whether the rat model of HF following MI can actually induce a reduction in functional capacity relative to controls. Despite its importance, to our knowledge, there are no literature reviews concerning the repercussions of the rat model of HF following MI on functional capacity variables, aside from the pitfalls of their evaluation.

Additionally, despite the importance of (animal) preclinical studies [24], several systematic reviews of animal studies have identified the low methodological quality of the primary studies include in this review [25-27]. An important review provides evidence that many peer-reviewed animal research publications fail to report important information regarding experimental and statistical methods [28]. Up to now, a methodological quality assessment of studies on the repercussions of the rat model of HF following MI on functional capacity variables has not been conducted.

Therefore, the aim of the present report was to evaluate the impact of a rat model of HF after MI following left coronary artery ligation on functional capacity (VO_{2max}, run time to fatigue and distance to run). As a secondary aim, we assessed the methodological quality of the studies on the repercussions of the rat model of HF after MI following left coronary artery ligation on functional capacity outcomes.

Methods

Criteria for considering studies for this review.

Types of studies

Controlled studies that evaluated the impact of a rat model of HF after MI in functional capacity variables were included in the review.

Types of participants

Laboratory rats of any strain, sex, weight or age were included. Studies performed in human patients or in laboratory animals other than rats (e.g. mice), were excluded.

Types of interventions

HF models induced by MI following coronary artery ligation were included. Other animal models of HF, such as ascending aortic constriction, ischemia/reperfusion, left ventricle radio-frequency ablation, cryo-injury, toxic models, Dahl salt-sensitive rats and spontaneously hypertensive heart failure-prone rats [8,5-7] were not included.

Types of outcome measures

The primary outcome was functional capacity assessment measured by maximal oxygen uptake (VO_{2max}; ml/kg/min or $ml \cdot kg^{-1} \cdot min^{-1}$). The secondary outcome was functional capacity assessment measured by run time to fatigue (run time; seconds or minutes) and distance run (distance; meters).

Search methods for identification of studies.

Data sources

A literature search for original research papers evaluating the impact of animal models of HF after MI on functional capacity in rats was performed by searching the bibliographic databases MEDLINE (Pubmed), Web of Science and Scielo (Scientific Electronic Library Online), reference lists of articles and private databases and carried out by two independent reviewers (V.H and L.C.).

Period and Language

No publication date or language restrictions were imposed.

Search terms

We developed a literature search strategy (search terms) based on population (“rats”), intervention (“myocardial infarction”) and outcomes (“exercise tolerance"; “oxygen consumption”), as follows: MEDLINE (Pubmed): ((("Rats"[Mesh] OR "Rats" OR “Rat” OR “Rattus” OR “Rattus norvegicus” OR “Rats, Laboratory” OR “Rats, Norway” OR “Laboratory Rat” OR “Laboratory

Rats” OR “Rat, Laboratory” OR “Rats, Sprague-Dawley” OR “Rats, Wistar”)) AND ("Myocardial Infarction" [Mesh] OR "Myocardial Infarction" OR “Infarction, Myocardial” OR “Infarctions, Myocardial” OR “Myocardial Infarctions” OR “Myocardial Infarct” OR “Infarct, Myocardial” OR “Infarcts, Myocardial” OR “Myocardial Infarcts”)) AND ("Exercise Tolerance"[Mesh] OR "Exercise Tolerance" OR “Tolerance, Exercise ”OR "Exercise Test" [Mesh] OR "Exercise Test" OR “Exercise Tests” OR “Test, Exercise” OR “Tests, Exercise” OR “Bicycle Ergometry Test” OR “Bicycle Ergometry Tests” OR “Ergometry Test, Bicycle” OR “Ergometry Tests, Bicycle” OR “Test, Bicycle Ergometry” OR “Tests, Bicycle Ergometry” OR “Treadmill Test” OR “Test, Treadmill” OR “Tests, Treadmill” OR “Treadmill Tests” OR “Step Test” OR “Step Tests” OR “Test, Step” OR “Tests, Step” OR “Stress Test” OR “Arm Ergometry Test” OR “Arm Ergometry Tests”OR “Ergometry Test, Arm” OR “Ergometry Tests, Arm” OR “Test, Arm Ergometry” OR “Tests, Arm Ergometry” OR “Cardiopulmonary Exercise Test” OR “Cardiopulmonary Exercise Tests” OR “Exercise Test, Cardiopulmonary” OR “Exercise Tests, Cardiopulmonary” OR “Test, Cardiopulmonary Exercise” OR “Tests, Cardiopulmonary Exercise” OR "Oxygen Consumption"[Mesh] OR "Oxygen Consumption" OR “Consumption, Oxygen” OR “Consumptions, Oxygen” OR “Oxygen Consumptions”). Web of Science: ("myocardial infarction" and "exercise tolerance" or "maximal oxygen consumption"), refined by “rats” and only articles types. Scielo: (rat) AND (myocardial infarction) AND (exercise test) OR (exercise tolerance) OR (maximal oxygen consumption). Date of bibliographic database search: 19 September, 2014.

Data collection and analysis

Study selection

After excluding duplicate and triplicate references, two independent reviewers (V.H and L.C.) evaluated the titles and abstracts of the identified references on the basis of inclusion and exclusion criteria. Clearly irrelevant references were excluded and all titles/abstracts that did not provide enough information about the inclusion and exclusion criteria were selected for review of the full text. In the second phase, the reviewers evaluated the full text and then selected references on the basis of the same inclusion and exclusion criteria. Any disagreements that arose during the study selection phase were settled by a third reviewer (J.P.).

Data collection process

The same two independent reviewers (V.H and L.C.) extracted data (see *"Data items"*) from each included study using a previously developed and tested data extraction sheet. Any disagreements that arose during the data collection process were settled by a third reviewer (J.P.). We also contacted authors of included studies for information that had not been reported or was reported unclearly. If the authors did not respond within two weeks, the information was considered "not reported". When necessary, data were extracted from published graphs and recorded using graph digitizing software when data were only present graphically in the included study (see "*Summary measures"*).

Data items

The items extracted from each included study were the following: reference information (authors, date, journal name, corresponding author name with contact information and affiliation); animal characteristics (strain, sex, age/ weight) and experimental groups with sample size; intervention/animal model of HF characteristics (type and technical characteristics of myocardial infarction induction and control group type) and MI size; methodology/functional capacity assessment characteristics (technical apparatus, protocol description, exhaustion criteria, air flow parameter, days after animal model induction, presence/absence and description of animal protocol adaptation, presence/absence and types of adverse effects); outcomes/functional capacity assessed variables (primary and secondary outcomes); results and values with units if available and study conclusion (*P* value between MI and control groups).

Risk of bias in individual studies

To assess the risk of bias in individual studies, we performed a quality assessment of the included studies. Four key areas were selected on the basis of a well-described survey of the literature on the quality of animal studies [29]: ethics, study design (allocation to groups/randomization and blinding), experimental animals (species, sex, age and group size) and sample size estimation/power calculations. Note that randomization, blinding and group size items were referred to as functional capacity assessment methodology.

Summary measures

Summary measures were the overall effect size of the HF animal model induced by MI following coronary artery ligation on functional capacity,

calculated as the change in the intervention group compared with the outcomes (primary outcome: maximal oxygen uptake; secondary outcome: run time and distance run) in the control group at maximal follow-up. To calculate this, we extracted for each group the mean and standard deviation (SD) of functional capacity variables evaluated and group size. If a SD was not reported directly, it was calculated by multiplying the reported standard error (SE) with the square root of the group size. If the numbers necessary for the calculation of summary effect measures were not adequately reported in the main text, data points were estimated by extraction from published graphs and recorded using graph digitizing software (GetData Graph Digitizer 2.24; S. Fedorov; http://getdata-graph-digitizer.com). All data values were extracted and converted, if necessary, to $ml \cdot kg^{-1} \cdot min^{-1}$ (maximal oxygen uptake), minutes (run time) and meters (distance run). The latter method was adapted from other well-designed systematic reviews of animal studies [30,31].

Synthesis of results

The meta-analyzed of the overall effect size (primary outcome: maximal oxygen uptake; secondary outcome: run time and distance run) was calculated using the random-effects model [32,31]. The random-effects model assumes that there is no common treatment effect for all included studies but rather that the variation of the effects across studies follows a particular distribution [33,32]. In a random effects model it is believed that the included studies represent a random sample from a larger population of studies addressing the question of interest [34,32]. For random-effects analyses we use the DerSimonian and Laird approach [34,32,31]. Statistical heterogeneity was investigated using the

inconsistency measure (I^2) [30,31,35,36]. Data was considered to be heterogeneous when the I^2 statistic was >50% [36]. When high heterogeneity was identified, we performed a subgroup analysis involving sex, strain and myocardial infarction size (see “Additional analysis” item*)*.

Statistical analysis

Values were expressed as percentages and descriptive statistics (mean and standard deviation; median and maximal/minimum) were calculated. Review Manager (RevMan. Version 5.3.0. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration) for Windows was used as a computational tool in the data analysis. GraphPad Prism 5 (Graph-Pad Software, San Diego, CA, USA) for Windows was used for complementary analysis and to construct charts. A *P* value <0.05 was considered statistically significant.

Additional analysis

Because the strain and sex of animals as well as MI area can interfere with functional capacity values, we performed a specific subgroup analysis involving these components.

Results

Study selection

Figure 1 shows the flowchart of search methods for the identification and selection of studies. A total of 377 possible references were identified (Pubmed: 226; Web of Science: 128; Scielo 15 and hand searching: 8). Eight duplicate studies and 313 references after title/abstract evaluation were excluded. Fifty-six remaining references were assessed in full text. Of these, a total of 25 references were excluded for various reasons (surgical reintervention (3), absence of a control group (7), other HF models (1), non-normal animals (1), incompatible outcome (7), outcome assessed in animals other than rats (1), outcome assessed in instrumented animals (4) and outcome assessed only before MI induction (1)). In the end, 31 controlled studies using HF rat models induced by MI following coronary artery ligation that assessed functional capacity (VO_{2max} and/or run time to fatigue and/or distance run) were included in this review.

Study characteristics

A characterization of the included studies related to the impact of the animal model of HF after MI on functional capacity in rats is presented in detail in Table 1.

Animals

The included studies involved 593 animals distributed among three different rat strains: Wistar (23/31 studies; ~74%), Sprague-Dawley (7/31

studies; ~23%) and Fischer 344 (1/31 study; 3%). Male rats were predominately used (20/31 studies; ~65%), range between 150-400 g and 4-12 weeks old. Studies that evaluated VO_{2max} included Wistar (15/21 studies; ~71%) and Sprague-Dawley (6/21 studies; ~29%) male (12/21; ~57%) rats between 180 and 400 g. Studies that evaluated run time to fatigue included Wistar (9/11 studies; ~82%), Sprague-Dawley (1/11 studies; ~9%) and Fischer 344 (1/11; ~9%) male (7/11; ~64%) rats between 150 and 400 g. Studies that evaluated distance run included Wistar (6/6 studies; ~100%) male (6/6; ~100%) rats between 150 and 300 g. The group size of all studies for the control group was mean ± SD = 9.5 ± 2.8 (median [min; max] = 9.0 [6; 17]) and for the intervention group was mean ± SD= 10.3 ± 4.2 (median [min; max] = 9.0 [5; 25]). Of studies that evaluated maximal oxygen uptake, the groups size for the control group was mean ± SD = 9.2 ± 2.9 (median [min; max] = 9.0 [6; 17]) and for the intervention group was mean ± SD= 10.3 ± 4.8 (median [min; max] = 9.0 [5; 25]). Of studies that evaluated run time to fatigue the groups size for the control group was mean ± SD= 9.1 ± 2.5 (median [min; max] = 8.0 [7; 14]) and for the intervention group was mean ± SD= 9.6 ± 3.4 (median [min; max] = 10.0 [5; 16]). Of studies that evaluated distance run the groups size for the control group was mean ± SD = 10.0 ± 2.3 (median [min; max] = 10.5 [6; 12]) and for the intervention group was (mean ± SD= 10.3 ± 3.1; median [min; max] = 11.5 [6; 14]).

Intervention (Animal model of Heart Failure)

All included studies used the animal model of HF after MI following left coronary artery (anterior descending coronary artery/left main coronary artery)

ligation. Regarding control groups, sham rats underwent the same procedures as those in MI groups except that myocardial ischemia was not induced when rats were used as controls in 29 studies (29/31; ~94%). Two other studies (2/31; ~6%) used normal rats as controls, without well-described methodology.

Some studies (21/31; ~68%) assessed myocardial infarction area (MI area, % of total left ventricular area). Of these, 9 (9/21; ~43%) used histology, 9 (9/21~43%) employed echocardiography and 3 (3/21; ~14%) did not report the methodology used for MI area evaluation. There was a wide range of MI area (%) measured in all included studies: mean ± SD = 36.8 ± 6.7; median [min; max] = 38.7 [26; 50]; for studies that evaluated maximal oxygen uptake (n = 17 studies), mean ± SD = 37.1 ± 5.7; median [min; max] = 38.7 [27; 46]; for studies that evaluated run time to fatigue (n = 4 studies), mean ± SD= 42.9 ± 5.3; median [min; max] = 41.5 [38.7; 50]; for studies that evaluated distance run (n = 4 studies), mean ± SD = 28.2 ± 2.1; median [min; max] = 27.9 [26; 31].

Methodology (functional capacity assessment)

For the assessment of VO_{2max}, the main apparatus involved was a metabolic chamber coupled to a gas analyser and the test was performed in a rodent treadmill. Additionally, two studies assessed maximal oxygen consumption using a metabolic mask coupled to a gas analyser and two studies showed swimming VO_{2max} assessment (measured while the rats swam with 4% of their body weight attached to the base of their tails). For run time to fatigue and distance run, all studies used a rodent treadmill.

We identified a wide range of protocols to assess functional capacity (e.g. constant workload treadmill, incremental workload treadmill and swimming),

including different criteria for exhaustion. For VO_{2max} assessment (n = 21 studies), we identified 12 different protocols; for run time to fatigue assessment (n = 11 studies), we identified 6 different protocols and for distance run assessment (n = 6 studies), we identified 6 different protocols. Table 1 summarizes the protocol characteristics for the assessment of functional capacity in rat models of HF after MI.

Additionally, we observed a large variance and a poor description of animal adaptation protocols, including pre- or postsurgical adaptation and different durations, days and velocities. See more details of animal adaptation in Table 1. Interestingly, none of the studies reported any adverse effects on functional capacity.

We observed a great variance in time-point evaluation (days after MI induction) in functional capacity variables: all outcome variables (n = 31 studies): mean ± SD= 74.7 ± 30.3; median [min; max] = 84.0 [20; 140]; for maximal oxygen uptake variable (n = 21 studies): mean ± SD = 76.3 ± 27.4; median [min; max] = 84.0 [20.0; 126.0]; for the run time to fatigue variable (n = 11studies): mean ± SD = 68.0 ± 36.8; median [min; max] = 70.0 [20; 140]; for distance run (n = 6 studies): mean ± SD= 79.3 ± 27.5; median [min; max] = 84.0 [28;112].

Additional information (e.g. calibration) was poorly or incompletely described in most of the selected studies. Calibration of the gas analyser (e.g. on every test day or before each test) was reported in only 6 studies [37-42] (6/21; ~29% studies that assessed maximal oxygen consumption) and used primarily a reference gas mixture (primary gas standards). Only 7 studies (7/31; ~23% of total studies) demonstrated a concern related to the time of day at

which functional capacity assessment was performed, for example, “all rats were subjected to the same experimental conditions of time and environment and the exercise test was done in the late afternoon” [43]; “all animals ran between 8:00 a.m. and 11:00 a.m.” [22]; “all exercise tests were initiated between 9 and 10 AM” [19,38,44,45]; “the tests were performed at the same time each day” [37].

Regarding VO_{2peak}/VO_{2max} assessment method, few methods were described and many methods were unclear. Methods for VO_{2peak}/VO_{2max} assessment included the following: “the VO2 reached at the highest workload during the treadmill test as peak VO_2” [39]; “the highest VO_{2max} measured at each workload was taken as a measure of each rat`s running economy ($VO_{2submax}$) for that workload and, at the last step, as VO_{2max}” [37,38]; “VO_{2peak} was defined as the highest VO_2 achieved before exhaustion” [46,47]. Additionally, only two studies related a sampling rate in oxygen consumption analyses: “oxygen fraction in effluent air was registered every second” [39] and “every 2 min of the protocol, oxygen consumption was measured” [48].

Regarding food and water intake before functional capacity assessment, there was little attention and discrepant methodologies, as rats were allowed food *ad libitum* before the test to maintain normal glycogen stores [22] and all rats were restricted from food for 12 h before the exercise test [44].

Similarly, there was little attention given to and information about reproducibility of the functional capacity test, especially in VO_2 assessment. For Musch, T.I. *et. al.* (1986) [41], Musch, T.I. *et. al.* (1988) [40] Musch, T.I. *et. al.* (1989) [42] VO_{2max} was determined at least twice in each rat to ensure reproducibility, with each maximal test separated by a minimum of 24 h or 72 h

of recovery, respectively. One other study described repeating the same protocol of functional assessment (VO_{2max} and total exercise time) after a 7-day interval in eight control animals to assure protocol reproducibility and reported a protocol reproducibility of r = 0.82 [48]. Interestingly, two studies reported a methodological approach related to reproducibility [45,19].

Regarding animal acclimatization and basal values prior to VO_2 analysis, some methodological approaches were described: during measurement of VO_2, baseline (rest) values were recorded for 200 s [49]; the experimental animals only started the exercise test when the levels of resting VO_2 were near 30 ± 5 $ml \cdot kg^{1} \cdot min^{-1}$ [50]; rats were allowed a 5-min period of acclimation in the treadmill and gas-analysis system before the protocol began [37,38] and each rat was given a 20 min rest period before the protocol began [39].

Observer blinding (blinded to rat identities or to surgery group - sham or MI) was described in only eight studies (8/31; ~26%) [19,38,40,44,45,51,52].

Outcomes

Regarding the types of outcome measured, 14 (14/31; ~45%) studies used VO_{2max}, 7 studies used run time to fatigue (7/31; ~24%), 3 studies used distance run (3/31; ~10%) and 7 studies used association (7/31; ~24%; 4 studies - maximal oxygen consumption plus run time to fatigue and 3 studies - maximal oxygen consumption plus distance run) as variables of functional capacity in the animal model of HF after MI following left coronary artery ligation.

Risk of bias within studies

Figure 1 shows a quality assessment of the included studies. Ethical oversight and approval via institutional review was reported in 81% (25/31) of studies. Randomization was reported in 61% (19/31) of included studies. Only 26% (8/31) described blinding in functional capacity methodological assessment. Strain and sex of the animals were reported in 100% (31/31) and 97% (30/31) of studies, respectively. Age of the animals was reported in 52% (16/31) of studies. Group size was described in 97% (30/31) of studies. No studies reported sample size estimation/power calculations (power or sample size analysis).

Results of individual studies

Table 2 shows the values of functional capacity variables (VO_{2max}, run time to fatigue and distance run) from select studies comparing the MI group with the control group.

The overall effect of MI induction on functional capacity variables (VO_{2max}, run time to fatigue and distance run) versus control groups is shown in forest plots (Figure 3, 4 and 5, respectively). It is important to note that in some studies, the MI group was subdivided into moderate and severe left ventricular dysfunction and groups were assessed by a swimming protocol. We considered in our meta-analyses only that data that were derived from the severe left ventricular dysfunction groups. Swimming VO_2 assessment (measured while the rats swam with 4% of their body weight attached to the base of their tails) represented ~60-80% of those values obtained by treadmill VO_2 assessment [53,54] and these data were not considered in our review.

Synthesis of results

None of the included studies showed 100% adequacy in all qualitative analysis categories. Ethics, strain, sex and group size were described in most of the included studies. Age and randomization of the animals were described in ~50-60% of studies. Only ~25% of studies described blinding in functional capacity methodological assessment. No studies reported sample size estimation/power calculations (power or sample size analysis).

The values of maximal oxygen consumption (ml· kg^{1}·min^{-1}) in the included studies in control and MI groups were, respectively, mean ± SD = 68.2 ± 19.5; median [min; max] = 66.0 [42.0; 109.0] and mean ± SD = 53.8 ± 16.9; median [min; max] = 52.1 [23.1; 81.0]. The values of run time to fatigue (minutes) in the included studies in control and MI groups were, respectively, mean ± SD = 31.7 ± 11.6; median [min; max] = 25.2 [17.9; 55.4] and mean ± SD= 20.6 ± 9.6; median [min; max] = 19.8 [3.3; 34.3]. The values of distance run (meters) in the included studies in control and MI groups were, respectively, mean ± SD = 597.8 ± 403.3; median [min; max] = 382.8 [339.6; 1339]; and mean ± SD= 270.3 ± 50.8; median [min; max] = 276.9 [177.6; 320.3].

Regarding summary results (statistical significance), 18 studies that assessed maximal oxygen consumption (18/21; ~86%), 10 that assessed run time to fatigue (10/11; ~91%) and 4 that assessed distance run (4/6; ~66%) showed significant differences ($p < 0.05$) between control and MI groups.

In the meta-analysis, the animal model of HF after MI following left coronary artery ligation was associated with significant reductions in VO_{2max} (ml· kg^{1}·min^{-1}) of 13.58 (95% CI, -16.96 to -10.20; $p < 0.00001$; I^2, 92%; *P* for heterogeneity <0.00001) (Figure 3), in time run to fatigue (minutes) of 9.60

(95% CI, -11.02 to -8.17; $p < 0.00001$; I^2, 24%; *P* for heterogeneity = 0.22) (Figure 4) and in distance run (meters) of -122.53 (95% CI, -200.80 to -44.25; P<0.002; I^2, 86%; *P* for heterogeneity <0.00001) compared with controls (Figure 5).

Additional analysis

A meta-analysis of VO_{2max} and distance run showed a high degree of heterogeneity (I^2 = 92% and 86%, respectively). Thus, we performed a subgroup analysis of VO_{2max} outcome involving sex (only studies that used male rats were selected because they were predominant), strain (only studies that used Wistar rats were selected because they were predominant) and myocardial infarction size (studies with MI size >40% and <40% were selected). Subgroup analysis of VO_{2max} of male rats did not reduce heterogeneity (I^2 = 94%; $p < 0.00001$). Similarly, subgroup analysis of the Wistar strain did not reduce heterogeneity (I^2 = 91%; $p < 0.00001$). Subgroup analysis of VO_{2max} outcome involving MI size did not reduce heterogeneity I^2 = 95% ($p < 0.00001$) (only studies with MI size >40% were selected) and I^2 = 88% ($p < 0.00001$) (only studies with MI size <40% were selected).

Subgroup analysis of distance run outcome involving strain, sex and MI size were not feasible because all include studies used male Wistar rats with a MI size above 40%. Visually in the forest plot, two studies were outliers [52,55] and were excluded, resulting in a reduction in heterogeneity (I^2 = 66%; p = 0.03), with no significant alteration in the overall effect ($p < 0.001$).

Discussion

This systematic review (31 included studies) and meta-analysis provided, for the first time, important conclusions about the impact of animal models of HF on functional capacity: a rat model of HF after MI following left coronary artery ligation reduced maximal oxygen uptake (-13.58 ml· kg^{1}·min^{-1}; 95% CI, -16.96 to -10.20; $p < 0.00001$), run time to fatigue (-9.60 minutes; 95% CI, -11.02 to -8.17; $p < 0.00001$) and distance run (-122.53 meters; 95% CI, -200.80 to -44.25; $p < 0.002$) relative to control rats (predominantly sham surgery), despite 1) a wide range of animal characteristics (strains, sex and weight/age), 2) several MI sizes, 3) various time-points (days after myocardial infarction induction) of assessment, 4) different functional capacity protocol characteristics, 5) methodological flaws (absence of blinding and sample size estimation/power calculations) and 6) high heterogeneity.

In meta-analyses, we observed a high heterogeneity ($I^2 > 50\%$) in VO_{2max} ($I^2 = 92\%$; $p < 0.00001$) and distance run ($I^2 = 86\%$; $p < 0.00001$). Contrarily, run time to fatigue showed a low heterogeneity ($I^2 = 24\%$; $p = 0.22$). The strain and sex of animals [56,57] as well left ventricular dysfunction (MI size) [19] interfered with functional capacity values. A subgroup analysis involving age, sex and MI size did not reduce the heterogeneity in VO_{2max} or distance run outcomes.

For scientific, ethical and economic reasons, experiments involving animals should be appropriately designed, correctly analysed and transparently reported [28]. Our study shows that, with exception of blinding and sample size estimation/power calculations, most of the studies reported ethics, randomization, strain, sex of the animals and group size. Two well-designed surveys assessed the quality of animal studies [29,28]. Our results concerning

the percentage of the quality-assessed items (ethics, strain, age, sex and group size) in the included studies are similar [29]. Additionally, our study reports that only ~26% of the studies reported blinding and the results are also similar (~20%) [29]. Of note in our study, randomization was described in ~61% of the included studies. This percentage was higher than that reported by other studies (10% and 20%) evaluating the methodological quality of animal research [29,28]. Sample size and power calculations are not described in our included studies, similar to the observations of others [29,28]. One important issue with animal studies is the widespread lack of transparent, quality reporting of study design and implementation [29]. Appropriate and efficient experimental design is a critical component of high-quality science [28]. We believe that the four key areas analysed using the quality assessment tools in this study include the assessment of the major types of bias that are known to influence the results of research (selection, performance, detection, and exclusion) [58].

Reproducibility is an important characteristic of diagnostic tools and is extremely important when investigators carry out sequential analyses over a time course in the same animals. In functional capacity assessment of MI rats, only few studies reported statistical analysis or tests of reproducibility [40,19,41,42,45,48]. Thus, the reproducibility of functional capacity assessment in the rat model of HF after MI is unknown. In normal rats, a classic study has developed a valid and reproducible protocol for measuring maximal oxygen uptake [57].

In this way, some studies have assessed the changes in functional capacity over time (sequential analyses) [50,52,59-63], with discrepant results. Thus, the time course of development and progression of functional capacity

deterioration in the rat model of HF has not been precisely determined. This follow-up design is very important to determine the initial time of development and the progression of functional capacity deterioration in the rat model of HF, especially, for example, to test the impact of potential therapies in this syndrome. More studies are necessary and the knowledge of the reproducibility of functional capacity assessment is essential in the context of changes in functional capacity over time in the rat model of HF after MI.

In humans, VCO_2 (CO_2 production) and respiratory exchange ratio (RER; VCO_2/VO_2) are well established during a cardiopulmonary test. In HF rats, the studies that evaluated the VCO_2 and RER status during the maximal exercise test post MI induction are scarce and are limited to one study group [64,40]. VCO_2 are reduced in MI rats compared to sham rats and no significant differences in RER during the maximal exercise test was detected between groups [40]. One other study reported different values in MI rats [64]. Additionally, other studies reported VCO2 and RER values in MI rats assessed during a submaximal swimming protocol [53,54] or in instrumented rats [42]. Two classical studies showed RER values in normal rats during different protocols [65,57]. Thus, despite its importance, little is known about the behaviour pattern of these variables (VCO_2 and RER) in the rat model of HF after MI.

Although MI rats classified as small MI/large MI or moderate and severe left ventricular dysfunction (based on left ventricular end diastolic pressure (LVEDP) [19,22] and infarct size [44]) present different values of functional variables (e.g. run time to fatigue) and different statistically significant effects when compared with sham (control) rats, we identified few studies that

correlated functional capacity variables (run time to fatigue and VO_{2max}) with hemodynamic variables (LVEDP) [19] and no studies revealed correlations with infarct size.

In this way, transthoracic echocardiography (TTE) emerges as a frequently employed and powerful noninvasive tool to serially assess different structural and functional cardiac parameters [66,17,67]. Thus, TTE is utilized as a noninvasive method to observe changes in cardiac function during the progression of the disease [68-71,67] or during therapeutic interventions [51,59,72,73] in different animal models of cardiovascular diseases. Nevertheless, only one study correlated functional capacity variables (distance run) with echocardiography variables [52]. We know that functional capacity assessment is long and strenuous (one limitation of the functional capacity assessment) and therefore, the identification of correlations with hemodynamic variables (invasive), especially with echocardiography (a practiced, noninvasive technique), can help to identify and extrapolate functional capacity alterations and to characterize the animal model of HF. Additionally, little is known about correlations between run time to fatigue, distance run and VO_{2max} in a rat model of HF induced by MI [19]. In healthy rats and other animal models, studies have shown correlations between functional capacity variables [65,57] and equations to predict VO_2 [74].

To our knowledge, there are no reference values for functional capacity variables that allow the classification of MI rats as HF+/HF- or as moderate/severe HF. This classification is important, for example, to observe the impact of novel therapies in different degrees of functional capacity impairment. We identified studies in which the selection of infarction animals

was based on a VO_{2max} of less than 70 ml· kg^{1}·min^{-1} [75-77], however, well-defined criteria are necessary.

Classically, MI in animals might be classified as large infarcts when the infarcted area >40% and is associated with severe left ventricular dysfunction [11]. In a subanalysis, our study showed that VO_{2max} (ml· kg^{1}·min^{-1}) was mean ± SD = 46.2 ± 17.3; median [min; max] = 49.1 [23.1; 73.7]; the overall difference between MI and control rats was -18.14 (95% IC, -26.48, -9.80) in studies that reported infarcted size/area >40% [47-50, 60, 78, 79]. In studies that reported infarcted size/area <40% [37-42, 51, 53, 63] our study showed that VO_{2max} (ml· kg^{1}·min^{-1}) was mean ± SD= 57.0±14.6; median [min; max] = 52.0 [39.9;81.0]; the overall difference between MI and control was -10.44 (95% IC, -14.02, -6.86). We propose that these mean values of VO_{2max} (46.2 ml· kg^{1}·min^{-1} for infarcted areas >40% and 57.0 ml· kg^{1}·min^{-1} for infracted areas <40%) be used as initial indicators to classify the animals as HF+/HF- or as moderate/severe HF. Since the assessment of functional capacity is a non-invasive tool, it may be useful in studies that that require prior classification of the animals by degree of functional capacity impairment. However, these initial values are derived from studies performed on rats of different strains, sexes and ages and with varying protocol characteristics; these differences must be strongly considered. Additional, studies are necessary to validate this approach. For other functional capacity variables (run time and distance to run), these subanalyses are not adequate, because few studies reported infarct size (4/11 studies) and those that did reported MI areas <40.

Moreover, the overall difference between MI and control rats was -13.58 ml· kg^{1}·min^{-1}, -9.60 minutes and -122.53 meters in maximal oxygen uptake, run

time to fatigue and distance run, respectively. This overall difference may be important for future sample size calculations and for the design of powerful studies to assess functional capacity in a rat model of HF after coronary artery ligation.

The assessment of functional capacity in the rat model of HF is a challenge for investigators. Various methodological concerns, such as blinding, adaptation, acclimatization, well-defined exhaustion criteria, temperature and day period during the functional capacity test and calibration of the gas analyser (for VO_2 and VCO_2 measurements), are necessary for an optimal, precise and reproducible assessment. In this context, we propose some basic suggestions and care before, during and after (in the data-management phase) functional capacity assessment in the rat model of HF following coronary artery ligation (see Table 3).

Limitations

The present study does have limitations that warrant discussion. Firstly, the data extraction process was unblinded. Additionally, data not adequately reported in the main text were estimated by extraction from published graphs and recorded using graph digitizing software (GetData Graph Digitizer 2.24; S. Fedorov), creating a potential source of bias. Secondly, meta-analysts have reported a high degree of heterogeneity and the causes are undefined. We speculated that the cause of the high heterogeneity we observed was complex and multifactorial involving methodological aspects such as different time points of functional evaluation after MI induction, protocol characteristics (e.g. velocity,

inclination, stages, exhaustion criteria, air flow through metabolic box) and associations with age, sex and strain of the select animals.

Conclusion

In conclusion, the rat model of HF after MI following left coronary artery ligation is systematically associated with functional capacity reduction (VO_{2max}, run time do fatigue and distance run) compared with control animals. Attention to methodological improvements is necessary to create optimal, precise and reproducible protocols for functional capacity assessment in this widely used animal model of HF, especially to explore and test novel therapies that have the biological potential to improve functional capacity in this high mortality-associated syndrome.

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Conflict of interest

None declared.

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79. Wisloff U, Loennechen JP, Currie S, Smith GL, Ellingsen O (2002) Aerobic exercise reduces cardiomyocyte hypertrophy and increases contractility, Ca2+ sensitivity and SERCA-2 in rat after myocardial infarction. Cardiovascular research 54 (1):162-174

80. Miyauchi T, Fujimori A, Maeda S, Iemitsu M, Sakai S, Shikama H, Tanabe T, Matsuda M, Goto K, Yamaguchi I (2004) Chronic administration of an endothelin-A receptor antagonist improves exercise capacity in rats with myocardial infarction-induced congestive heart failure. Journal of cardiovascular pharmacology 44 Suppl 1:S64-67

81. Yamaguchi F, Kawana K, Tanonaka K, Kamano I, Igarashi T, Gen E, Fujimoto Y, Maki T, Sanbe A, Nasa Y, Takeo S (1999) Improvement of exercise capacity of rats with chronic heart failure by long-term treatment with trandolapril. British journal of pharmacology 126 (7):1585-1592. doi:10.1038/sj.bjp.0702471

82. Jannig PR, Moreira JB, Bechara LR, Bozi LH, Bacurau AV, Monteiro AW, Dourado PM, Wisloff U, Brum PC (2014) Autophagy signaling in skeletal muscle of infarcted rats. PloS one 9 (1):e85820. doi:10.1371/journal.pone.0085820

Figure legends

Figure 1 - Flowchart of search methods for identification and selection of studies.

Figure 2 – Qualitative analysis of quality assessment of the included studies. Data presented as 100% of included studies. Numbers in the bars represent number of studies in each category (Adequate/Yes or Inadequate/No).

Figure 3 - Forest plot of a random-effects metanalysis of 20 included studies (comprising 391 rats) about the effect of myocardial infarction induction in maximal oxygen uptake ($ml \cdot Kg^{1} \cdot min^{-1}$) versus control.

Figure 4 - Forest plot of a random-effects metanalysis of 11 included studies (comprising 206 rats) about the effect of myocardial infarction induction in run time to fatigue (minutes) versus control.

Figure 5 - Forest plot of a random-effects metanalysis of 6 included studies (comprising 122 rats) about the effect of myocardial infarction induction in distance run (meters) versus control.

TABLE 1 – Characterization of studies related to the impact of animal model of heart failure after myocardial infarction in functional capacity of rats. Studies are divided according to the outcome assessed.

Study Identification	*Animals*	*Intervention (Animal model of Heart Failure)*	*Methodology (Functional Capacity assessment)*	*Notes on study design and findings*
Maximal Oxygen Uptake				
Batista, Jr. *et. al.* (2008)[37]	*Strain:* Wistar *Sex:* male *Age/weight:* 6 to 8 weeks/~250 g *Groups(n)*[1]*:* - control (n=6) - MI (n=7)	*Myocardial infarction induction:* following left main coronary artery was ligated. *MI area (%):* 35.7 (histological) *Control group:* sham rats, underwent the same procedure, except that the suture under the coronary artery was left untied.	*Technical apparatus:* treadmill and gas-analyzing system for small animals *Protocol:* stepwise increases in the treadmill speed as follows: 15-min period of acclimation, the treadmill was then started at 10 m/min, and the speed was incrementally increased 5 m/min every 3 min until the rat reached exhaustion. *Air flow*[2]*:* 4,500 ml/min *Exhaustion criteria:* defined as enduring the electrical stimulus without attempting to reengage the treadmill within 15 s. *Days after animal model induction:* 84 *Animal adaptation:* familiarization with the treadmill was maintained also in the groups by having each rat run on the treadmill (0% grade) for 10 min/d, 2 d/wk, at a speed of 15 m/min. *Adverse effects in functional capacity assessment:* NR *Additional information:* The gas analyzer was calibrated with a reference gas mixture before each test. The highest VO_{2max} measured at each workload was taken as a measure of each rat`s running economy ($VO_{2submax}$) for that workload and, at the last step, as VO_{2max}. The training sessions were performed at the same time each day, in order to avoid circadian interference.	NA

Johnsen, A.B. et. al. (2013)[78]	*Strain:* Sprague-Dawley *Sex:* female *Age/weight:* NR *Groups(n)[1]:* - control (n=9) - MI (n=9)	*Myocardial infarction induction:* following left coronary artery ligation. *MI area (%):* >40% (echocardiography) *Control group:* sham rats, underwent the same procedure that MI rats, except the suture around the coronary artery was not ligated.	*Technical apparatus:* customized treadmill in a metabolic chamber *Protocol:* warmed up by treadmill running at a 25° inclination for 20 min at 50–60% of VO2max. Thereafter, the treadmill velocity was increased by 0.03 m/s every 2 min until exhaustion criteria. *Air flow[2]:* 4,500 ml/min *Exhaustion criteria:* VO_2 plateaued despite of increased workload (a leveling off of oxygen uptake despite increased workload). *Days after animal model induction:* 84 *Animal adaptation:* NR *Adverse effects in functional capacity assessment:* NR *Additional information:* VO_2 was expressed as ($ml \cdot kg^{-0.75} \cdot min^{-1}$).	NA
Jorge, L. *et. al.* (2011)[59]	*Strain:* Wistar *Sex:* male *Age/weight:* adults/250-300g *Groups(n)[1]:* - control (n=8) - MI (n=8)	*Myocardial infarction induction:* following left anterior descending coronary artery was ligated. *MI area (%):* 34 (echocardiography) *Control group:* sham rats, same surgical procedure except that the coronary artery was not ligated.	*Technical apparatus:* treadmill, metabolic chamber and gas analyzer. *Protocol:* a progressive exercise ramp protocol, with 3 m/min increments every 3 min and no grade until exhaustion. *Air flow[2]:* ~6,000 ml/min *Exhaustion criteria:* NR *Days after animal model induction:* 98 *Animal adaptation:* rats were adapted to the treadmill (10 min per day; 0.3 km/h) for 1 week after MI. *Adverse effects in functional capacity assessment:* NR *Additional information:* NR	NA
Kemi *et. al.* (2007)[60]	*Strain:* Sprague–	*Myocardial infarction induction:* following left coronary artery was ligated.	*Technical apparatus:* treadmill, metabolic chamber and gas analyzer *Protocol:* The rats warmed up by treadmill running at a 25° inclination for	One week after the MI placebo was given in drinking water (2 g/L, ad libitum) in sham and

	Dawley *Sex:* female *Age/weight:* NR *Groups(n)*[1]*:* - control (n=NR) - MI (n=NR)	*MI area (%):* 40% (echocardiography) *Control group:* sham rats, subjected to the same surgical procedures except the coronary artery ligation.	20 min at 50–60% of VO2max. Thereafter, the treadmill velocity was increased by 0.03 m/s every 2 min until exhaustion criteria. *Air flow*[2]*:* 4,500 ml/min *Exhaustion criteria:* VO2 plateaued despite of increased workload (a leveling off of oxygen uptake despite increased workload). *Days after animal model induction:* 84 *Animal adaptation:* NR *Adverse effects in functional capacity assessment:* NR *Additional information:* VO_2 was express as ($ml \cdot kg^{-0.75} \cdot min^{-1}$). The authors reported that VO_2 was normalized to body mass raised to the power of 0.75 to avoid confounding factors related to different body weights.	myocardial infarction animals.
Kemi, O.L. *et. al.* (2011)[61]	*Strain:* Sprague-Dawley *Sex:* female *Age/weight:* 3-4 months *Groups(n)*[1]*:* - control (n=8) - MI (n=8)	*Myocardial infarction induction:* following left coronary artery ligation. *MI area (%):* 40 (echocardiography) *Control group:* sham rats, underwent the same procedure that MI rats, except the suture around the coronary artery was not ligated.	*Technical apparatus:* customized treadmill in a metabolic chamber *Protocol:* The rats warmed up by treadmill running at a 25° inclination for 20 min at 50–60% of VO2max. Thereafter, the treadmill velocity was increased by 0.03 m/s every 2 min until exhaustion criteria. *Air flow*[2]*:* 4,500 ml/min *Exhaustion criteria:* VO_2 plateaued despite of increased workload (a leveling off of oxygen uptake despite increased workload). *Days after animal model induction:* 77 *Animal adaptation:* NR *Adverse effects in functional capacity assessment:* NR *Additional information:* NA	

Musch, T. I. *et. al.* (1986)[41]	*Strain:* Sprague-Dawley *Sex:* male *Age/weight:* NR/~350g *Groups(n)*[1]*:* - control (n=11) - MI (n=11)	*Myocardial infarction induction:* following left main coronary artery was ligated. *MI area (%):* 36 (histological) *Control group:* sham rats, underwent the same procedure that MI rats, except the suture around the coronary artery was not ligated.	*Technical apparatus:* metabolic box that was designed to fit into a channel of the rodent treadmill and gas analyzer. *Protocol:* stage 1 (grade: 0°; speed: 8.2 m/min); stage 2 (grade: 5°; speed: 15.2 m/min); stage 3 (grade: 10°; speed: 19.3 m/min); stage 4 (grade: 10°; speed: 26.8 m/min); stage 5 (grade: 12.5°; speed: 26.8 m/min); stage 6 (grade: 12.5°; speed: 30.3 m/min); stage 7 (grade: 15°; speed: 30.3 m/min); stage 8 (grade: 15°; speed: 35.4 m/min); stage 9 (grade: 15°; speed: 40 m/min); stage 10 (grade: 15°; speed: 43.8 m/min). Each stage takes 3-4 min long. *Air flow*[2]*:* 5,000 ml/min *Exhaustion criteria:* less than a 5% increase in vo2 with an increase in work intensity or stop, spread eagle, jump on and off the grid, or sit on the grid when the grade was changed. *Days after animal model induction:* 112-126 *Animal adaptation:* rats were familiarized with running on a motordriven. The first stage of the test was used for familiarization and warm-up. *Adverse effects in functional capacity assessment:* NR *Additional information:* the oxygen analyzer was precalibrated. VO_2 was determined at least twice in each rat to ensure reproducibility, with each maximal test separated by a minimum of 24 hr of recovery.	NA

Musch, T. I. *et. al.* (1988) [40]	*Strain:* Wistar *Sex:* female *Age/weight:* NR/~300g *Groups(n)*[1]*:* - control (n=15) - MI (n=25)	*Myocardial infarction induction:* following left main coronary artery was ligated. expanded. *MI area (%):* 27 (histological) *Control group:* sham rats, underwent the same procedure that MI rats, except the suture around the coronary artery was not ligated.	*Technical apparatus:* treadmill, gas analyzer and metabolic box (designed to fit into a stall of a 10-channel rodent treadmill) or metabolic mask (A lightweight mask was constructed from a sheet of clear plastic and epoxy. The mask fit over the face of the rat and was attached behind the ears with a wire collar). *Protocol:* a 2-min warmup at 16 m/min, 0% grade followed by increases in treadmill speed and/or grade every 2 min. The increases in treadmill speed and grade were as follows: stage 1 was 19 m/min, 5% grade; stage 2 was 24 m/min, 10% grade; stage 3 was 31 m/min, 15% grade; and stage 4 was 37 m/ min, 20% grade. When rats reached stage 4, further increases in work load were produced. by increasing the treadmill speed 3-5 m/min every 60 s. *Air flow*[2]*:* ~5,300 ml/min *Exhaustion criteria:* VO_{2max} was defined as the point at which VO_2 did not increase with further increases in work load or when the rat was unable or unwilling to continue running. *Days after animal model induction:* 42 *Animal adaptation:* after the recovery from surgery, all rats were acclimatized to sitting and running on the treadmill in the metabolic box and to sitting and running on the treadmill while wearing the metabolic mask. Rats that refused to adapt to either the metabolic mask or box were eliminated from the study. *Adverse effects in functional capacity assessment:* NR *Additional information:* the gas analyzer was calibrated with two or three primary gas standards. Additionally, this study show data values of CO_2 production (VCO_2) and respiratory exchange ratio (R). Exercise tests were conducted in a blind fashion with regard to each rat´s identity.	The same rats (control, n=15 and MI, n=25) were studied at rest, during various levels of submaximal exercise, and during a maximal exercise and both techniques (mask and box) in a random order. We considered the data values obtained by the maximal exercise test, because this test provide VO_{2max} values.

Musch, T. I. (1988) [54]	*Strain:* Wistar *Sex:* female *Age/weight:* NR/~250g *Groups(n)*[1]*:* - control (n=17) - MI (n=16)	*Myocardial infarction induction:* following left main coronary artery ligation. *MI area (%):* NA *Control group:* sham rats, underwent the same procedure that MI rats, except the suture around following left main coronary artery was not ligated.	*Technical apparatus:* metabolic box designed to fit into a channel of the rodent treadmill and gas analyzer *Protocol:* stage 1 (grade: 0°; speed: 8.2 m/min); stage 2 (grade: 5°; speed: 15.2 m/min); stage 3 (grade: 10°; speed: 19.3 m/min); stage 4 (grade: 10°; speed: 26.8 m/min); stage 5 (grade: 12.5°; speed: 26.8 m/min); stage 6 (grade: 12.5°; speed: 30.3 m/min); stage 7 (grade: 15°; speed: 30.3 m/min); stage 8 (grade: 15°; speed: 35.4 m/min); stage 9 (grade: 15°; speed: 40 m/min); stage 10 (grade: 15°; speed: 43.8 m/min). Each stage takes 3-4 min long. *Air flow*[2]*:* 5,000 ml/min *Exhaustion criteria:* VO_{2max} was defined as the point in which VO_2 did not increase with further increases in work load. *Days after animal model induction:* 49 *Animal adaptation:* rats were familiarized with treadmill running by having them run on a motor-driven treadmill over a wide range of speeds and grades for ~5 min a day for a period of 1 wk. Additionally, the first stage of the test was used for familiarization and warm-up. *Adverse effects in functional capacity assessment:* NR *Additional information:* VO_{2max} was determined at least twice on each rat to ensure reproducibility, with each maximal test separated by a minimum of 72 h of recovery.	Additionally, the study showed a swimming VO_2 assessment (measured while the rats swam with 4 % of their body weight attached to the base of their tails). However, data values represent ~65-80% of those obtained in VO_2 assessment by treadmill. That data values were not considerate in our review.

Musch, T.I (1989)[42]	*Strain:* Sprague-Dawley *Sex:* male *Age/weight:* NR/350-400g *Groups(n)*[1]*:* - control (n=13) - MI (n=16)	*Myocardial infarction induction:* following left main coronary artery was ligated. *MI area (%):* 29 (histological) *Control group:* sham rats, underwent the same procedure that MI rats, except the suture around the coronary artery was not ligated.	*Technical apparatus:* metabolic box that was designed to fit into a channel of the rodent treadmill and gas analyzer. *Protocol:* stage 1 (grade: 0°; speed: 8.2 m/min); stage 2 (grade: 5°; speed: 15.2 m/min); stage 3 (grade: 10°; speed: 19.3 m/min); stage 4 (grade: 10°; speed: 26.8 m/min); stage 5 (grade: 12.5°; speed: 26.8 m/min); stage 6 (grade: 12.5°; speed: 30.3 m/min); stage 7 (grade: 15°; speed: 30.3 m/min); stage 8 (grade: 15°; speed: 35.4 m/min); stage 9 (grade: 15°; speed: 40 m/min); stage 10 (grade: 15°; speed: 43.8 m/min). Each stage takes 3-4 min long. *Air flow*[2]*:* 5,000 ml/min *Exhaustion criteria:* less than a 5% increase in vo2 with an increase in work intensity or stop, spread eagle, jump on and off the grid, or sit on the grid when the grade was changed. *Days after animal model induction:* 98-112 *Animal adaptation:* rats were familiarized with running on a motor-driven treadmill. The first stage of the test was used for familiarization and warm-up. *Adverse effects in functional capacity assessment:* NR *Additional information:* Familiarization of running on the treadmill was maintained in sedentary control rats by having each rat run on the treadmill (0% grade) 5 min/day, 3 days/ wk at a speed of 19 m/min. The oxygen analyzer was precalibrated. VO_2 was determined at least twice in each rat to ensure reproducibility, with each maximal test separated by a minimum of 24 hr of recovery.	NA

Musch, T. I. *et. al.* (1990)[53]	*Strain:* Wistar *Sex:* female *Age/weight:* NR *Groups(n)[1]:* - control (n=7) - MI (n=8)	*Myocardial infarction induction:* following left main coronary artery was ligated. *MI area (%):* 36.7 (histological) *Control group:* sham rats, underwent the same procedure that MI rats, except the suture around the coronary artery was not ligated.	*Technical apparatus:* treadmill, gas analyzer and metabolic box. *Protocol:* a 2-min warmup at 16 m/min, 0% grade followed by increases in treadmill speed and/or grade every 2 min. The increases in treadmill speed and grade were as follows: stage 1 was 19 m/min, 5% grade; stage 2 was 24 m/min, 10% grade; stage 3 was 31 m/min, 15% grade; and stage 4 was 37 m/ min, 20% grade. When rats reached stage 4, further increases in work load were produced. by increasing the treadmill speed 3-5 m/min every 60 s. *Air flow[2]:* ~5,300 ml/min *Exhaustion criteria:* VO_{2max} was defined as the point at which VO_2 did not increase with further increases in work load or when the rat was unable or unwilling to continue running. *Days after animal model induction:* 42 *Animal adaptation:* NR *Adverse effects in functional capacity assessment:* NR *Additional information:* NR	Additionally, the study showed a swimming VO_2 assessment (measured while the rats swam with 4 % of their body weight attached to the base of their tails). However, data values represent ~60-70% of those obtained in VO_2 assessment by treadmill. Even, the study showed VO_2 values assessed in instrumented rats (polyethylene catheter placed into the right carotid artery, advanced towards the heart and advanced subcutaneously to the dorsal aspect of the cervical region of the rat, where it was exteriorized). That data values were not considerate in our review.

Rodrigues, B. *et. al.* (2013)[50]	*Strain:* Wistar *Sex:* male *Age/weight:* adults/230–260g *Groups(n)[1]:* - control (n=8) - MI (n=8)	*Myocardial infarction induction:* following left anterior descending coronary artery was ligated *MI area (%):*46 (echocardiography) and 50 (millimeter graph paper stamps) *Control group:* sham rats, underwent the same procedures except that myocardial ischemia was not induced.	*Technical apparatus:* treadmill, metabolic chamber and gas analyzer. *Protocol:* progressive exercise ramp protocol, with 3m/min increments every 3min and no grade until exhaustion. *Air flow[2]:* ~6 l/min *Exhaustion criteria:* VO_2 max was reached (VO_2 max was defined as the VO_2 after which an increase in work rate was not associated with a further increase (± 5%) in continuously measured) O2 uptake. *Days after animal model induction:* 91 *Animal adaptation:* NR *Adverse effects in functional capacity assessment:* NR *Additional information:* The experimental animals only started the exercise test when the levels of resting VO_2 were near to 30±5 ml·Kg^{1}·min^{-1}.	Study demonstrated a criteria to started the exercise test.
Rolim, N.P.L. *et. al.* (2006)[49]	*Strain:* Wistar *Sex:* male *Age/weight:* 3-month/330 g *Groups(n)[1]:* - control (n=9) - MI (n=6)	*Myocardial infarction induction:* following left anterior descending coronary artery was ligated. *MI area (%):*40% (methodology NR) *Control group:* normal control (methodology NR)	*Technical apparatus:* treadmill using a metabolic mask and gas analysis was performed using an oxygen analyzer *Protocol:* ramp protocol of progressive exercise on a treadmill with 1-m/min increments every 40 s and no grade until exhaustion. *Air flow[2]:* NR *Exhaustion criteria:* NR *Days after animal model induction:* 35 *Animal adaptation:* rats were conditioned to the metabolic mask from 6 weeks of age to the time of the experimental procedure.	Study demonstrated a baseline (rest) record of VO2 data before started the exercise test. Additionally, the study report a temporal responses of oxygen consumption approach during progressive exercise in rats with ischemic heart failure and employed an algorithm to fit the sigmoidal equation to the experimental data points variables as VO_2

			Adverse effects in functional capacity assessment: NR *Additional information:* The mask fit over the face of the rat and was attached behind the ears with a latex collar. A small length of polyethylene tubing (PE-280, 3 mm ID) was attached to the top portion of the mask in such a way that ambient air was drawn unidirectionally into the mask from around the rat's head and exhausted through the tubing just above the rat's nose. VO2 was calculated using the measured flow through the metabolic mask, the expired fraction of effluent oxygen and the fraction of oxygen in room air. During measurement of VO2, a baseline (rest) values are recorded during 200s.	slope and ΔVO_2. Thus, the methodology permits a more detailed analysis of VO_2 kinetics during progressive exercise and showed the oxygen uptake kinetics in heart failure rats.
Rondon, E. *et al.* (2006)[47]	*Strain:* Wistar *Sex:* male *Age/weight:* NR/180-200g *Groups(n)*[1]*:* - control (n=8) - MI (n=9)	*Myocardial infarction induction:* following left coronary artery ligation. *MI area (%):* 40% (methodology NR) *Control group:* normal control (methodology NR)	*Technical apparatus:* metabolic chamber, motor treadmill and gas analyzers *Protocol:* progressive exercise protocol (5 m/min increments every 3 min and no grade). *Air flow*[2]*:* 5.000 ml/min *Exhaustion criteria:* rats could no longer maintain the running speed. *Days after animal model induction:* 84 *Animal adaptation:* rats were exposed to treadmill exercise (5 min), three times a week, to become accustomed to the exercise protocol and handling. *Adverse effects in functional capacity assessment:* NR *Additional information:* Peak VO_2 was defined as the highest VO_2 attained at the end of the exercise period when the rats could no longer maintain the running speed. The maximal exercise test was performed at the beginning, at the fourth week to adjust the training intensity, and at the end of exercise training period.	NA

Wisloff (2002) SERCA [79]	*Strain:* Sprague-Dawley *Sex:* female *Age/weight:* adults/300-325g *Groups(n)*[1]*:* - control (n=6) - MI (n=9)	*Myocardial infarction induction:* following left coronary artery ligation *MI area (%):* 44 (echocardiography) *Control group:* sham rats, same surgical procedure except that the left coronary artery was not ligated.	*Technical apparatus:* customized treadmill in a metabolic chamber *Protocol:* warmed up by treadmill running at a 25° inclination for 20 min at 50–60% of VO2max. Thereafter, the treadmill velocity was increased by 0.03 m/s every 2 min until exhaustion criteria. *Air flow*[2]*:* 4,500 ml/min *Exhaustion criteria:* VO_2 plateaued despite of increased workload (a leveling off of oxygen uptake despite increased workload). *Days after animal model induction:* 84 *Animal adaptation:* NR *Adverse effects in functional capacity assessment:* NR *Additional information:* In sedentary rats, running skill was maintained by treadmill running for 15 min at 08 inclination at 0.15 m.s-1 3 days per week. After each training or test session, each rat was rewarded with 0.5 g chocolate; sedentary rats were given the same amount.	NA
Run time to fatigue				
Aaker, A. *et. al.* (1996)[44]	*Strain:* Wistar *Sex:* female *Age/weight:* NR/~300g *Groups(n)*[1]*:* - control (n=12) - small MI (n=13) - large MI	*Myocardial infarction induction:* following left main coronary artery was ligated. *MI area (%):* - small MI: 33 - large MI: 50 (methodology NR) *Control group:* sham rats, underwent the same	*Technical apparatus:* motordriven treadmill *Protocol:* graded running test in which each rat initially ran up a 5% grade at a speed of 25 m/min for 15 min. Thereafter, the treadmill speed was increased 5 m/min every 15 min until each animal reached the point of fatigue. *Air flow*[2]*:* NA *Exhaustion criteria:* rat's inability to keep pace with the treadmill, which occurred even though each animal was encouraged to keep running with bursts of high-pressure air directed at the animal's hindquarters. At the end of each exercise test, fatigue was confirmed for each rat by the loss of the animal's righting reflex. *Days after animal model induction:* 70	The infracted animals were categorized as small M I (10% < M I < 40%) or large M I (M I > 40%). The control groups received water (placebo) by gastric gavage at 8:00 a.m. and at 4:00 p.m. for 28 days.

	(n=11)	procedure that MI animals , except the suture around the coronary artery was not ligated.	*Animal adaptation:* during the recovery period post MI (minimum of 6 weeks), each animal was familiarized with running on a motordriven treadmill. During these training sessions, each rat exercised for 5-10 min/day at a speed of 20 m/min up a 10% grade to ensure that they were proficient runners. *Adverse effects in functional capacity assessment:* NR *Additional information:* all rats were restricted from food for 12 h before the exercise test and all treadmill exercise tests were initiated between 9 and 10 a.m. to prevent the confounding effects from the diurnal variation in tissue glycogen concentrations. Time from the beginning of the exercise test to the removal of the animal from the treadmill was measured and recorded to the nearest half minute.	
Koh, S.G. *et. al.* (2003)[62]	*Strain:* Fischer 344 *Sex:* NR *Age/weight:* 9 wk/NR *Groups(n)*[1]*:* - control (n=7) - MI (n=12)	*Myocardial infarction induction:* following left coronary artery ligation *MI area (%):* NR *Control group:* sham rats, underwent the same procedure that MI rats, except the suture around the coronary artery was not ligated.	*Technical apparatus:* treadmill *Protocol:* initial speed of 15 m/min with 2 m/min increases every 2 min. The treadmill was maintained at an uphill grade of 15 degrees at all times. *Air flow*[2]*:* NA *Exhaustion criteria:* defined as the point when the rats could not run on the treadmill for longer than 5 sec. *Days after animal model induction:* 91 *Animal adaptation:* rats were acclimated to treadmill by walking at a speed of 10 m/min, 10 min/day for 2 weeks. *Adverse effects in functional capacity assessment:* NR *Additional information:* NA	NA

Louzada, R.A.N. *et. al.* (2010)[63]	*Strain:* Wistar *Sex:* male *Age/weight:* 8-10 weeks/200-250g *Groups(n)*[1]*:* - control (n=7) - MI (n=7)	*Myocardial infarction induction:* following left coronary artery ligation. *MI area (%)*[*]*:* 39 (echocardiography) *Control group:* sham rats, underwent the same procedure that MI rats, except the suture around following left main coronary artery was not ligated.	*Technical apparatus:* treadmill *Protocol:* starting velocity of 17 cm/s and a constant slope of 10°. Treadmill velocity was then increased by 2 cm/s every 2 min until exhaustion. *Air flow*[2]*:* NA *Exhaustion criteria:* when animals stayed in the steel grids despite increasing shocks *Days after animal model induction*: 70 *Animal adaptation:* each rat was first adapted to run on the treadmill for 5 min at a speed of 17 cm/s over a 3–4 day period. *Adverse effects in functional capacity assessment:* NR *Additional information:* stainless steel grids at the end of the treadmill provided an electrical stimulus to keep the rats running.	Four weeks post-MI rats received subcutaneously during seven consecutive days vehicle (5% of glucose). Additionally, the study assessed in other group of rats that received vehicle subcutaneously for 4 weeks the functional capacity in the same protocol time. We chose the first group analysis, because the short term treatment with vehicle.
Miyauchi *et. al.* (2004)[80]	*Strain:* Sprague-Dawley *Sex:* male *Age/weight:* NR/4–5 weeks *Groups(n)*[1]*:* - control (n=12) - MI (n=14)	*Myocardial infarction induction:* following the descending branch of the left coronary artery was ligated. *MI area (%):* NR *Control group:* sham rats, underwent an identical operative procedure without ligation of the coronary artery.	*Technical apparatus:* treadmill *Protocol:* tolerance of the animals was evaluated by measuring running time at a running speed of 25 m/min. *Air flow*[2]*:* NA *Exhaustion criteria:* NR *Days after animal model induction:* 140 *Animal adaptation:* rats were allowed to run on a treadmill apparatus preoperatively so that they became acclimatized to the test. Starting approximately 1 month after experimentally induced MI, rats were subjected to running once weekly for further acclimatization. *Adverse effects in functional capacity assessment:* NR	MI and sham groups received distilled water administered orally once daily, for approximately 25 weeks, 10 days after ligation of the coronary artery or sham operation.

			Additional information: NA	
Pfeifer, P. C. *et. al. (2001)[22]*	*Strain:* Wistar *Sex:* male *Age/weight:* NR/~400g *Groups(n)[1]:* - control (n=7) - small MI (n=7) - large MI (n=6)	*Myocardial infarction induction:* following left main coronary artery was ligated. *MI area (%):* NR *Control group:* sham rats, underwent the same procedures except that the suture around the coronary artery was not ligated.	*Technical apparatus:* customized rodent treadmill *Protocol:* run up a 5% grade, beginning at a speed of 25 m·min^{-1}. If the animal completed the required 15 min of running at this workload, the treadmill speed was increased to 30 m·min^{-1}. If the animal completed the required 15 min of running at this workload, then the treadmill speed was increased to 35 m·min^{-1}. This treadmill speed was then maintained until the rat reached the point of fatigue. *Air flow[2]:* NA *Exhaustion criteria:* rat could no longer keep pace with the treadmill, even when encouraged to continue with high-pressured bursts of air directed at the hindquarters of the animal. Fatigue was confirmed by a loss of the animal's righting reflex. *Days after animal model induction:* 42 *Animal adaptation:* NR *Adverse effects in functional capacity assessment:* NR *Additional information:* Rats were allowed food *ad libitum* before the test to maintain normal glycogen stores. All animals ran between 8:00 a.m.	Rats that had undergone a MI operation, with an LVEDP of >20 mm Hg, were assigned to a large MI group. Those rats that had undergone a MI operation, with an LVEDP below 20 mm Hg, were assigned to a small MI group.

			and 11:00 a.m. to prevent the confounding effects of the diurnal variation in glycogen concentrations. Time from the beginning of the test to removal from the treadmill was recorded to the nearest second.	
Soares da Silva, J. *et. al.* (2014)[43]	*Strain:* Wistar *Sex:* male *Age/weight:* NR/150–200g *Groups(n)*[1]*:* - control (*n*=8) - MI (*n*=8)	*Myocardial infarction induction:* following main left coronary artery was ligated. *MI area (%):* NR *Control group:* sham group, the same procedure was employed but the suture was not tied.	*Technical apparatus:* customized rodent treadmill *Protocol:* rats run up a 5% grade, beginning at a speed of 25 m/min. If the rat completed the required 15 min of running at this workload, the treadmill speed was increased to 30 m/min. If the animal completed the required 15 min of running at this workload, then the treadmill speed was increased to 35 m/min. This treadmill speed was then maintained until the rat reached the point of fatigue. *Air flow*[2]*:* NA *Exhaustion criteria:* rat could no longer keep pace with the treadmill, even when encouraged; fatigue was confirmed by loss of the animal righting reflex. *Days after animal model induction:* 21 *Animal adaptation:* NR *Adverse effects in functional capacity assessment:* NR *Additional information:* all rats were submitted to the same experimental conditions of time and environment and the exercise test was done in the late afternoon.	Sham and MI rats were treated with vehicle-injected (dimethyl sulfoxide – DMSO, i.p.) after surgery for 4 weeks.

Yamaguchi, F. *et. al.* (1999)[81]	*Strain:* Wistar *Sex:* male *Age/weight:* NR/220-250g *Groups(n)*[1]*:* - control (n=7) - MI (n=7)	*Myocardial infarction induction:* following left coronary artey ligation. *MI area (%):* NR *Control group:* sham rats, underwent the same surgical procedures except that no suture was tied around the coronary artery.	*Technical apparatus:* treadmill *Protocol:* slope of 5° and operated at a constant running speed of 25 m/min. *Air flow*[2]*:* NA *Exhaustion criteria:* The end point for each test was decided by the inability of the rat to run any more on the treadmill belt. After a certain period of running, the animals experienced difficulty in continuing the running to match the pace of the treadmill. This resulted in a landing on the electric shock grid at the rear of the continuous belt. When given the electric shock, the animals again started running. The end point for every test was marked by a rat's inability to return to the treadmill belt from the shock grid, despite additional manual encouragement. *Days after animal model induction:* 56 *Animal adaptation:* before starting the study, we selected rats that could run on a treadmill. Animals were subjected to treadmill exercise by the methods described in “protocol”. The rats that could run on the treadmill for more than 20 min (approximately 50% of the acclimatized animals) were used for coronary artery ligation or sham-operation. Acclimatized rats were subjected to the treadmill test. *Adverse effects in functional capacity assessment:* NR *Additional information:* The treadmill was equipped with a continuous belt. The running time from the start to the end point of each rat was recorded.	NA
Distance run				

Jannig *et. al.* (2014)[82]	*Strain:* Wistar *Sex:* male *Age/weight:* 8 weeks/250–300 g *Groups(n)*[1]*:* - control (n=12) - MI (n=14)	*Myocardial infarction induction:* following left anterior descending coronary artery was ligated *MI area (%):* 28 (histological) *Control group:* sham rats, underwent similar procedure, with the exception of left anterior descending coronary ligation.	*Technical apparatus:* treadmill *Protocol:* rats were submitted to a graded treadmill exercise test. The test started at 6 m/min and speed was increased by 3 m/min every 3 minutes until exhaustion criteria. *Air flow*[2]*:* NA *Exhaustion criteria:* rats were unable to run due to exhaustion. *Days after animal model induction:* 84 *Animal adaptation:* animals were adapted to treadmill exercise during five days (10 minutes each day) before test. *Adverse effects in functional capacity assessment:* NR *Additional information:* NR	NA
Trueblood, N. A. *et. al.* (2005)[52]	*Strain:* Wistar *Sex:* male *Age/weight:* NR/225-250g *Groups(n)*[1]*:* - control (n=11) - MI (n=11)	*Myocardial infarction induction:* following left main coronary artery ligation. *MI area (%):* 26 (histological) *Control group:* sham rats, underwent the same surgical procedures except for the coronary artery ligation.	*Technical apparatus:* rodent treadmill *Protocol:* the treadmill was set at a constant incline of 15°. The initial speed was 15 m/min and was increased by 1 m/min every minute. *Air flow*[2]*:* NA *Exhaustion criteria*: the point at which the animals could not keep pace with the treadmill and no longer avoided the electrical stimulus. *Days after animal model induction:* 112 *Animal adaptation:* animals were familiarized with running on the treadmill before surgery *Adverse effects in functional capacity assessment:* NR *Additional information:* the rodent treadmill equipped with an electric motivator grid. Total exercise time was recorded as the elapsed time to exhaustion and then converted to distance. Exhaustion was determined	NA

			by an observer blinded to surgery group (sham or MI).	
Zapata-Sudo, G. *et. al.* (2014)[55]	*Strain:* Wistar *Sex:* male *Age/weight:* NR/150-200g *Groups(n)*[1]*:* - control (n=6) - MI (n=6)	*Myocardial infarction induction:* following anterior descendent coronary was ligated *MI area (%):* NR *Control group:* sham rats, underwent the same procedure that MI rats, except the suture around the coronary artery was not ligated.	*Technical apparatus:* customized rodent treadmill *Protocol:* rats running in three steps, with a progressive increase in treadmill speed: 1) 8 m/min, 2) 12 m/min, and 3) 18 m/min. The two initial steps lasted for 3 min each, whereas rats continued running in the final step until they reached the point of fatigue. *Air flow*[2]*:* NA *Exhaustion criteria:* loss of the animal righting reflex *Days after animal model induction:* 28 *Animal adaptation:* NR *Adverse effects in functional capacity assessment:* NR *Additional information:* NA	NA
Association				
Batista, Jr. *et. al.* (2007)[38]	*Strain:* Wistar *Sex:* male *Age/weight:* 6 to 8 wk/~250 g *Groups(n)*[1]*:* - control (n=7) - MI (n=10)	*Myocardial infarction induction:* following left main coronary artery was ligated. *MI area (%):* 38.7 (histological) *Control group:* sham rats, underwent the same procedure that MI animals, except that the suture under the coronary artery was left untied.	*Technical apparatus:* *- Determination of Endurance Exercise Capacity (run time to fatigue):* treadmill *- Treadmill Testing (VO_2):* treadmill and gas-analyzing system for small animals *Protocol:* *- Determination of Endurance Exercise Capacity (run time to fatigue):* graded running test in which each rat ran initially at 0% grade, and at a speed of 15 m/min, for 10 min. Thereafter, the treadmill speed was increased 5 m/min every 10 min until each animal reached the point of fatigue. *- Treadmill Testing (V_{O2}):* stepwise increasing of the treadmill speed as follows: after a 15-min period of acclimation, the treadmill was then started at 10 m/min, and the speed was incrementally increased 5 m/min	NA

			every 3 min until the rat reached exhaustion. *Air flow*[2]*:* 4,500 ml/min *Exhaustion criteria:* *- Determination of Endurance Exercise Capacity (run time to fatigue):* rat's inability to keep pace with the treadmill, thus being unable to avoid the electric stimulus (volts) for a period longer than 15 s. *- Treadmill Testing (V_{O2}):* spending time on the shocker plate without attempting to reengage the treadmill within 15 s. *Days after animal model induction:* 98 *Animal adaptation:* all rats were exercised on the treadmill before the experiment at a speed of 20 m/min and 5° elevation for 5 min/day, 3 days/wk, for 2 wk. *Adverse effects in functional capacity assessment:* NR *- Additional information:* rats performed a endurance exercise capacity test initiated between 9:00 and 10:00 AM. The gas analyzer was calibrated with a reference gas mixture before each test. The highest V_{O2max} measured at each workload was taken as a measure of each rat's running economy ($V_{O2submax}$) for that workload, and at the last step, as V_{O2max}.	
Campos, J. C. *et. al.* (2012)[39]	*Strain:* Wistar *Sex:* male *Age/weight: 12 weeks /*250-300g *Groups(n)*[1]*:* - control (n=9)	*Myocardial infarction induction:* following left anterior descending coronary artery was ligated. *MI area (%):* 31 (histological) *Control group:* sham rats, left thoracotomy with	*Technical apparatus:* treadmill, metabolic chamber and gas analyzer. *Protocol:* Each rat had a twenty-minute rest period and a ten-minute warm-up at 3 m/min before the test protocol. Treadmill speed was increased by 3 m/min every 3 minutes until the animal was unable to run. *Air flow*[2]*:* 3,500 ml/min *Exhaustion criteria:* rat could no longer maintain the running speed over 3 min.	Additionally to the animal adaptation described previously, treadmill running skills were maintained in HF and sham rats by treadmill running for 5 min, twice a week during 8 weeks of experimental protocol. This procedure was performed in order

	- MI (n=7)	equal procedure duration to that of heart failure group, but without left anterior descending coronary artery ligation.	*Days after animal model induction:* 84 *Animal adaptation:* rats were adapted to treadmill exercises and the test environment for over one week (10 minutes each session). *Adverse effects in functional capacity assessment:* NR *Additional information:* Oxygen fraction in effluent air was registered every second. The analyzer was calibrated with known gas mixtures every day of tests. We considered the VO2 reached at the highest workload during the treadmill test as peak VO2.	to avoid any interference of treadmill stress on the variables studied. This latter activity did not seem to alter maximal exercise capacity.
Wernec k-de-Castro, J.P.S *et. al.* (2006)[48]	*Strain:* Wistar *Sex:* male *Age/weight:* 8-10w/200-250g *Groups(n)*[1]*:* - control (n=9) - MI (n=10)	*Myocardial infarction induction:* following left coronary artery ligation *MI area (%):* 44 (echocardiography) *Control group:* sham rats, underwent the same procedure that MI rats, except the suture around the coronary artery was not ligated.	*Technical apparatus:* motordriven treadmill chamber and gas analyzer. *Protocol:* starting velocity of 17 cm/s and a constant slope of 10°. Treadmill velocity was then increased by 2 cm/s every 2 min and each rat ran until exhaustion. *Air flow*[2]*:* 680 ml/min *Exhaustion criteria:* VO_2 leveled off despite increasing running velocity or when animals stayed in the steel grids despite increasing shocks. *Days after animal model induction:* 19-20 *Animal adaptation:* each rat was first adapted to run on the treadmill for 5 min at a speed of 17 cm/s over a 3–4 day period. *Adverse effects in functional capacity assessment:* NR *Additional information:* every 2 min of the protocol, oxygen consumption was measured. Stainless steel grids at the end of the treadmill provided an electrical stimulus to keep the rats running. Authors described a protocol reproducibility (r=0.82).	Three hours after permanent occlusion, rats received vehicle twice a day during seven consecutive days subcutaneously. Additionally, authors assessed maximal oxygen uptake and total exercise time in normal rats (without sham surgery). No differences compared to sham rats were detected.

Gomes-Santos, I.L. et al. (2014)[46]	*Strain:* Wistar *Sex:* male *Age/weight:* 2 mo/NR *Groups(n)*[1]*:* - control (n=10) - MI (n=12)	*Myocardial infarction induction:* following main descending branch of the left coronary artery ligation *MI area (%):* NR *Control group:* sham rats, underwent the same procedure that MI rats, except the suture around the coronary artery was not ligated.	*Technical apparatus:* treadmill inside of a metabolic chamber coupled to a gas analyzer *Protocol:* starting at 6 m/min and 3 m/min increments every 3 minutes. *Air flow*[2]*:* 3,500 ml/min *Exhaustion criteria:* NR *Days after animal model induction:* 84 *Animal adaptation:* rats were previously adapted to treadmill exercise before testing. *Adverse effects in functional capacity assessment:* NR *Additional information:* Peak oxygen uptake (peak VO2) was defined as the highest VO2 achieved before exhaustion.	Additionally to animal adaptation, all rats were exposed to treadmill running for 5 minutes, once a week, to maintain their running skills during 8 weeks of exercise training period.
Helwig *et. al.* (2003)[45]	*Strain:* Wistar *Sex:* female *Age/weight:* NR *Groups(n)*[1]*:* - control (n=10) - MI (n=16)	*Myocardial infarction induction:* following left main coronary artery was ligated. *MI area (%):* NR *Control group:* sham rats, same surgical procedure except that the coronary artery was not ligated.	*Technical apparatus:* *- Determination of endurance exercise capacity:* treadmill *- Determination of VO_2:* metabolic chamber (14.5 x 43 x 7 cm) designed to fit into a stall of a 10-channel rodent treadmill and gas analyzer *Protocol:* *- Determination of endurance exercise capacity:* graded running test in which each rat initially ran up a 5% grade at a speed of 25 m/min for 15 min. Thereafter, the treadmill speed was increased 5 m/min every 15 min until each animal reached the point of fatigue. *- Determination of VO_2:* a 2-min warm-up at a treadmill grade and speed of 0% and 15 m/min, respectively. The treadmill speed and/or grade was increased every 2 min. However, confirmation that VO_2 max was truly attained in each animal was demonstrated by having each rat perform a subsequent maximal exercise test after 48 h of recovery from the initial test. With the second test, each rat was given a 2-min warm-up at a treadmill grade and speed of 0% and 15 m/min. The treadmill grade and speed were then increased to the highest workload each animal was able to sustain during the initial maximal test. VO_2 and VCO_2 were	NA

			recorded. The treadmill speed was then increased by 3–5 m/min, and VO_2 and VCO_2 were recorded. If the measured VO_2 was similar between the two workloads, the animal was considered to be at VO_2 max, and the exercise test was terminated. If the rat demonstrated an increase in VO_2 during the second exercise test, the test was terminated and the same procedure repeated after 48 h of recovery. This procedure was repeated until comparable VO_2 values were found between the initial and second (greater) workloads during each subsequent maximal exercise test, thus ensuring an accurate assessment of $VO_{2\,max}$ in each animal. *Air flow[2]:* ~5,300 ml/min *Exhaustion criteria:* - *Determination of endurance exercise capacity:* rat's inability to keep pace with the treadmill, even though the animal was encouraged to run by applying bursts of high-pressure air at the hindquarters. At the end of each exercise test, the end point of fatigue was confirmed by the loss of the animal's righting reflex. - *Determination of VO_2:* VO_2 max was defined as the point at which the VO_2 did not increase with further increases in workload or when the rat was unable to or unwilling to continue running. *Days after animal model induction: 84-98* *Animal adaptation:* familiarity with treadmill running was maintained in both groups by having each animal run on the treadmill for 5 min/day at a treadmill speed of 20 m/min up a 10% grade. *Adverse effects in functional capacity assessment:* NR - *Additional information:* Time from the beginning of the exercise to the removal of the rat from the treadmill was measured and recorded to the nearest half minute. All exercise tests were initiated between 9 and 10 AM to prevent the confounding effects from the diurnal variation in tissue glycogen. The exercise test was administered by an observer blinded to the animal's condition. Therefore, the observer did not know whether the animal being tested was a MI rat with CHF or a noninfarcted sham	

			control rat.	
Moreira J.B.N *et. al.* (2013)[51]	*Strain:* Wistar *Sex:* male *Age/weight:* 8 wk/NR *Groups(n)*[1]*:* - control (n=6-12) - MI (n=6-12)	*Myocardial infarction induction:* following left anterior descending coronary artery ligation. *MI area (%):* 27.9 (echocardiography) *Control group:* sham rats, underwent the same procedure that MI rats, except the suture around following left anterior descending coronary artery was not ligated.	*Technical apparatus:* treadmill mounted into a metabolic chamber and gas analyzer. *Protocol:* 15° inclination; the speed started at 6 m/min and was increased by 3 m/min every 3 min until rats were unable to run. *Air flow*[2]*:* 3,500 ml/min *Exhaustion criteria:* VO_{2max} was considered achieved when oxygen uptake no longer increased despite an increase in workload (i.e., intensity at which oxygen uptake leveled off) and rats were no longer able to run *Days after animal model induction:* 84 *Animal adaptation:* were adapted to treadmill exercise over 5 days (10 min each day) before tests. *Adverse effects in functional capacity assessment:* NR *Additional information: t*ests were performed by an experienced observer who was blinded to rat identities.	To distance run assessment *n*=12 for both groups. For VO_2 assessment *n*=6 for both groups. Additionally to animal adaptation, animals were placed on the treadmill twice a week for 10 min each day at 40% VO_2 to maintain running skills, during 8 weeks of exercise training period.
Musch, T. I. *et. al.* (2002)[19]	*Strain:* Wistar *Sex:* female *Age/weight:* NR/NR *Groups(n)*[1]*:* - control (n=9-14): - MI (moderate left ventricular dysfunction, n= 10-18) - MI (severe	*Myocardial infarction induction:* following left main coronary artery ligation. *MI area (%):* NR *Control group:* sham rats, same surgical procedure except that the coronary artery was not ligated.	*Technical apparatus:* - *Determination of endurance exercise capacity:* treadmill - *Determination of VO_2:* metabolic chamber (14.5 x 43 x 7 cm) designed to fit into a stall of a 10-channel rodent treadmill and gas analyzer *Protocol:* - *Determination of endurance exercise capacity:* graded running test in which each rat initially ran up a 5% grade at a speed of 25 m/min for 15 min. Thereafter, the treadmill speed was increased 5 m/min every 15 min until each animal reached the point of fatigue. - *Determination of VO_2:* test consisted of a 2-min warm-up at a treadmill grade and speed of 0% and 15 m/min, respectively. The treadmill speed and/or grade were increased every 2 min. Confirmation that VO_2 max was truly attained in each animal was demonstrated by having each rat perform a subsequent maximal exercise test after 48 h of recovery from	Rats that received an MI were categorized as having moderate LV dysfunction (LVEDP < 20 mmHg) or as having severe LV dysfunction (LVEDP > 20 mmHg). For run time to fatigue assessment: control (n=14), MI (moderate left ventricular dysfunction, n=18), MI (severe left ventricular dysfunction rats, n=5). For VO2 assessment: control (n=9), MI

	left ventricular dysfunction rats, n=5)		the initial maximal test. With the second maximal test, each rat was given a 2-min warm-up at a treadmill grade and speed of 0% and 15 m/min. The treadmill grade and speed were then increased to the highest workload each animal was able to sustain during the initial maximal test. VO_2 and VCO_2 were recorded. The treadmill speed was then increased by 3–5 m/min, and VO_2 and VCO_2 were recorded. If the measured VO_2 was similar between the two workloads, the animal was considered to be at VO_2 max, and the exercise test was terminated. If the rat demonstrated an increase in VO_2 during the second maximal exercise test, the test was terminated and the same procedure was repeated after 48 h of recovery. This procedure was repeated until comparable VO2 values were found between the initial and second (greater) workloads during each subsequent maximal exercise test, thus ensuring an accurate assessment of VO2 max in each animal. *Air flow[2]: ~5,300 ml/min* *Exhaustion criteria:* - *Determination of endurance exercise capacity:* rat's inability to keep pace with the treadmill, even though the animal was encouraged to run by application of bursts of high-pressure air at the hindquarters. At the end of each exercise test, the end point of fatigue was confirmed by loss of the animal's righting reflex. - *Determination of VO_2:* as the point at which the VO_2 did not increase with further increases in workload or when the rat was unable to or unwilling to continue running. *Days after animal model induction:* 42 *Animal adaptation:* NR *Adverse effects in functional capacity assessment:* NR *Additional information:* Time from the beginning of the exercise to the removal of the rat from the treadmill was measured and recorded to the nearest half minute. All exercise tests were initiated between 9 and 10	(moderate left ventricular dysfunction, n= 10), MI (severe left ventricular dysfunction rats, n=5)

			AM to prevent the confounding effects from the diurnal variation in tissue glycogen. The exercise test was administered by an observer blinded to the animal's condition. Therefore, the observer did not know whether the animal being tested was an MI rat with CHF or a noninfarcted sham control rat.	

NA: not applicable; NE: not evaluated; NR: not reported; MI: myocardial infarction; VO_2: maximal oxygen uptake or maximal oxygen consumption; [1] Groups of interest in this review; selected studies may have more experimental groups in their study design based on their aims; *n* represents the amount of animals in each group related to assessment of functional capacity variables; [2] gas analyzer air flow during VO2 assessment; [3] some studies describe as VO_2 peak; [*] data only presented graphically and then estimated by extracted from published graphs using graph digitizing software (GetData Graph Digitizer 2.24).

TABLE 2 - Functional capacity variables (maximal oxygen uptake, run time to fatigue and distance run) values in selected studies comparing myocardial infarction group to control group after myocardial infarction or control intervention.

Study Identification	Control	MI	%	*p*
VO_2[1] ($ml \cdot kg^{-1} \cdot min^{-1}$)				
Rondon, E. *et al.* (2006)[47]	66.0±8.5	53.0±6.0	20	<0.05
Rolim, N.P.L. *et. al.* (2006)[49]	109.0±27.9	73.7±7.4	32	<0.05
Rodrigues, B. *et. al.* (2013)[50]	77.1±4.7*	49.1±2.3*	36	<0.05
Musch, T. I. *et. al.* (2002)[3][19]	77.1±5.4*	70.2±2.3*/55.7±4.9*	9/28	>0.05/<0.05
Kemi, O. J. *et. al.* (2007)[60]	44.9±1.4#†*	28.0±1.0#†*	38	?
Helwig, B. *et. al.* (2003)[45]	82.0±4.4*	71.3±13.8*	13	<0.05
Jorge, L. *et. al.* (2011)[59]	77.0±8.5	49.0±2.8	36	<0.05
Campos, J. C. *et. al.* (2012)[39]	58.7±7.5	47.8±6.3	19	<0.05
Batista, Jr. *et. al.* (2008)[37]	55.9±3	46.4± 2.0	17	<0.05
Batista, Jr. *et. al.* (2007)[38]	46.1±3.4*	39.9±2.1*	13	0.076
Musch, T. I. *et. al.* (1988)I [40][4]	87.7±3.6*/85.3±3.6*	77.2±5.8*/77.0±4.6*	12/10	<0.05
Musch, T. I. *et. al.* (1986)[41]	57.0±3.3	52±3.3	9	<0.05

Musch, T. I. *et. al.* (1990)[53]	98.0±2.6	81±5.7	17	<0.05
Werneck-de-Castro, J.P.S. *et. al.* (2006)[48]	42.0±5.1*	23.1±4.8*	45	<0.05
Johnsen, A.B. et. al. (2013)[78]	43.4±3.1#*	40.1±3.7#*	7.6	<0.05
Gomes-Santos, I.L. et al. (2014)[46]	65.9±7.1	56.0±2.6	15	>0.05
Moreira J.B.N *et. al.* (2013)[51]	54.8±9.9*	52.1±4.1*	5	>0.05
Musch, T. I. (1988)II [54]	95.0±4.1	81.0±8.0	15	<0.05
Kemi, O.L. *et. al.* (2011)[61]	44.8±10.9#*	28.1±5.5#*	37	<0.05
Wisloff (2002)[79]	75.4±2.9*	56.4±5.5*	25	<0.05
Musch (1989)[42]	74.0±3.4*	68.0±3.8*	8	<0.05
Run time to fatigue (min)				
da Silva, J. S. *et. al.* (2014)[43]	17.9±7.3	3.3±2.3	82	<0.05
Pfeifer, P. C. *(2001)*[2][22]	20±2.6	- 25±7.9/11±4.90	25/45	>0.05/<0.05
Musch, T. I. *et. al.* (2002)[3][19]	55.4±9.1*	52.4±9.2*/33.5±13.9*	5/39	>0.05/<0.05

Miyauchi *et. al.* (2004)[80]	42.8±15.2	26.4±14.6	38	<0.05
Helwig *et. al.* (2003)[45]	36.9±2.6*	26.7±11.1*	28	<0.05
Batista, Jr. *et. al.* (2007)[38]	33.8±3.1*	25.0±2.0*	25	<0.05
Aaker, A. *et. al.* (1996)[2][44]	42.5±5.3*	40.9±4.0*/34.3±12.0*	4/19	>0.05/<0.05
Werneck-de-Castro, J.P.S *et. al.* (2006)[48]	24.3±2.2*	14.0±2.2*	42	>0.05
Koh, S.G. *et. al.* (2003)[62]	25.2±2.6*	19.8±6.9	21	<0.05
Louzada, R.A.N. *et. al.* (2010)[63]	25.1±2.8*	16.0±2.8*	36	<0.05
Yamaguchi, F. *et. al.* (1999)[81]	25.1±4.2	16.3±3.2	35	<0.05
Distance run (meters)				
Jannig *et. al.* (2014)	367.0±54.9*	308.6±52.3*	16	<0.05
Campos, J. C. *et. al.* (2012)[39]	398.6±51.4*	261.4±68.0*	34	<0.05
Zapata-Sudo, G. *et. al.* (2014)[55]	1339.0±677.5	177.6±38.7	87	<0.05
Gomes-Santos, I.L. et al.	339.6±53.7*	267.4±73.5*	21	>0.05

(2014)[46]				
Moreira J.B.N *et. al.* (2013)[51]	344.6±56.2*	320.3±56.2*	7	>0.05
Trueblood, N. A. *et. al.* (2005)[52]	797.7±315.0*	286.3±302.9*	64	<0.05

Values expressed as mean±SD. MI: myocardial infarction group; control: control group; %: percentage change to control; VO_2: maximal oxygen uptake or maximal oxygen consumption; *p*: control group x MI group; [1] some studies describe as VO_{2peak}; [2] myocardial infarction group was subdivided in small and large myocardial infarction (small myocardial infarction/large myocardial infarction). [3] myocardial infarction group was subdivided in moderate and severe left ventricular dysfunction (moderate left ventricular dysfunction/severe left ventricular dysfunction). [4] groups were assessment by metabolic box and metabolic mask (box/mask). * data only presented graphically and then estimated by extracted from published graphs using graph digitizing software (GetData Graph Digitizer 2.24); # data expressed as $ml \cdot kg^{-0.75} \cdot min^{-1}$. † data expressed as mean±SEM (sample size was not reported).

TABLE 3 - Basic suggestions and care before, during and posterior (management of data) the functional capacity assessment in the rat model of heart failure following coronary artery ligation.

Before test
- investigator blinding (blinded to rat identities or to surgery group: sham or MI);
- precalibration of the gas analyzer (for VO_2/VCO_2 measurements) every day of the test;
- animal adaptation with a well-defined protocol and identification of no proficient animals for functional capacity test.
During test
- room temperature control and perform the functional capacity test in the same day period (e.g initiated the test between 9 and 10 AM);
- acclimatization of animal before test (15-30 min) and stabilization and recording of basal values (for VO_2/VCO_2 measurements);
- well defined protocol characteristics as constant/incremental workload test and speed/grade increments;
- well defined exhaustion criteria;
- identification of the reproducibility of the test.
Posterior test
- pattern of acquisition and data extraction (e.g. peak oxygen uptake was defined as the highest VO2 achieved before exhaustion);

Figure 1.

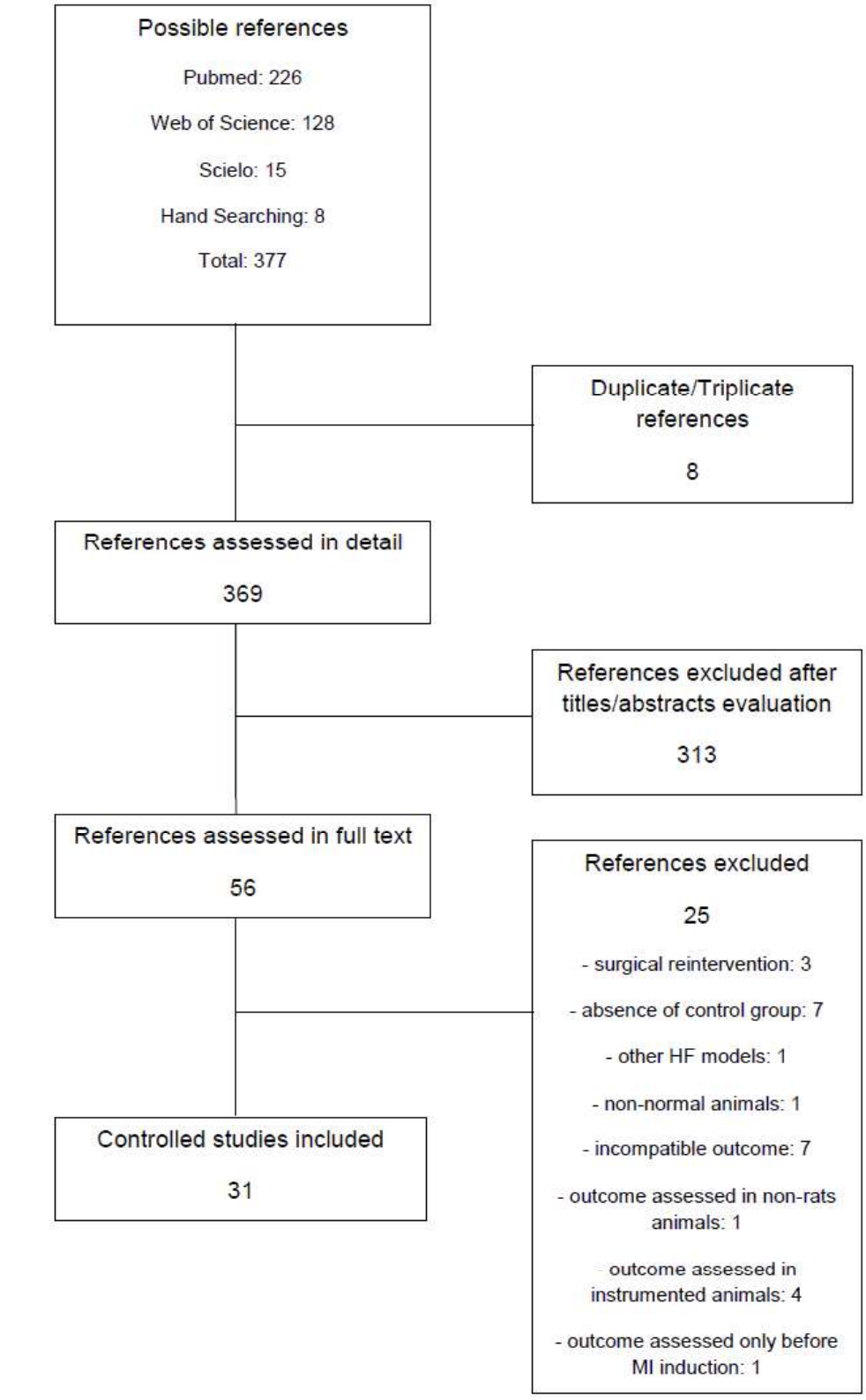

Possible references
Pubmed: 226
Web of Science: 128
Scielo: 15
Hand Searching: 8
Total: 377
Duplicate/Triplicate references
8
References assessed in detail
369
References excluded after titles/abstracts evaluation
313
References assessed in full text
56
References excluded
25
- surgical reintervention: 3
- absence of control group: 7
- other HF models: 1
- non-normal animals: 1
- incompatible outcome: 7
- outcome assessed in non-rats animals: 1
- outcome assessed in instrumented animals: 4
- outcome assessed only before MI induction: 1
Controlled studies included
31

Figure 2.

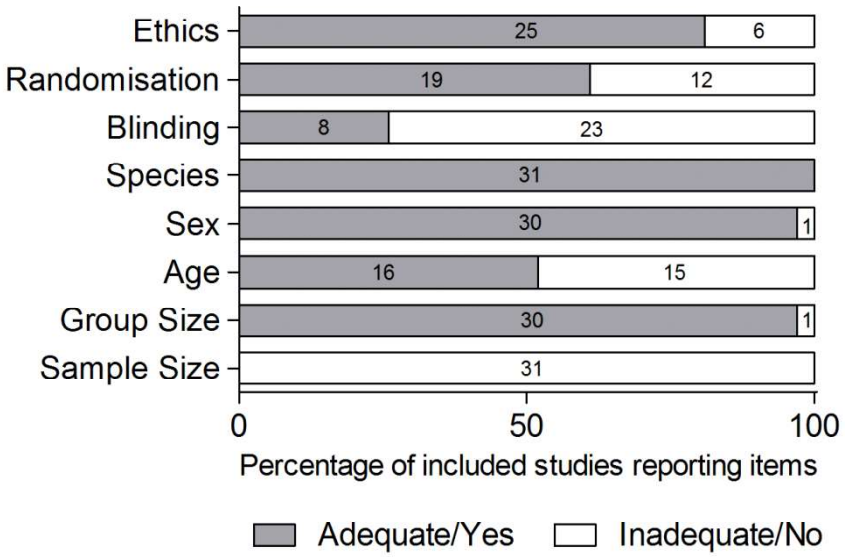

Ethics
Randomisation
Blinding
Species
Sex
Age
Group Size
Sample Size
25
6
19
12
8
23
31
30
1
16
15
30
1
31
0
50
100
Percentage of included studies reporting items
Adequate/Yes
Inadequate/No

Figure 3.

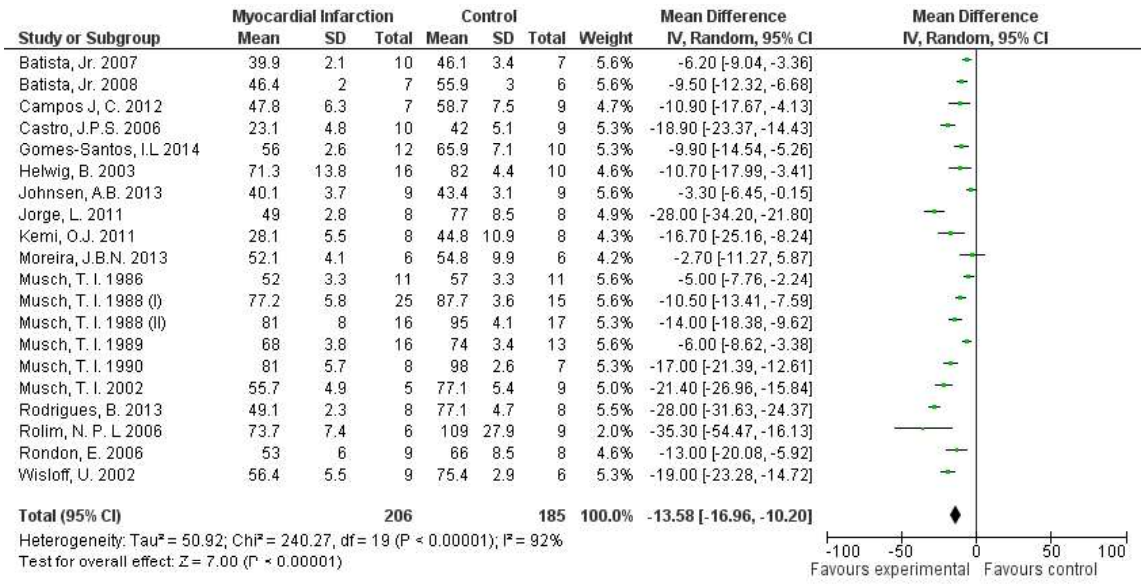

Myocardial Infarction
Control
Mean Difference
Mean Difference
Study or Subgroup Mean SD Total Mean SD Total Weight IV, Random, 95% CI
IV, Random, 95% CI
Batista, Jr. 2007 39.9 2.1 10 46.1 3.4 7 5.6% -6.20 [-9.04, -3.36]
Batista, Jr. 2008 46.4 2 7 55.9 3 6 5.6% -9.50 [-12.32, -6.68]
Campos J, C. 2012 47.8 6.3 7 58.7 7.5 9 4.7% -10.90 [-17.67, -4.13]
Castro, J.P.S. 2006 23.1 4.8 10 42 5.1 9 5.3% -18.90 [-23.37, -14.43]
Gomes-Santos, I.L 2014 56 2.6 12 65.9 7.1 10 5.3% -9.90 [-14.54, -5.26]
Helwig, B. 2003 71.3 13.8 16 82 4.4 10 4.6% -10.70 [-17.99, -3.41]
Johnsen, A.B. 2013 40.1 3.7 9 43.4 3.1 9 5.6% -3.30 [-6.45, -0.15]
Jorge, L. 2011 49 2.8 8 77 8.5 8 4.9% -28.00 [-34.20, -21.80]
Kemi, O.J. 2011 28.1 5.5 8 44.8 10.9 8 4.3% -16.70 [-25.16, -8.24]
Moreira, J.B.N. 2013 52.1 4.1 6 54.8 9.9 6 4.2% -2.70 [-11.27, 5.87]
Musch, T. I. 1986 52 3.3 11 57 3.3 11 5.6% -5.00 [-7.76, -2.24]
Musch, T. I. 1988 (I) 77.2 5.8 25 87.7 3.6 15 5.6% -10.50 [-13.41, -7.59]
Musch, T. I. 1988 (II) 81 8 16 95 4.1 17 5.3% -14.00 [-18.38, -9.62]
Musch, T. I. 1989 68 3.8 16 74 3.4 13 5.6% -6.00 [-8.62, -3.38]
Musch, T. I. 1990 81 5.7 8 98 2.6 7 5.3% -17.00 [-21.39, -12.61]
Musch, T. I. 2002 55.7 4.9 5 77.1 5.4 9 5.0% -21.40 [-26.96, -15.84]
Rodrigues, B. 2013 49.1 2.3 8 77.1 4.7 8 5.5% -28.00 [-31.63, -24.37]
Rolim, N. P. L 2006 73.7 7.4 6 109 27.9 9 2.0% -35.30 [-54.47, -16.13]
Rondon, E. 2006 53 6 9 66 8.5 8 4.6% -13.00 [-20.08, -5.92]
Wisloff, U. 2002 56.4 5.5 9 75.4 2.9 6 5.3% -19.00 [-23.28, -14.72]
Total (95% CI) 206 185 100.0% -13.58 [-16.96, -10.20]
Heterogeneity: Tau² = 50.92; Chi² = 240.27, df = 19 (P < 0.00001); I² = 92%
Test for overall effect: Z = 7.00 (P < 0.00001)
-100 -50 0 50 100
Favours experimental Favours control

Figure 4.

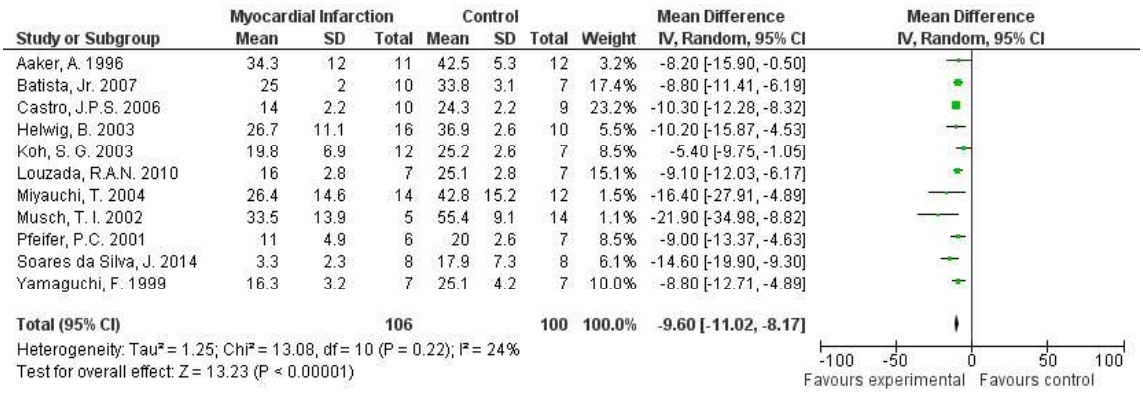
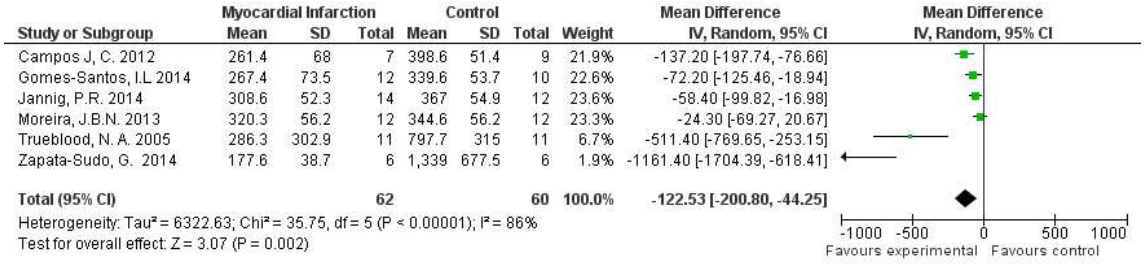

Myocardial Infarction
Control
Mean Difference
Mean Difference
Study or Subgroup Mean SD Total Mean SD Total Weight IV, Random, 95% CI
IV, Random, 95% CI
Aaker, A. 1996 34.3 12 11 42.5 5.3 12 3.2% -8.20 [-15.90, -0.50]
Batista, Jr. 2007 25 2 10 33.8 3.1 7 17.4% -8.80 [-11.41, -6.19]
Castro, J.P.S. 2006 14 2.2 10 24.3 2.2 9 23.2% -10.30 [-12.28, -8.32]
Helwig, B. 2003 26.7 11.1 16 36.9 2.6 10 5.5% -10.20 [-15.87, -4.53]
Koh, S. G. 2003 19.8 6.9 12 25.2 2.6 7 8.5% -5.40 [-9.75, -1.05]
Louzada, R.A.N. 2010 16 2.8 7 25.1 2.8 7 15.1% -9.10 [-12.03, -6.17]
Miyauchi, T. 2004 26.4 14.6 14 42.8 15.2 12 1.5% -16.40 [-27.91, -4.89]
Musch, T. I. 2002 33.5 13.9 5 55.4 9.1 14 1.1% -21.90 [-34.98, -8.82]
Pfeifer, P.C. 2001 11 4.9 6 20 2.6 7 8.5% -9.00 [-13.37, -4.63]
Soares da Silva, J. 2014 3.3 2.3 8 17.9 7.3 8 6.1% -14.60 [-19.90, -9.30]
Yamaguchi, F. 1999 16.3 3.2 7 25.1 4.2 7 10.0% -8.80 [-12.71, -4.89]
Total (95% CI) 106 100 100.0% -9.60 [-11.02, -8.17]
Heterogeneity: Tau² = 1.25; Chi² = 13.08, df = 10 (P = 0.22); I² = 24%
Test for overall effect: Z = 13.23 (P < 0.00001)
-100 -50 0 50 100
Favours experimental Favours control

Figure 5.


Myocardial Infarction
Control
Mean Difference
Mean Difference
Study or Subgroup Mean SD Total Mean SD Total Weight IV, Random, 95% CI
IV, Random, 95% CI
Campos J, C. 2012 261.4 68 7 398.6 51.4 9 21.9% -137.20 [-197.74, -76.66]
Gomes-Santos, I.L 2014 267.4 73.5 12 339.6 53.7 10 22.6% -72.20 [-125.46, -18.94]
Jannig, P.R. 2014 308.6 52.3 14 367 54.9 12 23.6% -58.40 [-99.82, -16.98]
Moreira, J.B.N. 2013 320.3 56.2 12 344.6 56.2 12 23.3% -24.30 [-69.27, 20.67]
Trueblood, N. A. 2005 286.3 302.9 11 797.7 315 11 6.7% -511.40 [-769.65, -253.15]
Zapata-Sudo, G. 2014 177.6 38.7 6 1,339 677.5 6 1.9% -1161.40 [-1704.39, -618.41]
Total (95% CI) 62 60 100.0% -122.53 [-200.80, -44.25]
Heterogeneity: Tau² = 6322.63; Chi² = 35.75, df = 5 (P < 0.00001); I² = 86%
Test for overall effect: Z = 3.07 (P = 0.002)
-1000 -500 0 500 1000
Favours experimental Favours control

5 ARTIGO 2

Functional capacity in a rat model of heart failure: impact of myocardial infarction size.

(Artigo formatação *American Journal of Physiology: Heart and Circulatory Physiology*; Fator de impacto: 3.838)

Functional capacity in a rat model of heart failure: impact of myocardial infarction size.

Running head: Myocardial infarction size and functional capacity in rats.

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ABSTRACT

Oxygen uptake (VO_2) and exercise tolerance in rats classified by myocardial infarction (MI) size are underexplored. The aim of this study was to evaluate VO_2/carbon dioxide production (VCO_2) and exercise tolerance in rats that have undergone MI. Fourteen weeks after MI or sham surgery, rats underwent an integrated approach to left ventricular function and VO_2/VCO_2, exercise tolerance and skeletal muscle mass evaluation. Based on MI size determination, rats were assigned to sham (Sham, n = 12), small myocardial infarction (SMI, n = 8), and large myocardial infarction (LMI, n = 5) groups. LMI rats showed lower systolic (ejection fraction and fractional shortening) and diastolic (E/A ratio) left ventricular function compared with SMI. VO_{2max} (~24%, $P < 0.05$), $VO_{2reserve}$ (~30%, $P < 0.05$), time to exhaustion (~36%, $P < 0.05$) and maximal velocity (~30%, $P < 0.05$) was lower in LMI compared with sham rats, with no difference between SMI rats and controls. VCO_{2max} and respiratory exchange ratio (RER) showed no significant difference between MI rats and sham rats. LMI rats demonstrated lower gastrocnemius mass (~12%, $P < 0.05$) and soleus mass (~19%, $P = 0.07$) compared with sham rats. Significant correlations between MI size, left ventricular end-diastolic pressure, right ventricle hypertrophy, pulmonary congestion, ejection fraction, and fractional shortening with VO_{2max} and run distance were observed. O_2 uptake and exercise intolerance are MI size dependent. The classification of MI rats based on MI size can distinguish rats with functional capacity impairment, and then differentiate MI rats with or without HF.

Keywords: Maximal oxygen uptake. Carbon dioxide production. Echocardiography. Hemodynamic evaluation. Skeletal muscle.

INTRODUCTION

The most frequent symptoms of heart failure (HF) are dyspnea and muscle fatigue, which may limit exercise tolerance (23, 38, 39, 52). Exercise intolerance is the cardinal clinical manifestation of HF and an accurate estimation of functional capacity is imperative (46). Cardiopulmonary exercise testing with metabolic monitoring is established as a foundation of the objective assessment of functional ability in HF and the key measurement is peak oxygen uptake (VO_{2peak}) (12), which has been used as good short-term predictor of mortality in HF patients (32).

Animal models of HF have been used to evaluate novel therapies for the treatment of HF (15, 36). Myocardial infarction (MI) following coronary artery ligation in rats is an extensively characterized animal model used in therapeutic studies of HF (4, 14, 16, 34), with advantages and disadvantages including high variability in the resulting size of the MI, cardiac remodeling, and left ventricular dysfunction (3, 13, 22, 53).

Our research group and others have observed that not all rats undergoing MI develop features of HF, and some only present moderate HF signals and symptoms. Therefore, post-infarction rats can be classified as nonfailing MI or heart failure-MI (40), MI/HF- or MI/HF+ (22), moderate left ventricular dysfunction or severe ventricular dysfunction HF (31), and small, moderate or large infarcts (11). This classification is based on different variables(10, 22, 31, 37, 40) and especially on infarcted size (1, 11). However, the behaviors of oxygen uptake (e.g. VO_{2max} and $VO_{2reserve}$) and exercise tolerance (e.g. distance to run and time to exhaustion) in rats classified by infarcted size are underexplored (1). Still, it is unknown whether this classification based on MI

size can distinguish MI rats with or without functional capacity impairment and also MI/HF- from MI/HF+ rats.

In HF treatment studies, it is essential to define whether infarcted rats present HF or not to accurately assess the effects of treatment (22). In this context, it is important to determine the repercussions of functional capacity in this animal model essentially for two aims: to characterize of the HF syndrome in general, and to test novel therapies that could potentially modify variables of functional capacity in MI rats with prior oxygen uptake and exercise tolerance impairment in particular.

In humans, carbon dioxide production (VCO_2) and respiratory exchange ratio (RER; VCO_{2max}/VO_{2max}) are well established during a cardiopulmonary test. In HF rats, studies that evaluated VCO_2 and RER status during a maximal exercise test post MI induction are limited (26, 27). Thus, despite its importance, little is known about the behavior pattern of VCO_2 and RER in the rat model of HF after MI.

To the best of our knowledge, no studies have evaluated VO_2/VCO_2 and exercise tolerance in rats that have undergone MI classified by MI size. Therefore, the aim of this study was to evaluate VO_2/VCO_2 and exercise tolerance in rats undergoing MI following descending coronary artery ligation and classified by MI size. In this context, we proposed an integrated approach of infarcted size, left ventricular function (invasive and non-invasive), VO_2/VCO_2 and exercise tolerance evaluation in sham, small myocardial infarction size (MI <40%) and large myocardial infarction size (MI >40%) rats. We formulated the hypothesis that O_2/CO_2 gas exchange and exercise tolerance variables are MI

size dependent and the classification of MI rats based on MI size can distinguish rats with impairment of functional capacity.

MATERIALS AND METHODS

Ethical Approval

All experimental procedures were conducted in accordance with the *Guide for the Care and Use of Experimental Animals* published by the National Institutes of Health (NIH publication no. 85–23, revised in 1996) and were approved by the UFCSPA Animal Ethic Committee (protocol 059/11).

Animals

This animal experimental controlled study was performed on 36 male Wistar rats weighting between 220 and 300 g (~70-90 days of age), obtained from the Animal Breeding Unit of the Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA). Rats were housed two or three per cage, and received food and water *ad libitum* in an animal room under a 12:12 h light–dark cycle, at 22 °C.

Experimental design

Initially, the 36 rats were assigned to one of two experimental groups: sham surgery rats (n = 12) and myocardial infarction surgery rats (n = 24). After

initial allocation in experimental groups, the animals were subjected to experimental procedures.

Animal model of heart failure

The rat model of HF was induced by MI following coronary artery ligation as previously described in other studies by our laboratory (2, 6, 14, 16, 34). Briefly, animals were anesthetized with xylazine (12 mg/kg, ip) and ketamine (90 mg/kg, ip), intubated, and artificially ventilated (SamWay VR 15) with a breathing rate of 60 breaths/min and an oxygen inspired fraction of 100%. Then, the heart was briefly exposed through left thoracotomy between the fourth and fifth ribs. In animals in which MI was induced, a mononylon suture 6–0 was passed around the main left descending coronary artery. As previously described, to obtain a range of infarction sizes, the location of the coronary ligation varied between 1 and 4 mm distal to its origin between the pulmonary trunk and left atrial appendage. Without exception, distal ligations resulted in small infarct sizes and proximal ligations resulted in large infarctions (48). Sham-operated animals underwent the same procedures, without ligation of the coronary artery, and served as control rats. The thorax was closed, the skin was sutured with mononylon suture 3–0, and the pneumothorax was drained using a continuous aspiration system. To reduce algic and inflammatory effects after surgery, rats received Turbogesic® (butorfanol) (0.5 mg/kg; 12/12; i.p.) during the first 24 h. To prevent infection, rats received a single dose of penicillin (20 000 U; ip). Rats were observed (vocalization, piloerection, abnormal posture,

act of licking and increased aggression) after drug administration to determine the necessity of analgesic supplementation.

After MI, rats were allowed a minimum of 14 weeks to recover. Sham-operated rats were allowed the same recovery period.

Maximal oxygen uptake and exercise tolerance tests

Description of the apparatus: The protocol for maximal oxygen uptake and exercise tolerance was conducted in a small animal treadmill equipped with a metabolic chamber with an expired gas analyzer (AVS Projects, São Carlos, SP, Brazil). The metabolic chamber was connected to an air pump that generated an air flow ~2500 ml/min and permitted gas aspiration. Maximal oxygen uptake (VO_{2max}) and maximal carbon dioxide production (VCO_{2max}) were continuously monitoring during maximal exercise tests by an O_2 and CO_2 analyzer (AVS Projects, São Carlos, SP, Brazil). The VO_2 ($ml\,kg^{-1}min^{-1}$) was calculated by the air flow measured through the metabolic chamber ~2500 ml/min, the ambient oxygen fraction (A) and the body mass of the rat (M [kg]), using the formula: $VO_2 = [2500 \times (A-E)]/M$, as previously described (9). The gas analyzer and treadmill data were computed by software (AQCAD version 2.3.9.0, AVS Projects, São Carlos, SP, Brazil), which allowed the collection of the following data: oxygen uptake (VO_2, $ml\,min^{-1}kg^{-1}$), carbon dioxide production (VCO_2, $ml\,min^{-1}kg^{-1}$), respiratory exchange ratio (RER; VCO_2/VO_2) , distance to run (m), time to exhaustion (s) and maximal velocity (m/min).

Maximal oxygen uptake and exercise tolerance test adaptation protocol: Thirteen weeks after MI or sham surgery, rats were adapted to the apparatus for one week (3 min/d, 10 m/min, 3x/wk) (20, 43, 50).

Maximal oxygen uptake and exercise tolerance test protocol: Fourteen weeks after MI or sham surgery, the rats underwent a maximal oxygen uptake and an exercise tolerance test was carried out based on an incremental speed protocol of exercise to exhaustion, as previously described (5). First, each animal was acclimatized for 15 min in a stopped treadmill, in which baseline gas exchange levels were measured (VO_{2basal}). Thereafter, initial speed was stated in 10 m/min and an increment of 5 m/min was applied in 3-min intervals, until the animals reached exhaustion. Animal exhaustion was established at the time at which the animal was unable to run for at least 15 s, even when receiving an electrical shock (1.5 μA). On each test day, the gas analyzer was calibrated with a known gas mixture.

Data extracted: From the extracted data, the highest value measured at the last step was taken as a measure of $VO_{2max,}$ $VCO_{2max,}$ and RER. The VO_{2basal} was extracted as the mean of 30 points (sampling rate 1 Hz; 30 s) up to the initial speed of the treadmill. $VO_{2reserve}$ was calculated as: $VO_{2reserve} = VO_{2max} - VO_{2basal.}$ The distance to run, time to exhaustion, and maximal velocity were defined as the maximal distance, time, and velocity at the end of the protocol.

Echocardiography

Twenty-four hours after the maximal oxygen uptake and exercise tolerance tests, the animals underwent non-invasive cardiac function evaluation

using a commercially available echocardiograph (GE Vivid I; GE Medical Systems, Israel) equipped with an 8-13 MHz electronic transducer. The echocardiography was performed by a trained operator with experience in small animal echocardiography. All echocardiographic evaluations were performed by the same examiner. Echocardiographic examination followed the recommendations of the American College of Echocardiography (18) and was guided by an adapted protocol previously reported for rats with MI (9, 22, 24, 44, 47) and for animal guidelines (8, 41). Rats were anesthetized with xylazine (12 mg/kg, ip) and ketamine (90 mg/kg, ip) and the animals were positioned in a lateral decubitus position (45° angle). An ultrasound transmission gel was applied to a previously shaved chest and M-mode tracings were derived from a 2D-mode obtained from parasternal short-axis views of the left ventricle (LV) at three levels: basal (at the tip of the mitral valve leaflets), middle (at the papillary muscle level) and apical (distal from the papillary muscle but before the final curve cavity). For cardiac structural parameters, the following variables were measured: interventricular septum in diastole (IVSd, mm), interventricular septum in systole (IVSs, mm), left ventricular end-diastolic diameter (LVEdD, mm), left ventricular end-systolic diameter (LVEsD, mm), left ventricular posterior wall in diastole (LVPWd, mm), and left ventricular posterior wall in systole (LVPWs, mm). The measurements obtained were the means of at least three cardiac cycles on each of the three levels and the final value for each rat was the mean of all three described planes. Then, the following secondary variables of left ventricular systolic function were obtained: end-diastolic volume (LVEdV (ml) = $1.047(LVEdD)^3$ and end-systolic volume (LVEdV (ml) = $1.047(LVEsD)^3$) by cubic or ellipsoid model [38, 39], left ventricular ejection

fraction (EF (%) = [(LVEdV- LVEsV) – LVEdV] x 100), left ventricular fractional shortening (FS (%) = [(LVEsD –LVEsD)/ LVEdD] x 100), and relative wall thickness (RWT = (IVSd + LVPWd)/LVEdD).

Left ventricular diastolic function was guided by an adapted protocol previously reported for MI rats (33). Briefly, mitral diastolic inflow measurement by pulsed Doppler was obtained from the four-chamber view. The sample volume was positioned at the tip of the mitral valve to obtain the mitral diastolic flow velocity, which was used to measure the peak E and A wave velocities (cm/s) and the ratio between them (E/A ratio). The heart rate was calculated using an average of three consecutive cycles intervals.

Hemodynamic evaluation

Forty-eight hours after echocardiography, the animals underwent hemodynamic evaluation as previously described by our research group (2, 6, 14, 16, 34). Briefly, animals were anesthetized with xylazine (12 mg/kg, ip) and ketamine (90 mg/kg, ip), and a small incision was made in the anterior cervical region in order to insert a polyethylene catheter (PE-50) connecting to a pressure transducer (strain gauge; Narco Byosystem Miniature Pulse Transducer RP-155, Houston, TX, USA), coupled to a pressure amplifier (Stemtech), into the right carotid artery. After that, the catheter was positioned inside the left ventricle, and the pulse wave was monitored by graphical registration of ventricular pressure for 5 min. Analogical pressure signals were digitized by a data acquisition system (CODAS-Data Acquisition System) with a sampling rate of 2000 Hz. These recordings were used for left ventricular

systolic pressure (LVSP), left ventricular maximum change in pressure over time (dP/dt_{max}), left ventricular minimum change in pressure over time (dP/dt_{min}) and left ventricular end-diastolic pressure (LVEDP). The last parameter was determined manually by detecting the point of inflection to the end of diastole via analysis of the ventricular pressure wave.

Skeletal muscle sample collection

Immediately after hemodynamic evaluation, rats were euthanized by decapitation and the gastrocnemius, soleus and plantaris (right side) were collected and weighed. The skeletal muscle index (muscle mass/body mass) was calculated for each muscle and rat.

Heart hypertrophy, pulmonary and hepatic congestion

After skeletal muscle sample collection, the lungs, liver and heart were removed and weighed. The lungs and liver of each animal were dehydrated (80 °C) for 48 h and then reweighed to determine their water content. Lung and liver wet-to-dry mass ratios were used to determine the percentage of water in those tissues, as an indication of congestion. The right ventricle (RV) and left ventricle (LV) were dissected, separated and weighed. The heart-to-body mass (H/BM), LV-to-body mass (LV/BM) and RV-to-body mass (RV/BM) were determined and used as an indication of heart hypertrophy (14, 45).

Infarcted size determination

Immediately after heart hypertrophy determination, left ventricles were filled with a latex cushion and placed in 10% buffered formaldehyde for 24 h for subsequent analysis of infarction size. Infarcted size was determined by histological study adapted from the method previously described in Martinez, P.F *et. al.* (22). Measurements were taken from midventricular slices (5–6 mm from the apex), assuming that slices from this point showed a close linear relation with the sum of the measurements from all heart slices (35). For this, 10-μm thick sections of the midventricular slice were cut and stained with Masson's trichrome stain. ImageJ 1.47 software (freeware available at http://rsbweb.nih.gov/ij/download.html) was used to obtain the length of the entire endocardial circumference and the segment of the endocardial circumference made up by the infarcted portion from two midventricular slices of the left ventricle by manually planimetry. The infartected fraction of the left ventricle was calculated from these measurements. A researcher blinded to the hemodynamic data made the infarct size measurements.

Statistical analysis

Data are expressed as mean ± SD for each variable and group. The Shapiko-Wilk test was performed to evaluate normality for all variables. One-way ANOVA followed by Tukey's *post hoc* test was used to compare variables (body mass, infarct size, heart hypertrophy, pulmonary and hepatic congestion, hemodynamic and echocardiography variables, VO_{2max}, VCO_{2max}, RER,

VO_{2basal}, $VO_{2reserve}$, distance to run, time to exhaustion, maximal velocity, and skeletal muscle mass) among groups. Pearson's correlation test was used to examine the relationship between infarcted size, right ventricular hypertrophy, pulmonary congestion, and hemodynamic and echocardiography variables with VO_{2max} and distance to run in sham and HF rats. A *P* value <0.05 was considered statistically significant. GraphPad Prism 5 (Graph-Pad Software, San Diego, CA, USA) for Windows was used as a computational tool in data analysis and to create charts.

RESULTS

Mortality and experimental groups (MI size classification)

The mortality in myocardial infarction surgery groups was ~46% (11/24). No deaths were observed after sham surgery (0/12) or during other experimental procedures.

Based on infarcted size determination, the remaining 25 rats were assigned to one of three experimental groups: sham surgery rats, small myocardial infarction rats (SMI) and large myocardial infarction rats (LMI). Small myocardial infarction was defined as MI size between 10% and 40% of the left ventricle (10% < MI < 40%) and large myocardial infarction, as MI size greater than 40% of the left ventricle (MI > 40%) (1). The final sample sizes of the experimental groups were as follows: Sham (n = 12), SMI (n = 8) and LMI (n = 5).

Body mass, infarct size, heart hypertrophy, pulmonary and hepatic congestion

Body mass, infarct size, heart hypertrophy, and pulmonary and hepatic congestion data are shown in Table 1. No significant differences were observed in initial and final body mass among groups. MI size was larger in LMI rats than in SMI rats. LMI and SMI rats demonstrated higher H/BM compared with controls. SMI rats demonstrated higher LV/BM compared with sham rats. LMI rats demonstrated higher RV/BM and pulmonary congestion compared with sham rats. No significant differences were observed in hepatic congestion among groups.

Echocardiography

Table 2 presents the values for the evaluation of non-invasive left ventricular function. MI rats demonstrated higher left ventricular internal diameters (LVEdD, LVEsD e LVEdD/BW) and lower FS, EF, and RWT compared with sham rats. LMI rats showed lower IVSs and diastolic function impairment (E/A ratio) compared with sham rats. SMI showed lower LVPWs compared with sham rats. Interestingly, LMI rats showed lower systolic (FS and EF) and diastolic function (E/A ratio) compared with SMI, suggesting a decreased in systolic and diastolic function dependent on MI size.

Hemodynamic variables

Table 2 presents the values of hemodynamic variables. MI rats demonstrated left ventricular systolic and diastolic dysfunction. This fact is supported by lower LVSP and dP/dt_{min} and higher LVEDP in SMI and LMI compared with sham rats. Only LMI demonstrated a dP/dt_{max} reduction relative to sham rats. No significant differences were observed between SMI and LMI.

Oxygen and Carbon Dioxide Uptake

Figure 1 shows the values of oxygen uptake and carbon dioxide production in sham and MI rats. Only rats with LMI demonstrated oxygen uptake impairment compared with sham control rats, suggesting that MI size is predictive of gas exchange impairment. The LMI group demonstrated a reduction in VO_{2max} (Figure 1B) and $VO_{2reserve}$ (Figure 1C) relative to sham rats. Also, LMI rats demonstrated a reduction in VO_{2max} relative to SMI rats. No significant differences were observed in SMI rats in any of the VO_2 and VCO_2 variables. VO_{2basal} (Figure 1A), VCO_{2max} (Figure 1D), and RER (Figure 1E) showed no significant difference in HF rats compared with sham rats, independently of MI size.

Exercise tolerance

Values of run distance, time to exhaustion, and maximal velocity of sham and MI rats are presented in Figure 2. LMI rats showed lower run distance

(Figure 2A), time to exhaustion (Figure 2B), and maximal velocity (Figure 2C) compared with sham rats. Also, LMI rats demonstrated lower maximal velocity compared with SMI rats. SMI rats showed lower distance to run compared with sham rats.

Skeletal muscle mass

Table 3 shows the values of gastrocnemius, soleus and plantaris muscle mass, expressed as muscle index (muscle mass/body mass), in sham and MI rats. LMI rats demonstrated lower gastrocnemius mass compared with sham rats. A trend toward differences were observed in soleus mass among groups (P = 0.0730). No significant differences were observed in plantaris mass among groups. These data suggest that mainly skeletal muscle composed of slow-twitch fiber type (mixed gastrocnemius and soleus muscle) was affected in rats with MI, principally in LMI. No MI effects in fast-twitch muscles were observed.

Correlations between infarcted size, pulmonary congestion, hemodynamic, echocardiography parameters with VO_{2max} and distance to run in sham and heart failure rats

Table 4 shows the correlation between MI size, LVEDP, right ventricle hypertrophy, pulmonary congestion, EF and FS with VO_{2max} and run distance. Significant correlations were observed between all independent variables with VO_{2max} and run distance. Trend towards correlation (P = 0.0733) were observed between pulmonary congestion (%) with distance to run.

DISCUSSION

This is the first report to show that O_2 uptake and exercise tolerance is impaired only in LMI rats relative to controls. Evidence of this was provided by lower VO_{2max}, $VO_{2reserve}$, time to exhaustion and maximal velocity in LMI compared with sham rats, with no significant difference in SMI rats compared with controls. These data suggest that O_2 uptake and exercise tolerance variables are MI size dependent and that the classification of MI rats based on MI size can distinguish rats with functional capacity impairment.

As previously described in a well-designed study (31), decrements in exercise performance (reductions in running time to the point of fatigue and VO_{2max}) were found in rats with severe LV dysfunction (LVEDP >20.0 mmHg), with no decrements in rats with moderate LV dysfunction (LVEDP <20.0 mmHg). Our study demonstrated similar results with VO_{2max} and exercise tolerance impairment only in LMI. In therapeutics studies, left ventricular function (e.g. LVEDP) can be modified; additionally, these measurements are invasive and can performed only as terminal procedures (MI evaluation can be performed by echocardiography at different time points, showing a good correlation with histology (49)). For these reasons, the classification of MI based on LVEDP is somewhat limited. Musch, T. I. *et. al*. (31) classified MI rats based on LVEDP and our study showed similar results when MI rats were classified based on MI size, suggesting that the classification based on MI size can be an alternative to selecting animals with O_2 uptake and exercise tolerance impairments and to test novel therapies that can modify this status.

Our study demonstrated an inverse correlation between LVEDP and VO_{2max} ($r = -0.47$; $P < 0.05$) and distance to run ($r = -0.61$; $P < 0.01$) in all rats,

but only LMI demonstrated functional capacity impairment. Similar results have previously demonstrated that indexes of exercise performance (VO_{2max} and time to exhaustion) were significantly correlated with LVEDP, but only rats with severe LV dysfunction (LVEDP >20.0 mmHg) demonstrated functional capacity impairment (31). Additionally, we demonstrated a significant correlation between other cardiac function variables evaluated noninvasively by echocardiography (EF and FS) with VO_{2max} and distance to run. To the best of our knowledge, only one previous study was able to show a correlation between functional capacity variables (distance to run) with echocardiography variables (48). In this way, echocardiography emerges as a powerful noninvasive tool to serially assess different structural and functional cardiac parameters (19, 22, 41) and was utilized as a method to observe changes in cardiac function during therapeutic interventions (17, 24, 25, 47) in different animal models of cardiovascular diseases. We know that functional capacity assessment is long and strenuous and therefore, the identification of correlations with echocardiography (a practiced noninvasive technique) can help to extrapolate functional capacity alterations and characterize the animal model of HF.

Many variables have been proposed to distinguish MI animals with and without HF. The classification is based on the presence of pleural effusion (40), a combination of clinical and pathologic HF features, and new proposals, such as echocardiography classification (22), lung congestion and right ventricular hypertrophy (10), infracted size (% of the left ventricle) (11) and mainly by left ventricular end diastolic pressure after hemodynamic evaluation (>20.0 mmHg) (31, 37). Our study corroborates this studies and shows that some variables are significantly altered only in LMI or can distinguish SMI and LMI, as follows: right

ventricular hypertrophy, pulmonary congestion, ejection fraction, fractional shortening, E/A ratio, and gastrocnemius mass. Additionally, in this context, our study shows that VO_{2max}, $VO_{2reserve}$, time to exhaustion, and maximal velocity are altered only in LMI and may be good parameters to differentiate MI rats with and without HF.

Our study demonstrated that, despite a marked left ventricular systolic (e.g. EF, FS, LVSP and dP/dt_{min}) and diastolic (e. g. LVEDP) dysfunction in SMI and LMI rats, only LMI demonstrated an impairment in O_2 uptake and exercise tolerance. Interestingly, this impairment was associated with lower skeletal muscle mass, mainly in the skeletal muscle composed of slow-twitch fibers (mixed gastrocnemius and soleus muscle) in LMI rats. Skeletal muscle composed of slow-twitch fibers are affected in HF (21). Evidence indicates that HF provokes a muscle fiber type shift from type I to type II and a reduction in muscle mass and these alterations can explain, at least in part, the reduced maximal VO_2 in heart failure (42).

VCO_2 and RER values during the maximal exercise test are poorly described in HF rats. One study showed that VCO_2 was reduced in MI rats relative to sham rats (~92 ml· kg^1·min^{-1} *versus* ~81 ml· kg^1·min^{-1}, $P < 0.05$; respectively) and no significant differences in RER during the maximal exercise test was detected between groups (~1.05 for both groups) (27). Another study reported a VCO_2 of 74 ± 3 ml· kg^1·min^{-1} and a RER of 1.10 ± 0.01 in MI rats (26). Additionally, other studies reported VCO_2 and RER values in MI rats assessed during a submaximal swimming protocol (28, 29) or in instrumented rats (30). Two classical studies showed RER values in normal rats during different protocols (7, 51). Thus, despite its importance, little is known about the

pattern of behavior of VCO_2 and RER in the rat model of HF after MI and the effects of MI size. Our results demonstrated no significant difference in VCO_2 and RER and no effects of MI size.

The present study does have limitations that warrant discussion. We used only one maximal exercise test protocol. In our opinion, different results could be obtained using an alternative protocol (e.g. inclination) (51). Additionally, analyzes of O_2/CO_2 gas exchange and exercise tolerance over time and in different post-MI times are important to determine the time course and the impact of chronicity of the rat model of MI on these parameters.

In conclusion, despite the left ventricular systolic and diastolic dysfunction seen in both SMI and LMI, only LMI demonstrated O_2 uptake and exercise tolerance impairment. These results confirm our hypothesis that O_2 uptake and exercise tolerance variables were MI size dependent. VCO_2 and RER impairment do not seem to be dependent on MI size. Additionally, our study suggests that the classification of MI rats based on MI size can distinguish rats with functional capacity impairment, and further differentiate MI rats with or without HF. These findings are of particular importance for the testing of novel therapies that have the potential to modify the variables of functional capacity in MI rats with prior oxygen uptake and exercise tolerance impairment. LMI rats (>40% of the left ventricle) may be a good model for this approach.

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DISCLOSURES

None.

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FIGURE LEGENDS

Figure 1. Oxygen uptake and dioxide carbon variables of sham and myocardial infarction (MI) rats. **A:** Baseline oxygen uptake (VO_{2basal}). **B:** Maximal oxygen uptake (VO_{2max}). **C:** Oxygen uptake reserve ($VO_{2reserve}$). **D:** Maximal dioxide carbon production (VCO_{2max}). **E:** RER (VCO_{2max}/VCO_{2max}). Sham, sham surgery group (*n*=12); SMI, small myocardial infarction size (MI<40%) (*n*=8); LMI, large myocardial infarction size (MI>40%) (*n*=5). Values are means ± SD. Statistical analysis: one-way ANOVA followed by the post hoc test of Tukey. *P* ANOVA, *P* value for ANOVA. Symbols represent the comparison among groups by the post hoc analysis: **P*<0.05 compared to Sham; †*P*<0.05 compared to SMI.

Figure 2. Exercise tolerance variables of sham and myocardial infarction (MI) rats. **A:** Distance to run (m, meters). **B:** Time to exhaustion (s, seconds). **C:** Maximal velocity (m/min, meters per minute). Sham, sham surgery group (*n*=12); SMI, small myocardial infarction size (MI<40%) (*n*=8); LMI, large myocardial infarction size (MI>40%) (*n*=5). Values are means ± SD. Statistical analysis: one-way ANOVA followed by the post hoc test of Tukey. *P* ANOVA, *P* value for ANOVA. Symbols represent the comparison among groups by the post hoc analysis: **P*<0.05 compared to Sham; †*P*<0.05 compared to SMI.

Table 1. Body weight, infarct size, heart hypertrophy, pulmonary and hepatic congestion

	Sed-Sham	SMI	LMI	*P* One-Way ANOVA
Initial Body Mass, g	260.2±18.3	265.6±21.2	260.6±29.5	0.8480
Final Body Mass, g	324.8±21.4	341.9±32.7	354.0±35.7	0.1422
Infarcted Area, %	-	29.3±8.0	47.0±5.3†	0.0012 (t-test)
H/BM, mg/g	3.0±0.3	3.6±0.6*	3.9±0.6*	0.0022
LV/BM, mg/g	2.2±0.2	2.6±0.4*	2.5±0.2	0.0240
RV/BM, mg/g	0.7±0.2	1.0±0.4	1.4±0.4*	0.0022
Pulmonary Congestion, %	72.4±2.0	75.0±4.1	78.0±2.0*	0.0041
Hepatic Congestion, %	71.4±2.0	71.4±2.0	71.6±1.2	0.9762

Values are means ± SD. Sham, sham surgery group (*n*=12); SMI, small myocardial infarction (myocardial infarction < 40% of the total left ventricle) group (*n*=8) and LMI, small myocardial infarction (myocardial infarction > 40% of the total left ventricle) group (*n*=5). H/BM, Heart-to-body mass ratio; LV/BM, Left ventricular-to-body mass ratio; RV/BM, Right ventricular-to-body mass ratio. Statistical analysis: one-way ANOVA followed by the post hoc test of Tukey (note: t-test was performance to infarcted size

analysis). Symbols represent the comparison among groups by the post hoc analysis: *P<0.05 compared to Sham group; †P<0.05 compared to SMI group.

Table 2. Echocardiography and hemodynamic and cardiac function evaluation of experimental groups.

	Sham	SMI	LMI	*P* One-Way ANOVA
Echocardiography				
HR, bpm	268.1±63.5	217.0±29.5	271.7±27.4	0.2746
IVSd, mm	1.7±0.4	1.8±0.4	1.4±0.2	0.2866
IVSs, mm	3.0±0.4	2.4±0.8	1.8±0.4*	0.0012
LVEdD, mm	8.1±0.8	11.3±1.0*	10.9±1.2*	<0.0001
LVEsD, mm	4.8±0.8	8.7±0.7*	9.1±1.1*	<0.0001
LVPWd, mm	1.5±0.3	1.3±0.3	1.7±0.4	0.1514
LVPWs, mm	2.8±0.4	2.3±0.3*	2.4±0.5	0.0201
LVEdD/BM	25.2±2.5	31.7±4.5*	30.9±3.4*	0.0014
EF, %	79.4±4.9	54.5±3.9*	40.1±8.0*†	<0.0001
FS, %	41.3±4.9	23.1±2.2*	15.8±3.7*†	<0.0001

RWT	0.4±0.1	0.3±0.0*	0.3±0.0*	0.0018
E, cm/s	0.6±0.1	0.6±0.2	0.7±0.2	0.2282
A, cm/s	0.3±0.1	0.3±0.1	0.2±0.0	0.2361
E/A	2.0±0.5	2.0±0.7	4.4±0.4*†	<0.0001
Hemodinamic evaluation				
LVEDP, mmHg	4.5±1.6	14.4±11.1*	23.4±7.6*	0.0001
LVSP, mmHg	124.3±18.2	97.5±9.4*	99.6±6.4*	0.0010
dP/dt_{max}, mmHg/s	7390.0±2383.0	5292.0±1260.0	4721.0±582.6*	0.0189
dP/dt_{min}, mmHg/s	-4740.0±955.5	-3471.0±721.9*	3158.0±356.7*	0.0013

Values are means ± SD. Hemodynamic variables were not measured in 1 rat that had small MI. Sham, sham surgery group (*n*=12); SMI, small myocardial infarction (myocardial infarction < 40% of the total left ventricle) group (*n*=7) and LMI, small myocardial infarction (myocardial infarction > 40% of the total left ventricle) group (*n*=5). For echocardiography evaluation *n*=12; 5; 4 rats, respectively. LVEDP, left ventricular end-diastolic pressure; LVSP, left ventricular systolic pressure; dP/dt_{max}, left ventricular maximum change in pressure over time; dP/dt_{min}, left ventricular minimum change in pressure over time; IVSd, interventricular septum in diastole; IVSs, interventricular septum in systole; LVEdD, left ventricular end-diastolic diameter; LVEsD, left ventricular end-systolic diameter; LVPWd, left ventricular posterior wall in diastole; LVPWs, left ventricular posterior wall in systole; EF, left ventricular ejection fraction; LVEdD/BM, left ventricular end-diastolic diameter-to-body ratio; FS, left ventricular fractional shortening; RWT, relative wall-thickness; E, maximal early diastolic peak velocity; A, late peak velocity. Statistical analysis: one-way ANOVA,

followed by the post hoc test of Tukey. Symbols represent the comparison between groups by the post hoc analysis: *P<0.05 compared to Sham; †P<0.05 compared to SMI.

Table 3. Skeletal muscle mass.

	Sham	SMI	LMI	*P* One-Way ANOVA
Gastrocnemius mass/BM, mg/g	5.84±0.30	5.35±0.54	5.16±0.19*	0.0127
Soleus mass/BM, mg/g	0.57±0.08	0.49±0.08	0.47±0.09	0.0730
Plantaris mass/BM, mg/g	1.08±0.18	0.97±0.09	1.11±0.15	0.3309

Values are means ± SD. Sham, sham surgery group (*n*=9); SMI, small myocardial infarction (myocardial infarction < 40% of the total left ventricle) group (*n*=5) and LMI, small myocardial infarction (myocardial infarction > 40% of the total left ventricle) group (*n*=4). BM, body mass. Statistical analysis: one-way ANOVA, followed by the post hoc test of Tukey. Symbols represent the comparison among groups by the post hoc analysis: *$P<0.05$ compared to Sham.

Table 4. Correlations between infarcted size, pulmonary congestion, hemodynamic, and echocardiography parameters with VO_{2max} and distance to run in sham and myocardial infarction (MI) rats.

	n	VO_{2max}, ml· kg^{-1}·min^{-1}		Distance to run, meters	
		r	*P*	*r*	*P*
Infarcted Size, %	13	-0.6366	0.0193	-0.7255	0.0050
LVEDP, mmHg	24	-0.4725	0.0197	-0.6128	0.0015
RV/BM, mg/g	25	-04377	0.0287	-0.4944	0.0120
Pulmonary Congestion, %	25	-0.5867	0.0021	-0.3644	0.0733
EF, %	21	0.5829	0.0056	0.6688	0.0009
FS, %	21	0.5904	0.0048	0.6604	0.0011

Values are means ± SD. LVEDP, left ventricular end-diastolic pressure; RV/BM, Right ventricular-to-body mass ratio; EF, left ventricular ejection fraction; FS, left ventricular fractional shortening. Statystical analysis: Pearson´s correlation test. Note: Correlation between infarcted area with VO_{2max} and distance to run was performed only in MI rat. Other correlations were performed in all rats with available data.

Figure 1.

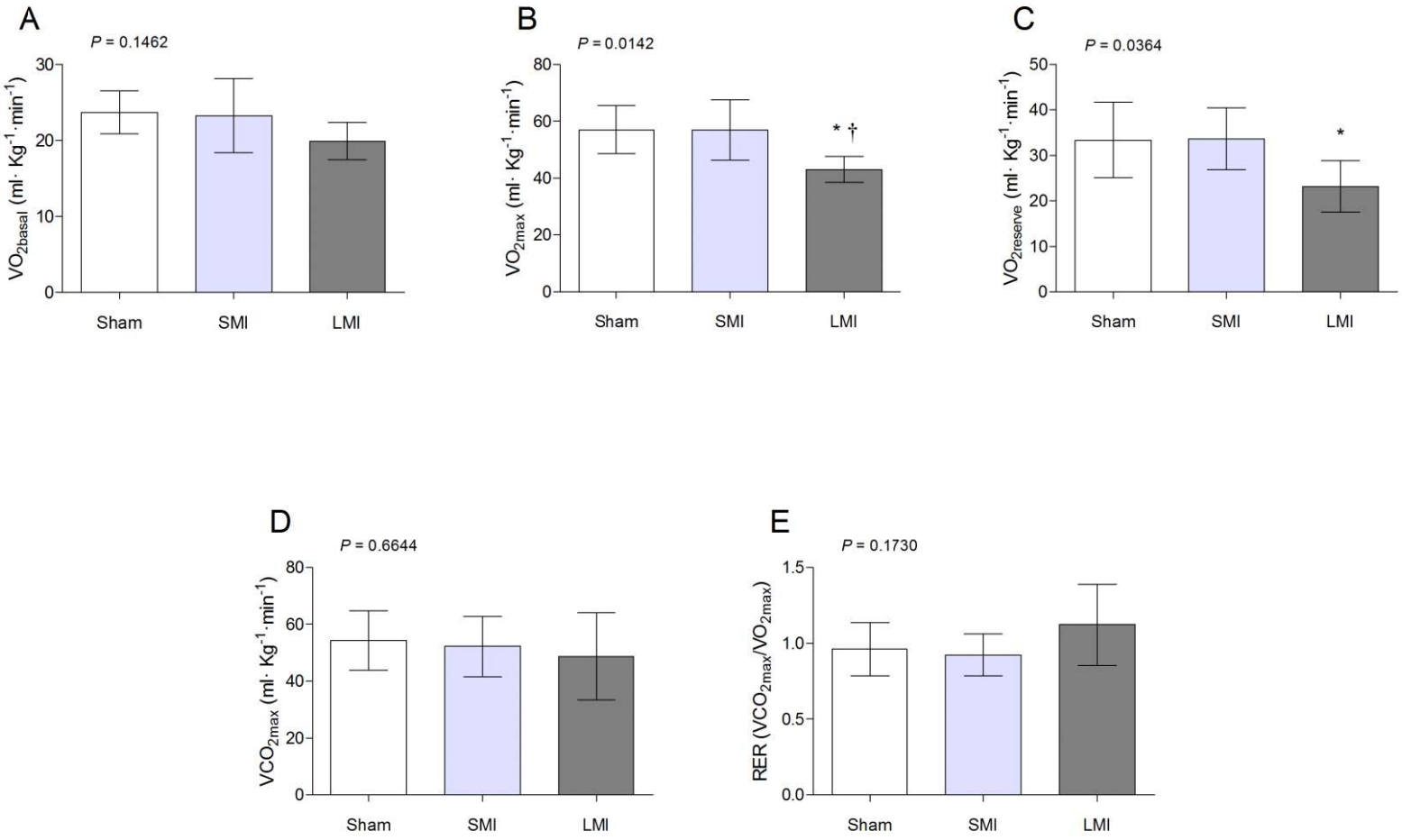
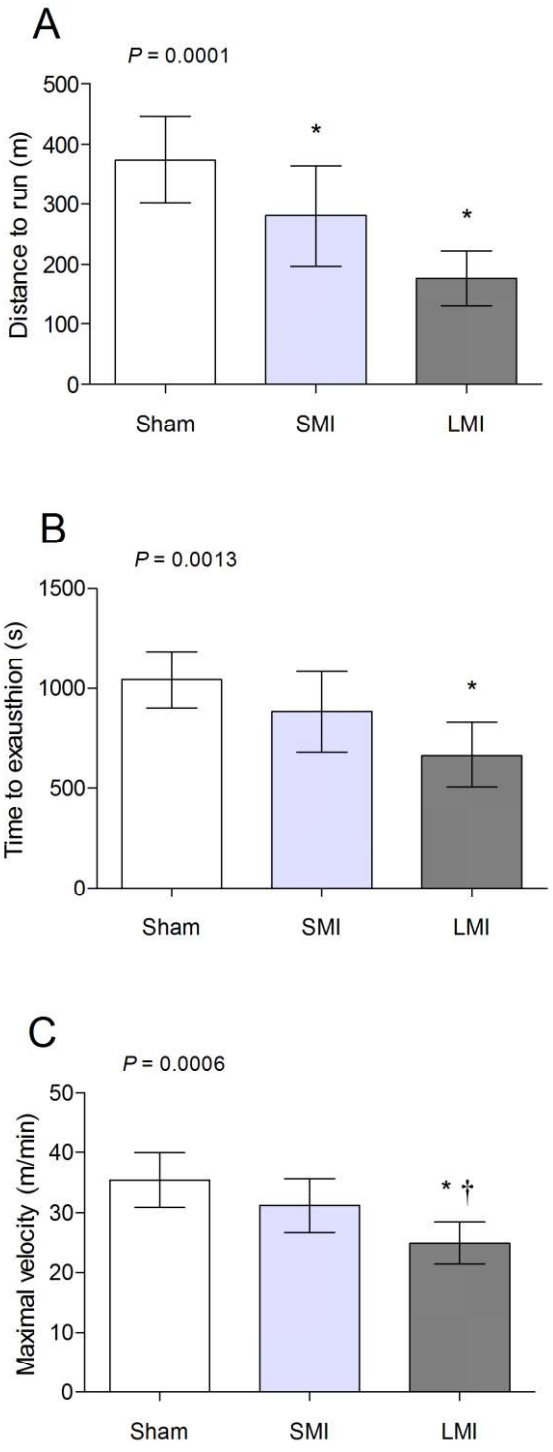

A
P = 0.1462
VO2basal (ml· Kg-1·min-1)
30
20
10
0
Sham
SMI
LMI
B
P = 0.0142
VO2max (ml· Kg-1·min-1)
80
60
40
20
0
* †
Sham
SMI
LMI
C
P = 0.0364
VO2reserve (ml· Kg-1·min-1)
50
40
30
20
10
0
*
Sham
SMI
LMI
D
P = 0.6644
VCO2max (ml· Kg-1·min-1)
80
60
40
20
0
Sham
SMI
LMI
E
P = 0.1730
RER (VCO2max/VO2max)
1.5
1.0
0.5
0.0
Sham
SMI
LMI

Figure 2.


A
P = 0.0001
Distance to run (m)
500
400
300
200
100
0
*
*
Sham
SMI
LMI
B
P = 0.0013
Time to exausthion (s)
1500
1000
500
0
*
Sham
SMI
LMI
C
P = 0.0006
Maximal velocity (m/min)
50
40
30
20
10
0
* †
Sham
SMI
LMI

6 ARTIGO 3

Maximal oxygen uptake and exercise tolerance are improved in rats with heart failure subjected to low-level laser therapy associated with resistance training.

(Artigo formatação *Lasers in Surgery and Medicine*; Fator de impacto: 2.619)

Maximal oxygen uptake and exercise tolerance are improved in rats with heart failure subjected to low-level laser therapy associated with resistance training.

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ABSTRACT

Background and Objective: Exercise tolerance and maximal oxygen uptake (VO_{2max}) are reduced in heart failure (HF). The influence of combined resistance training (RT) and low-level laser therapy (LLLT) on exercise tolerance and VO_{2max} in HF has not yet been explored. The aim of this study was to evaluate the influence of combined RT and LLLT on VO_{2max} and exercise tolerance in rats with HF induced by myocardial infarction (MI).

Study Design/Materials and Methods: Rats were allocated to sedentary sham (Sed-Sham, n = 12), sedentary heart failure (Sed-HF, n = 9), RT heart failure (RT-HF, n = 7) and RT associated with LLLT heart failure (RT + LLLT-HF, n = 7) groups. After MI or sham surgery, rats underwent a RT and LLLT protocol (applied immediately after RT) for eight weeks. VO_{2max} and exercise tolerance were evaluated at the end of protocol.

Results: HF rats subjected to LLLT combined with RT showed higher VO_{2basal} (41%), VO_{2max} (40%), $VO_{2reserve}$ (39%), run distance (46%), time to exhaustion (30%) and maximal velocity (22%) compared with HF rats that underwent RT alone.

Conclusions: LLLT associated with RT improved oxygen uptake and exercise tolerance compared with RT alone in HF rats.

Keywords: Myocardial infarction. Phototherapy. Maximal exercise test. Functional capacity.

INTRODUCTION

Despite remarkable progress in the therapeutic approach to patients with heart failure (HF), exercise intolerance remains one of the hallmarks of this syndrome (1). Maximal oxygen uptake (VO_{2max}/VO_{2peak}) measured during cardiopulmonary exercise testing is a key measurement of functional ability (2) and is used as a good short-term predictor of mortality in HF patients (3). Many organs and factors contribute to exercise intolerance in HF (4), with the focus on skeletal muscle. In this context, the symptoms that characterize HF, including fatigue, are often directly related to skeletal muscle abnormalities in HF (5).

Cardiac rehabilitation programs can be useful in clinically stable patients with HF to improve functional capacity, exercise tolerance, health-related quality of life, and mortality (6). Aerobic training (AT) has more beneficial effects on aerobic power and cardiac structure and function than resistance training (RT), while the latter is more effective for increasing muscle strength and endurance (7). RT is a muscle contraction performed against a specific opposing force, thereby generating resistance, such as lifting weights, and is recommend for cardiac rehabilitation programs (8). However, the effects of RT on VO_{2max} in HF patients are not well established (9), with some studies describing no effects (10-13).

While AT and RT will undoubtedly continue to be at the centre of a well-formulated rehabilitation program, other adjunctive interventions, which are presently underutilized in clinical practice, may prove beneficial in patients with HF (14). Emerge, in the therapeutic context, other non-pharmacological

therapies that may be an alternative to physical exercise for patients with HF (15).

Low level laser therapy (LLLT) is the application of light (usually a low-power laser or LED in the range of 1 mW – 500 mW) to a disease to promote tissue regeneration, reduce inflammation and relieve pain (16,17). Presently, a new approach to LLLT had been explored as increased performance during exercise (17,18). Interestingly, it was evidenced that LLLT increased exercise performance (VO_{2max} and time to exhaustion) in progressive-intensity running to exhaustion (19). Recently, LLLT combined with AT increased VO_{2max} by reducing proinflammatory cytokines (IL-6 and TNF-α) in aged rats (20). Our research group showed that LLLT applied to skeletal muscle improves the inflammatory profile by reducing TNF- α and IL-6 and augmenting IL-10 (21). Also, we have demonstrated that LLLT attenuates oxidative stress parameters (24) in HF rats.

To the best of our knowledge, no studies have evaluated the influence of combined RT and LLLT on VO_{2max} and exercise tolerance in HF rats. In this context, we formulated the hypothesis that combined RT and LLLT can increase VO_{2max} and exercise tolerance compared with RT alone in rats with HF. Therefore, the aim of this study was to evaluate the influence of combined RT and LLLT on VO_{2max} and exercise tolerance in rats with HF induced by myocardial infarction (MI) following descending coronary artery ligation.

MATERIALS AND METHODS

Animals and experimental groups

This animal experimental controlled study was performed on 48 male Wistar rats weighing between 220 and 290 g (~70 days of age), obtained from the Animal Breeding Unit of the Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA). The rats were housed two or three per cage, and received food and water *ad libitum* in an animal room under a 12:12 h light:dark cycle at 22 °C. All experimental procedures were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health (NIH publication no. 85–23, revised in 1996) and were approved by the UFCSPA Animal Ethic Committee (protocol 059/11).

Rats were initially allocated to one of four experimental groups: sedentary sham (Sed-Sham, n = 12), sedentary heart failure (Sed-HF, n = 12), resistance training heart failure rats (RT-HF, n = 12) and resistance training associated with low-level laser therapy heart failure rats (RT + LLLT-HF, n = 12).

Myocardial infarction induction surgery

A myocardial infarction (MI) rat model was induced following coronary artery ligation as previously described in other studies from our laboratory (21,23-25). Briefly, rats were anaesthetized with xylazine (12 mg/kg, ip) and ketamine (90 mg/kg, ip), intubated, and artificially ventilated (SamWay VR 15) with a breathing rate of 60 breaths/min and an oxygen inspired fraction of 100

%. Then, the heart was briefly exposed through left thoracotomy between the fourth and fifth ribs. In the animals in which MI was induced, a mononylon suture 6–0 was passed around the main left descending coronary artery, at a point between ~1 and ~2 mm distal to the edge of the left atrium, and the left coronary was ligated. Sham-operated animals underwent the same procedure without tying the suture and served as control rats. The thorax was closed, the skin was sutured with mononylon suture 3–0, and the pneumothorax was drained using a continuous aspiration system. To reduced algic and inflammatory effects after surgery process, rats received Turbogesic® (butorfanol) (0.5 mg/kg; 12/12; i.p.) during the first 24 h. To prevent infection, rats received a single dose of penicillin (20 000 U; ip). Rats were observed (vocalization, piloerection, abnormal posture, act of licking and increased aggression) after drug administration to determine the necessity of analgesic application.

Experimental design

After MI, rats were allowed a minimum of six weeks to recover (time necessary to develop the HF state). Sham-operated rats were allowed the same recovery period (23,26,27). Six weeks after MI or sham surgery, rats underwent a RT and LLLT protocol for 8 weeks and a final evaluation of VO_{2max} and exercise tolerance (14 weeks after MI or sham surgery).

Resistance Training protocol

Training groups underwent a RT protocol previously described by our research group for HF (23) and normal rats (28,29) in the adapted squat apparatus for rats (30). Five weeks after MI, training groups underwent a familiarization period. Animals were placed in the apparatus and performed 5 to 10 repetitions with 40% to 60% of their body mass, three times per week for one week. This load was considered low intensity as it had already been demonstrated that non-trained rats could lift up to three times their body mass upon first contact with the above-mentioned apparatus (31). After familiarization, the rats were placed in a neoprene vest, which was left in an upright position on their lower limbs. An electrical stimulus (4–5 mA, 1 s duration, with a 3 s interval between each repetition) was applied to the rat's tail through a surface electrode. As a result, the animals extended their legs repeatedly, which lifted the weight on the exercise apparatus. As this stimulus is considered low intensity, it is does not cause any physical injury to the animals (32). All training sessions were performed in a dark room. The RT program was based on four sets of 10–12 repetitions, at 65 to 75% of the one repetition maximum (1RM), a rest period of 90 s between sets, four times per week, for a total of eight weeks. The animals were exercised at 65% of the 1RM in the first week and the workload was increased in the next week to 75% of the 1RM. The intensities of this protocol are classified as moderate and high intensity for the percentage of 65 to 75% of 1RM according to the guidelines of the American College of Sports Medicine (33).

To determine the training workload, all rats were subjected to a 1RM test. The 1RM was determined as the maximum weight lifted in one complete repetition using the exercise apparatus with the rat having no capacity to complete the second repetition in full range of motion. The 1RM test was performed every two weeks.

Low-level laser protocol

Concurrently with the RT adaptation protocol, the rats underwent LLLT adaptation for one week. For this, each animal was subjected to gentle handling procedures that mimicked the LLLT application (30 seconds per day, three days per week).

Six weeks after MI or sham operation, the rats were started on the LLLT protocol or placebo. We developed a new method of low-level laser therapy in rats that permits the irradiation of large anatomical regions in lower limbs, as follows. A previously calibrated continuous infrared wave diode laser GaAlAs - 850nm (ISO: 13485, modelo 2779, Chattanooga Group - Intelect® Mobile Laser) was used. The animals received the irradiation bilaterally (medially and laterally) at 6 points corresponding to the belly of biceps femoris, gluteus, lateral and medial gastrocnemius, iliopsoas and adductor longus (Figure 1), totalling 12 points with a total energy of 8.8 J. Table 1 summarizes the LLLT protocol characteristics, LLLT parameters and the application mode. The energy chosen (0.735J per point) in this study was based on previously studies by our research group using similar parameters that found that LLLT improves the inflammatory profile (21) and reduces oxidative stress (24) in post-MI Wistar rats. All

experimental animals were irradiated with LLLT immediately after the end of the RT session, respecting the RT protocol frequency (once per day) and period (four times per week for eight weeks). The LLLT probe was held stationary in contact with the skin at a 90° angle, maintaining slight pressure, and the skin was been shaved and cleaned beforehand (Figure 1). Hair removal minimized reflection and refraction and consequently, increased the laser's effectiveness. Placebo LLLT-exposed animals underwent the same handling procedures, probe contact and treatment time (~30 s), although the device was switched off and these animals were used as controls.

Maximal oxygen uptake and exercise tolerance tests

Description of the apparatus: The protocol for maximal oxygen uptake and exercise tolerance was carried realized in a small animal treadmill equipped with a metabolic chamber with expired gas analysis (AVS Projetos, São Carlos, SP, Brazil). The metabolic chamber was connected to an air pump that generated an air flow of ~2500 ml/min and permitted gas aspiration. Maximal oxygen uptake (VO_{2max}) and carbon dioxide production (VCO_{2max}) was continuously monitored during the maximal oxygen uptake and exercise tolerance tests by an O_2 and CO_2 analyser (AVS Projetos, São Carlos, SP, Brasil). The VO_2 ($ml\,kg^{-1}min^{-1}$) was calculated by the air flow measured through the metabolic chamber at ~2500 ml/min, the ambient oxygen fraction (A) and the body mass of the rat (M [kg]), using the formula: $VO_2 = [2500 \times (A-E)]/M$, as previously described (34). The gas analyser and treadmill data was computed using software (AQCAD version 2.3.9.0, AVS Projetos, São Carlos, SP, Brazil)

allowing the collection of the following data: maximal oxygen uptake (VO_{2max}, ml $min^{-1}kg^{-1}$), basal oxygen uptake (VO_{2basal}, ml $min^{-1}kg^{-1}$), oxygen uptake reserve ($VO_{2reservel}$, ml $min^{-1}kg^{-1}$), distance run (m), time to exhaustion (s) and maximal velocity (m/min).

Maximal oxygen uptake and exercise tolerance test protocol: Thirteen weeks after MI or sham surgery, rats were adapted to apparatus (3 min/d, 10 m/min, 3x/wk) by adapted protocol (35-37). Twenty-four hours after the last RT and LLLT session, rats underwent maximal oxygen uptake and exercise tolerance tests carried out based on an incremental speed protocol of exercise until time to animal exhaustion, as previously described (38). Firstly, each animal was acclimatized for 15 min in a stopped treadmill, in which basal gas exchange levels were measured (VO_{2basal}). Thereafter, initial speed was stated in 10 m/min and an increment of 5 m/min was applied at every 3-min interval, until the animal reached exhaustion. Animal exhaustion was established as the time at which the animal was unable to run for at least 15 s, even while receiving an electrical shock (1,5 μA). Each test day, the gas analyser was calibrated with a known gas mixture. The highest values of extracted data measured at the last step was taken as a measure of $VO_{2max.}$ The VO_{2basal} was extracted as the mean of 30 points (30 s) up to initial speed of the treadmill. The $VO_{2reserve}$ was calculated by the formula: VO_{2max}-VO_{2basal}. The running distance, time to exhaustion and maximal velocity were defined as the maximal distance, time and velocity at the end of the protocol.

Echocardiography

Forty-eight hours after the last RT and LLLT session, rats underwent non-invasive cardiac function evaluation using a commercially available echocardiograph (GE Vivid I; GE Medical Systems, Israel) equipped with an 8-13 MHz electronic transducer by a trained operator with experience in small animal echocardiography. All echocardiographic evaluations were performed by the same researcher. Echocardiographic examination followed the recommendations of the American College of Echocardiography (39) and was guided by an adapted protocol previously reported for resistance training (40) and myocardial infarction in rats (34,41-44) and for animal guidelines (45,46). The rats were anaesthetized with xylazine (12 mg/kg, ip) and ketamine (90 mg/kg, ip) and the animals were positioned in lateral decubitus position (45° angle). An ultrasound transmitted gel was applied in a previously shaved chest and M-mode tracings were derived from a 2D-mode obtained from parasternal short-axis views of the LV at three levels: basal (at the tip of the mitral valve leaflets), middle (at the papillary muscle level) and apical (distal to the papillary muscle but before the final curve cavity). To cardiac structural parameters the following structural variables were measured: interventricular septum in diastole (IVSd, mm), interventricular septum in systole (IVSs, mm), left ventricular end-diastolic diameter (LVEdD, mm), left ventricular end-systolic diameter (LVEsD, mm), left ventricular posterior wall in diastole (LVPWd, mm) and left ventricular posterior wall in systole (LVPWs, mm). The measurements obtained were the means of at least three cardiac cycles on each of the three levels and the final value of each rat was the mean of all three described planes. Then, the

following secondary variables of left ventricular systolic function were obtained: end diastolic volume (LVEdV (ml) = $1.047(LVEdD)^3$ and end-systolic volume (LVEsV (ml) = $1.047(LVEsD)^3$) by cubic or ellipsoid model [45, 46]; left ventricular ejection fraction (EF (%) = [(LVEdV- LVEsV) – LVEdV] x 100), left ventricular fractional shortening (FS (%) = [(LVEsD –LVEsD)/ LVEdD] x 100), relative wall-thickness (RWT = (IVSd + LVPWd)/LVEdD) and left ventricular end-diastolic diameter-to-body mass (LVEDD/body mass, mm/kg).

Left ventricular diastolic function was guided by an adapted protocol previously reported for rats with MI (47). Briefly, mitral diastolic inflow measurement by pulsed Doppler was obtained from the four-chamber view and the sample volume was positioned at the tip of the mitral valve to obtain the mitral diastolic flow velocity, which was used to measure the peak E and A wave velocities (cm/s) and the ratio between them (E/A ratio). Additionally, the heart rate was calculated using an average of three consecutive cycle intervals.

Hemodynamic evaluation

Forty-eight hours after the echocardiography, rats underwent hemodynamic evaluation as previously described by our research group (21,23-25). Briefly, the animals were anaesthetized with xylazine (12 mg/kg, ip) and ketamine (90 mg/kg, ip), and a small incision was made in the anterior cervical region in order to insert a polyethylene catheter (PE-50) connected to a pressure transducer (strain gauge; Narco Byosystem Miniature Pulse Transducer RP-155, Houston, TX, USA), coupled to a pressure amplifier (Stemtech), into the right carotid artery. Then, the catheter was positioned

inside the left ventricle, and the pulse wave was monitored by graphical registration of ventricular pressure for 5 min. Analogical pressure signals were digitized by a data acquisition system (CODAS-Data Acquisition System) with a sampling rate of 2000 Hz. These recordings were used to assess left ventricular systolic pressure (LVSP), left ventricular maximum change in pressure over time (dP/dt_{max}), left ventricular minimum change in pressure over time (dP/dt_{min}) and left ventricular end-diastolic pressure (LVEDP). The last parameter was determined manually by detecting the point of inflection to the end of diastole via analysis of the ventricular pressure wave.

Heart hypertrophy, pulmonary and hepatic congestion

Immediately after hemodynamic evaluation, the lungs, liver and heart were removed and weighed. The lungs and liver of each animal were dehydrated (80 °C) for 48 h and then reweighed to determine the water content. Lung and liver wet-to-dry mass ratios were used to determine the percentage of water in those tissues, as an indication of congestion. The right ventricle (RV) and left ventricle (LV) were dissected, separated and weighed. The heart-to-body mass (H/BM), LV-to-body mass (LV/BM) and RV-to-body mass (RV/BM) were determined and used as indicators of heart hypertrophy (21).

Infarcted size determination

Immediately after heart hypertrophy determination, left ventricles were filled with a latex cushion and placed in 10% buffered formaldehyde for 24 h for

subsequent analysis of the size of the infarction area. Infarcted size was determined by histological study adapted from previously a described method from Martinez, P.F *et. al.* (41). Measurements were performed in the midventricular slices (5–6 mm from the apex), assuming that slices from this point showed a close linear relation with the sum of the measurements from all heart slices (48). Accordingly, 10-µm thick sections of the midventricular slice were cut and stained with Masson's trichrome stain. The ImageJ 1.47 software (freeware available at http://rsbweb.nih.gov/ij/download.html) was used to obtain the length of the entire endocardial circumference and of the segment of the endocardial circumference made up by the infarcted portion from two midventricular slices of the left ventricle by manually planimetry. The fraction of the left ventricle that was infarcted was calculated from these measurements. The infarct size was made without knowledge of hemodynamic data.

Statistical analysis

Data are expressed as mean ± SD for each variable and group. The Shapiko-Wilk test was performed to evaluate normality for all variables. One-way ANOVA followed by Tukey's *post hoc* test was used to compare variables (body mass, infarct size, heart hypertrophy, pulmonary and hepatic congestion, echocardiography, hemodynamic, oxygen consumption and exercise tolerance variables) between groups. A P value <0.05 was considered statistically significant. GraphPad Prism 5.0 (Graph-Pad Software, San Diego, CA, USA) for Windows was used as a computational tool in data analysis and to construct charts.

RESULTS

Mortality and adverse effect

Mortality of MI-induce surgery was approximately 30% (11/36). No deaths were observed in sham surgery (12/12). Two HF rats that underwent RT died during the RT protocol (undefined cause). In the sham groups, there were no deaths during the study period. No deaths or behaviours associated with stress were identified in rats that participated in the LLLT protocol.

The final sample size was Sed-Sham (n = 12), Sed-HF (n = 9), RT-HF (n = 7) and RT + LLLT-HF (n = 7).

Body mass, infarct size, heart hypertrophy, pulmonary and hepatic congestion

No significant differences were observed in initial body mass and final body mass among experimental groups. Infarcted size was similar in all HF rats, suggesting a homogenous sample among HF rats. HF rats showed an increased in H/BM and LV/BM compared to sham rats. RV/BM was higher in Sed-HF rats than in Sed-Sham rats. No significant difference was observed in RV/BM between resistance training HF rats and sham rats, suggesting that RT normalized right ventricular hypertrophy in HF rats. Pulmonary congestion was higher in the Sed-HF group than in the Sed-Sham group. No significant difference was observed in pulmonary congestion among resistance trained HF rats and sham rats, suggesting that RT normalized pulmonary congestion in HF

rats. No significant differences were observed in hepatic congestion between experimental groups. No LLLT effects were observed in body mass, heart hypertrophy or pulmonary and hepatic congestion among groups. Table 2 summarizes the body mass, infarct size, heart hypertrophy, pulmonary and hepatic congestion data.

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Echocardiography

HF rats demonstrated an obvious left ventricular systolic and diastolic dysfunction (Table 3). HF rats demonstrated an increase in left ventricular internal diameters (LVEsD and LVEdD) and LVEdD/BW and a decrease in interventricular septum (IVSs and IVSd) and LVEPWTs compared with sham rats, resulting in a decreased in RWT. Additionally, FS and EF were higher in HF rats than in sham rats. RT was capable of increasing interventricular septum (IVSs and IVSd) and posterior wall thickness (LVEPWTs and LVEPWTd) and RWT in HF rats compared with sedentary HF rats. No RT effects were observed in left ventricular internal diameters, FS or EF. Related to diastolic function, Sed-HF rats demonstrated an increased in E/A ratio compared with sham rats. RT was capable to decrease E/A in HF rats compared to sedentary ones.

Hemodynamic data

Table 4 shows the data for invasive cardiac function evaluation in experimental groups. HF rats demonstrated higher LVEDP and lower LVSP, dP/dt_{max} and dP/dt_{min} compared with Sed-Sham rats. RT was capable of

reducing LVEDP in HF rats relative to sedentary HF rats, with no effects on LVSP, dP/dt_{max} and dP/dt_{min}. No LLLT effects were observed on hemodynamic variables.

Oxygen Uptake

VO_{2basal}, VO_{2max} and $VO_{2reserve}$ values of sedentary, RT and LLLT rats are demonstrated in Figure 2. After the protocol, RT + LLLT-HF rats showed higher VO_{2basal} compared to Sed-HF and RT-HF rats (Figure 2A). No significant difference was observed in RT-HF compared with Sed-HF rats in the VO_{2basal} variable. VO_{2max} was lower in Sed-HF and RT-HF rats than in Sed-Sham rats. Interestingly, RT + LLLT-HF rats showed higher VO_{2max} compared with Sed-HF and RT-HF rats (Figure 2B). $VO_{2reserve}$ was higher in RT + LLLT-HF rats than in Sed-HF rats. No significant difference was observed in RT-HF compared with Sed-HF rats (Figure 2C). In summary, these data suggested that RT adaptations alone showed no effects on VO_{2basal}, VO_{2max}, and $VO_{2reserve}$; however, when LLLT was combined with RT, VO_{2max} increased in HF rats.

Exercise Tolerance

Figure 3 shows the values of run distance (Figure 3A), time to exhaustion (Figure 3B) and maximal velocity (Figure 3C) in sedentary, RT and LLLT heart failure and sham rats. Sed-HF and RT-HF rats showed lower distance run, time to exhaustion and maximal velocity compared to Sed-Sham rats. Interestingly, RT + LLLT-HF rats demonstrated longer run distance, time to exhaustion and

maximal velocity compared with Sed-HF and RT-HF rats. In summary, these data suggest that RT alone presented no effects on exercise tolerance variables (distance run, time to exhaustion and maximal velocity), but when LLLT was added to RT, exercise tolerance in HF rats increased.

DISCUSSION

This is the first study to show that LLLT combined with RT improves oxygen uptake and exercise tolerance in HF rats. The evidence of this effect was provided by higher O_2 uptake (VO_{2basal}, VO_{2max} and $VO_{2reserve}$) and exercise tolerance (run distance, time to exhaustion and maximal velocity) variables in HF rats subjected to eight weeks of LLLT in combination with a RT protocol compared with sedentary and RT alone groups.

In the present study, an increase in O_2 uptake and exercise tolerance variables was evident after a LLLT plus RT protocol. A lack of similar studies on the influence of LLLT on O_2 uptake and exercise tolerance variables in HF greatly limits the comparison of our results. One study has shown that the use of LLLT before progressive-intensity running exercise increases exercise performance (VO_{2max} and time to exhaustion) without changing the aerobic and anaerobic thresholds in healthy subjects (19). A previous pilot study in our research laboratory shows that LLLT improves VO_{2max} and distance to run in healthy Wistar rats in a dose-dependent manner (data not published). In other diseases, phototherapy with a combination of super-pulsed lasers and LEDs prior to exercise also led to decreased dyspnea and fatigue in the lower limbs in patients with chronic obstructive pulmonary disease (COPD) (49). Additionally,

one clinical trial was registered to explore the effects of light-emitting diodes on muscle fatigue and exercise tolerance in patients with COPD (50). In this context, an increasing body of evidence has been reported regarding the positive effects of phototherapy on the increase in VO_{2max} and aerobic physiological variables.

Our understanding of the mechanisms behind the effects of LLLT on exercise performance has progressed in recent years. These mechanisms are related to energy metabolism (mitochondrial bioenergetics, enzymatic modulation), reactive oxygen species and reactive nitrogen species, repair of muscle damage and gene expression effects (expression and/or suppression of essential specific genes for augmented physical performance) (18). Specifically, LLLT and LEDT have been used to neutralize ROS produced during physical exercise. This neutralization might improve mitochondrial function, which contributes to the reduction of muscle fatigue and could therefore increase muscle performance (18). In this context, a clear improvement was reported in muscle performance, energy metabolism, defence against oxidative stress and repair/proliferation with different regimens of LEDT applied to muscles in conjunction with a training regimen in healthy mice (51). In addition, a clinical study suggested that modulation of the redox system by LLLT might be related to the delay in skeletal muscle fatigue observed after the use of LLLT (19).

Another mechanistic approach showed that TNF-α, IL-1β and IL-6 gene expression are reduced in skeletal muscles of a Duchene muscular dystrophy mouse model after skeletal muscle LLLT irradiation (52). Recently, it has been shown that LLLT in conjunction with AT may increase VO_{2max} by reducing the inflammatory markers (IL-6 and TNF-α) in aged rats (20). Our research group

demonstrated previously that LLLT improves the inflammatory profile (reduction in TNF-α and IL-6 and increase in IL-10 in skeletal muscle) (21) and reduces oxidative stress (24) in HF rats. Since LLLT showed no effect in systolic or diastolic left ventricular function or in pulmonary congestion, we speculated that the mechanism of action beyond the improvement in exercise tolerance of HF rats is primarily peripheral and is possibly related to inflammatory and oxidative stress profile imbalance. This mechanism is strengthened by the fact that the reduction in exercise tolerance in HF, aside from central mechanisms, is related to changes in peripheral skeletal muscle (5), increased cytokine levels (53) and oxidative stress (54), which contributes to HF myopathy. Also, a recent study revealed that LLLT induces a fibre type-dependent increase in cytochrome *c* oxidase in muscle fibres, showing that LLLT is particularly effective at enhancing the aerobic capacity of intermediate and red fibres (55). It is likely that these adaptations may explain, at least in part, the VO_{2max} improvement observed in the present study.

MI following coronary artery ligation in rats is an extensively used animal model in pathophysiological (56) and therapeutic studies of HF (21,25). However, the general observation of studies that evaluate the functional capacity variables (run time to fatigue, distance run and VO_{2max}) in post-MI rats is controversial (38,57). These results make it difficult to conclude whether or not the rat model of HF following MI can truly induce a reduction in functional capacity. In the present study, HF rats demonstrated a marked reduction in VO_{2max} and exercise tolerance. Additionally, HF rats demonstrated a distinct reduction in VO_{2basal} and $VO_{2reserve}$. These last parameters are poorly described in the rat model of MI (58) and represent a new finding obtained from our study.

Here, MI rats demonstrated marked left ventricular systolic (reduced PSV, dP/dt_{max}, $dP/dt_{min,}$ EF and FS) and diastolic (elevated LVEDP and E/A ratio) dysfunction together with a reduction in O_2 uptake and exercise tolerance variables. The large myocardial infarction (approximately 40%) (59,60) shown in the present study allows us to conclude that the rats presented severe HF.

The effects of RT on VO_{2max} and exercise capacity are not well established. Positive effects have been described (61-63) and reported (8), with some studies describing no effects (10-13) or indicating small or no changes (64). Specifically, RT in HF patients showed no effect on VO_{2peak} and a trend toward treadmill time to exhaustion (13). Similarly, our RT protocol alone showed no effects on VO_{2max} and exercise tolerance variables (e.g. time to exhaustion) in HF rats.

Previously, our research group demonstrated that eight weeks of moderate-to-high intensity RT (65–75% of 1RM) promotes an improvement in cardiac function and strength gain in HF rats (23). The present study was based on the same protocol and showed that RT was capable of increasing interventricular septum (IVSs and IVSd) and posterior wall thickness (LVEPWTs and LVEPWTd) and RWT in HF rats compared with sedentary HF rats, with no effects on left ventricular internal diameters, FS and EF. Likewise, in a study that evaluated the ventricular function and cardiac hypertrophy in healthy rats undergoing a RT program, an increase was observed in the interventricular septum and the free posterior wall mass with no reduction in the end-diastolic left ventricular internal diameter, and no significant difference in systolic function during the RT period. In this case, RT seems to induce the development of concentric cardiac hypertrophy without ventricular dysfunction (40). In HF

patients, RT shows no change in the left ventricular diameter or ejection fraction (13). Related to diastolic function, in addition to decreased LVEDP, our study show that RT was capable of decreasing E/A in HF rats compared with sedentary rats. Similar results were reported in another study by our group that observed an improvement in diastolic function expressed as a reduction in LVEDP (23). No LLLT effects were observed in diastolic or systolic function.

The present study does have limitations that warrant discussion, but do not invalidate the results. The absence of biomarker analysis precludes more profound conclusions regarding the mechanisms of action of LLLT behind the improvement in VO_{2max} and exercise tolerance in HF rats. Our research group and others have published evidence in support of mechanisms that underlie improvements in VO_2 and exercise tolerance (e. g. inflammatory and oxidative stress modulation), which strengthens our main findings.

In conclusion, this study is the first report on the effects of phototherapy in combination with resistance training in VO_{2max} and exercise tolerance in HF syndrome. The addition of LLLT to a RT protocol improved VO_{2max} and exercise tolerance in HF rats compared with RT alone. Non-pharmacological therapies other than exercise may be an alternative (15) and we demonstrated that a simple and inexpensive strategy such as LLLT might be associated with a resistance training protocol as a new nonpharmacological way to improve VO_{2max} parameters and exercise tolerance in HF rats.

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CONFLICT OF INTEREST

None declared.

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FIGURE LEGENDS

Figure 1. Low-level laser therapy cluster application in heart failure rats. The phototherapy was applied medially (A) and laterally (B) of both hind limbs in three sites of each region corresponding to iliopsoas, adductor longus and medial gastrocnemius (C) and biceps femoris, gluteus and lateral gastrocnemius, (D) respectively.

Figure 2. Oxygen uptake variables of sedentary, resistance training and low-level laser therapy rats. **A:** Basal oxygen uptake (VO_{2basal}). **B:** Maximal oxygen uptake (VO_{2max}). **C:** Oxygen uptake reserve ($VO_{2reserve}$). Sed-Sham, sedentary sham group (*n*=12); Sed-HF, sedentary heart failure group (*n*=9); RT-HF, resistance training heart failure group (*n*=7); and RT+LLLT-HF, resistance training plus low level laser therapy heart failure group (*n*=7). Values are in mean ± SD. Statistical analysis: one-way ANOVA followed by the post hoc test of Tukey. *P* value for ANOVA. Symbols represent the comparison among groups by the post hoc analysis: **P*<0.05 compared to Sed-Sham; †*P*<0.05 compared to Sed-HF; ‡*P*<0.05 compared to RT-HF.

Figure 3. Functional capacity variables in sedentary, resistance training and low-level laser therapy rats. **A:** Distance run (m, meters). **B:** Time to exhaustion (s, seconds). **C:** Maximal velocity (m/min, meters/minute). Sed-Sham, sedentary sham group (*n*=12); Sed-HF, sedentary heart failure group (*n*=9); RT-HF, resistance training heart failure group (*n*=7); and RT+LLLT-HF, resistance training plus low level laser therapy heart failure group (*n*=7). Values are in

mean ± SD. Statistical analysis: one-way ANOVA followed by the post hoc test of Tukey. *P* value for ANOVA. Symbols represent the comparison among groups by the post hoc analysis: **P*<0.05 compared to Sed-Sham; †*P*<0.05 compared to Sed-HF; ‡*P*<0.05 compared to RT-HF.

Table 1. Low-level laser therapy protocol characteristics.

Model	Laser GaAlAs (850nm)
Number of laser diodos	3
Pulse frequency	Continuous output
Output power (mW)	300 (100/diode)
Spot size (cm^2)	0.012
Power density (W/cm^2)	8.33
Protocol regime	Immediately after resistance training protocol; bilaterally in hind limbs.
Irradiation sites per application	3 (simultaneously)
Irradiation regions per hind limb	2 (one application medially and one application laterally)
Irradiation sites per hind limb	6 (3 sites medially and 3 sites laterally)
Irradiation sites per animal	12
Dose per treatment (J/cm^2)	61.25
Total energy per point (J)	0.735
Total energy per region (J)	2.205
Total energy per hind limb (J)	4.410
Total energy per animal (J)	8.820
Time per point (s)	7.3
Time per region (s)	7,3
Time per hind limb (s)	14,6
Time per animal (s)	29.2
Frequency treatment	1x/day
Number of treatment per week	4
Protocol duration (weeks)	8
Total number of treatments	32
Application mode	Spot held stationary in skin contact at 90^o angle with slight pressure

Table 2. Body weight, infarct size, heart hypertrophy, pulmonary and hepatic congestion

	Sed-Sham	Sed-HF	RT-HF	RT+LLLT-HF	*P* One-Way ANOVA
Initial Body Weight, g	254.9±17.5	257.6±19.0	246.0±13.2	240.0±6.2	0.1195
Final Body Weight, g	322.6±22.9	333.4±19.8	306.7±49.8	307.4±21.0	0.2764
Infarcted Area, %	-	43.4±6.9	48.2±4.4	46.6±3.8	0.2219
H/BM, mg/g	3.0±0.3	4.0±0.5*	4.0±0.5*	3.5±0.4*	<0.0001
LV/BM, mg/g	2.2±0.2	2.7±0.3*	2.6±0.2*	2.6±0.4*	0.0035
RV/BM, mg/g	0.7±0.2	1.4±0.4*	1.2±0.5	1.2±0.6	0.0084
Pulmonary Congestion, %	71.9±1.9	78.3±1.6*	74.4±6.6	74.9±1.5	0.0016
Hepatic Congestion, %	71.1±1.3	71.6±0.9	70.9±0.7	70.4±0.2	0.1447

Values are means ± SD. Sed-Sham, sedentary sham group (*n*=12); Sed-HF, sedentary heart failure group (*n*=9); RT-HF, resistance training heart failure group (*n*=7); and RT+LLLT-HF, resistance training plus low level laser therapy heart failure group (*n*=7). H/BM, Heart-to-body mass ratio; LV/BM, Left ventricular-to-body mass ratio; RV/BM, Right ventricular-to-body mass ratio. Statistical

analysis: one-way ANOVA followed by the post hoc test of Tukey. Symbols represent the comparision between groups by the post hoc analysys: *P<0.05 compared to Sed-Sham.

Table 3. Non-invasive cardiac function evaluation of experimental groups.

	Sed-Sham	Sed-HF	RT-HF	RT+LLLT-HF	*P* (ANOVA)
Echocardiography					
HR, bpm	272.8±67.3	254.5±39.7	225.7±14.6	249.0±19.1	0.2518
IVSd, mm	1.8±0.4	1.4±0.1*	1.9±0.2†	1.9±0.1†	0.0018
IVSs, mm	3.0±0.4	1.5±0.4*	2.8±0.8†	2.5±0.5†	<0.0001
LVEdD, mm	7.9±0.9	10.8±1.4*	9.7±0.7*	10.3±0.7*	<0.0001
LVEsD, mm	4.5±1.0	9.0±1.4*	7.4±1.1*	8.2±1.0*	<0.0001
LVPWd, mm	1.6±0.3	1.6±0.4	3.0±0.4*†	2.9±0.2*†	<0.0001
LVPWs, mm	2.9±0.4	2.4±0.5*	4.0±0.3*†	4.4±0.2*†	<0.0001
LVEdD/BW	24.6±2.5	31.8±4.1*	31.7±6.7*	33.7±3.5*	0.0001
EF, %	80.6±6.2	38.2±9.0*	53.1±17.0*	49.7±12.1*	<0.0001
FS, %	42.92±7.0	14.98±4.1*	23.4±10.3*	20.9±6.3*	<0.0001

RWT	0.4±0.0	0.3±0.04*	0.5±0.1†	0.5±0.0†	<0.0001
E, cm/s	0.6±0.1	0.8±0.1	0.6±0.1	0.7±0.1	0.1143
A, cm/s	0.3±0.1	0.2±0.0	0.3±0.1	0.4±0.1	0.0918
E/A	2.0±0.4	3.9±0.6*	2.2±0.6†	1.7±0.6†	<0.0001

Values are in mean ± SD. Sed-Sham, sedentary sham group (*n*=12); Sed-HF, sedentary heart failure group (*n*=8); RT-HF, resistance training heart failure group (*n*=6); and RT+LLLT-HF, resistance training plus low level laser therapy heart failure group (*n*=7). For left ventricular diastolic function n=11; 5; 6; 6 rats, respectively. IVSd, interventricular septum in diastole; IVSs, interventricular septum in systole; LVEdD, left ventricular end-diastolic diameter; LVEsD, left ventricular end-systolic diameter; LVPWd, left ventricular posterior wall in diastole; LVPWs, left ventricular posterior wall in systole; EF, left ventricular ejection fraction; LVEdD/BW, left ventricular end-diastolic diameter-to-body ratio; FS, left ventricular fractional shortening; RWT, relative wall-thicness; E, maximal early diastolic peak velocity; A, late peak velocity. Statistical analysis: one-way ANOVA, followed by the post hoc test of Tukey. Symbols represent the comparison among groups by the post hoc analysis: *P<0.05 compared to Sed-Sham; †P<0.05 compared to Sed-HF.

Table 4. Invasive cardiac function evaluation of experimental groups.

	Sed-Sham	Sed-HF	RT-HF	RT+LLLT-HF	*P* (ANOVA)
LVEDP, mmHg	4.161±1.2	23.81±5.9*	13.23±6.2*†	11.01±6.0*†	<0.0001
LVSP, mmHg	120.3±19.9	97.35±8.9*	90.09±16.8*	90.97±11.1*	0.0003
dP/dt_{max}, mmHg/s	7266.0±2459.0	4787.0±675.1*	4201.0±1001.0*	3913.0±747.1*	0.0002
dP/dt_{min}, mmHg/s	-4590.0±955.9	-3187.0±387.4*	-2926.0±392.9*	-3353.0±939.4*	<0.0001

Values are in mean ± SD. Sed-Sham, sedentary sham group (*n*=12); Sed-HF, sedentary heart failure group (*n*=9); RT-HF, resistance training heart failure group (*n*=7); and RT+LLLT-HF, resistance training plus low level laser therapy heart failure group (*n*=7). LVEDP, left ventricular end-diastolic pressure; LVSP, left ventricular systolic pressure; dP/dt_{max}, left ventricular maximum change in pressure over time; dP/dt_{min}, left ventricular minimum change in pressure over time. Statistical analysis: one-way ANOVA, followed by the post hoc test of Tukey. Symbols represent the comparison among groups by the post hoc analysis: *P<0.05 compared to Sed-Sham; †P<0.05 compared to Sed-HF.

Figure 1.

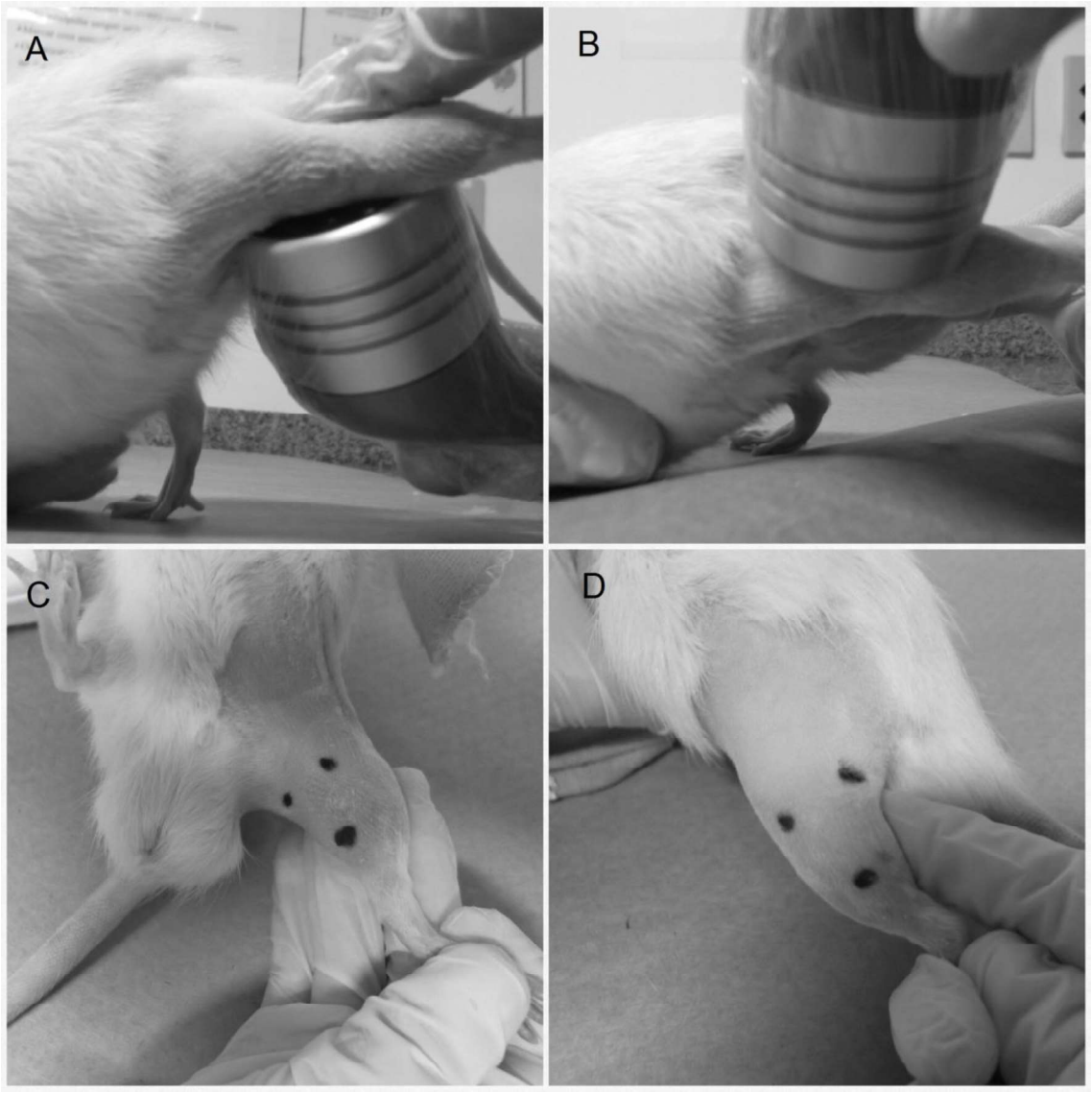


Figure 2.

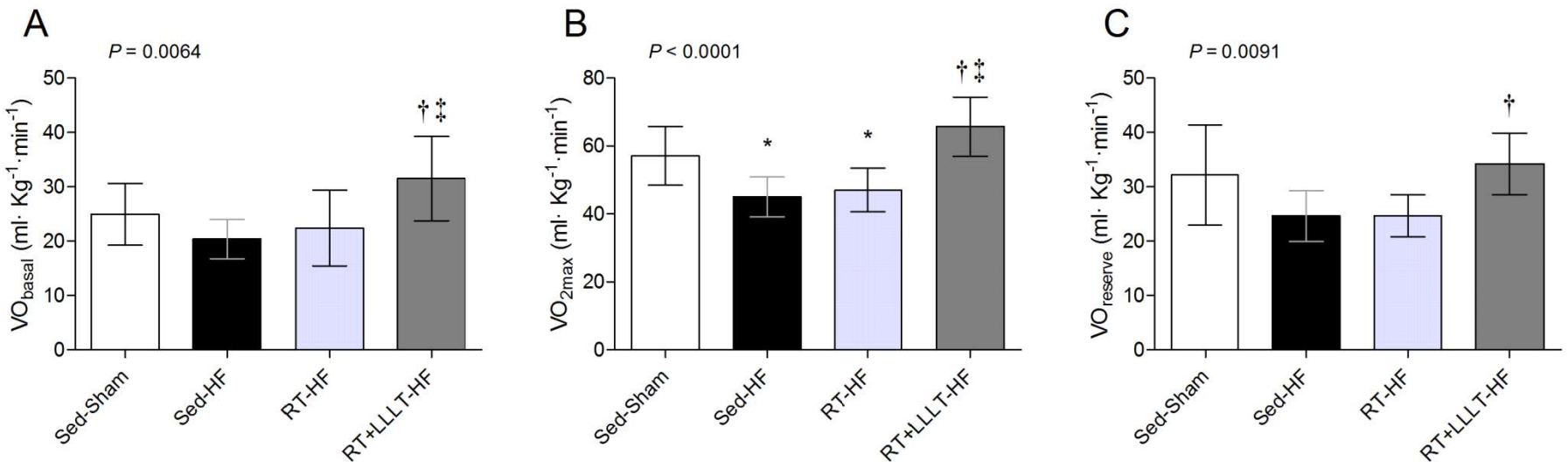
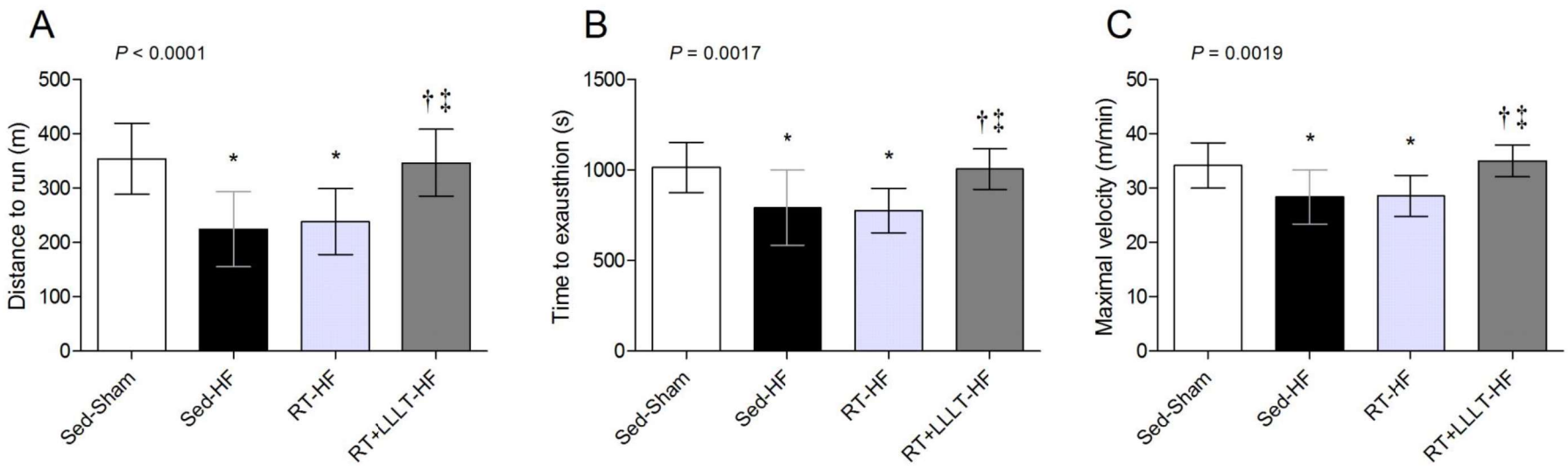

A
P = 0.0064
VO basal (ml· Kg-1·min-1)
50
40
30
20
10
0
†‡
Sed-Sham
Sed-HF
RT-HF
RT+LLLT-HF
B
P < 0.0001
VO2max (ml· Kg-1·min-1)
80
60
40
20
0
*
*
†‡
Sed-Sham
Sed-HF
RT-HF
RT+LLLT-HF
C
P = 0.0091
VO reserve (ml· Kg-1·min-1)
50
40
30
20
10
0
†
Sed-Sham
Sed-HF
RT-HF
RT+LLLT-HF

Figure 3.


A
P < 0.0001
Distance to run (m)
500
400
300
200
100
0
*
*
†‡
Sed-Sham
Sed-HF
RT-HF
RT+LLLT-HF
B
P = 0.0017
Time to exausthion (s)
1500
1000
500
0
*
*
†‡
Sed-Sham
Sed-HF
RT-HF
RT+LLLT-HF
C
P = 0.0019
Maximal velocity (m/min)
50
40
30
20
10
0
*
*
†‡
Sed-Sham
Sed-HF
RT-HF
RT+LLLT-HF

7 ARTIGO 4

Low-level Laser Therapy Enhances the Skeletal Muscle Strength Gain Promoted by Resistance Training in Rats with Heart Failure after Large Myocardial Infarction

(Artigo formatação *Circulation: Heart Failure*; Fator de impacto: 5.867)

Low-level Laser Therapy Enhances the Skeletal Muscle Strength Gain Promoted by Resistance Training in Rats with Heart Failure after Large Myocardial Infarction

Hentschke et. al. LLLT and RT on Maximal Strength in Heart Failure.

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ABSTRACT

Background: The aim of this study was to evaluate the influence of combined resistance training (RT) and low-level laser therapy (LLLT) on maximal strength in rats with heart failure (HF) induced by myocardial infarction (MI).

Methods and Results: Wistar rats were assigned to groups as follows: sedentary sham rats (Sed-Sham, n = 8), sedentary rats with HF (Sed-HF, n = 7), rats with HF subjected to RT (RT-HF, n = 7) and rats with HF subjected to combined RT and LLLT (RT + LLLT-HF, n = 7). Six weeks after MI, rats underwent a RT (4 days per week) and LLLT protocol (GaAlAs 850 nm, after RT, bilaterally in hind limbs) for 8 weeks. HF rats demonstrated a large MI size (~46%). RT improved diastolic function, expressed as a reduction in left ventricular end-diastolic pressure (~50%), and normalized the E/A ratio in HF compared with sedentary rats. Maximal strength gain was higher in RT + LLLT-HF rats (~71%) than in RT-HF rats (~52%, $P < 0.05$). Gastrocnemius mass was higher in the HF animals that underwent RT than the in the Sed-HF animals (~11%, $P < 0.05$). Maximal strength gain was correlated with left ventricular end-diastolic pressure (r = -0.69, $P < 0.001$) and gastrocnemius mass (r = 0.47, $P < 0.04$).

Conclusions: LLLT combined with RT increases maximal strength gain compared with RT alone in large MI-HF rats.

Keywords: Phototherapy. Strength training. One maximum repetition test. Echocardiography.

INTRODUCTION

Heart failure (HF) has become a worldwide epidemic of the 21st century with an increasing impact on healthcare systems [1]. Although initially viewed as a pure cardiac problem, HF in fact becomes a multi-system disorder as the disease progresses [2, 3]. Skeletal muscle HF myopathy plays a key role in the syndrome and loss of skeletal muscle mass occurs early in the disease course and is clinically relevant [3]. HF can reduce whole-body muscle strength, which can affect patient quality of life [4]. Additionally, muscle strength is an important predictor of long-term survival in severe HF [5].

It has been demonstrated that resistance training (RT) was able to increase quadriceps strength, which was accompanied by an improvement in clinical status, quality of life and exercise capacity in patients with stable HF [6]. These findings support the hypothesis that muscle weakness is a determinant of physical disability in HF and shows that non-pharmacological interventions that augment muscle strength, such as RT, may reduce physical disability [4]. Consequently, therapies that improve maximal strength during rehabilitation programs in HF patients are remarkably beneficial.

Low-level laser therapy (LLLT) has emerged as a tool to improve exercise performance in humans [7, 8]. A clinical study demonstrated that RT associated with LLLT applied to the quadriceps muscle of both lower limbs immediately after the end of each training session can increase muscle strength compared with strength training only in healthy subjects [9]. Additionally, LLLT applied before eccentric training sessions seems to improve the hypertrophic response and muscular strength gains in healthy subjects [10]. Recently, an animal study

demonstrated that phototherapy applied over the leg, gluteus and lower-back muscles increased muscle performance in mice subjected to strength training, suggesting a potential application of phototherapy in training programs [11].

Previous reports by our research group provided evidence that LLLT applied to skeletal muscle for 10 consecutive days improves the inflammatory profile [12] and oxidative stress [13] in HF rats. However, no functional evaluation was performed to observe the impact of inflammatory and oxidative stress modulation in the functional capacity of an HF rat model following myocardial infarction (MI).

To the best of our knowledge, no studies have evaluated the influence of combined RT and LLLT on maximal strength gain in the rat model of HF induced by MI. Therefore, we hypothesized that RT followed by LLLT could increase maximal strength gain compared to RT alone in rats with HF. Thus, the aim of this study was to evaluate the influence of combined RT and LLLT on maximal strength gain in rats with HF induced by large MI.

MATERIALS AND METHODS

This animal experimental controlled study was based on the ARRIVE (Animals in Research: Reporting *In Vivo* Experiments) guideline [14].

Sample Size Calculation, Animals and Ethical Approval

For sample size calculation, we used the relative values for one repetition maximal test (the main outcome of this study) obtained from a previous study by

our research group in HF rats [15] as follows: minimum detectable difference in means of 1.7; expected standard deviation of residuals of 1.0; desired power of 0.80; and type 1 error rate of 0.05. The final sample size was 9 animals per experimental group. Considering an expected mortality of ~35-40% in MI surgery [12, 15, 16] and of ~10% in sham surgery (data not reported) the final sample size was 15 animals per HF group and 10 animals per sham group.

Therefore, this study was performed with 55 male Wistar rats weighing between 220 and 300 g (~70 days of age), obtained from the Animal Breeding Unit of the Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA). The rats were housed two or three animals to a cage, and received food and water *ad libitum* in an animal room under a 12:12-h light–dark cycle, at 22 °C. The study protocol followed the ethical rules established by the *Guide for Care and Use of Experimental Animals* published by the National Institutes of Health (NIH publication no. 85–23, revised in 1996). All procedures outlined in this study were approved by the UFCSPA Animal Ethic Committee (protocol 059/11).

Experimental Design

Fifty-five rats were assigned to one of four experimental groups: sedentary sham rats (Sed-Sham, n = 10), sedentary rats with heart failure (Sed-HF, n = 15), rats with heart failure subjected to resistance training (RT-HF, n = 15) and rats with heart failure subjected to resistance training combined with low-level laser therapy (RT + LLLT-HF, n = 15). After allocation to experimental groups,

the animals were subjected to experimental procedures. Figure 1 shows the time-line diagram of the study design.

Animal Model of Heart Failure

The rat model of HF was induced by MI, following ligation of the coronary artery as previously described in our laboratory [12, 13, 15-17]. Briefly, rats were anaesthetized with xylazine (12 mg/kg, ip) and ketamine (90 mg/kg, ip), intubated, and artificially ventilated (SamWay VR 15) with a breathing rate of 60 breaths per minute and an oxygen inspired fraction of 100%. Then, the heart was briefly exposed through a left thoracotomy between the fourth and fifth ribs. In this study, we intended to develop a large MI (>40% of the left ventricle). As previously described, without exception, distal ligations resulted in small infarct sizes and proximal ligations resulted in large infarctions [18]. Therefore, in the animals in which MI was induced, a mononylon suture 6–0 was passed around the main left descending coronary artery, at a point between ~1 and ~2 mm distal to the edge of the left atrium, and the left coronary was ligated in order to produce large MI areas [19]. Sham-operated animals underwent the same procedure without tying the suture and served as control rats. The thorax was closed, the skin was sutured with mononylon suture 3–0, and the pneumothorax was drained using a continuous aspiration system. To reduce pain and inflammatory effects after surgery, rats received Turbogesic® (butorfanol) (0.5 mg/kg; 12/12; i.p.) during the first 24 h. To prevent infection, rats received a single dose of penicillin (20 000 U; ip). Rats were observed (vocalization,

piloerection, abnormal posture, act of licking and increased aggression) after drug administration to determine the necessity of analgesic supplementation.

After MI, rats were allowed a minimum of 6 weeks to recover to develop the HF state. Sham operation rats were allowed the same recovery period [15, 20, 21].

Resistance Training Protocol

Training groups underwent RT according to the protocol previously described for heart failure [15] and normal rats [22, 23] in the adapted apparatus for RT [24]. Five weeks after MI, training groups underwent a familiarization in the adapted apparatus. The animals were placed in the apparatus and performed 5 to 10 repetitions with a resistance of 40% to 60% of their body weight, three times per week for one week. This load was considered low intensity as it has already been demonstrated that non-trained rats can lift up to three times their body weight upon first contact with the referred apparatus [25]. After familiarization and 6 weeks after MI, the rats were placed in a neoprene vest leaving it in an upright position on their lower limbs. An electrical stimulus (4–5 mA, of duration 1 s, with a 3 s interval between repetitions) was applied to the rat's tail through a surface electrode. As a result, the animals extended their legs repeatedly, which lifted the weight on the exercise apparatus. As this electrical stimulus is considered low intensity, it does not cause any physical injury to the animals [26]. All training sessions were performed in a dark room. The RT program was based on 4 sets of 10–12 repetitions, 65% to 75% of the one repetition maximum test (1RM) (after the 1RM test, the animals were

exercised at 65% of the 1RM in the first week and the workload was increased in the next week to 75% of the 1RM), a rest period of 90 s between sets, 4 times per week, for a total of 8 weeks. The intensities of this protocol are classified as moderate and high intensity for the percentage of 65% to 75% of 1RM according to the guidelines of the American College of Sports Medicine [27].

Training Workload and Maximal Strength Determination

To determine the training workload and the maximal strength, we reproduced a methodology previously described for HF [15] and normal rats [23]. All rats were subjected to a 1RM test. The 1RM was determined as the maximum weight lifted in one repetition with the exercise apparatus while being unable to lift the second repetition in full range of motion. The 1RM test was performed every 2 weeks as follows: first 1RM (1st 1RM), 6 weeks after MI and 1° week of RT; second 1RM (2nd 1RM), 8 weeks after and 3° week of RT; third 1RM (3rd 1RM), 10 weeks after and 5° week of RT; fourth 1RM (4th 1RM), 12 weeks after and 7° week of RT. All 1RM tests were normalized to body mass 1RM/BM (grams lifted per gram of BM) and used as the maximal strength parameter. Maximal strength gain was calculated using the formula: Δ1RM/BM (%) = [(4° 1RM - 1° 1RM) / 1° 1RM] x 100; and expressed the percentage change in 1RM/BM after 6 weeks of resistance training and LLLT protocol. All training workload and maximal strength determinations were made by a single researcher, who was blinded to the allocation group of each animal.

Low-Level Laser Irradiation Protocol

On the same days and immediately after the RT adaptation protocol, the rats underwent LLLT adaptation for one week. For this step, each animal was gently submitted to handling procedures that simulated the LLLT application (30 seconds per day, 3 days per week).

Six weeks after MI or sham operation, the rats were started on the LLLT protocol or placebo. A previously calibrated continuous infrared wave diode laser GaAlAs - 850nm (ISO: 13485, model 2779, Chattanooga Group - Intelect® Mobile Laser) was used. The animals received irradiation bilaterally (medially and laterally) at 6 points that corresponded to the belly of the biceps femoris, gluteus, lateral and medial gastrocnemius, iliopsoas and adductor longus, totalizing 12 points with a total energy of 8.8 J. Table 1 summarizes the LLLT protocol characteristics, LLLT parameters and application mode. The energy chosen (0.735 J per point) in this study was based on previous studies by our research group using similar parameters that showed a beneficial impact in post-MI Wistar rats [12, 13]. All experimental animals were irradiated with LLLT immediately after the end of RT session, respecting the RT protocol frequency (once per day) and period (4 times per week for 8 weeks). The LLLT probe was held stationary in contact with the skin at a 90° angle, maintaining slight pressure, after shaving and cleaning of the skin. Hair removal minimized reflection and refraction, and consequently, increased the laser's effectiveness. Placebo LLLT animals underwent the same handling procedures and probe contact although the device was switched off during the same time of treatment (~30 s). These animals were used as controls.

Echocardiography

Forty-eight hours after the last RT and LLLT sessions, rats underwent non-invasive cardiac evaluation using a commercially available echocardiograph (GE Vivid I; GE Medical Systems, Israel) equipped with an 8-13 MHz electronic transducer by a trained operator with experience in small animal echocardiography. All echocardiographic evaluations were performed by the same operator. Echocardiographic examination followed the recommendations of the American College of Echocardiography [28] and used a sequence of measurements adapted from protocols previously reported for RT [29] and myocardial infarction rats [30-34] and for animal guidelines [35, 36]. The rats were anaesthetized with xylazine (12 mg/kg, ip) and ketamine (90 mg/kg, ip) and positioned thereafter in the lateral decubitus position (45° angle). An ultrasound transmission gel was applied to the previously shaved chest and M-mode tracings were derived from 2D-mode images obtained from parasternal short-axis views of the LV at three levels: basal (at the tip of the mitral valve leaflets), middle (at the papillary muscle level) and apical (distal from the papillary muscle but before the final curve of the LV cavity). The following structural variables were measured: interventricular septum in diastole (IVSd, mm), interventricular septum in systole (IVSs, mm), left ventricular end-diastolic diameter (LVEdD, mm), left ventricular end-systolic diameter (LVEsD, mm), left ventricular posterior wall in diastole (LVPWd, mm) and left ventricular posterior wall in systole (LVPWs, mm). The measurements obtained were the mean of at least three cardiac cycles on each of the three levels and the final value of each rat was the mean of all three described planes. Then, the following secondary

variables were computed: end diastolic volume (LVEdV (ml)= $1.047(LVEdD)^3$ and end-systolic volume (LVEdV (ml) $=1.047(LVEsD)^3$) by cubic or ellipsoid model [35, 36]; left ventricular mass (LV mass = $(((LVEdD + IVSd + LVPWd)^3 - LVEdD^3) \times 1.04) \times 0.8 + 0.14$ g.) by cubic formula using the correction factor implemented by Devereux [36]; left ventricular ejection fraction (EF (%) = [(LVEdV – LVEsV) – LVEdV] x 100); left ventricular fractional shortening (FS (%) = [(LVEsD – LVEsD) / LVEdD] x 100); and relative wall-thickness (RWT = (IVSd + LVPWd) / LVEdD).

Cardiac remodelling was evaluated by the LV mass to volume ratio (LV mass / LVEdV). Briefly, when an increase in LV mass occurs, it can be caused by eccentric hypertrophy (increased mass and increased LVEdV, without an increase in LV mass-to-volume ratio) or by concentric hypertrophy (increased mass without an increased LVEdV, increasing LV mass-to-volume ratio [28].

Left ventricular diastolic function was evaluated using a previously reported protocol [37]. Briefly, mitral diastolic inflow measurements were obtained by pulsed Doppler from the four-chamber view, with sample volume positioned at the tip of the mitral valve to obtain the early (E-wave, cm/sec) and late (A-wave, cm/sec) mitral diastolic flow velocities, both waves were used to calculate the E/A ratio. Additionally, the heart rate was calculated using an average of three consecutive cycle intervals.

Hemodynamic Evaluation

Forty-eight hours after the echocardiogram, the rats underwent hemodynamic evaluation as previously described [12, 13, 15-17]. Briefly, the animals were anaesthetized with xylazine (12 mg/kg, ip) and ketamine (90 mg/kg, ip), and a small incision was made in the anterior cervical region in order to insert a polyethylene catheter (PE-50) connected to a pressure transducer (strain gauge; Narco Byosystem Miniature Pulse Transducer RP-155, Houston, TX, USA), coupled to a pressure amplifier (Stemtech), into the right carotid artery. Then, the catheter was positioned inside the left ventricle, and the pulse wave was monitored by graphical registration of ventricular pressure for 5 min. Analogical pressure signals were digitalized by a data acquisition system (CODAS-Data Acquisition System) with a sampling rate of 2000 Hz. These recordings were used to compute left ventricular systolic pressure (LVSP), left ventricular maximum change in pressure over time (dP/dt_{max}), left ventricular minimum change in pressure over time (dP/dt_{min}), left ventricular end-diastolic pressure (LVEDP) and left ventricular developed-pressure (LVPdev = LVSP - LVEDP). LVEDP was determined manually by detecting the point of inflection in the end of diastole via analysis of the ventricular pressure wave.

Skeletal Muscle Sample Collection

Immediately after hemodynamic evaluation, the rats were euthanized by decapitation and the gastrocnemius and soleus (right side) were collected and

weighed. The skeletal muscle index (muscle mass/body mass) was calculated for each muscle.

Cardiac Hypertrophy, Pulmonary and Hepatic Congestion

After skeletal muscle sample collection, the lungs, liver and heart were removed and weighed. The lungs and liver of each animal were dehydrated (80 °C) for 48 h and then reweighed to determine their water content. Lung and liver wet-to-dry weight ratios were used to determine the percentage of water in those tissues, as an indication of congestion. The right ventricle (RV) and left ventricle (LV) were dissected, separated and weighed. The heart-to-body mass (H/BM), LV-to-body mass (LV/BM) and RV-to-body mass (RV/BM) were determined and used as an indication of cardiac hypertrophy [12].

Infarcted Size Determination

Immediately after heart weighing, left ventricles were filled with a latex cushion and placed in 10% buffered formaldehyde for 24 h for subsequent analysis of the size of the infarction area. Infarcted size was determined by histological evaluation adapted from the method previously described by Martinez, P.F. *et al.* [31]. Measurements were performed in the midventricular slices (5–6 mm from the apex), assuming that slices from this point show a close linear relation with the sum of the measurements from all heart slices [38]. Accordingly, 10 μm thick sections of the midventricular slice were cut and stained with Masson's trichrome stain. ImageJ 1.47 software was used to obtain

the length of the entire endocardial circumference and that segment of the endocardial circumference made up by the infarcted portion from two midventricular slices of the left ventricle by manual planimetry. The fraction of the LV that was infarcted was calculated from these measurements. All infarct size measurements were made by a single technician who was blinded to hemodynamic data and group assignments.

Statistical Analysis

Data are expressed as mean ± SD for each variable and group. The Shapiro-Wilk test was performed to evaluate normality for all variables. One-way ANOVA followed by Tukey's *post hoc* test was used to compare variables among groups (body mass, infarct size, cardiac hypertrophy, pulmonary and hepatic congestion, hemodynamic and echocardiography variables, maximal strength percentage change after RT and LLLT protocol and skeletal muscle mass). Two-way repeated measures ANOVA followed by Tukey's *post hoc* test was used to compare maximal strength among groups at different experimental times. Pearson's correlation test was used to examine the relationship between muscle mass parameters, maximal strength and maximal strength gain and hemodynamic variables in heart failure rats. A P value <0.05 was considered statistically significant. GraphPad Prism 5.0 (Graph-Pad Software, San Diego, CA, USA) for Windows was used as a computational tool in the data analysis and to construct charts. SigmaPlot 11.0 (Systat Software Inc., San Jose, CA, USA) and SPSS 21.0 (IBM Corporation, Armonk, NY, USA) for Windows were used for complementary analysis.

RESULTS

Mortality, Exclusion Criteria and Adverse Effects

The mortality in MI-induced surgery was ~40% (18/45) and in sham surgery was ~10% (1/10), with a total mortality after surgeries in all groups of ~35% (19/55). Only animals that concluded all experimental procedures were included in the statistical analysis. We consider in this study only animals with large MI (>40%) [19, 39]. Therefore, left ventricular hemodynamic parameters were not measured in two rats: Sham-Sed (1) and HF-Sed (1); three rats displayed an MI area <40%: HF-Sed (1) and HF-RT (2); two rats did not complete 75% of the RT protocol due to the presence of respiratory distress: HF-RT (1) and HF-RT + LLLT (1); and were excluded from the study. The remaining rats formed the experimental group as follows: Sham-Sed (n = 8), HF-Sed (n = 7), HF-RT (n = 7) and HF-RT + LLLT (n = 7). No deaths were identified in rats from RT or LLLT groups.

Body Mass, Infarct Size, Cardiac Hypertrophy, Pulmonary and Hepatic Congestion

Table 2 summarizes these data. Initial and final body masses were similar between groups, as were infarct sizes, suggesting that all infarcted groups developed HF syndrome similarly. Rats with HF had a higher H/BM and LV/BM compared with sham rats. RV/BM, pulmonary and hepatic water percentage

were increased in the Sed-HF rats relative to the Sed-Sham rats. The HF-RT and HF-RT + LLLT groups showed RV/BM, pulmonary and hepatic water percentages that were similar to those of the Sham-Sed group. These data suggest that RT and LLLT prevented the right ventricle hypertrophy and pulmonary and hepatic congestion that occurred in the HF-Sed group. However, the interventions did not prevent the increase in heart-to-body mass and left ventricle-to-body mass ratios generated by HF.

Echocardiography and Hemodynamic Evaluation

Rats with HF demonstrated an increase in LV cavity (LVEdV and LVEsV) and a reduction in IVSs and RWT (Table 3), without an increase in LV mass-to-volume ratio, indicating a pattern of eccentric hypertrophy (Figure 2B). The HF model generated a significant left ventricular systolic dysfunction, expressed by a reduced LVSP, dP/dt_{max}, dP/dt_{min}, LVPdev, EF and FS (Table 3), and severe left ventricular diastolic dysfunction, expressed by a markedly increased LVEDP (>20 mmHg) and E/A ratio (Figure 2A and 2C, respectively). RT reversed the IVS and PW thinning and increased the RWT (Table 3) and LV mass/volume ratio (Figure 2B), indicating that RT can protect against eccentric hypertrophy in the HF group. RT showed a trend toward improvement in systolic function (EF and FS), but without statistical significance (Table 3). Additionally, RT decreased the LVEDP and E/A ratio compared with the HF group, indicating an improvement in diastolic dysfunction (Figure 2A and 2C, respectively). No RT effects were observed in other hemodynamic parameters, such LVSP, dP/dt_{max}

and d*P*/d$t_{min.}$ HF-RT + LLLT showed no additional effect on structural and left ventricular systolic and diastolic function, as compared with RT alone.

Maximal Strength Over Time

Figure 3A shows the maximal strength (1RM/BM) changes over time (1st 1RM, 2nd 1RM, 3rd 1RM and 4th 1RM) during the RT and LLLT protocol in the experimental groups. No significant differences were observed in the first test of 1RM among all groups. In the second 1RM test, intervention groups (RT-HF and RT + LLLT-HF) showed higher maximal strength compared to sedentary groups (Sed-Sham and Sed-HF). In intragroup analysis, RT-HF and RT + LLLT-HF showed a progressive increase in maximal strength in all experimental times.

Individual Performance of Animals and Groups Before and After Interventions in Maximal Strength

The individual performance of animals/groups in maximal strength tests before (1st 1RM) and after (4th 1RM) sedentary, resistance training and LLLT periods are presented in Figure 3B. No significant differences were observed in Sed-Sham and Sed-HF groups between 1st 1RM and 4th 1RM. The RT-HF group showed higher values in the last 1RM compared with the first 1RM and a significant relative gain. The RT + LLLT-HF group showed higher values in the last 1RM compared with the first 1RM and a more pronounced percentage relative gain compared with the RT-HF group.

Maximal Strength Gain (percentage change after resistance training and LLLT protocol)

Figure 4 shows the maximal strength gain expressed in percentage change of 1RM/BM after 8 weeks of resistance training and LLLT protocol (Δ1RM/BM) in the experimental groups. No significant difference was observed between Sed-Sham and Sed-HF groups. Resistance training (HF-RT and HF-RT+LLLT) groups showed a higher maximal strength gain compared with sedentary groups (Sed-HF and Sed-Sham). Interestingly, the percentage change in RM/BM after intervention was higher in the HF-RT + LLLT group (~71%) than in the HF-RT group (~52%), suggesting that LLLT associated with resistance was able to increase the maximal strength gain compared to resistance training alone in heart failure rats.

Skeletal Muscle Mass

Figure 5 shows the values of skeletal muscle index (muscle mass/body mass) of gastrocnemius (Fig. 5A) and soleus (Fig. 5B). Gastrocnemius index is lower in the Sed-HF animals than the Sed-Sham animals. Gastrocnemius mass index was higher in the HF animals that underwent RT (RT-HF and RT+LLLT-HF groups) than in the Sed-HF animals. No additional effects were observed in the RT + LLLT-HF group compared with RT alone in gastrocnemius mass index. There were no significant differences in soleus index among groups.

Correlations Between Central (hemodynamic) and Peripheral (muscle mass) Parameters with Maximal Strength in Rats with Heart Failure

Figure 6 shows the correlation between LVEDP and skeletal muscle index (gastrocnemius) with maximal strength measured by the last RM/BM and maximal strength gain measured by percentage change after the RT protocol and LLLT protocol in HF rats. We observed a significant correlation between LVEDP and the last 1RM/BM (Figure 6A; r = -0.59, $P < 0.01$) and between LVEDP and maximal strength gain (Figure 6B; r = -0.69; $P < 0.001$). Additionally, we observed a significant correlation between the gastrocnemius index and the last 1RM/BM (Figure 6C; r = 0.78, $P < 0.001$) and the maximal strength gain (Figure 6D; r = 0.47; $P = 0.04$).

DISCUSSION

To the best of our knowledge, this is the first experimental study to describe an increase in maximal strength gain after LLLT combined with RT, versus RT alone in large MI-HF rats. The evidence of this effect was demonstrated by a higher percentage change in 1RM/BM after 6 weeks of intervention in the LLLT associated with RT rats (~71%) than in RT alone rats (~52%). In addition, the present study demonstrated that RT improves diastolic function, expressed as a reduction in LVEDP (~50%) and normalization of E/A ratio, in HF rats compared to sedentary rats. Similar results were observed in a previously study published by our research group, in which a decrease of 68% in LVEDP in HF rats subjected to an identical RT protocol was demonstrated [15].

Our results are similar to those reported in animal studies, which demonstrated that LLLT associated with RT increased values of the maximal resistance test in normal rats [40] and muscle performance in normal mice [11]. Furthermore, clinical studies demonstrated that LLLT in combination with RT increased muscle strength when compared with RT alone in healthy subjects [9]. The results of maximal strength gain in HF rats subjected to RT and LLLT have important implications in the context of HF therapy. Muscle strength is remarkably reduced in HF patients [4] and is a predictor of long-term survival in this syndrome [5]. Daily activities, such as lifting objects, rising from a seated position and climbing stairs are strongly dependent on muscle strength [4] and thus, muscular strength impacts the capacity of HF patients to perform daily tasks [41]. Since muscle weakness is a determinant of physical disability in HF patients, interventions that increase muscle strength diminish physical disability in this population [4]. In this context, therapies that improves maximal strength during rehabilitation programs in HF patients should be beneficial.

A significant negative correlation between LVEDP with maximal strength ($r = -0.59$, $P < 0.01$) and with maximal strength gain ($r = -0.69$; $P < 0.001$) was observed. The data suggest that an increase in maximal strength can improve diastolic function in HF rats. Additionally, a significant correlation between gastrocnemius index with maximal strength ($r = 0.47$, $P = 0.04$) and maximal strength gain ($r = 0.65$, $P < 0.01$) was observed. Our findings suggest that the higher maximal strength in HF rats that underwent RT may be, in part, explained by the increase in gastrocnemius mass.

Our initial hypothesis was that LLLT improves skeletal muscle mass in a fibre type-dependent manner. Soleus muscle is composed mainly of slow-twitch

fibres (i.e., type I) [32, 42] and gastrocnemius is composed mainly of fast-twitch fibres (i.e., type IIa e IIb) [43-45]. Some studies have further classified fast-twitch gastrocnemius into oxidative red fibre (Gastrocnemius red), glycolytic white fibre (Gastrocneiumus white) and mixed red and white fibre (Gastrocnemius mixed) [43]. Hayworth, C. R. *et al.* [46] showed that *in vivo,* LLLT induced a dose- and fibre type-dependent increase in cytochrome oxidase activity in muscle fibres and LLLT was particularly effective at enhancing the aerobic capacity of intermediate and red fibres. However, no additional LLLT effects were observed in skeletal muscle mass.

Similar to our results, a clinical study demonstrated that RT associated with LLLT can increase muscle performance compared with RT only, without increases in thigh perimeter between groups [9]. On the other hand, a clinical study demonstrated that subjects who underwent LLLT applied before eccentric training sessions reached significantly higher percent changes in quadriceps muscle thickness assessed by ultrasonography compared with subjects who underwent eccentric training only [10]. An animal study demonstrated that the skeletal muscle mass of the LLLT group was 37.5% higher than that of the control group [47]. The authors concluded that LLLT has the potential to decrease the progression of myocyte apoptosis in sarcopenic muscles in aged rats. Another animal study showed that significant increases were noted in the muscle volume of the phototherapy group compared with the control group in ovarectomized rats [48].

As shown, no additional LLLT effects to RT were observed in systolic/diastolic left ventricular function or skeletal muscle mass in HF rats. We speculated that the higher muscle strength gains observed in the HF-RT + LLLT

group versus the RT only group was due peripheral mechanisms, such as inflammation and oxidative stress. Our research group has demonstrated previously that LLLT improved the inflammatory profile, expressed as a reduction in TNF-α and IL-6 and an increase in IL-10 myokines in the skeletal muscle of MI rats [12]. Additionally, we demonstrated that LLLT changed the oxidative balance in the skeletal muscle of HF rats [13]. Other studies demonstrated the ability of LLLT to modulate inflammatory [49, 50] and oxidative stress biomarkers [51-53]. Inflammation [54, 55] and reactive oxygen species [56] play a key role in skeletal muscle myopathy in HF syndrome, which may be highly important to the development of mitochondrial dysfunction [57]. The enhancement in muscle performance by phototherapy may be also explained by the increase in cytochrome c oxidase activity and ATP concentration in skeletal muscle [11], as well mechanisms involving phosphocreatine resynthesis and lactate oxidation by mitochondria [7]. In this context, we believe that the increase in maximal strength gain after LLLT associated with RT compared with RT alone in HF rats, in the absence of skeletal muscle mass improvement, are strongly associated with inflammatory and oxidative stress processes and also with the modulation of energy metabolism and an improvement in mitochondrial function.

Additionally, our study demonstrated that RT was capable of increasing interventricular septum (IVSs and IVSd) and posterior wall thickness (LVEPWTs and LVEPWTd) and RWT in HF rats compared with sedentary HF rats. Related to systolic function, our study shows no effects in left ventricular internal diameters, FS and EF, LVSP, dP/dt_{max}, dP/dt_{min}. However, a previous study performed in our laboratory demonstrated an increase in LVSP, dP/dt_{max}, dP/dt_{min} in HF rats subjected to an 8 week RT protocol [15]. We speculated that

this discrepant result is due to the MI size. We selected only animals with large MI (>40%) and it seems that the RT is not capable of normalizing systolic function parameters in rats with severe HF.

CONCLUSIONS

In conclusion, this study demonstrates that LLLT associated with RT increases maximal strength gain compared with RT alone in large MI-HF rats, which confirms our hypothesis that RT followed by LLLT increases maximal strength gain. Considering that skeletal muscle strength is reduced and directly impacts functional capacity, interventions that improve maximal strength are beneficial in HF.

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DISCLOSURES

None.

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FIGURE LEGENDS

Figure 1. Time-line diagram of the study design. MI: myocardial infarction; RT: resistance training; LLLT: low-level laser therapy; 1st 1RM: first one repetition maximum test; 2nd 1RM: second one repetition maximum test; 3rd 1RM: third one repetition maximum test; 4th 1RM: fourth one repetition maximum test.

Figure 2. **A:** Left ventricular end-diastolic pressure (LVEDP); **B:** Left ventricular mass to volume ratio (LV mass/volume); and **C:** Early (E wave) and late (A wave) ratio from mitral flow (E/A ratio). Sed-Sham, sedentary sham group (*n*=8); Sed-HF, sedentary heart failure group (*n*=7); RT-HF, resistance training heart failure group (*n*=7); and RT+LLLT-HF, resistance training associated with low level laser therapy heart failure group (*n*=7). Values are means ± SD. Statistical analysis: one-way ANOVA and Tukey as post hoc. **P*<0.05 compared to Sed-Sham; †*P*<0.05 compared to Sed-HF.

Figure 3. **A:** Maximal strength changes over the time. Values are means ± SD. Statistical analysis: two-way repeat measure ANOVA and Tukey as post hoc. **P*<0.05 RT groups compared to own baseline; †*P*<0.05 RT groups compared to all own times; ‡*P*<0.05 RT groups compared Sed groups at the same time point. **B:** Individual performance of animals/groups in maximal strength before and after interventions. *P* value represent the within group comparison obtained by two-way repeated measure ANOVA. Sed-Sham, sedentary sham group (*n*=8); Sed-HF, sedentary heart failure group (*n*=7); RT-HF, resistance training heart failure group (*n*=7); and RT+LLLT-HF, resistance training associated with low level laser therapy heart failure group (*n*=7).

Figure 4. Maximal strength gain after 6 weeks of resistance training and LLLT protocol. Sed-Sham, sedentary sham group (*n*=8); Sed-HF, sedentary heart failure group (*n*=7); RT-HF, resistance training heart failure group (*n*=7); and RT+LLLT-HF, resistance training associated with low level laser therapy heart failure group (*n*=7). Values are means ± SD. Statistical analysis: one-way ANOVA and Tukey as post hoc. **P*<0.05 compared to Sed-Sham; †*P*<0.05 compared to Sed-HF; ‡*P*<0.05 compared to RT-HF.

Figure 5. Skeletal muscle mass of **A:** Gastrocnemius and **B:** Soleus. Sed-Sham, sedentary sham group (*n*=8); Sed-HF, sedentary heart failure group (*n*=6); RT-HF, resistance training heart failure group (*n*=6); and RT+LLLT-HF, resistance training associated with low level laser therapy heart failure group (*n*=7) for all skeletal muscles. Values are means ± SD. Statistical analysis: one-way ANOVA and Tukey as post hoc. **P*<0.05 compared to Sed-Sham; †*P*<0.05 compared to Sed-HF.

Figure 6. Correlations between hemodynamic and muscle mass parameters with maximal strength in Sed-HF, black circles (●), RT-HF, light gray circles (●) and RT+LLLT-HF, dark gray circles (●) rats. **A:** LVEDP with 4th RM/BM. **B:** LVEDP with maximal strength gain. **C:** Gastrocnemius index with 4th RM/BM. **D:** Gastrocnemius index with maximal strength gain. Statistical analysis: Pearson´s correlation test.

Table 1. Low-level laser therapy parameters, protocol characteristics and application mode.

Model	Laser GaAlAs (850nm)	
Number of laser diodos	3	
Pulse frequency	Continuous output	
Output power (mW)	300 (100/diodo)	
Spot size (cm^2)	0.012	
Power density (W/cm^2)	8.33	
Protocol regim	Immediately after resistance training protocol; bilaterally in hind limbs.	
Irradiation sites per application	3 (simultaneously)	
Irradiation regions per hind limb	2 (one application medially and one application laterally)	
Irradiation sites per hind limb	6 (3 sites medially and 3 sites laterally)	
Irradiation sites per animal	12	
Groups	Placebo groups	LLLT groups
Dose per treatment (J/cm^2)	-	61.25
Total energy per point (J)	-	0.735
Total energy per region (J)	-	2.205
Total energy per hind limb (J)	-	4.410
Total energy per animal (J)	-	8.820
Time per point (s)	7.3	7.3
Time per region (s)	7.3	7.3
Time per hind limb (s)	14.6	14.6
Time per animal (s)	29.2	29.2
Frequency treatment	1x/day	1x/day
Number of treatment per week	4	4
Protocol duration (weeks)	8	8
Total number of treatments	32	32
Application mode	Spot held stationary in skin contact at 90^o angle with slight pressure	

Table 2. Body mass, infarct size, heart hypertrophy, pulmonary and hepatic congestion

	Sed-Sham	Sed-HF	RT-HF	RT+LLLT-HF	*P* One-Way ANOVA
Initial Body Mass (g)	254.6±17.8	264.0±24.7	245.7±20.1	236.7±10.7	0.0683
Final Body Mass (g)	333.0±20.2	346.9±32.3	302.7±47.7	301.1±20.5	0.0275
Infarcted Area (%)	-------	46.1±4.7	46.6±3.9	45.7±2.8	0.9139
H/BM (mg/g)	2.9±0.1	3.9±0.5*	3.8±0.4*	3.9±1.0*	0.0036
LV/BM (mg/g)	2.2±0.1	2.6±0.2*	2.8±0.3*	2.7±0.4*	0.0009
RV/BM (mg/g)	0.6±0.08	1.3±0.4*	1.0±0.3	1.0±0.4	0.0042
Pulmonary Congestion (water %)	71.4±1.7	77.9±1.7*	73.8±6.6	74.8±1.2	0.0125
Hepatic Congestion (water %)	70.3±0.4	71.5±1.0*	71.3±1.1	70.3±0.3	0.0121

Values are means ± SD. Sed-Sham, sedentary sham group (*n*=8); Sed-HF, sedentary heart failure group (*n*=7); RT-HF, resistance training heart failure group (*n*=7); and RT+LLLT-HF, resistance training associated with low level laser therapy heart failure group (*n*=7). H/BM, Heart-to-body mass ratio; LV/BM, Left ventricular-to-body mass ratio; RV/BM, Right ventricular-to-body mass ratio.

Statistical analysis: one-way ANOVA followed by the Tukey post hoc test. Symbols represent the comparison among groups by the post hoc analysis: *P<0.05 compared to Sed-Sham.

Table 3. Hemodynamic and echocardiography variables of experimental groups.

	Sed-Sham	Sed-HF	RT-HF	RT+LLLT-HF	*P* One-Way ANOVA
Hemodynamic evaluation					
LVSP, mmHg	124.3±18.5	98.2±6.7*	95.1±19.0*	92.1±9.7*	0.0016
LVPdev, mmHg	116.0±21	77±9*	83±20*	80±12*	<0.001
dP/dt_{max}, mmHg/s	7924.0±2577.0	4692.0±510.2*	4497.0±1080.0*	4159.0±678.0*	0.0001
dP/dt_{min}, mmHg/s	-4888.0±1012.0	-3145.0±319.2*	-3075.0±452.7*	-3687.0±954.2*	0.0003
Echocardiography					
HR, bpm	283.4±57.1	259.2±35.6	224.3±14.4	241.0±9.5	0.0724
IVSd, mm	1.6±0.2	1.4±0.2	2.0±0.2*†	1.8±0.1†	0.0004
IVSs, mm	2.9±0.3	1.6±0.5*	2.7±0.9†	2.6±0.6†	0.0037
LVEdD, mm	8.1±0.8	10.4±1.5*	10.3±1.6*	10.5±1.0*	0.0027

LVEsD, mm	4.8±0.9	8.8±1.6*	7.9±2.3*	8.5±1.4*	0.0002
LVPWd, mm	1.5±0.3	1.6±0.5	2.8±0.4*†	3.0±0.2*†	<0.0001
LVPWs, mm	2.8±0.3	2.3±0.4	4.1±0.3*†	4.4±0.2*†	<0.0001
EF, %	78.6±5.4	40.4±11.4*	53.7±19.9*	50.3±11.6*	<0.0001
FS, %	40.6±5.5	16.1±5.4*	24.3±12.4*	19.7±6.9*	<0.0001
RWT	0.4±0.1	0.3±0.1*	0.5±0.1†	0.4±0.1†	0.0012
E, cm/s	0.6±0.1	0.8±0.1	0.6±0.1	0.7±0.1	0.0822
A, cm/s	0.3±0.1	0.2±0.1	0.3±0.1	0.4±0.1	0.0855

Values are means ± SD. Sed-Sham, sedentary sham group (*n*=8); Sed-HF, sedentary heart failure group (*n*=7); RT-HF, resistance training heart failure group (*n*=7); and RT+LLLT-HF, resistance training associated with low level laser therapy heart failure group (*n*=7). For left ventricular diastolic function n=8; 4; 6; 7 rats, respectively. LVEDP, left ventricular end-diastolic pressure; LVSP, left ventricular systolic pressure; dP/dt_{max}, left ventricular maximum change in pressure over time; dP/dt_{min}, left ventricular minimum change in pressure over time; LVPdev, left ventricular pressure developed; IVSd, interventricular septum in diastole; IVSs, interventricular septum in systole; LVEdD, left ventricular end-diastolic diameter; LVEsD, left ventricular end-systolic diameter; LVPWd, left ventricular posterior wall in diastole; LVPWs, left ventricular posterior wall in systole; EF, left ventricular ejection fraction; FS, left ventricular fractional shortening; RWT, relative wall-thicness; LVmass/volume, left ventricular mass to volume ratio; E, maximal early diastolic peak velocity; A, late peak velocity. Statistical analysis: one-way ANOVA, followed by the Tukey post hoc test. Symbols represent the comparison among groups by the post hoc Tukey analysis: *P<0.05 compared to Sed-Sham; †P<0.05 compared to Sed-HF.

Figure 1.

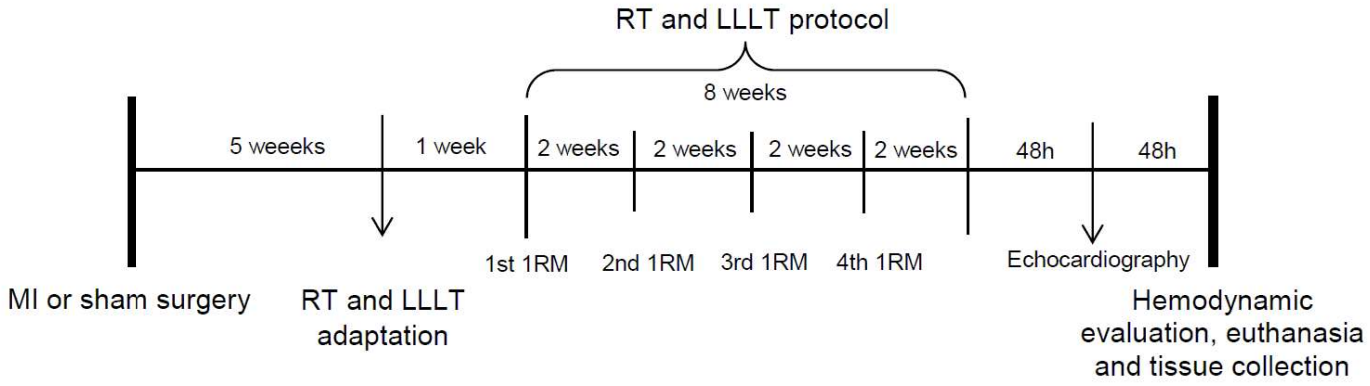

RT and LLLT protocol
8 weeks
5 weeeks
1 week
2 weeks
2 weeks
2 weeks
2 weeks
48h
48h
1st 1RM
2nd 1RM
3rd 1RM
4th 1RM
Echocardiography
MI or sham surgery
RT and LLLT adaptation
Hemodynamic evaluation, euthanasia and tissue collection

Figure 2.

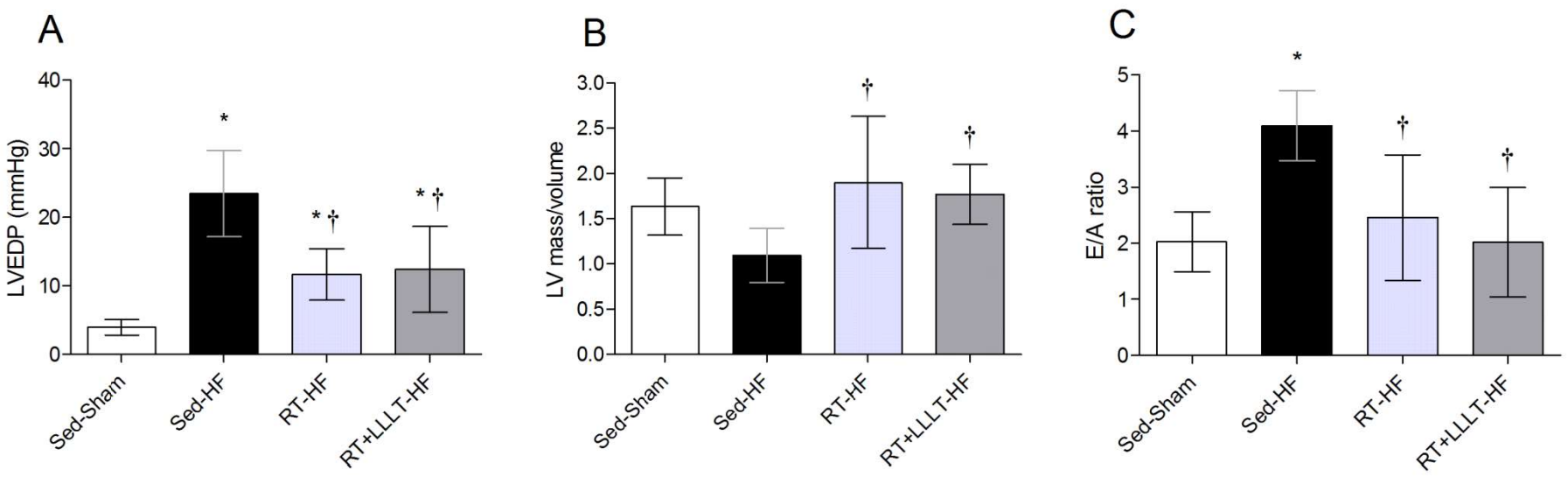

A
LVEDP (mmHg)
40
30
20
10
0
*
* †
* †
Sed-Sham
Sed-HF
RT-HF
RT+LLLT-HF
B
LV mass/volume
3.0
2.5
2.0
1.5
1.0
0.5
0.0
†
†
Sed-Sham
Sed-HF
RT-HF
RT+LLLT-HF
C
E/A ratio
5
4
3
2
1
0
*
†
†
Sed-Sham
Sed-HF
RT-HF
RT+LLLT-HF

Figure 3.

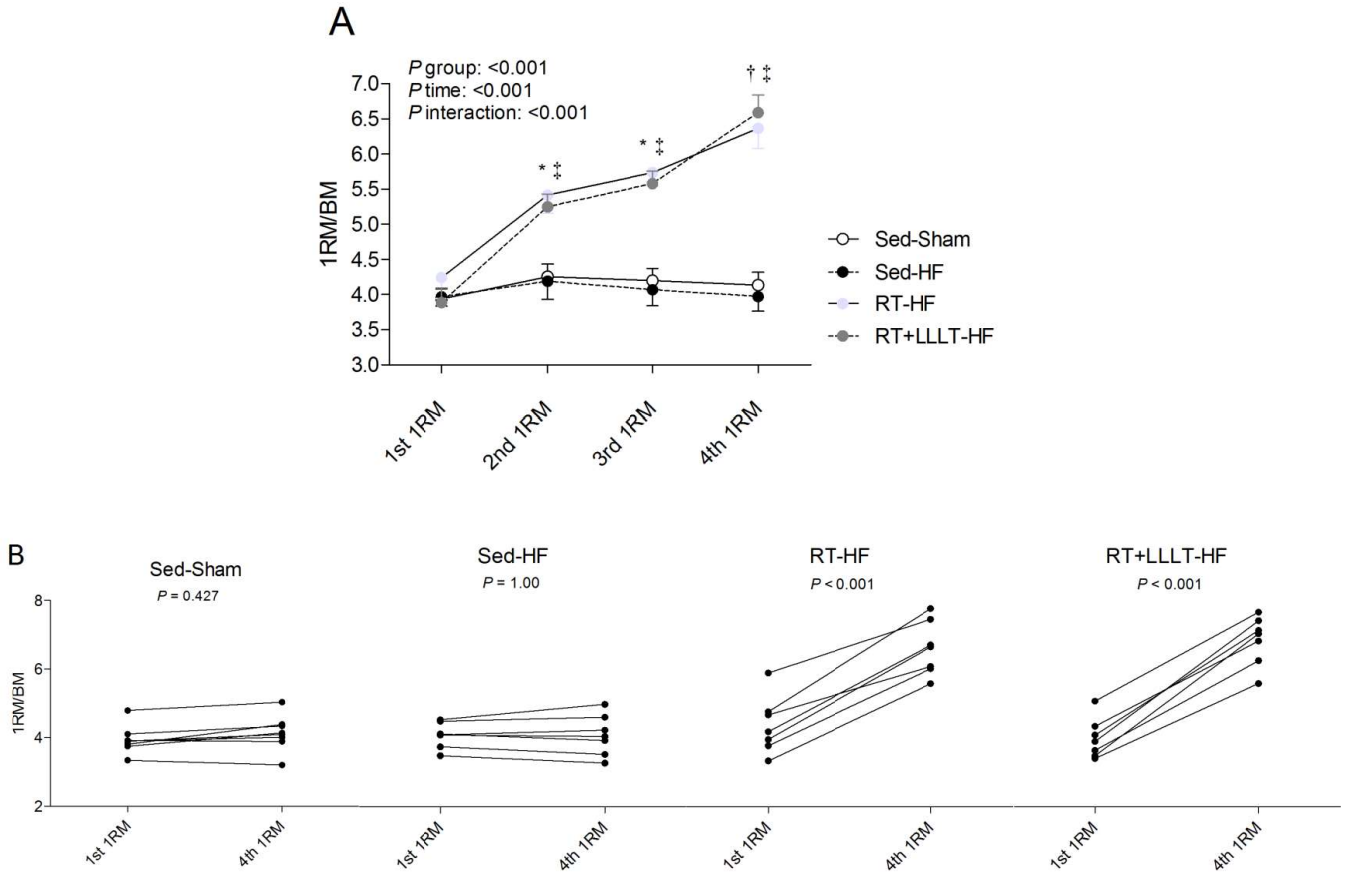

A
P group: <0.001
P time: <0.001
P interaction: <0.001
1RM/BM
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
* ‡
* ‡
† ‡
1st 1RM
2nd 1RM
3rd 1RM
4th 1RM
Sed-Sham
Sed-HF
RT-HF
RT+LLLT-HF
B
Sed-Sham
P = 0.427
Sed-HF
P = 1.00
RT-HF
P < 0.001
RT+LLLT-HF
P < 0.001
1RM/BM
8
6
4
2
1st 1RM
4th 1RM

Figure 4.

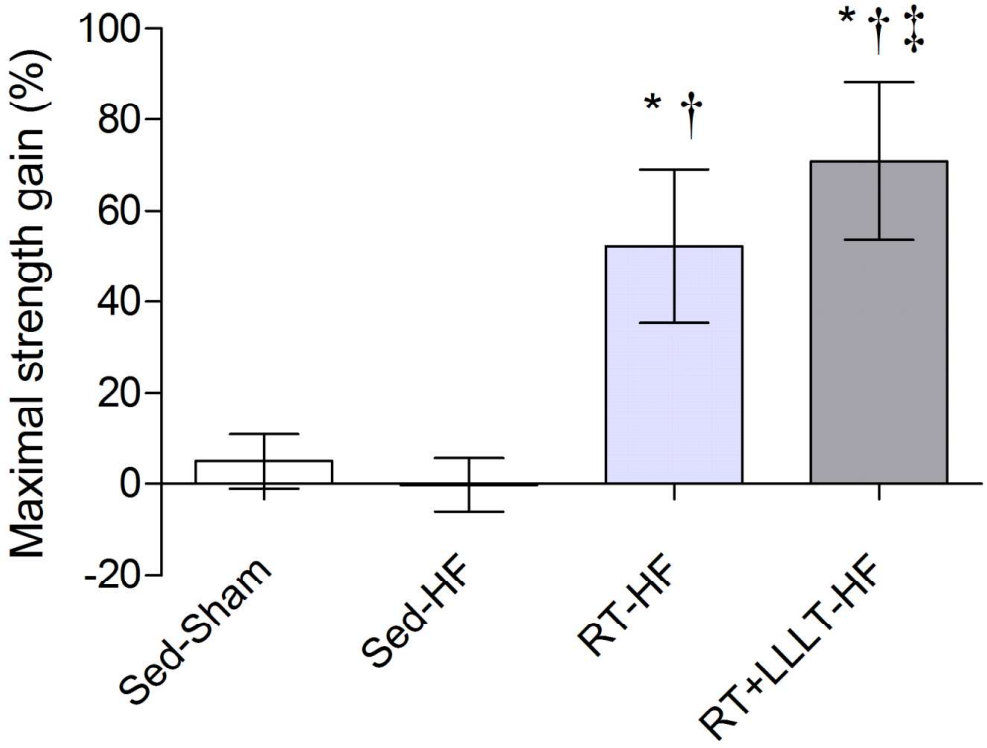

Maximal strength gain (%)
100
80
60
40
20
0
-20
* †
* † ‡
Sed-Sham
Sed-HF
RT-HF
RT+LLLT-HF

Figure 5.

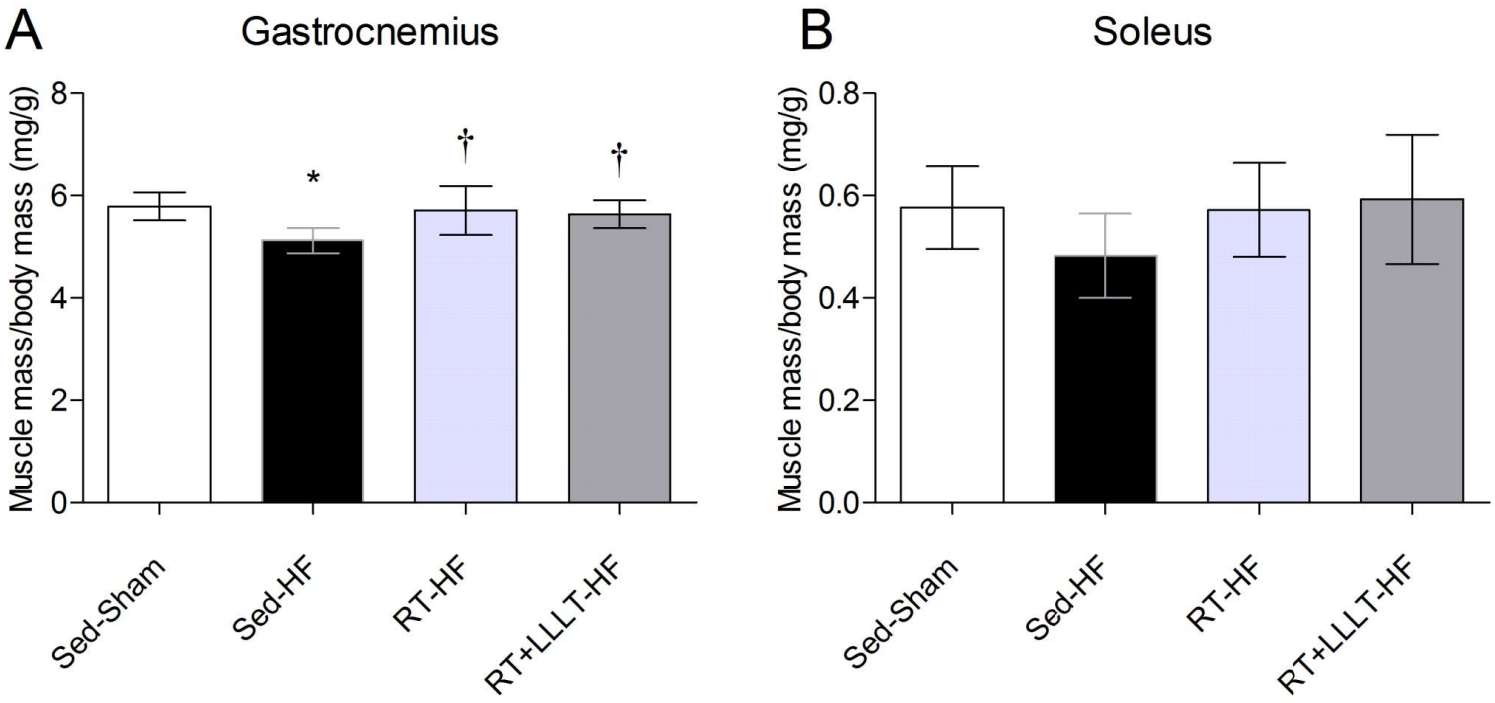
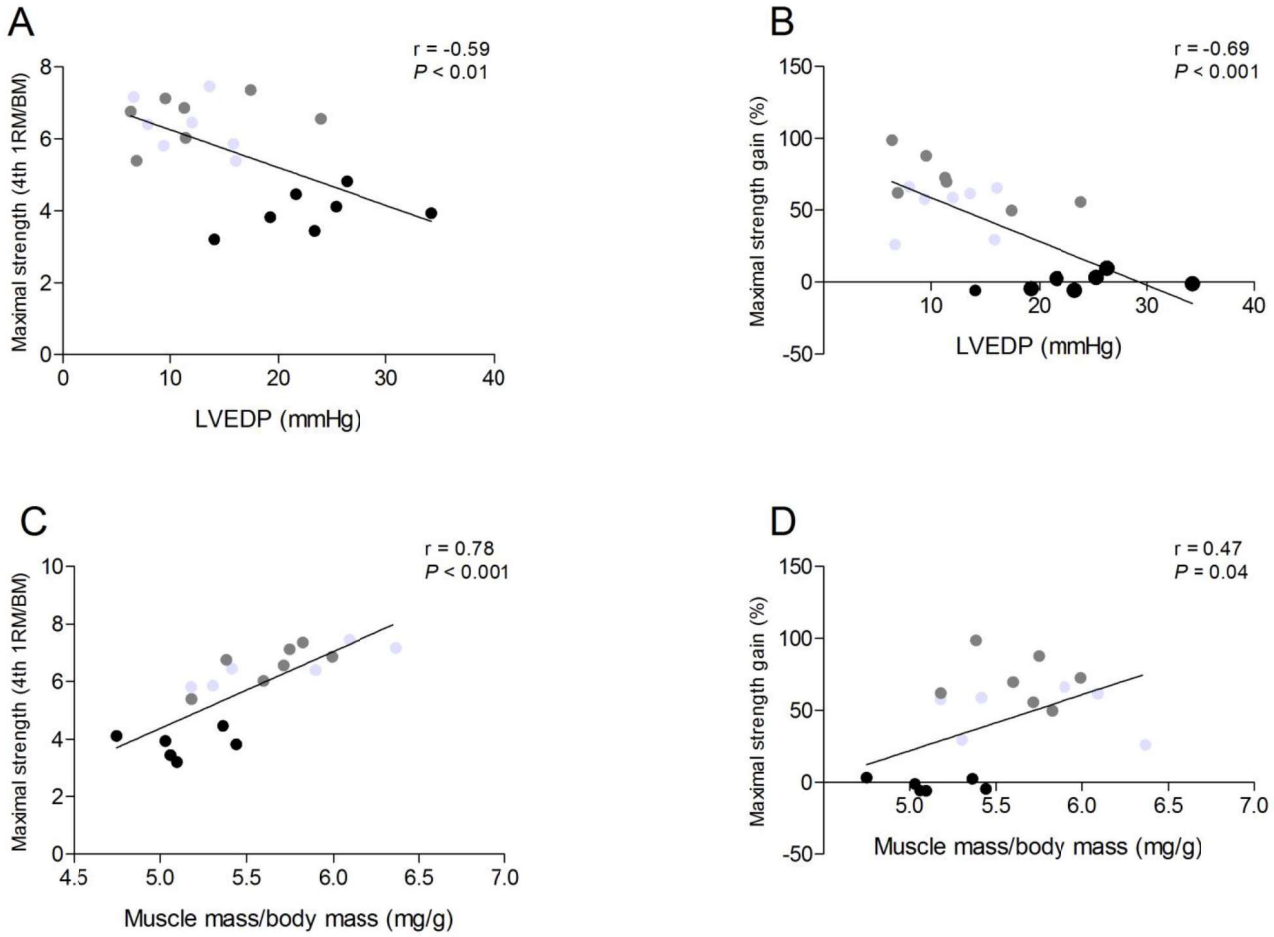

A
Gastrocnemius
Muscle mass/body mass (mg/g)
8
6
4
2
0
*
†
†
Sed-Sham
Sed-HF
RT-HF
RT+LLLT-HF
B
Soleus
Muscle mass/body mass (mg/g)
0.8
0.6
0.4
0.2
0.0
Sed-Sham
Sed-HF
RT-HF
RT+LLLT-HF

Figure 6.


A
r = -0.59
P < 0.01
Maximal strength (4th 1RM/BM)
LVEDP (mmHg)
B
r = -0.69
P < 0.001
Maximal strength gain (%)
LVEDP (mmHg)
C
r = 0.78
P < 0.001
Maximal strength (4th 1RM/BM)
Muscle mass/body mass (mg/g)
D
r = 0.47
P = 0.04
Maximal strength gain (%)
Muscle mass/body mass (mg/g)

8 CONCLUSÕES

A presente tese, por meio de três estudos experimentais inéditos e uma revisão sistemática com meta-análise em animais, conclui que:

- o modelo animal (ratos) de IC induzida por IAM reduz sistematicamente o VO_{2max}, o tempo de permanência em esteira e a distância percorrida quando comparado a ratos controle;

- o VO_2 e a intolerância ao exercício são dependentes da área de infarto do miocárdio e a classificação de ratos baseada no tamanho da área de infarto do miocárdio pode distinguir ratos com comprometimento da capacidade funcional e, assim, diferenciar ratos com IC ou sem IC;

- a TLBI (aplicada na musculatura esquelética) associada ao TF aumenta o VO_{2max} e a tolerância ao exercício (distância percorrida e tempo de permanência em esteira) comparada ao TF isolado em ratos com IC após indução do IAM;

- a TLBI (aplicada na musculatura esquelética) associada ao TF aumenta o ganho de força máximo comparada ao TF isolado em ratos com IC e grande área de IAM.

De forma genérica, nossos resultados mostram que as variáveis de VO_2 e de tolerância ao exercício são dependentes do tamanho do infarto do miocárdio. Assim, animais com grandes tamanhos de infarto (>40%) são um bom modelo para testar novas terapias que possam promover alterações em variáveis de capacidade funcional em ratos primariamente com intolerância ao exercício.

Em destaque, nossos resultados contribuem de forma significativa para o entendimento dos efeitos da TLBI associada ao TF nos parâmetros de capacidade funcional na IC. Nossa hipótese de que a TLBI pode ser adicionada a protocolos de TF com o objetivo de aumentar a força máxima, o VO_{2max} e a tolerância ao exercício na IC, foi comprovada em animais. Estudos clínicos são necessários para testar os efeitos da combinação da TLBI e o TF como uma nova terapia não farmacológica capaz de aumentar a capacidade funcional na IC.

9 ANEXO 1 - PARECER CONSUBSTANCIADO DE PROJETO DE PESQUISA E ENSINO.

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MINISTÉRIO DA EDUCAÇÃO

UFCSPA
UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE

CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

PARECER CONSUBSTANCIADO DE PROJETO DE PESQUISA E ENSINO

1) PROTOCOLO Nº: 059-11 **Parecer 103/11**

2) DATA DO PARECER: 13/12/11 (2º parecer)

3) TÍTULO DO PROJETO:

A influência da terapia laser de baixa intensidade associada a um protocolo de treinamento de força nos biomarcadores inflamatórios, de estresse oxidativo, do metabolismo do óxido nítrico e de ativação do NF-kB e nas variáveis morfométricas no músculo esquelético de ratos com insuficiência cardíaca

4) PESQUISADOR RESPONSÁVEL:

Dr. Pedro Dal Lago

5) SUMÁRIO DO PROJETO:

A insuficiência cardíaca está associada a um estado de ativação imune, de estresse oxidativo, de alteração no metabolismo do óxido nítrico e de alterações na ativação do NF-kB, com importantes repercussões no músculo esquelético. O treinamento de força (TF) tem sido utilizado como estratégia de tratamento para diferentes cardiapatias e a terapia de laser de baixa potencia parece potencializar as ações do exercício físico. Portando iremos avaliar a associação de exercício físico e terapia de laser sobre diferentes biomarcadores.

6) OBJETIVOS DO PROJETO:

Avaliar a influência da terapia laser de baixa intensidade associada a um protocolo de treinamento de força, nos biomarcadores inflamatórios, de estresse oxidativo, do metabolismo do óxido nítrico e de ativação do fator de transcrição NF-kB e nas variáveis morfométricas no músculo esquelético de ratos com insuficiência cardíaca subseqüente ao infarto do miocárdio

7) FINALIDADE DO PROJETO: [ ] Ensino [X] Pesquisa

8) ITENS METODOLÓGICOS E ÉTICOS DO PROJETO:

Título	[X] Adequado	[ ] Comentários

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Introdução	[X] Adequada	[ ] Comentários
Objetivos	[X] Adequados	[ ] Comentários
Relevância e Justificativa	[X] Adequados	[ ] Comentários
Materiais e Métodos	[X] Adequados	[ ] Comentários
Cronograma para execução da pesquisa	[X] Adequado	[ ] Comentários

Cronograma incompreensível. Deve ser reapresentado, pois não apresenta as atividades e nem o período de tempo inicial.

Orçamento e fonte financiadora	[X] Adequados	[ ] Comentários
Referências Bibliográficas	[X] Adequadas	[ ] Comentários

9) O PROJETO ESTÁ ADEQUADO À LEGISLAÇÃO VIGENTE:
[X] Sim [ ] Não

10) INFORMAÇÕES RELATIVAS AOS ANIMAIS:

Grau de dor/estresse: **B** | **C** [ ] **D** [X] **E** [ ]
Justifique:

Espécie: Rattus norvegicus - Wistar **Número Amostral:** 107

Redução Amostral: [ ] Sim [X] Não
Justifique:

Substituição de Metodologia: [ ] Sim [X] Não
Se achar necessário, justifique e sugira uma nova metodologia:

Aprimoramento da Metodologia: [ ] Sim [X] Não
Se achar necessário, justifique e sugira aprimoramentos da metodologia:

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Acomodação e manutenção dos animais: [X] Adequada [ ] Inadequada
Se achar inadequada cite abaixo as melhorias necessárias:

Manipulação dos animais: [X] Adequada [ ] Inadequada
Se achar inadequada cite abaixo as melhorias necessárias:

Analgesia dos animais (se aplicável): [x] Adequada [ ] Inadequada
Se achar inadequada cite abaixo as melhorias necessárias com analgésico substituto:

Anestesia dos animais (se aplicável): [x] Adequada [ ] Inadequada
Se achar inadequada cite abaixo as melhorias necessárias com anestésico substituto:

Eutanásia dos animais (se aplicável): [X] Adequada [ ] Inadequada

Local de Realização (Biotério/Labotarório):
Laboratório de Fisiologia da UFCSPA, salas 10, 12 e 14 do Prédio da Pós-Graduação

11) CRONOGRAMA DE UTILIZAÇÃO DE ANIMAIS

Data	Espécie	Sexo	Quantidade

12) RECOMENDAÇÃO:

[x] Aprovado

[ ] Com Pendência

[ ] Não aprovado

Comentários gerais

Aprovado após inclusão do cronograma solicitado no parecer anterior.

10 ANEXO 2 - NORMAS DA REVISTA *HEART FAILURE REVIEWS*

MANUSCRIPT SUBMISSION

Manuscript Submission

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

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Online Submission

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

TITLE PAGE

Title Page

The title page should include:

- The name(s) of the author(s)
- A concise and informative title
- The affiliation(s) and address(es) of the author(s)
- The e-mail address, telephone and fax numbers of the corresponding author

Abstract

Please provide an abstract of 150 to 250 words. The abstract should not contain any undefined abbreviations or unspecified references.

Keywords

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

TEXT

Text Formatting

Manuscripts should be submitted in Word.

- Use a normal, plain font (e.g., 10-point Times Roman) for text.
- Use italics for emphasis.
- Use the automatic page numbering function to number the pages.
- Do not use field functions.

- Use tab stops or other commands for indents, not the space bar.
- Use the table function, not spreadsheets, to make tables.
- Use the equation editor or MathType for equations.
- Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).

Manuscripts with mathematical content can also be submitted in LaTeX.

- LaTeX macro package (zip, 182 kB)

Headings

Please use no more than three levels of displayed headings.

Abbreviations

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

Footnotes

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

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

Always use footnotes instead of endnotes.

Acknowledgments

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

SCIENTIFIC STYLE

- Please always use internationally accepted signs and symbols for units (SI units).
- Nomenclature: Insofar as possible, authors should use systematic names similar to those used by Chemical Abstract Service or IUPAC.
- Genus and species names should be in italics.
- Generic names of drugs and pesticides are preferred; if trade names are used, the generic name should be given at first mention.
- Please use the standard mathematical notation for formulae, symbols, etc.:

  Italic for single letters that denote mathematical constants, variables, and unknown quantities

  Roman/upright for numerals, operators, and punctuation, and commonly defined functions or abbreviations, e.g., cos, det, e or exp, lim, log, max, min, sin, tan, d (for derivative)

  Bold for vectors, tensors, and matrices.

REFERENCES

Citation

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

1. Negotiation research spans many disciplines [3].
2. This result was later contradicted by Becker and Seligman [5].

3. This effect has been widely studied [1-3, 7].

Reference list

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

The entries in the list should be numbered consecutively.

- Journal article

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

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

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

- Article by DOI

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

- Book

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

- Book chapter

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

- Online document

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

- Dissertation

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

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

- ISSN.org LTWA

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

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

- EndNote style (zip, 2 kB)

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- For vector graphics, the preferred format is EPS; for halftones, please use TIFF format. MSOffice files are also acceptable.
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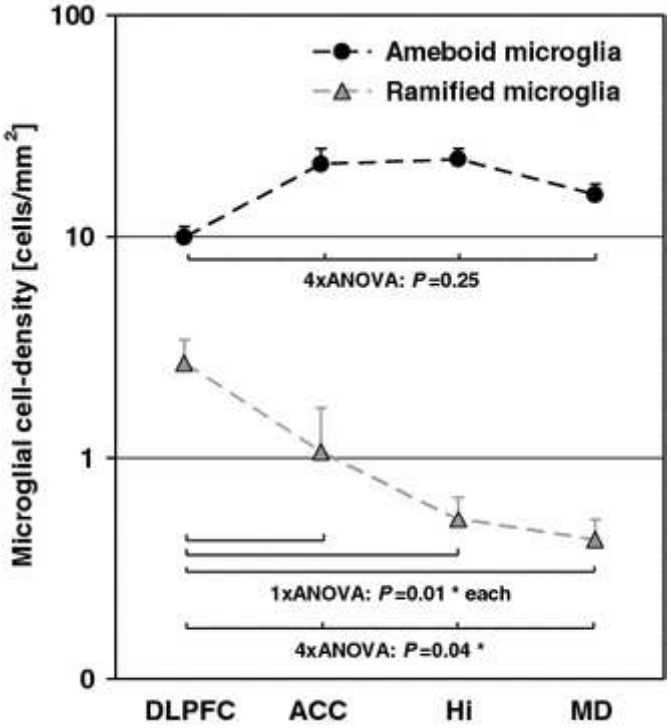

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- Do not use faint lines and/or lettering and check that all lines and lettering within the figures are legible at final size.
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- Scanned line drawings and line drawings in bitmap format should have a minimum resolution of 1200 dpi.
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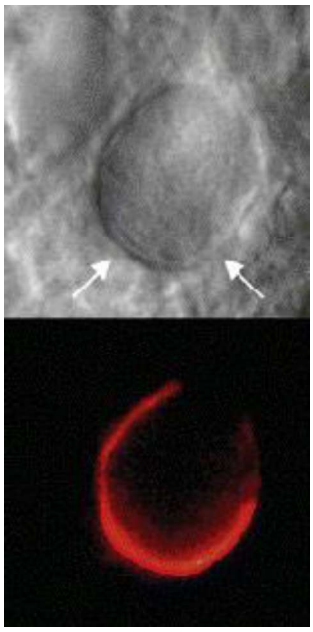

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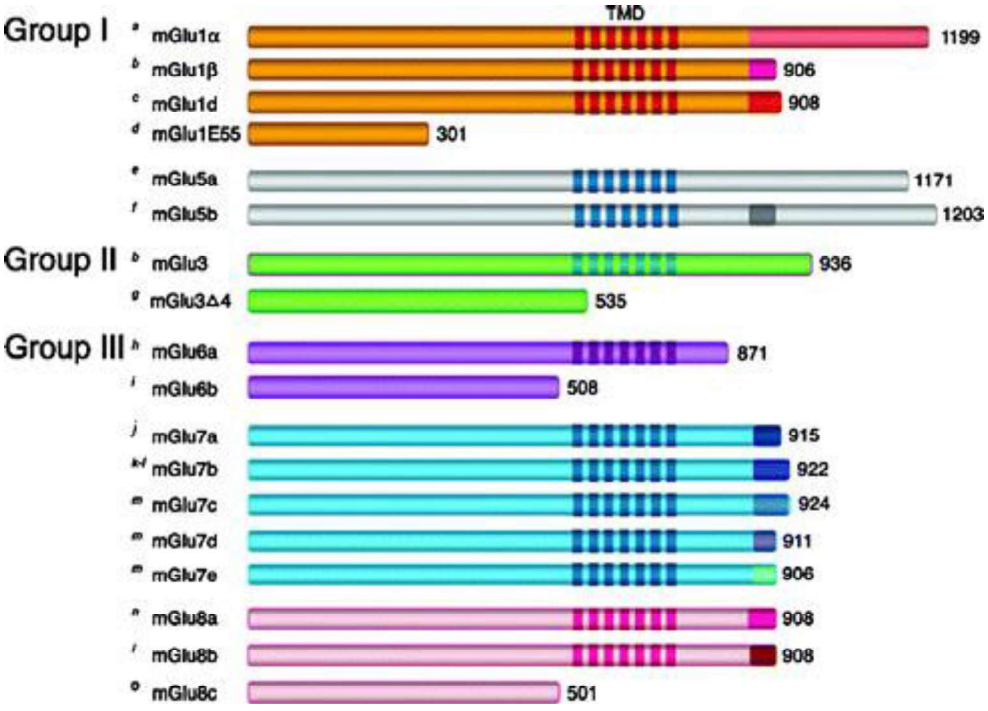

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11 ANEXO 3 - NORMAS DA REVISTA *AMERICAN JOURNAL OF PHYSIOLOGY: HEART AND CIRCULATORY PHYSIOLOGY*

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12 ANEXO 4 - NORMAS DA REVISTA *LASERS IN SURGERY AND MEDICINE*

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C. All submissions where animal subjects are involved must have Institutional Animal Care and Use Committee approval (IACUC) where applicable by country

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Key words used for indexing the article should appear in alphabetical order and should not repeat terms already in the title.

Abstract.

Provide a structured abstract in which the following sections are delineated: Background and Objective: Gives brief overview of the topic and in this context states the main objective of the study; Study Design/Materials and Methods: Describes the basic design, subjects, and scientific methods (for case reports, section title is Study Design/Patients and Methods); Results: Gives main results of the study including confidence intervals and exact level of statistical significance, whenever appropriate; Conclusion: States only those conclusions supported by the data obtained, and, whenever appropriate, the direct clinical application of the findings (avoid speculation). Include the headings Background and Objective, Study Design/Materials and Methods, Results, and Conclusions in the text of the abstract.

Introduction.

The introduction should not be an extensive review of the literature, but only of that portion which is pertinent to the purpose of the study and its relationship to work in the same field.

Materials and Methods.

Materials and methods should be written clearly and in such detail that the work can be duplicated by others. Particular care should be taken in specifying laser wavelength, pulsewidth or exposure duration, focal spot, and treatment energy, power, irradiance, or fluence.

Results.

Results must be described concisely. Text, tables and figures must be consistent and not repetitious.

Discussion.

Discussion should be concise, explaining the significance of the experimental findings and their relation to previous investigations.

References.

List references in consecutive numerical order (not alphabetically). Number references consecutively in the order in which they are first mentioned in the text. Identify references in text, tables, and legends by arabic numerals (in parentheses). Once a reference is cited, all subsequent citations should use the original reference number.

Authors are responsible for bibliographic accuracy and must check every reference in the manuscript and proofread each reference in the page proofs. Abbreviations of journal titles should follow those used in *Index Medicus* . Do not use periods after abbreviations of journal titles. References must contain names of all authors, complete titles, and first and last page numbers. Unpublished observations and personal communications should be cited parenthetically in the text and not be included in the References.

Examples:

Journal Article:
Tan OT, Stafford TJ, Murray S, Kurban AK. Histologic comparison of the pulsed dye laser and copper vapor laser effects on pig skin. Lasers Surg Med 1990; 10:551-558.

Chapter in a Book:
Golenhofen K, Finger K, Foster B, Mandrek K, Noack T. Light-induced relaxation of smooth muscle after treatment with BAY K 8644 is related to release of nitric oxide. In: Sperelakis N, Wood J, eds. Frontiers in Smooth Muscle Research. New York: Wiley-Liss, Inc. 1990:595-604.

Legends.

Legends must be typed double-spaced, beginning on a separate sheet of paper. The legend should allow the illustration to be fully understandable without recourse to the text. Use arrows, letters, etc., for enhanced understanding of features being illustrated.

Tables.

Tables must be typed on separate pages, be numbered in order of appearance with sequential arabic numerals, have a title, and be cited in the text.

Acknowledgments.

Illustrations from other publications must be acknowledged. Include the following when applicable: author(s), title of article, title of journal or book, volume number, page(s), month and year. The publisher's and author's permission to reprint must accompany the manuscript.

Primary Data Retention.

Primary data used in the preparation of articles published in *Lasers in Surgery and Medicine*should be retained in readily accessible and interpretable form.

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Each author of a manuscript submitted to *Lasers in Surgery and Medicine* must have made a significant contribution to the research and must assume responsibility for the content of the article. (See Huth EJ: Guidelines on authorship of medical papers. Ann Intern Med 1986; 104:269-274.)

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13 ANEXO 5 - NORMAS DA REVISTA *CIRCULATION: HEART FAILURE*.

Circulation: Heart Failure will include articles related to research into the pathophysiology, evaluation, and management of heart failure, including observational studies, clinical trials, epidemiology, and advances in applied (translational) research.

Manuscripts are examined by the editorial staff and usually evaluated by expert reviewers assigned by the editors. Both clinical and basic articles will also be subject to statistical review, when appropriate. Provisional or final acceptance is based on originality, scientific content, and topical balance of the journal. Decisions are communicated by email, generally within six weeks. The editors will not discuss a decision about a manuscript over the phone. All rebuttals must be submitted in writing to the editorial office.

- How to Contact the Journal
- How to Prepare a Manuscript
- How to Submit a Manuscript
- Revised Manuscripts
- Accepted Manuscripts
- Permissions and Rights
- Subject Codes
- Journal Policies

How to Contact the Journal:

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Suite 502
Boston MA 02118
Phone: 617-542-5100
Fax: 617-542-6539
E-mail: circ@circulationjournal.org

[Top]

How to Prepare a Manuscript:

Circulation: Heart Failure publishes several types of manuscripts under the umbrella of full-length articles. A brief description of each type follows:

Original Research Articles

Circulation: Heart Failure considers all types of original research articles, including experiments conducted in human subjects, laboratory animals, and in vitro.

Review Series

Please note that the editors invite most review articles. However, unsolicited material will be considered for publication.

- **Advances in Heart Failure:** Reviews will focus on topics of contemporary interest to the clinician and researcher. Overviews of new concepts in pathophysiology, natural history, diagnostic strategies, and treatment approaches will be included in this series. In addition, the series will also include cutting edge reviews of the scientific basis of disease.

- **Development of Therapeutics for Heart Failure:** Articles in this series will focus attention on how therapeutic strategies are developed for heart failure. The series will follow therapeutic development of drugs, devices and regenerative strategies from animal models through human studies, and critically review clinical trial methodologies as well as regulatory issues influencing heart failure therapeutics.
- **Controversies in Heart Failure:** Controversial topics in the practice of heart failure will be presented in this series. Opposite viewpoints will be presented in tandem, with rebuttal responses by both authors included.
- **Challenges for the Basis of Practice:** This series provides a forum for presentation and discussion of decisions arising commonly during the care of individual patients. We invite clinicians whose practice includes heart failure patients to summarize in less than 500 words a difficult clinical situation that requires a decision regarding therapy. As it is anticipated that this situation will have arisen multiple times, the presentation should not describe an individual patient, but rather be as general as possible. A Challenge to Practice article may be submitted by up to 5 clinicians, who can include physicians and/or nurses and will be cited as authors. After selection and initial publication of the article, an expert consultant will be invited to review the relevant literature and his or her own practical approach to the situation. Readers will be invited to submit brief responses, from which representative views will be published.
- **Forum for Early Career Clinical Investigation:** This section is devoted to publishing primary clinical research conceived and performed by early career investigators during training or during the first 3 years on faculty. This research should be performed by the primary author or in some cases an investigative cohort of authors, beginning with the initial articulation of a relevant question, through development of a strategy to address it, the personal collection of the primary data, analysis with rigorous statistical guidance as needed, and thorough interpretation of the results, limitations, and implications. Hypothesis-driven research is ideal, but reasonable questions for these exercises might also be "…what are the reasons given by clinicians in a heart failure clinic for their patients being on lower than target doses of beta-blockers? or "…how many and which patients with heart failure describe thirst as a major symptom?" We would consider publication even without a clear answer to support or refute a hypothesis, if the question and strategy were appropriate and the data and limitations were cogently analyzed. Novelty of the question and the results will be less critical in the evaluation of these manuscripts than for those submitted through the standard journal review process. However, authors are expected to demonstrate familiarity with previous studies in order to describe how their population or strategy differs, and how their results either confirm or challenge what is already known. Submissions should follow the same formatting guidelines as original research submissions but should not exceed 4,500 words in length.

Special Sections

- **Book Reviews:** Reviews of selected books on heart failure, including books that present innovative concepts, books that describe state-of-the-art diagnostic and therapeutic methods or important advances, and textbooks will be reviewed in this section. Unsolicited book reviews will be considered for publication online. In addition, authors or publishers may submit books, as well as a list of suggested reviewers, to the editorial office at the address noted above.
- **Correspondence:** Letters to the Editor, which pertain directly to an article published in the journal within the preceding 8 weeks, will be considered for publication online. A letter must not exceed 500 words in length and must be limited to three authors and five references. They should not have tables or figures. Unpublished observations are not considered legitimate references and letters citing unpublished data will not be accepted. Authors of the original article cited in the letter will be invited to reply. Letters to the editor should be submitted via the online manuscript submission process as described below.
- **Images and Case Reports in Heart Failure:** Clinical or basic science images (including motion studies) that illustrate either important "classic" or novel findings, provide insight into basic mechanisms responsible for cardiovascular disease, emphasize an abnormality, r elucidate a new therapy will be considered for publication in online format. The written portion of the submission should include a title page, descriptive text of no more than two pages with up to 4 references (if appropriate) and a figure legend. Movie clips are encouraged and may be submitted in any of the standard formats (e.g. avi, mov, etc) and codecs.
- ***Please note that all manuscripts must conform to one of the above article types.***

General Preparation Instructions:

- Maximum Word Length: 6000 words
- Word Count includes title page, abstract, text, references, tables, and figure legends

- Maximum Number of References: 50
- Maximum Number of Figures and Figure Legends: 8
- Manuscript should be typed double-spaced, including title page, abstract, text, references, figure legends, and tables. Text should only appear on one side of the page. Acceptable formats are Word or WordPerfect.
- Leave a 1-inch margin on all sides. Do not use justified margins.
- Cite references, figures, and tables in numeric order. For review, acceptable figure formats are GIF, TIFF, EPS, JPEG, and single slides of Power Point.
- Formats NOT supported are as follows: Object Linking and Embedding (OLE), Bitmap (.bmp), PICT (.pict), Excel (.xls), Photoshop (.psd), Canvas (.cnv), CorelDRAW (.cdr), and locked or encrypted PDFs. For publication, see acceptable figure requirements under "Accepted Manuscripts " below.
- Use SI units of measure. A more conventionally used measurement may follow in parentheses. Make all conversions before manuscript submission.
- Please provide sex-specific and/or racial/ethnic-specific data when appropriate, in describing the outcomes of epidemiologic analyses or clinical trials; or specifically state that no sex-based or racial/ethnic-based differences were present.
- Consult the American Medical Association Manual of Style, 9th ed, Baltimore, Md, Williams & Wilkins, 1998, for style.
- Manuscripts must conform to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals"http://www.icmje.org/.
- Assemble the manuscript in this order: Title Page, Abstract, Text, Acknowledgments, Funding Sources, Disclosures, References, Figure Legends, Tables, and Figures.

Title Page

- The title page (page 1, do not number) should contain these elements:
  1. Full title
  2. First author's surname and short title (not to exceed 50 characters, including spaces)
  3. Authors' names, academic degrees, and affiliations
  4. Name and complete address for correspondence (include street name and address as well as post office box, and address for reprints if different from correspondence)
  5. Fax number, telephone number and email address
  6. The total word count of the manuscript, including the title page, abstract, text, references, tables and figures legends
  7. The Journal Subject Codes pertaining to the article. Please refer to the subject code list.

Abstract and Key Words

- Do not cite references in the abstract
- Limit use of acronyms and abbreviations. Define at first use acronym or abbreviation in parenthesis.
- Be concise (250 words maximum)
- Use the following headings:
  1. Background - rationale for study
  2. Methods and Results - brief presentation of methods and presentation of significant results; please include sample size
  3. Conclusions - succinct statement of data interpretation
  4. When applicable, include a fourth heading: "Clinical Trial Registration". Please list the URL, as well as the Unique Identifier, for the publicly accessible website on which the trial is registered.
- Insert three to five Key Words after abstract. Please refer to the key word list.

Text

- Typical main headings include Methods, Results, and Discussion
- Number pages
- Abbreviations must be defined at first mention

Methods

- **Please note that the manuscript version of the Methods and Results should be able to stand alone and should provide sufficient information for the reader to understand the basic methods of the study and to review the fundamental findings in a mechanistic way.**
- Experimental animals: State the species, strain, number used, and pertinent descriptive characteristics. When describing surgical procedures, identify the preanesthetic and anesthetic agents used and the amounts, concentrations, routes, and frequency of administration of each. Paralytic agents are not considered acceptable substitutes for anesthetics. For other invasive procedures on animals, report the analgesic or tranquilizing drug used. If none were used, provide justification for exclusion.
- Human studies: Indicate that the study was approved by an institutional review committee and that the subjects gave informed consent.
- Drugs and Devices: In the Methods, the complete name and location of the manufacturer must be supplied for all reagents, equipment, and devices used. In all other instances, the generic rather than trademark names of all drugs and devices.
- Independent Data Access and Analysis: The Editors consider it preferable for investigators to have direct access to the primary data in a clinical trial (raw and derived datasets) when reporting results of the trial. Alternatively, an independent party with an academic affiliation who has access to the primary data may serve as the analyst for the investigators. It is recognized that for logistical reasons these options may not be possible in all instances. At a minimum, the authors should have the ability to query any aspect of the data either directly or through an independent analysis. However, the Editors reserve the right to ask for additional information from the corresponding author regarding measures that were taken to minimize bias and verify the integrity of the primary data and any analyses performed.

Guidelines for Clinical Trials

- In accordance with the Clinical Trial Registration Statement from the International Committee of Medical Journal Editors (Circulation. 2005;111:1337) and (http://content.nejm.org/cgi/content/full/NEJMe078110), all clinical trials in ***Circulation: Heart Failure*** must be registered in a public trials registry at or before the onset of participant enrollment. This requirement applies to all clinical trials that began enrollment after July 1, 2005.
- Research is considered to be a clinical trial if it involves prospective assignment of human subjects to an intervention or comparison group to study the relation between a health-related intervention and a health outcome. Health-related interventions include any intervention used to modify a biomedical or health-related outcome (for example, drugs, surgical procedures, devices, behavioral treatments, dietary interventions, and process-of-care changes). Health outcomes include any biomedical or health-related measures obtained in patients or participants, including pharmacokinetic measures and adverse events. As previously, purely observational studies (those in which the assignment of the medical intervention is not at the discretion of the investigator) will not require registration. ***We will start to implement the expanded definition of clinically directive trials for all trials that began enrollment on or after July 1, 2008. Those who are uncertain whether their trial meets the expanded ICMJE definition should err on the side of registration if they wish to seek publication.***
- The registry must be accessible to the public at no charge, searchable, open to all prospective registrants, and managed by a not-for-profit organization. The registry must include the following information: a unique identifying number, a statement of the intervention(s), study hypothesis, definition of primary and secondary outcome measurements, eligibility criteria, target number of subjects, funding source, contact information for the principal investigator, and key dates (registration date, start date, and completion date). **The registry sponsored by the United States National Library of Medicine (http://www.clinicaltrials.gov) meets these requirements and is recommended by the editors.**
- Other registries are acceptable if they meet these requirements. In addition to **www.clinicaltrials.gov**, the following registries are recommended by the ICMJE:
    1. http://isrctn.org
    2. http://www.umin.ac.jp/ctr/index/htm/
    3. http://www.anzctr.org.au/Default.aspx
    4. http://www.trialregister.nl/trialreg/index.asp
- In accordance with the ICMJE's recommendation, we will also accept registration of clinical trials in any of the primary registers that participate in the World Health Organization's International Clinical Trial Registry Platform. Primary registers are WHO selected registers managed by not-for-profit entities that will accept

registrations for any interventional trials, delete duplicate entries from their own register, and provide data directly to the WHO. Please note that registration in any WHO partner registers is insufficient.

- The authors will be requested to provide the exact URL and unique identification number for the trial registration at the time of submission. Since this information will be published, we ask that you include a fourth heading in your abstract: "Clinical Trial Registration Information". Please list the URL, as well as the unique identifier, for the publicly accessible website on which the trial is registered in this section.
- Clinical trial reports should also comply with the Consolidated Standards of Reporting Trials (CONSORT) and include a flow diagram presenting the enrollment, intervention allocation, follow-up, and data analysis with number of subjects for each (http://www.consort-statement.org/?o=1011). Please also refer specifically to the CONSORT Checklist of items to include when reporting a randomized clinical trial.
- Results posted in the same clinical trials registry in which the primary registration resides will not be considered prior publication if they are presented in the form of a brief abstract (500 words or less) or a table.

Guidelines for Meta-Analyses

- See "Meta-analysis of Observational Studies in Epidemiology: A Proposal for Reporting," *JAMA*. 2000; 283: 2008-2012.

Guidelines for Studies on Diagnostic Tests

- See "The STARD Statement for Reporting Studies of Diagnostic Accuracy: Explanation and Elaboration," *Ann Intern Med*. 2003; 138: 40-44.

Guidelines for Human Phenotype-Genotype Association or Linkage Studies

- Reporting issues:
  1. Report process for selecting genes and SNPs.
  2. Report Hardy-Weinberg statistics or p-values and method of calculating same.
  3. Refer to existing public domain websites for the Human Gene Ontology name and the rs number for SNPs:http://www.ncbi.nlm.nih.gov/projects/SNP/ and http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Snp
  4. Describe genotyping methods. If numerous primers have been used, please include them in an online supplement.
- False positive and false negative concerns. Given well-described problems with both false positive and false negative associations, phenotype-genotype association studies should meet some or all of the criteria below:
  1. Phenotype is clearly defined, is heritable, and if a quantitative phenotype is reported, reproducibility data are provided.
  2. The sample size is adequate to detect a SNP or haplotype with a modest effect. For genotype-trait associations, provide an estimate of the effect size that could be detected with power 0.80 or higher with the allele frequency and sample size reported.
  3. Since multiple statistical testing methods are frequently used in genotyping-phenotyping studies, please include specifics of the primary model(s) tested. Nonessential secondary models may be published as electronic data supplements. Clinically relevant confounders should be included in multivariable models or residuals.
- Review criteria for human linkage studies. Manuscripts should include the following:
  1. Identifying plausible candidate genes under the linkage peak.
  2. Follow-up fine mapping to narrow the region of linkage, and/or genotyping some of the candidate genes under the linkage peak.
  3. Replication data from another sample.

Guidelines for Genomic and Proteomic Studies

- Preparation of Data Submitted: Data should follow the MIAME checklist (for more information seehttp://www.mged.org/Workgroups/MIAME/miame_checklist.html).
- Accessibility of Data: Authors of papers that include genomic, proteomic, or other high-throughput data are required to make their data easily accessible for the reviewers and the editors during the review process.
- You may submit your data to the NCBI gene expression and hybridization array data repository (GEO,http://www.ncbi.nlm.nih.gov/geo/) and provide the GEO accession number; or,

- You may provide a link to a secure or publicly accessible website which hosts the data. Prior to publication, the data must be submitted and an accession number obtained. Access to the information in the database must be available at the time of publication. GEO has a web-based submission route, suitable for a small number of samples, or a batch submission tool (called SOFT). GEO is accessible from http://www.ncbi.nlm.nih.gov/geo/. The submission FAQ is available at http://www.ncbi.nlm.nih.gov/projects/geo/info/faq.html.

Guidelines for Proteins and Nucleic Acid Sequences

- Newly reported nucleotide or protein sequences must be deposited in GenBank or EMBL databases, and an accession number must be obtained. Access to the information in the database must be available at the time of publication. Authors are responsible for arranging release of data at the time of publication. The authors must also provide a statement in the manuscript that this sequence has been scanned against the database and all sequences with significant relatedness to the new sequence identified (and their accession numbers included in the text of the manuscript).
- **GenBank**
  GenBank Submissions
  National Center for Biotechnology Information
  8600 Rockville Pike, Building 38A
  Room 8N-805
  Bethesda, MD 20894
  Tel: (301) 496-2475
  On the web at: http://www.ncbi.nlm.nih.gov/Genbank/index.html
- **EMBL Nucleotide Sequence Submissions**
  European Bioinformatics Institute
  Hinxton Hall
  Hinxton, Cambridge CB10 1SD, UK
  Tel.: 44-1223-494401; Fax: 44-1223-494472
  e-mail: support@ebi.ac.uk
  On the web at: http://www.ebi.ac.uk
- **DNA Data Bank of Japan**
  Center for Information Biology
  National Institute of Genetics
  Mishima, Shizuoka, 411, Japan
  Tel.: 81-559-81-6853; Fax: 81-559-81-6849
  On the web at: http://www.ddbj.nig.ac.jp
- **Submission to any data bank is sufficient to ensure entry in all.**

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Disclosures

- All potential conflicts of interest must be stated within the text of the manuscript, under this heading. This pertains to relationships with pharmaceutical companies, biomedical device manufacturers, or other corporations whose products or services are related to the subject matter of the article. Such relationships include, but are not

limited to, employment by an industrial concern, ownership of stock, membership on a standing advisory council or committee, being on the board of directors, or being publicly associated with the company or its products. Other areas of real or perceived conflict of interest could include receiving honoraria or consulting fees or receiving grants or funds from such corporations or individuals representing such corporations. ***Please state "None" if no conflicts exist.***

References

- Accuracy of reference data is the responsibility of the author
- Verify all references against original sources
- List all authors for each reference; do not use "et al. "
- Example reference: 1. Smith HJ, Allen S, Yu W, Fard S. This is the title. Circulation. 2004; 104:276-308.
- *Please note that if you use reference software tools (e.g. EndNote or Reference Manager), they do not always match our style and you may need to manually correct your references.*.
- Cite references in numeric order according to first mention in the text. In the text, ensure accuracy of spelling and details of publication, i.e., the text citation should match the reference information.
- Personal communications, unpublished observations, and submitted manuscripts are not legitimate references. They must be cited in the text only (not in the reference list) as follows: author name, degree(s) held, unpublished data, year.
- Abstracts may be cited only if they are the sole source and must be identified in the reference as "Abstract."
- References must be from a full length publication in a peer reviewed journal.
- "In press" citations must have been accepted for publication and the name of the journal or book publisher must be included.

Figures

- Figure parts should be clearly labeled. Letters and locants must be uniform in size and style within each figure, and when possible, between figures. (The font size must be 10 point or higher.)
- Avoid headings on the figure. Heading information should appear in the figure legend.
- Line art should not contain hair lines, which are hard to reproduce
- Supply a scale bar with photomicrographs
- Provide double-spaced copy for figure legends on a separate page
- Symbols and abbreviations must be defined in the figure or its legend
- Limit white space between the panel and panel label
- Figures should be sized as close as possible to their final publication size. Please note that very few figures qualify for a 2-column format.

Tables

- Begin each table on a separate page, double-spaced. Please remember that tables prepared with Excel are not accepted unless embedded within your text document.
- The table number should be Arabic, followed by a period and brief title
- Use same size type as in text
- Supply a brief heading for each column
- Indicate footnotes in this order: *, †, ‡, §, | |, #, * *
- Do not use vertical lines between columns. Use horizontal lines above and below the column headings and at the bottom of the table only. Use extra space to delineate sections within the table.
- Abbreviations used in the table must be defined in a footnote to the table.

Online Data Supplements

- Online Data Supplements are encouraged as an enhancement to the manuscript Methods section. This optional section provides an opportunity to present supporting materials to the manuscript. Please note that all supplements undergo peer review and must be submitted with the original submission of the manuscript.
- Online Data Supplements can consist of the following:
    1. Expanded Methods and Results
    2. Additional Figures
    3. Additional Tables

4. Video Files

- If citations are made in an Online Data Supplement, the supplement must contain its own Reference Section, with references numbered sequentially beginning with the number 1. Please try to keep the individual file size to 10 MB or less to facilitate easier downloading for readers.
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Supplemental Materials Required for Review

- A copy of all submitted manuscripts mentioned in the article must be submitted as part of the review process
- A copy of all manuscripts, either in preparation or submitted, that potentially overlap with your ***Circulation: Heart Failure*** submission. Please note that failure to include such material is a violation of the journal's Ethical Policy, below.
- A copy of all in press articles cited in the Reference section must be supplied for review by the editors and the reviewers

How to Submit a Manuscript:

General Online Submission Information -

All manuscripts must be submitted electronically. Before proceeding to the online submission site, please prepare your manuscript according to the instructions listed above under the heading, "How to Prepare a Manuscript." When your manuscript is ready for submission, please go to http://circheartfailure-submit.aha-journals.org. The instructions below are meant to help guide you through the submission process.

- Submission Files:
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  - Tables – either embedded within the manuscript or submitted separately. Please note that, for publication purposes, all tables must be provided in an editable text format. Excel or PDF files will not be accepted.
  - Figures – either embedded or submitted separately. Please note that only PPT, EPS, or TIFF files will be allowed for publication.
  - Supplemental Files: When submitting supplemental files, please note whether or not they are intended for review purposes only or if they are intended for publication as an online data supplement.
  - For all revisions, invited de novo submissions, and uninvited resubmissions, please note that three additional files are required:
    - Response to Reviewers
    - Tracked Changes version of the manuscript
    - Clinical Perspective Summary
- All Author Information, including
  - First and Last Names
  - Affiliations
  - E-mail Addresses

Submission Process

The four steps of the submission process are: Files, Manuscript Information, Validate, and Submit. The four steps each contain sub-steps that can be accessed by clicking on their respective tabs. Navigating through this "Tab View" will save any entered information each time a new tab is clicked (or the boxes "Save and Continue" and "Next" are clicked). Each step and sub-step is listed below:

- Title and Running Title (you can cut and paste this from your manuscript).Please note that you will not be allowed to exceed a 50 character limit for the short title.
- Abstract (you can cut and paste this from your manuscript) – For Original Research articles only.
- Subject Codes, Key Words, and Categories (Original Articles only) – A minimum of 1 subject code and key word is required. Only 1 category may be selected; please note that, if accepted, your manuscript will be published under the category selected here.

- Clinical Trial Registration – All clinical trials should have been registered prior to patient enrollment. If your manuscript is considered a clinical trial but has not been registered, it will not be considered for publication in the journal. You will be asked to provide the URL and identification number for the trial registration.
  - Files
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