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

Rodrigo Boemo Jaenisch

Efeitos do Treinamento Muscular Ventilatório na Função Hemodinâmica, na Mecânica Respiratória, no Quimiorreflexo Periférico, na Variabilidade da Frequência Cardíaca e no Dano em DNA de Ratos com Insuficiência Cardíaca

Porto Alegre
2015

Rodrigo Boemo Jaenisch

Efeitos do Treinamento Muscular Ventilatório na Função Hemodinâmica, na Mecânica Respiratória, no Quimiorreflexo Periférico, na Variabilidade da Frequência Cardíaca e no Dano em DNA de Ratos com Insuficiência Cardíaca

Tese apresentada ao Programa de Pós-Graduação em Ciências da Saúde da Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, como requisito para a obtenção do grau de Doutor.

Orientador: Prof. Dr. Pedro Dal Lago

Porto Alegre
2015

Catalogação na Publicação

Boemo Jaenisch, Rodrigo
Efeitos do Treinamento Muscular Ventilatório na Função Hemodinâmica, na Mecânica Respiratória, no Quimiorreflexo Periférico, na Variabilidade da Frequência Cardíaca e no Dano em DNA de Ratos com Insuficiência Cardíaca / Rodrigo Boemo Jaenisch. -- 2015.
115 p. : 30 cm.

Tese (doutorado) -- Universidade Federal de Ciências da Saúde de Porto Alegre, Programa de Pós-Graduação em Ciências da Saúde, 2015.

Orientador(a): Pedro Dal Lago.

1. Insuficiência cardíaca. 2. Treinamento muscular ventilatório. 3. Infarto do miocárdio. 4. Estresse oxidativo. 5. DNA. I. Titulo.

Sistema de Geração de Ficha Catalográfica da UFCSPA com os dados fornecidos pelo(a) autor(a).

AGRADECIMENTOS

À minha família, **Mãe, Pai** e **Irmãs**, muito obrigado por compartilhar o amor, ensinar a lealdade e a sinceridade, assim como a vontade de superar os desafios.

À minha noiva **Joanna Tatith Pereira**. Palavras não descrevem o amor e a gratidão que sinto. Fomos feitos um para o outro, desde a primeira dança**.**

À **família Tatith Pereira**, pelo carinho, torcida, amizade e companheirismo.

Agradeço ao meu orientador Professor **Pedro Dal Lago**, pela oportunidade, ensinamentos e incentivo.

Em especial, agradeço aos meus colegas e amigos **Candissa Silva, Edson Quagliotto, Giuseppe Potrick, Jadson Alves, Leonardo Calegari, Luis Fernando Deresz, Marlus Karsten e Ramiro Barcos Nunes.** Muito obrigado pelos ensinamentos diários.

Aos alunos de iniciação científica **Camila Durante, Lucas Krolikowski, Betina Foscarina** e, em especial, à **Chalyne Chechi.** O auxílio de vocês foi fundamental para o desenvolvimento deste trabalho.

Ao **GPIC** pelo aprendizado e convívio.

Ao Professor **Alberto Rasia Filho**, por ser fonte inspiradora de conhecimento e integridade.

À Professora **Cláudia Ramos Rhoden** e aos seus alunos do Laboratório de Poluição Atmosférica, pelo companheirismo e troca constante.

Ao laboratório de Patologia da UFCSPA, especialmente à **Teresinha Stein, Rosalva Theresa Meuer** e à Professora **Marilda da Cruz Fernandes** pelo suporte técnico.

À querida **Inês** pelo apoio técnico.

À secretaria e aos funcionários da pós-graduação, especialmente à **Luciane Spencer, Maristela Pasin** e **Cristiane Mondadori.**

Aos meus colegas, amigos e incentivadores da **ABRAFICA**, **Michel Silva Reis, Solange Guizilini, Vera Lúcia dos Santos Alves, Iracema Ioco Kikuchi Umeda, Renata Trimer, Valéria Papa, Aparecida Maria Catai** e **Robison Quitério.**

Aos meus irmãos do peito **Everton dos Santos, Fábio Zanoni, Fabrício Beck, Felipe Scherer, Gustavo Barth, Leandro Pahim**, **Leandro Sauer**, **Luciano Gabbi**, **Maurício Hansel, Pedro Bajotto Filho, Renan Stein e Tiago Gonçalves**, que apesar da distância, sempre estão presentes nas minhas conquistas.

Aos queridos amigos **Cláudio Paiva**, **Rodrigo Zanotta** e **Emerson Boschi**.

Aos professores que compuseram a banca examinadora, **Antônio Marcos Vargas da Silva**, **Audrey Borghi e Silva**, **Jenifer Saffi** e **Marlus Karsten**. Muito obrigado pela gentileza e generosidade.

À minha querida Professora e amiga **Maria Elaine Trevisan**, pelos conselhos tão fraternos e torcida constante.

¨Aplico meu coração no
caminho, baseio-me na virtude,
confio na benevolência para
apoio e encontro entretenimento
nas artes.¨

Confúcio (551-479 a.C.)

Resumo

Introdução: A insuficiência cardíaca (IC) é uma síndrome clínica resultante de inúmeras doenças que afetam o coração, tornando-o incapaz de manter níveis adequados de suprimento sanguíneo para os tecidos. O treinamento muscular ventilatório (TMV) contribui para a melhora de desfechos clínicos relacionados às manifestações fisiopatológicas da IC. Contudo, alguns mecanismos fisiológicos que possam explicar os potenciais efeitos do TMV na IC ainda precisam ser esclarecidos.
Objetivos: Testar os efeitos do TMV sobre a função hemodinâmica, o dano ao DNA, o quimiorreflexo periférico, a variabilidade da frequência cardíaca e a mecânica respiratória em ratos com IC.
Métodos: Foram realizados dois estudos experimentais. Ratos Wistar machos (250-290g) foram alocados em: grupo *sham* - sedentários (Sham-Sed; n=8); grupo *sham* com TMV (Sham-TMV; n=8); grupo IC - sedentários (IC-Sed; n=8) e grupo IC com TMV (IC-TMV; n=8). Os animais treinados foram submetidos a um protocolo de TMV (30min/dia, 5 dias/semana, durante seis semanas), enquanto os sedentários não realizaram o protocolo. Os dados foram comparados pelo teste ANOVA de duas vias, seguida pelo *post hoc* de Tukey (α=5%).
Resultados: O TMV em ratos com IC promoveu a redução da congestão pulmonar, da pressão diastólica final do ventrículo esquerdo, da hipertrofia do ventrículo direito e do dano ao DNA no músculo diafragma. Além disso, reduziu a resposta pressórica quimiorreflexa e melhorou a função simpatovagal, verificada pela redução da modulação simpática e do balanço simpatovagal, e aumento da modulação parassimpática em ratos com IC. Ainda, o TMV reduziu a resistência do sistema respiratório e tecidual, a elastância do sistema respiratório, tecidual e estática em ratos com IC.
Conclusões: O protocolo de TMV, com a duração de seis semanas, promoveu a melhora na função hemodinâmica e reduziu a genotoxicidade no músculo diafragma de ratos com IC. Além disso, o TMV em ratos com IC melhorou a resposta pressórica quimiorreflexa, a atividade simpatovagal, assim como a mecânica respiratória em ratos com IC.
Palavras-chave: insuficiência cardíaca, treinamento muscular ventilatório, mecânica respiratória, quimiorreflexo, atividade simpática, dano ao DNA.

Abstract

Introduction: Heart failure (HF) is a syndrome resulting from the inability of the cardiac pump to meet the energy requirements of the body. Respiratory muscle training (RMT) has been shown to improve clinical outcomes related to pathophysiological manifestations of HF. However, some physiological mechanisms need to be clarified.
Objectives: To test the effects of RMT on the hemodynamic function, DNA damage, peripheral chemoreflex, heart rate variability and respiratory mechanics of rats with HF.
Methods: Two experimental studies were performed. Wistar rats were allocated into four groups: sedentary sham (Sed-Sham, *n*=8), trained sham (RMT-Sham, *n*=8), sedentary HF (Sed-HF, *n*=8) and trained HF (RMT-HF, *n*=8). The animals underwent a respiratory muscle training protocol performed a 30 min training per day, 5 days/wk and 6 wks, whereas sedentary animals did not exercise. Groups were compared by a two-way ANOVA and Tukey´s post hoc tests (α = 5%).
Results: In rats with HF, RMT promoted reduction in pulmonary congestion, left ventricular end diastolic pressure, right ventricular hypertrophy and DNA damage on diaphragm. Moreover, reduction in pressure response during chemoreflex activation and improvement in autonomic function, as verified by the reduced sympathetic modulation, reduction in sympathetic-vagal balance and increase parasympathetic modulation after RMT in rats with HF. Furthermore, respiratory mechanics was enhanced in RMT-HF group, demonstrated by reduction in the respiratory system resistance, tissue resistance, respiratory system elastance, tissue elastance and quasistatic elastance.
Conclusions: These findings show that a 6-wk of RMT protocol in rats with HF promotes an improvement in hemodynamic function, respiratory mechanics, peripheral chemoreflex and sympathetic and parasympathetic activity. Also, there is a reduction of diaphragm DNA damage in HF rats.
Keywords: heart failure, respiratory muscle training, respiratory mechanics, chemoreflex, sympathetic activity, DNA damage.

Lista de figuras dos estudos

Estudo 1

Estudo 2

Lista de tabelas dos estudos

Estudo 1

Estudo 2

Lista de abreviaturas da contextualização

AP	Arterial pressure
BP	Blood pressure
BRS	Baroreflex sensitivity
BW	Body weight
DAP	Diastolic arterial pressure
DVC	Doença cardiovascular
$+dP/dt_{max}$	Left ventricular maximum change in pressure over time
$-dP/dt_{max}$	Left ventricular minimum change in pressure over time
DP6	Distância percorrida em 6 minutos
ECR	Ensaio clínico randomizado
EF	Exercício físico
ERO	Espécies reativas de oxigênio
Ers	Respiratory system elastance
Est	Static elastance
Gti	Tissue resistance
HF	Heart failure
HR	Heart rate
HRV	Heart rate variability
Hti	Tissue elastance
HW	Heart weight
HW/BW	Heart weight-to-body weight ratio
I_{aw}	Inertance
IC	Insuficiência cardíaca
LF	Low frequency
LF/HF	Sympathetic-vagal balance
LVEDP	Left ventricular end-diastolic pressure
LVSP	Left ventricular systolic pressure
MAP	Mean arterial pressure
MI	Myocardial infarction
MtDNA	Mitochondrial DNA
NU	Normalized units
OTM	Olive tail moment

peakVO_2	oxygen consumption peak
PI	Pulse interval
$PI_{máx}$	Pressão inspiratória máxima
R_{aw}	Airway resistance
RCV	Reabilitação cardiovascular
RMT	Respiratory muscle training
ROS	Reactive oxygen species
RV	Right ventricle
SAP	Systolic arterial pressure
SBP	Systolic arterial blood pressure
SNS	Sympathetic nervous system
TM	Tail moment
TMI	Treinamento muscular inspiratório
TMV	Treinamento muscular ventilatório
VO_2máx	Consumo máximo de oxigênio
Zrs	Respiratory system impedance

Anexos

SUMÁRIO

CONTEXTUALIZAÇÃO

1. CONTEXTUALIZAÇÃO

Apesar dos grandes avanços no tratamento de praticamente todas as desordens cardíacas, a insuficiência cardíaca (IC) é uma exceção, visto que a sua prevalência vem aumentando com o passar dos anos e pouco se tem evoluído frente à sobrevivência de pacientes acometidos por essa síndrome (Braunwald, 2013).

A IC é uma síndrome clínica complexa, que resulta da deficiência estrutural ou funcional do enchimento ou ejeção ventricular de sangue. As manifestações cardinais da IC são a dispneia e a fadiga, que podem limitar a tolerância ao exercício, e a retenção de líquidos, que pode gerar a congestão pulmonar, esplânica e o edema periférico (Yancy *et al.*, 2013). Essas alterações causam sintomas depressivos e baixa qualidade de vida em pacientes com IC (Lesman-Leegte *et al.*, 2009).

A IC é uma pandemia global que afeta um número estimado de 26 milhões de pessoas em todo o mundo, e que resulta em mais de 1 milhão de hospitalizações ao ano nos Estados Unidos e na Europa (Ambrosy *et al.*, 2014). É a segunda causa mais comum de internações hospitalares (Roger *et al.*, 2012) e relaciona-se com mais de 275.000 mortes por ano (Joffe *et al.*, 2013). No Brasil, a IC representa a principal causa de internação no Sistema Único de Saúde, a partir dos 65 anos (Araujo *et al.*, 2005).

Sabe-se que 70% dos custos gerados por pacientes com IC são produzidos em suas reinternações nas unidades hospitalares e que, frequentemente, acontecem não somente pela evolução da síndrome, mas pela baixa adesão ao tratamento proposto, seja farmacológico ou não-farmacológico (Dickstein *et al.*, 2008).

A visão tradicional de que a IC seja um conjunto de sinais e sintomas causados pelo inadequado funcionamento cardíaco, concentra-se em apenas um aspecto da fisiopatologia envolvida nessa síndrome. Atualmente, o cenário visto na progressão da IC se forma por meio do somatório de alterações estruturais, funcionais e biológicas dos sistemas músculo esquelético, respiratório e nervoso que, somadas as do sistema cardiovascular estão envolvidas na diminuição da capacidade funcional de indivíduos com IC (Jessup e Brozena, 2003).

O comprometimento multissistêmico verificado na IC, em virtude da disfunção miocárdica, pode ser observado pelas seguintes alterações:

- Anormalidades hemodinâmicas (Braunwald, 2013), com a diminuição do volume sistólico e do débito cardíaco (Shammas *et al.*, 2007), aumento da pressão diastólica final do ventrículo esquerdo (Flaherty *et al.*, 2009) e da pressão do átrio

esquerdo, assim como da circulação pulmonar, o que pode resultar em distensão do átrio esquerdo e congestão pulmonar (Finsen *et al.*, 2005);

- Hiperatividade neurohumoral (Lymperopoulos *et al.*, 2013; Florea e Cohn, 2014; Sousa-Pinto *et al.*, 2014), com o aumento da atividade simpática e redução da parassimpática (Parati e Esler, 2012), aumento da atividade do sistema renina angiotensina aldosterona, elevados níveis plasmáticos de norepinefrina (Lymperopoulos *et al.*, 2013), alterações no controle do reflexo cardiopulmonar (Modesti *et al.*, 2004), dessensibilização do barorreflexo arterial (Iellamo *et al.*, 2007) e o aumento da sensibilidade do quimiorreflexo periférico (Ciarka *et al.*, 2006; Sousa-Pinto *et al.*, 2014);

- Aumento da produção de espécies reativas de oxigênio que, por sua vez, pode resultar no estresse oxidativo, com consequente oxidação de proteínas, lipoperoxidação e dano em DNA, o que contribui para a disfunção celular (Munzel *et al.*, 2015);

- Disfunção endotelial e musculoesquelética, caracterizada pela menor liberação ou ação do óxido nítrico (Katz *et al.*, 1992) e diminuição da força e massa muscular periférica (Georgiadou e Adamopoulos, 2012; Fulster *et al.*, 2013);

- Resposta inflamatória, com o aumento circulante de citocinas próinflamatórias (TNF-α, IL-1β, IL-6 e a PCR) e diminuição dos níveis de mediadores antiinflamatórios (Braunwald, 2008; Bozkurt *et al.*, 2010; Askevold *et al.*, 2014);

- Alterações no sistema respiratório, como a redução da complacência e o aumento da resistência pulmonar (Hizume *et al.*, 2007; Jaenisch *et al.*, 2011), a diminuição da capacidade vital (Gehlbach e Geppert, 2004) e, a fraqueza da musculatura ventilatória (Meyer *et al.*, 2001).

Apesar do considerável progresso no conhecimento da gênese da IC, não há consenso na literatura quanto ao maior responsável pelos principais sintomas em pacientes com IC. Parece haver, de fato, um somatório de eventos, tanto centrais como periféricos, que conduzem esses pacientes para um estado de incapacidade funcional e piora na qualidade de vida.

Pacientes cardiopatas após um evento agudo, intervenção ou diagnóstico de doença cardíaca crônica merecem atenção especial no sentido de restaurar a sua qualidade de vida, manter ou melhorar a capacidade funcional (Piepoli *et al.*, 2010). Nas últimas quatro décadas, tem se reconhecido a reabilitação cardiovascular (RCV) como um instrumento importante no cuidado dos pacientes com doença

cardiovascular (DCV) (Herdy *et al.*, 2014). A RCV é uma intervenção multifacetada e multidisciplinar, que promove a melhora da capacidade funcional, a recuperação e o bem-estar psicológico. Além disso, é uma intervenção custo-efetiva em pacientes com IC (Piepoli *et al.*, 2004), uma vez que melhora o prognóstico, reduz internações hospitalares e gastos com a saúde, além de prolongar a vida (Piepoli *et al.*, 2010).

Um recente posicionamento da Sociedade Europeia de Cardiologia (*European Society of Cardiology*) aconselha fortemente a participação de pacientes com IC estável em programas de exercício físico (EF) estruturado ou programas de RC (Piepoli *et al.*, 2011). Três modalidades de EF são propostos para pacientes com IC: 1) exercício aeróbio (contínuo e intervalado), 2) exercício de força e 3) treinamento muscular ventilatório (Labate e Guazzi, 2015). Independentemente da modalidade de EF, a maioria dos estudos demonstraram claramente melhorias significativas na capacidade funcional, qualidade de vida e função ventricular esquerda de pacientes com IC (Labate e Guazzi, 2015).

Na IC, a disfunção dos músculos ventilatórios é frequentemente observada, caracterizada por atrofia de fibras musculares ventilatórias, desoxigenação tecidual e prejuízo na capacidade oxidativa mitocondrial (Meyer *et al.*, 2001; Supinski e Callahan, 2005; Wong *et al.*, 2011). Essas mudanças conduzem à redução da força dos músculos inspiratórios e expiratórios, além da diminuição da resistência muscular inspiratória, definida como a capacidade de sustentar uma tarefa específica ao longo do tempo. Essas variáveis correlacionam-se com a tolerância ao exercício, nível de dispneia e capacidade funcional (Meyer *et al.*, 2001; Wong *et al.*, 2011; Labate e Guazzi, 2015). Nishimura et al. (1994) demonstraram que o consumo máximo de oxigênio ($VO_{2máx}$) está diretamente correlacionado com a força muscular inspiratória, e que a fraqueza muscular inspiratória está intimamente ligada à deterioração da função cardíaca, ao prejuízo da capacidade ao exercício e à gravidade da doença (Nishimura *et al.*, 1994), sendo considerada um preditor independente de mau prognóstico em pacientes com IC (Meyer *et al.*, 2001).

Deste modo, por meio dos achados que relacionaram os músculos ventilatórios e sua influência na síndrome IC, alguns investigadores levantaram a seguinte questão: o aumento da força e/ou da resistência muscular ventilatória pode determinar potenciais efeitos nas alterações promovidas pela IC?

O primeiro estudo que utilizou o treinamento da musculatura inspiratória como ferramenta terapêutica em pacientes com IC foi desenvolvido por Mancini et al. (1995).

Os autores comprovaram um aumento, tanto no VO_2 de pico como na tolerância ao exercício, contudo, o estudo não foi randomizado nem controlado por placebo (Mancini *et al.*, 1995).

O nosso grupo de pesquisa conduziu um ensaio clínico randomizado (ECR) controlado por placebo, que avaliou o efeito do treinamento muscular inspiratório (TMI) em pacientes com IC e fraqueza da musculatura ventilatória (Dall'ago *et al.*, 2006). Com protocolo realizado em 12 semanas, 7x/semana, 30 min/dia e carga de 30% da pressão inspiratória máxima ($PI_{máx}$), este estudo verificou um aumento da $PI_{máx}$ em 115%, do VO_2 de pico em 17%, da distância percorrida em 6 minutos (DP6) em 19% e da potência circulatória em 24%. Além disso, houve melhora da qualidade de vida, da eficiência ventilatória e da cinética de recuperação do consumo de oxigênio, além do decréscimo das oscilações ventilatórias (Dall'ago *et al.*, 2006).

Na tentativa de verificar o efeito do TMI em pacientes com IC e fraqueza muscular ventilatória sobre a modulação simpatovagal e a atividade nervosa simpática periférica, Melo et al. (2012) (Mello *et al.*, 2012) desenvolveram um ECR e duplo cego. Os autores verificaram que o TMI, além de aumentar a capacidade funcional e a $PI_{máx}$, promoveu a redução da atividade simpática periférica e da modulação simpática cardíaca, assim como o aumento da modulação parassimpática cardíaca. Ainda, confirmou a melhora da qualidade de vida e da eficiência ventilatória (Mello *et al.*, 2012).

O TMI tem demonstrado melhorar muitos desfechos clínicos relacionados com as manifestações fisiopatológicas da IC (Cahalin *et al.*, 2013), incluindo dispneia, qualidade de vida, equilíbrio, fluxo sanguíneo periférico, atividade nervosa simpática muscular, frequência cardíaca, frequência respiratória, $VO_{2máx}$, DP6, eficiência ventilatória, potência circulatória, cinética de recuperação e índices de desempenho cardíaco (Mancini *et al.*, 1995; Dall'ago *et al.*, 2006; Chiappa *et al.*, 2008; Winkelmann *et al.*, 2009; Bosnak-Guclu *et al.*, 2011; Mello *et al.*, 2012; Laoutaris *et al.*, 2013; Adamopoulos *et al.*, 2014). Apesar desses efeitos, o TMI ainda não está incluído em diretrizes relacionadas ao tratamento de pacientes com IC (Cahalin *et al.*, 2013). Atualmente, os resultados das revisões sistemáticas e metanálises sobre o TMI em pacientes IC têm sido favoráveis, considerando principalmente as manifestações clínicas da IC (Cahalin e Arena, 2015).

Lin et al. (2012) (Lin *et al.*, 2012) realizaram uma revisão sistemática sobre o TMI em pacientes com IC, composta de 12 estudos. Os autores concluíram que o TMI

promove o aumento da força muscular inspiratória e da capacidade funcional, assim como a melhora da dispneia em pacientes com IC estável e fraqueza muscular ventilatória (Lin *et al.*, 2012).

Em uma metanálise de estudos randomizados, Plentz et. al (2012) (Plentz *et al.*, 2012) revisaram sistematicamente os efeitos do TMI comparado a grupo controle (TMI placebo ou outra intervenção) em pacientes com IC. A busca incluiu as bases MEDLINE, PEDro e Cochrane CENTRAL, além de referências de estudos publicados, de 1960 a 2011. Dos 119 artigos identificados, sete estudos foram incluídos, que evidenciaram um aumento da DP6 (evidência muito baixa) e da $PI_{máx}$ (evidência baixa) no grupo TMI. Além disso, o TMI promoveu o aumento significativo no $VO_{2máx}$, embora esse benefício tenha sido verificado somente nos estudos que realizaram o protocolo de 12 semanas em indivíduos com fraqueza muscular inspiratória, quando comparados ao grupo placebo. Dessa forma, os autores concluíram que o TMI melhora a capacidade funcional e a força muscular inspiratória, contudo, estudos com um maior tamanho amostral e com melhor qualidade metodológica são necessários para o maior esclarecimento dos reais benefícios dessa forma terapêutica em pacientes com IC (Plentz *et al.*, 2012).

O conhecimento sobre a influência do treinamento dos músculos ventilatórios em pacientes com IC foi determinada por alguns pesquisadores (Mancini *et al.*, 1995; Dall'ago *et al.*, 2006; Chiappa *et al.*, 2008; Winkelmann *et al.*, 2009; Bosnak-Guclu *et al.*, 2011; Mello *et al.*, 2012; Laoutaris *et al.*, 2013; Adamopoulos *et al.*, 2014), contudo, alguns mecanismos fisiológicos envolvidos na fisiopatogênese da IC, que justifiquem os reais benefícios dessa forma terapêutica, ainda precisam ser esclarecidos.

O modelo experimental de infarto do miocárdio após a ligação da artéria coronária em ratos tem sido amplamente utilizado (Pfeffer *et al.*, 1979; Jaenisch *et al.*, 2011; Alves *et al.*, 2014). A oclusão da artéria coronária descendente esquerda leva à isquemia miocárdica e, consequentemente, à IC após 3 a 6 semanas ao ato cirúrgico (Francis *et al.*, 2001). A disfunção hemodinâmica, relacionada à àrea de necrose miocárdica após a cirurgia da oclusão da artéria coronária descendente esquerda, foi demonstrada de forma mais detalhada no estudo de Pfeffer *et al.* (1979) (Pfeffer *et al.*, 1979). Os resultados demonstraram aumento da pressão diastólica final do ventrículo esquerdo e do peso do ventrículo direito, consistentes com a elevação da pressão da artéria pulmonar devido à indução da insuficiência ventricular esquerda, caracterizando o modelo de IC (Pfeffer *et al.*, 1979).

A disfunção ventricular esquerda e a falência miocárdica, no modelo experimental de IC, também estão associadas às alterações da mecânica respiratória (Hizume *et al.*, 2007) e da musculatura diafragmática (Van Hees *et al.*, 2010), ao estresse oxidativo (Silva *et al.*, 2005; Supinski e Callahan, 2005), ao aumento da atividade simpática (Gao *et al.*, 2005) e à sensibilidade dos quimiorreceptores periféricos (Schultz e Li, 2007).

Embora estudos tenham demonstrado algumas manifestações promovidas pela IC em diferentes órgãos-alvo no modelo experimental de IC, alguns aspectos permanecem desconhecidos. Assim, a possibilidade da utilização de um protocolo de treinamento muscular ventilatório (TMV), em animais com IC, poderia responder alguns questionamentos pouco elucidados em pacientes cardiopatas.

Bisschop *et al.* (1997) (Bisschop *et al.*, 1997) realizaram um estudo que investigou o efeito do TMV em ratos hígidos e sua influência na hipertrofia diafragmática, caracterizando o efeito do treinamento. Os animais treinados foram submetidos a um protocolo de TMV por um período de 8 semanas, 5 dias/semana, e respiraram por meio de uma válvula de Hans-Rudolph. Resistores alineares, que variaram de 0,8 a 0,3 mm de diâmetro interno, com redução gradativa do diâmetro e consequente aumento de carga, foram conectados em um cilindro. Os animais que não realizaram o protocolo de TMV respiraram por essa válvula sem resistência. Os resultados evidenciaram um aumento da massa diafragmática, com hipertrofia de fibras do tipo IIa e IIb no grupo treinado, quando comparado ao grupo não treinado.

Partindo da premissa que o modelo experimental de IC pode simular as alterações promovidas pela síndrome, e que, o protocolo de TMV em animais é factível, o nosso grupo desenvolveu um estudo que agregou o modelo experimental animal de IC e o treinamento muscular ventilatório (TMV) (Jaenisch *et al.*, 2011) (Figura 1). Foram avaliados os efeitos do TMV na função hemodinâmica, no tônus e efeito vagal e simpático, na sensibilidade dos barorreceptores arteriais e na mecânica respiratória em ratos com IC. O TMV promoveu a redução da pressão diastólica final do ventrículo esquerdo, o aumento da pressão sistólica do ventrículo esquerdo e a redução da hipertrofia do ventrículo direito nos animais com IC. Foi observada a redução do tônus simpático, o aumento do efeito vagal e do ganho barorreflexo. Além disso, verificou-se que os animais com IC que realizaram o TMV tiveram a melhora da mecânica respiratória, verificada pela diminuição da resistência do sistema respiratório e tecidual, assim como da elastância do sistema respiratório, tecidual e

estática. Esses achados demonstraram que, o TMV em ratos com IC, gerou a melhora da função hemodinâmica, do balanço simpatovagal e da mecânica respiratória (Jaenisch *et al.*, 2011).

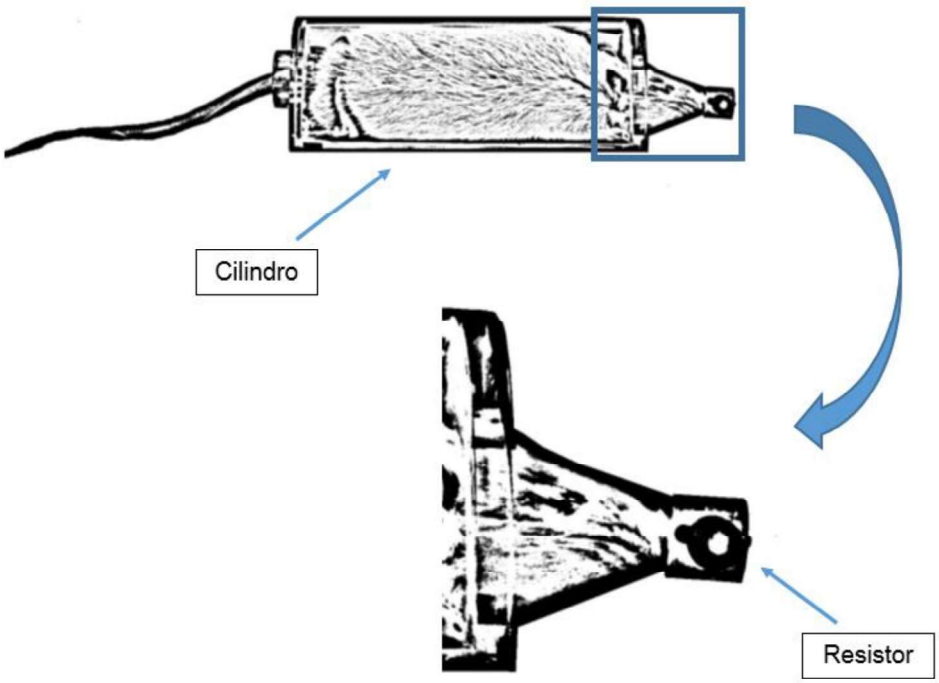

Figura 1. Equipamento de treinamento muscular ventilatório para pequenos animais, com resistores alineares conectados ao cilindro.

O estresse oxidativo, que representa o desequilíbrio entre a produção de espécies reativas de oxigênio (ERO) e endógenos antioxidantes, têm sido implicado em condições clínicas e experimentais da IC (Keith *et al.*, 1998; Grieve e Shah, 2003). Ainda, biomarcadores de estresse oxidativo têm sido correlacionados com a gravidade da disfunção miocárdica e gravidade da IC (Keith *et al.*, 1998; Grieve e Shah, 2003).

O excesso de ERO causa disfunção celular, proteica e lipoperoxidação, além de dano ao DNA. Esses fatores podem conduzir a dano e morte celular irreversíveis, com implicações em condições patológicas cardiovasculares (Tsutsui *et al.*, 2011). Elevados níveis de dano oxidativo ao DNA foram verificados em pacientes com DCV (Botto *et al.*, 2002), além de ter uma correlação positiva com a gravidade da IC (Kobayashi *et al.*, 2011).

Alguns estudos demonstraram anormalidades nos músculos esqueléticos de pacientes com IC (Fulster *et al.*, 2013; Kitzman *et al.*, 2014), e, em particular, no músculo diafragma (Meyer *et al.*, 2001; Coirault *et al.*, 2007; Van Hees *et al.*, 2010). Especificamente, foi verificado um aumento na produção de radicais livres (Supinski e Callahan, 2005), estresse oxidativo (Silva *et al.*, 2005), com consequente redução na força do diafragma (Supinski e Callahan, 2005) em ratos com IC. No entanto, ainda não foi demonstrado dano ao DNA no músculo diafragma em ratos com IC.

Nesse contexto, o melhor entendimento sobre as anormalidades do músculo diafragma no modelo experimental de IC e o efeito do treinamento muscular ventilatório nessa síndrome, foi realizado o estudo 1 dessa tese, intitulado: "*Respiratory muscle training decreases DNA damage on diaphragm in rats with heart failure"*, com o objetivo de testar o efeito de um protocolo de seis semanas de treinamento muscular ventilatório na função hemodinâmica e no dano ao DNA do diafragma de ratos com IC.

A hiperatividade simpática (Zucker *et al.*, 2012) e a atenuação vagal (Bibevski e Dunlap, 2011) estão presentes na IC, tanto em estudos clínicos quanto experimentais, presumivelmente como consequência de alterações hemodinâmicas associadas à disfunção cardíaca (Florea e Cohn, 2014). O padrão ventilatório anormal, que caracteriza a IC avançada, tem sido associado com a congestão pulmonar devido à disfunção hemodinâmica ou resposta autônoma e quimiorreflexa anormais (Passino *et al.*, 2006; Malfatto *et al.*, 2015).

Em pacientes e em animais com IC o aumento da ativação dos quimiorreceptores determina a hiperexcitação simpática (Schultz e Li, 2007), e correlaciona-se com a exacerbação da resposta ventilatória, desencadeando dispneia precoce (Ciarka *et al.*, 2006). Diversos mecanismos fisiopatológicos têm sido propostos para explicar as modificações no padrão ventilatório e desregulação quimiorreflexa em pacientes com IC (Tumminello *et al.*, 2007), sendo que a fraqueza muscular ventilatória parece estar presente. Foi verificado que, pacientes com IC e fraqueza muscular ventilatória apresentaram maior ativação dos quimiorreceptores quando comparados à pacientes com IC sem fraqueza muscular ventilatória (Callegaro *et al.*, 2010).

Assim sendo, com o intuito de verificar o efeito do treinamento muscular ventilatório na função cardiovascular, sua regulação por meio de ativação reflexa e determinar o melhor entendimento da interação cardiopulmonar no modelo

experimental de IC, foi realizado o estudo 2 dessa tese, intitulado: "*Respiratory muscle training improves hemodynamic, chemoreflex, autonomic function and respiratory mechanics in heart failure rats"*, com o objetivo de testar o efeito de um protocolo de seis semanas de treinamento muscular ventilatório na função hemodinâmica, mecânica respiratória, ativação quimiorreflexa e variabilidade da frequência cardíaca em ratos com IC.

Dessa forma, em linhas gerais, após o desenvolvimento dos estudos supracitados, aspiramos progredir no conhecimento sobre a influência do TMV no modelo experimental de IC. Os resultados obtidos e suas possíveis implicações contribuem para o melhor entendimento sobre as alterações hemodinâmicas e metabólicas dos sistemas músculo-esquelético, respiratório e nervoso, somadas às do sistema cardiovascular. Ainda, promove a possibilidade do desenvolvimento de novas abordagens terapêuticas no tratamento das modificações decorrentes da IC.

Deste modo, os estudos desenvolvidos são apresentados a seguir, em formato de artigo científico, de acordo com as normas das revistas para os quais foram submetidos, com os seguintes objetivos:

Estudo 1:

- Testar o efeito do TMV em ratos com IC sobre as características morfológicas e função hemodinâmica, representadas pela hipertrofia cardíaca, congestão pulmonar e pressão diastólica final do ventrículo esquerdo;

- Verificar a influência do TMV na genotoxicidade do músculo diafragma em ratos com IC, por meio do % do DNA da cauda, momento da cauda e momento da cauda de Olive.

Estudo 2:

- Avaliar se o TMV melhora a resposta quimiorreflexa periférica e a variabilidade da frequência cardíaca de ratos com IC;

- Testar a influência do TMV em ratos com IC sobre a mecânica respiratória, por meio da resistência do sistema respiratório e tecidual, elastância do sistema respiratório, tecidual e estática.

Referências

ADAMOPOULOS, S. et al. Combined aerobic/inspiratory muscle training vs. aerobic training in patients with chronic heart failure: The Vent-HeFT trial: a European prospective multicentre randomized trial. **Eur J Heart Fail,** v. 16, n. 5, p. 574-82, May 2014. ISSN 1879-0844 (Electronic) 1388-9842 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24634346 >.

ALVES, J. P. et al. Resistance training improves hemodynamic function, collagen deposition and inflammatory profiles: experimental model of heart failure. **PLoS One,** v. 9, n. 10, p. e110317, 2014. ISSN 1932-6203 (Electronic) 1932-6203 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25340545 >.

AMBROSY, A. P. et al. The global health and economic burden of hospitalizations for heart failure: lessons learned from hospitalized heart failure registries. **J Am Coll Cardiol,** v. 63, n. 12, p. 1123-33, Apr 1 2014. ISSN 1558-3597 (Electronic) 0735-1097 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24491689 >.

ARAUJO, D. V. et al. [Cost of heart failure in the Unified Health System]. **Arq Bras Cardiol,** v. 84, n. 5, p. 422-7, May 2005. ISSN 0066-782X (Print) 0066-782X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/15917977 >.

ASKEVOLD, E. T. et al. Interleukin-6 signaling, soluble glycoprotein 130, and inflammation in heart failure. **Curr Heart Fail Rep,** v. 11, n. 2, p. 146-55, Jun 2014. ISSN 1546-9549 (Electronic) 1546-9530 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24477903 >.

BIBEVSKI, S.; DUNLAP, M. E. Evidence for impaired vagus nerve activity in heart failure. **Heart Fail Rev,** v. 16, n. 2, p. 129-35, Mar 2011. ISSN 1573-7322 (Electronic) 1382-4147 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20820912 >.

BISSCHOP, A. et al. Intermittent inspiratory muscle training induces fiber hypertrophy in rat diaphragm. **Am J Respir Crit Care Med,** v. 155, n. 5, p. 1583-9, May 1997. ISSN 1073-449X (Print) 1073-449X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/9154861 >.

BOSNAK-GUCLU, M. et al. Effects of inspiratory muscle training in patients with heart failure. **Respir Med,** v. 105, n. 11, p. 1671-81, Nov 2011. ISSN 1532-3064 (Electronic) 0954-6111 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21621993 >.

BOTTO, N. et al. Elevated levels of oxidative DNA damage in patients with coronary artery disease. **Coron Artery Dis,** v. 13, n. 5, p. 269-74, Aug 2002. ISSN 0954-6928 (Print) 0954-6928 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12394651 >.

BOZKURT, B.; MANN, D. L.; DESWAL, A. Biomarkers of inflammation in heart failure. **Heart Fail Rev,** v. 15, n. 4, p. 331-41, Jul 2010. ISSN 1573-7322 (Electronic) 1382-4147 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19363700 >.

BRAUNWALD, E. Biomarkers in heart failure. **N Engl J Med,** v. 358, n. 20, p. 2148-59, May 15 2008. ISSN 1533-4406 (Electronic) 0028-4793 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18480207 >.

_____. Heart failure. **JACC Heart Fail,** v. 1, n. 1, p. 1-20, Feb 2013. ISSN 2213-1787 (Electronic) 2213-1779 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24621794 >.

CAHALIN, L. P. et al. Inspiratory muscle training in heart disease and heart failure: a review of the literature with a focus on method of training and outcomes. **Expert Rev Cardiovasc Ther,** v. 11, n. 2, p. 161-77, Feb 2013. ISSN 1744-8344 (Electronic) 1477-9072 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23405838 >.

CAHALIN, L. P.; ARENA, R. A. Breathing exercises and inspiratory muscle training in heart failure. **Heart Fail Clin,** v. 11, n. 1, p. 149-72, Jan 2015. ISSN 1551-7136 (Print) 1551-7136 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25432483 >.

CALLEGARO, C. C. et al. Augmented peripheral chemoreflex in patients with heart failure and inspiratory muscle weakness. **Respir Physiol Neurobiol,** v. 171, n. 1, p. 31-5, Apr 15 2010. ISSN 1878-1519 (Electronic) 1569-9048 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20096381 >.

CHIAPPA, G. R. et al. Inspiratory muscle training improves blood flow to resting and exercising limbs in patients with chronic heart failure. **J Am Coll Cardiol,** v. 51, n. 17, p. 1663-71, Apr 29 2008. ISSN 1558-3597 (Electronic) 0735-1097 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18436118 >.

CIARKA, A. et al. Increased peripheral chemoreceptors sensitivity and exercise ventilation in heart transplant recipients. **Circulation,** v. 113, n. 2, p. 252-7, Jan 17 2006. ISSN 1524-4539 (Electronic) 0009-7322 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16401774 >.

COIRAULT, C. et al. Oxidative stress of myosin contributes to skeletal muscle dysfunction in rats with chronic heart failure. **Am J Physiol Heart Circ Physiol,** v. 292, n. 2, p. H1009-17, Feb 2007. ISSN 0363-6135 (Print) 0363-6135 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17040975 >.

DALL'AGO, P. et al. Inspiratory muscle training in patients with heart failure and inspiratory muscle weakness: a randomized trial. **J Am Coll Cardiol,** v. 47, n. 4, p. 757-63, Feb 21 2006. ISSN 1558-3597 (Electronic) 0735-1097 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16487841 >.

DICKSTEIN, K. et al. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2008: the Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2008 of the European Society of Cardiology. Developed in

collaboration with the Heart Failure Association of the ESC (HFA) and endorsed by the European Society of Intensive Care Medicine (ESICM). **Eur Heart J,** v. 29, n. 19, p. 2388-442, Oct 2008. ISSN 1522-9645 (Electronic) 0195-668X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18799522 >.

FINSEN, A. V.; CHRISTENSEN, G.; SJAASTAD, I. Echocardiographic parameters discriminating myocardial infarction with pulmonary congestion from myocardial infarction without congestion in the mouse. **J Appl Physiol (1985),** v. 98, n. 2, p. 680-9, Feb 2005. ISSN 8750-7587 (Print) 0161-7567 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/15475595 >.

FLAHERTY, J. D. et al. Acute heart failure syndromes in patients with coronary artery disease early assessment and treatment. **J Am Coll Cardiol,** v. 53, n. 3, p. 254-63, Jan 20 2009. ISSN 1558-3597 (Electronic) 0735-1097 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19147042 >.

FLOREA, V. G.; COHN, J. N. The autonomic nervous system and heart failure. **Circ Res,** v. 114, n. 11, p. 1815-26, May 23 2014. ISSN 1524-4571 (Electronic) 0009-7330 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24855204 >.

FRANCIS, J. et al. Progression of heart failure after myocardial infarction in the rat. **Am J Physiol Regul Integr Comp Physiol,** v. 281, n. 5, p. R1734-45, Nov 2001. ISSN 0363-6119 (Print) 0363-6119 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/11641147 >.

FULSTER, S. et al. Muscle wasting in patients with chronic heart failure: results from the studies investigating co-morbidities aggravating heart failure (SICA-HF). **Eur Heart J,** v. 34, n. 7, p. 512-9, Feb 2013. ISSN 1522-9645 (Electronic) 0195-668X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23178647 >.

GAO, L. et al. Augmented input from cardiac sympathetic afferents inhibits baroreflex in rats with heart failure. **Hypertension,** v. 45, n. 6, p. 1173-81, Jun 2005. ISSN 1524-4563 (Electronic) 0194-911X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/15897358 >.

GEHLBACH, B. K.; GEPPERT, E. The pulmonary manifestations of left heart failure. **Chest,** v. 125, n. 2, p. 669-82, Feb 2004. ISSN 0012-3692 (Print) 0012-3692 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/14769751 >.

GEORGIADOU, P.; ADAMOPOULOS, S. Skeletal muscle abnormalities in chronic heart failure. **Curr Heart Fail Rep,** v. 9, n. 2, p. 128-32, Jun 2012. ISSN 1546-9549 (Electronic) 1546-9530 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22430147 >.

GRIEVE, D. J.; SHAH, A. M. Oxidative stress in heart failure. More than just damage. **Eur Heart J,** v. 24, n. 24, p. 2161-3, Dec 2003. ISSN 0195-668X (Print) 0195-668X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/14659766 >.

HERDY, A. H. et al. South American guidelines for cardiovascular disease prevention and rehabilitation. **Arq Bras Cardiol,** v. 103, n. 2 Suppl 1, p. 1-31, Aug 2014. ISSN

1678-4170 (Electronic) 0066-782X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25387466 >.

HIZUME, D. C. et al. Effects of positive end-expiratory pressure in an experimental model of acute myocardial infarct in wistar rats. **Shock,** v. 27, n. 5, p. 584-9, May 2007. ISSN 1073-2322 (Print) 1073-2322 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17438466 >.

IELLAMO, F. et al. Spontaneous baroreflex control of heart rate during exercise and muscle metaboreflex activation in heart failure. **Am J Physiol Heart Circ Physiol,** v. 293, n. 3, p. H1929-36, Sep 2007. ISSN 0363-6135 (Print) 0363-6135 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17604328 >.

JAENISCH, R. B. et al. Respiratory muscle training improves hemodynamics, autonomic function, baroreceptor sensitivity, and respiratory mechanics in rats with heart failure. **J Appl Physiol (1985),** v. 111, n. 6, p. 1664-70, Dec 2011. ISSN 1522-1601 (Electronic) 0161-7567 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21903877 >.

JESSUP, M.; BROZENA, S. Heart failure. **N Engl J Med,** v. 348, n. 20, p. 2007-18, May 15 2003. ISSN 1533-4406 (Electronic) 0028-4793 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12748317 >.

JOFFE, S. W. et al. Improved survival after heart failure: a community-based perspective. **J Am Heart Assoc,** v. 2, n. 3, p. e000053, Jun 2013. ISSN 2047-9980 (Electronic) 2047-9980 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23676294 >.

KATZ, S. D. et al. Impaired endothelium-mediated vasodilation in the peripheral vasculature of patients with congestive heart failure. **J Am Coll Cardiol,** v. 19, n. 5, p. 918-25, Apr 1992. ISSN 0735-1097 (Print) 0735-1097 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/1552112 >.

KEITH, M. et al. Increased oxidative stress in patients with congestive heart failure. **J Am Coll Cardiol,** v. 31, n. 6, p. 1352-6, May 1998. ISSN 0735-1097 (Print) 0735-1097 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/9581732 >.

KITZMAN, D. W. et al. Skeletal muscle abnormalities and exercise intolerance in older patients with heart failure and preserved ejection fraction. **Am J Physiol Heart Circ Physiol,** v. 306, n. 9, p. H1364-70, May 2014. ISSN 1522-1539 (Electronic) 0363-6135 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24658015 >.

KOBAYASHI, S. et al. Urinary 8-hydroxy-2'-deoxyguanosine reflects symptomatic status and severity of systolic dysfunction in patients with chronic heart failure. **Eur J Heart Fail,** v. 13, n. 1, p. 29-36, Jan 2011. ISSN 1879-0844 (Electronic) 1388-9842 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20965876 >.

LABATE, V.; GUAZZI, M. Past, present, and future rehabilitation practice patterns for patients with heart failure: the European perspective. **Heart Fail Clin,** v. 11, n. 1, p.

105-15, Jan 2015. ISSN 1551-7136 (Print) 1551-7136 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25432479 >.

LAOUTARIS, I. D. et al. Benefits of combined aerobic/resistance/inspiratory training in patients with chronic heart failure. A complete exercise model? A prospective randomised study. **Int J Cardiol,** v. 167, n. 5, p. 1967-72, Sep 1 2013. ISSN 1874-1754 (Electronic) 0167-5273 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22658571 >.

LESMAN-LEEGTE, I. et al. Quality of life and depressive symptoms in the elderly: a comparison between patients with heart failure and age- and gender-matched community controls. **J Card Fail,** v. 15, n. 1, p. 17-23, Feb 2009. ISSN 1532-8414 (Electronic) 1071-9164 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19181289 >.

LIN, S. J. et al. Inspiratory muscle training in patients with heart failure: a systematic review. **Cardiopulm Phys Ther J,** v. 23, n. 3, p. 29-36, Sep 2012. ISSN 2374-8907 (Electronic) 1541-7891 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22993500 >.

LYMPEROPOULOS, A.; RENGO, G.; KOCH, W. J. Adrenergic nervous system in heart failure: pathophysiology and therapy. **Circ Res,** v. 113, n. 6, p. 739-53, Aug 30 2013. ISSN 1524-4571 (Electronic) 0009-7330 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23989716 >.

MALFATTO, G. et al. Pulmonary congestion at rest and abnormal ventilation during exercise in chronic systolic heart failure. **J Am Heart Assoc,** v. 4, n. 5, May 2015. ISSN 2047-9980 (Electronic) 2047-9980 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25944875 >.

MANCINI, D. M. et al. Benefit of selective respiratory muscle training on exercise capacity in patients with chronic congestive heart failure. **Circulation,** v. 91, n. 2, p. 320-9, Jan 15 1995. ISSN 0009-7322 (Print) 0009-7322 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/7805234 >.

MELLO, P. R. et al. Inspiratory muscle training reduces sympathetic nervous activity and improves inspiratory muscle weakness and quality of life in patients with chronic heart failure: a clinical trial. **J Cardiopulm Rehabil Prev,** v. 32, n. 5, p. 255-61, Sep-Oct 2012. ISSN 1932-751X (Electronic) 1932-7501 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22785143 >.

MEYER, F. J. et al. Respiratory muscle dysfunction in congestive heart failure: clinical correlation and prognostic significance. **Circulation,** v. 103, n. 17, p. 2153-8, May 1 2001. ISSN 1524-4539 (Electronic) 0009-7322 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/11331255 >.

MODESTI, P. A. et al. Impairment of cardiopulmonary receptor sensitivity in the early phase of heart failure. **Heart,** v. 90, n. 1, p. 30-6, Jan 2004. ISSN 1468-201X (Electronic) 1355-6037 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/14676236 >.

MUNZEL, T. et al. Pathophysiological role of oxidative stress in systolic and diastolic heart failure and its therapeutic implications. **Eur Heart J**, Jul 4 2015. ISSN 1522-9645 (Electronic) 0195-668X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26142467 >.

NISHIMURA, Y. et al. Respiratory muscle strength and hemodynamics in chronic heart failure. **Chest,** v. 105, n. 2, p. 355-9, Feb 1994. ISSN 0012-3692 (Print) 0012-3692 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/8306727 >.

PARATI, G.; ESLER, M. The human sympathetic nervous system: its relevance in hypertension and heart failure. **Eur Heart J,** v. 33, n. 9, p. 1058-66, May 2012. ISSN 1522-9645 (Electronic) 0195-668X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22507981 >.

PASSINO, C. et al. Neuro-hormonal activation predicts ventilatory response to exercise and functional capacity in patients with heart failure. **Eur J Heart Fail,** v. 8, n. 1, p. 46-53, Jan 2006. ISSN 1388-9842 (Print) 1388-9842 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16112902 >.

PFEFFER, M. A. et al. Myocardial infarct size and ventricular function in rats. **Circ Res,** v. 44, n. 4, p. 503-12, Apr 1979. ISSN 0009-7330 (Print) 0009-7330 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/428047 >.

PIEPOLI, M. F. et al. Exercise training in heart failure: from theory to practice. A consensus document of the Heart Failure Association and the European Association for Cardiovascular Prevention and Rehabilitation. **Eur J Heart Fail,** v. 13, n. 4, p. 347-57, Apr 2011. ISSN 1879-0844 (Electronic) 1388-9842 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21436360 >.

PIEPOLI, M. F. et al. Secondary prevention through cardiac rehabilitation: from knowledge to implementation. A position paper from the Cardiac Rehabilitation Section of the European Association of Cardiovascular Prevention and Rehabilitation. **Eur J Cardiovasc Prev Rehabil,** v. 17, n. 1, p. 1-17, Feb 2010. ISSN 1741-8275 (Electronic) 1741-8267 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19952757 >.

PIEPOLI, M. F. et al. Exercise training meta-analysis of trials in patients with chronic heart failure (ExTraMATCH). **BMJ,** v. 328, n. 7433, p. 189, Jan 24 2004. ISSN 1756-1833 (Electronic) 0959-535X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/14729656 >.

PLENTZ, R. D. et al. Inspiratory muscle training in patients with heart failure: meta-analysis of randomized trials. **Arq Bras Cardiol,** v. 99, n. 2, p. 762-71, Aug 2012. ISSN 1678-4170 (Electronic) 0066-782X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22964897 >.

ROGER, V. L. et al. Heart disease and stroke statistics--2012 update: a report from the American Heart Association. **Circulation,** v. 125, n. 1, p. e2-e220, Jan 3 2012.

ISSN 1524-4539 (Electronic) 0009-7322 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22179539 >.

SCHULTZ, H. D.; LI, Y. L. Carotid body function in heart failure. **Respir Physiol Neurobiol,** v. 157, n. 1, p. 171-85, Jul 1 2007. ISSN 1569-9048 (Print) 1569-9048 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17374517 >.

SHAMMAS, R. L. et al. Diastolic heart failure and left ventricular diastolic dysfunction: what we know, and what we don't know! **Int J Cardiol,** v. 115, n. 3, p. 284-92, Feb 14 2007. ISSN 1874-1754 (Electronic)0167-5273 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16904774 >.

SILVA, F. L. et al. Infarto do miocárdio experimental e aumento do estresse oxidativo em diafragma de ratos. **Jornal Brasileiro de Pneumologia,** v. 31, p. 506-510, 2005. ISSN 1806-3713. Disponível em: < http://www.scielo.br/scielo.php?script=sci_arttext&pid=S180637132005000600008&nrm=iso >.

SOUSA-PINTO, B. et al. Central nervous system circuits modified in heart failure: pathophysiology and therapeutic implications. **Heart Fail Rev,** v. 19, n. 6, p. 759-79, Nov 2014. ISSN 1573-7322 (Electronic) 1382-4147 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24573960 >.

SUPINSKI, G. S.; CALLAHAN, L. A. Diaphragmatic free radical generation increases in an animal model of heart failure. **J Appl Physiol (1985),** v. 99, n. 3, p. 1078-84, Sep 2005. ISSN 8750-7587 (Print) 0161-7567 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16103520 >.

TSUTSUI, H.; KINUGAWA, S.; MATSUSHIMA, S. Oxidative stress and heart failure. **Am J Physiol Heart Circ Physiol,** v. 301, n. 6, p. H2181-90, Dec 2011. ISSN 1522-1539 (Electronic) 0363-6135 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21949114 >.

TUMMINELLO, G. et al. Exercise ventilation inefficiency in heart failure: pathophysiological and clinical significance. **Eur Heart J,** v. 28, n. 6, p. 673-8, Mar 2007. ISSN 0195-668X (Print) 0195-668X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17124197 >.

VAN HEES, H. W. et al. Heart failure decreases passive tension generation of rat diaphragm fibers. **Int J Cardiol,** v. 141, n. 3, p. 275-83, Jun 11 2010. ISSN 1874-1754 (Electronic) 0167-5273 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19150150 >.

WINKELMANN, E. R. et al. Addition of inspiratory muscle training to aerobic training improves cardiorespiratory responses to exercise in patients with heart failure and inspiratory muscle weakness. **Am Heart J,** v. 158, n. 5, p. 768 e1-7, Nov 2009. ISSN 1097-6744 (Electronic) 0002-8703 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19853695 >.

WONG, Y. Y. et al. Right ventricular failure in idiopathic pulmonary arterial hypertension is associated with inefficient myocardial oxygen utilization. **Circ Heart Fail,** v. 4, n. 6, p. 700-6, Nov 2011. ISSN 1941-3297 (Electronic) 1941-3289 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21900188 >.

YANCY, C. W. et al. 2013 ACCF/AHA guideline for the management of heart failure: executive summary: a report of the American College of Cardiology Foundation/American Heart Association Task Force on practice guidelines. **Circulation,** v. 128, n. 16, p. 1810-52, Oct 15 2013. ISSN 1524-4539 (Electronic) 0009-7322 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23741057 >.

ZUCKER, I. H.; PATEL, K. P.; SCHULTZ, H. D. Neurohumoral stimulation. **Heart Fail Clin,** v. 8, n. 1, p. 87-99, Jan 2012. ISSN 1551-7136 (Print) 1551-7136 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22108729 >.

ESTUDO 1

Respiratory muscle training decreases DNA damage on diaphragm in rats with heart failure

Artigo submetido no periódico ***Journal of Cardiac Failure***

Respiratory muscle training decreases DNA damage on diaphragm in rats with heart failure

Running title: Respiratory muscle training in heart failure

Article for submission to **Journal of Cardiac Failure**

Rodrigo B. Jaenisch[1], Giuseppe P. Stefani[1,2], Camila Durante[1], Chalyne Chechi[1], Vítor S. Hentschke[1], Douglas D. Rossatto[1], Anelise Sonza[3], Cláudia R. Rhoden[2], Pedro Dal Lago[1,3,4*]

[1] Laboratory of Experimental Physiology, Post Graduation Program in Health Sciences, Universidade Federal de Ciências da Saúde de Porto Alegre, Brazil

[2] Laboratory of Atmospheric Pollution and Oxidative Stress, Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, Brazil

[3] Post Graduation Program in Rehabilitation Sciences, Universidade Federal de Ciências da Saúde de Porto Alegre, Brazil

[4] Physical Therapy Department, Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, RS, Brazil

* Corresponding author: Universidade Federal de Ciências da Saúde de Porto Alegre, Sarmento Leite,245, 90050-170, Porto Alegre, RS, Brazil. Fax: +55 51 3303-8751.

E-mail address: pdallago@ufcspa.edu.br (P. Dal Lago, PT, ScD)

Abstract

Background: Respiratory muscle training (RMT) promotes beneficial effects on respiratory mechanics, on heart and lung morphological changes, and on hemodynamic variables in rats with heart failure (HF). However, the relation between RMT effects and diaphragm oxidative stress remains unclear. Therefore, the aim of this study was to evaluate the RMT effects on diaphragm DNA damage in HF rats.

Methods and Results: Wistar rats were allocated into four groups: sedentary sham (Sed- Sham, *n*=8), trained sham (RMT-Sham, *n*=8), sedentary HF (Sed-HF, *n*=8) and trained HF (RMT-HF, *n*=8). The animals underwent a RMT protocol (30 min/day, 5 days/wk, for 6 wks), whereas sedentary animals did not exercise. Groups were compared by a two-way ANOVA and Tukey´s post hoc tests. In rats with HF, RMT promoted reduction in pulmonary congestion ($p< .0001$) and left ventricular end diastolic pressure ($p< .0001$). Moreover, RMT produced a decrease in the DNA damage on diaphragm in HF rats. This was demonstrated through the reduction in the percent tail DNA ($p< .0001$), tail moment ($p< .01$) and Olive tail moment ($p< .001$).

Conclusion: These findings show that a 6-wk RMT protocol in rats with HF promotes an improvement in hemodynamic function and reduces diaphragm DNA damage.

Keywords: respiratory muscles, hemodynamic function, exercise.

Introduction

Respiratory muscle weakness is present in heart failure (HF), in humans and animals [1, 2], and represents an independent predictor of poor prognosis [1]. Diaphragm has reduced function following MI-induced HF in rats [2], and is likely a key mediator in the overall pathogenesis of HF. However, little is still known about the mechanisms that could lead to diaphragm dysfunction and its influence in the HF syndrome.

Oxidative stress, that represents an imbalance between the productions of reactive oxygen species (ROS) and endogenous antioxidants, has long been implicated in experimental [3] and clinical [4] HF conditions. Furthermore, biological markers of oxidative stress have been correlated with myocardial dysfunction and overall severity of HF [4, 5].

Excessive ROS cause cellular dysfunction, protein and lipid peroxidation and DNA damage; all these factors can lead to irreversible cell damage and death, which have been implicated in a wide range of pathological cardiovascular conditions [6]. The findings, from a previous study, show that DNA damage contributes to the pathogenesis of atherosclerosis and elevated levels of oxidative DNA damage in HF patients [7]. Moreover, the severity of oxidative DNA damage was positively associated with the end stage of HF [8].

The oxidatively induced DNA damage occurs mainly due to the ROS formation that most reacts with DNA strands [9,10]. The main mechanisms involving ROS causing DNA damage are the single and double strand breaks [10], the generation of modified bases as 8-oxo-7,8-dihydroguanine (8oxoG) which plays major role in mutagenesis [9], among others metabolic dysfunctions. The DNA strand breaks can be accessed by the comet assay

technique [11]. The comet assay evaluates global genotoxic damage to the DNA, therefore, the results can mirror the total extent of nuclear DNA damage of the tissue of interest [12].

In HF, oxidative stress in skeletal muscle has been linked to peripheral hypoperfusion as a consequence of low cardiac output and peripheral endothelial dysfunction [13, 14]. Several studies have reported intrinsic muscle abnormalities affecting skeletal muscles [15] and, in particular, in the diaphragm muscle of HF patients and animal models with this syndrome [2, 16, 17]. Specifically, it was demonstrated an increase in free radical production [18], oxidative stress [19] and a reduction in force generated by the diaphragm [18] in rats with HF. However, to the best of our knowledge, there is no published evidence showing if the specified HF model for rats causes DNA damage on the diaphragm.

Respiratory muscle training (RMT) in patients with HF increased the inspiratory muscle strength and resistance, which leads to improvement in peak VO_2, dyspnea, and quality of life [20, 21]. In a study completed in our laboratory [22], RMT in rats with HF showed an improvement in hemodynamics, autonomic function, baroreceptor sensitivity, and respiratory mechanics. However, the effects of RMT on the diaphragm DNA damage, a marker of oxidative stress, in rats with HF had not been evaluated.

Therefore, we conducted the present study to test the hypothesis that a 6-wk protocol of RMT could improve the hemodynamic function and decrease the DNA damage on diaphragm of HF rats.

Methods

Animals

Thirty-two male Wistar rats (250 to 290 g) were obtained from the Animal

Breeding Unit of the Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA; Brazil). Animals were housed in groups of three per cage, receiving food and water *ad libitum* and were maintained in a room under standard conditions at 22°C of temperature and at 12:12-h light-dark cycle of illumination. The investigation followed the ethical rules established by the Guide for Care and Use of Experimental Animals published by the National Institutes of Health (publication no. 85-23, revised in 1996). All procedures outlined in this study were approved by the UFCSPA Ethics and Research Committee (protocol 070/11).

Surgery to induce MI

Rats were anesthetized with xylazine (12 mg/kg ip) and ketamine (90 mg/kg ip), intubated and artificially ventilated. Ligation of the left coronary artery and sham operations were performed as previously described [22, 23]. After the surgery, the animals received a single injection of monofenew (0.05 ml/100 g) and gentamicin (0.05 ml/100 g).

Experimental design

After MI, rats were allowed a minimum of 4 wk to recover (time necessary to develop the HF state) [23] and were assigned into four experimental groups: sedentary sham rats (Sed-Sham; n=8), trained sham rats (RMT-Sham; n=8), sedentary HF rats (Sed-HF; n=8), or trained HF rats (RMT-HF; n=8).

RMT protocol

Four weeks after the MI or sham surgery, the rats assigned to the RMT groups were submitted to a 5-day adaption protocol. The animals were daily conditioned to breathe through an orifice, without resistance, attached to a rigid mask while restrained in a

whole-body cylinder [22, 24]. The RMT protocol began in the 5th wk. It comprised 30 min/day, 5 days/wk for 6 wks. The progress of the protocol was achieved by a progressive increase in resistance by reducing the internal diameter of the hole through which the animal breathed. During the first wk of the training, the orifice at the inspiratory port was set at an internal diameter of 0.8 mm and was progressively decreased, reaching after 2 wk of training a final internal diameter of 0.3 mm (maximal resistance) [22, 24].

Cardiac hemodynamic evaluation

In the week following the RMT period, animals were anesthetized with xylazine (12 mg/kg ip) and ketamine (90 mg/kg ip). A small incision in the anterior cervical region was performed for the insertion of a polyethylene catheter (PE-50) into the right carotid artery. The arterial pressure was first recorded during a 5-min period through a connection of the arterial cannula to a strain-gauge pressure transducer (Narco Biosystem Miniature Pulse Transducer RP-155, TX, USA), coupled to a pressure amplifier (General Purpose Amplifier 4 - model 2, Stemtech Inc. WI, USA). Then the catheter was positioned into the left ventricle, and pulse wave was monitored by the typical graphic registration of ventricular pressure and was recorded for 5 min. Pressure analogical signals were digitalized by a data-acquisition system (Windaq - AT/CODAS, Dataq Instruments Inc., OH, USA) with sampling rate of 2.000 Hz. These data were used to determine mean arterial pressure (MAP), heart rate (HR), left ventricular systolic pressure (LVSP), left ventricular maximum change in pressure over time ($+dP/dt_{max}$) and left ventricular minimum change in pressure over time ($-dP/dt_{max}$), and left ventricular end-diastolic pressure (LVEDP). This last parameter was determined manually by the detection of the point of inflection to the end of diastoles from the analysis of the wave of ventricular pressure.

Diaphragm collection and preparation

After hemodynamic evaluation, animals were euthanized with a thiopental overdose (80 mg/kg, ip). A laparotomy and thoracotomy were performed to allow the complete excision of the diaphragm. The right and left costal diaphragm muscle were weighed and immediately snap-frozen in liquid N_2, and stored at -80°C for subsequent molecular analysis.

Morphological characteristics, pulmonary and hepatic congestion

Heart, lungs, and liver were removed and weighed. The right ventricle (RV) and LV were dissected and weighed. The LVs were filled with an insufflating latex balloon and placed in 10% formaldehyde for a minimum of 3 days before being cut into two equal transverse sections, as previously described [22]. The total left ventricle area and myocardial infarction scar were manually drawn on scanned images and measured automatically using a computer program (IMAGE Pro-plus 6.1, Media Cybernetics, Silver Spring, USA). The percentage infarction area was calculated by dividing the sum of the infarcted area from all sections by the sum of the area of the LV (including tissue without infarction) multiplied by 100 [23]. The heart weight-to-body weight ratio (HW/BW), LV/BW, and RV/BW values were determined. Lungs and liver were dehydrated (80°C) for 48h and then weighed again to evaluate the water percentage.

Comet Assay (alkaline version)

The comet assay was performed in alkaline conditions (pH > 13.0), according to Singh and colleagues [25]. All the procedures were performed avoiding any direct incidence of light. For the assay, it was first made a cell suspension of diaphragm in PBS buffer (pH = 7.40) with standard and gentle manual homogenization. For this step, it

was necessary to observe the density of cells that would be used in each slide. It was used Neubauer's chamber to count, approximately 7.3 x 10^5 cells/slide. The suspension of diaphragm cells (40 µl) was added to agarose of low melting point (90 µl). After gently mixed, this material was carefully superimposed over a slide previously covered with a thin agarose gel layer with a coverslip, and kept in a humid chamber at 4°C for 10 minutes, in order to further secure the suspension of tissue cells in the gel. Then, the coverslip was carefully removed and the slide, conditioned in a vertical cuvette containing lysis solution for at least 1 hour at 4°C. The following step consisted in the unfolding of the cells, for 30 minutes in an alkaline buffer (pH > 10.0). Thereafter, was followed by the process of electrophoresis, where the lysed cells contained in the agarose gel were subjected to a voltage of 25 mV and 300 mA for 15 minutes in alkaline buffer solution (pH > 10.0). Then the plate was neutralized, stained with silver nitrate, rinsed and kept at room temperature to dry for later analysis. The slides of each animal were made in duplicate and a positive control of DNA damage with hydrogen peroxide (30 µl/slide). The analysis was conducted under an optical microscope with a 20-x increase by quantifying the size of the comet's tail in 100 to 200 cells, according to the lengths, diameters, radii and dimensions of individual comets. To quantify the damage it was used as parameters obtained percentage of tail DNA, tail moment and Olive tail moment.

Quantification of DNA Damage

Using the CASP software (CASP Labs®, Poland) [26], three different types of markers of genotoxic damage was measured. This included percentage of tail DNA, tail moment (represents the distance of DNA migration from the head of the comet) and olive tail moment (represents the product of the tail length and the fraction of DNA in the tail), using the following formulas:

$$\% \text{ Tail DNA} = \frac{100 \quad x \quad DNA_{Tail}}{DNA_{Head} + DNA_{Tail}}$$

$$\text{Tail Moment} = \text{Tail Length} \times \% \text{ Tail DNA}$$

$$\text{OTM} = \frac{\text{Center of Gravity}_{Tail} - \text{Center of Gravity}_{Head}}{\% \text{ Tail DNA}}$$

Statistical analysis

The mean values and the standard deviation (± SD) were calculated for all the analyzed data. The normality of the data distribution was assessed using the Kolmogorov-Smirnov test. To compare the effects between the groups (HF or Sham) and intervention (RMT or Sed), it was used the two-way ANOVA, followed by the Tukey post hoc test. Pearson's correlation analysis was performed to test the corresponding associations. A $p < .05$ was considered statistically significant. The GraphPad Prism 6 program (GraphPad Software, CA, USA) was used in the data analysis.

Results

Mortality, morphological characteristics, pulmonary and hepatic congestion

The mortality in the HF rats submitted to the MI-induced, during or after surgery, was 36%. In the sham groups, there were no deaths during the study. The Sed-HF group presented pulmonary congestion compared with sham groups; RMT reduced the pulmonary congestion ($p< .0001$ for group, and $p< .001$ for training effects). There was no difference in hepatic congestion between groups. The HW/BW and RV/BW were higher in the Sed-HF group compared with sham groups. RV hypertrophy was similar between RMT-HF and sham groups. There were no differences in the LV/BW. All of these data are summarized in Table 1.

Hemodynamic variables

In sham rats, RMT had no effects on LVEDP, LVSP, $+dP/dt_{max}$, or $-dP/dt_{max}$. The LVEDP was higher in the sedentary HF rats, however, the LVSP, $+dP/dt_{max}$ and $-dP/dt_{max}$ was lower. RMT in HF rats lower LVEDP ($p< .0001$ for group, $p< .0001$ for training, and $p< .0001$ for interaction effects). LVSP and $+ dP/dt_{max}$ were similar between RMT-HF and sham groups. There were no differences in HR, MAP, systolic arterial pressure (SAP) and diastolic arterial pressure (DAP) between all groups. All of these data are summarized in Table 2.

DNA damage

When DNA damage in the diaphragm was evaluated, there was a significant increase in sedentary rats with HF in all parameters; % Tail DNA, TM and OTM compared with sham groups. RMT in rats with HF lower % Tail DNA ($p< .001$ for group, training and interaction effects), TM ($p< .05$ for group, $p< .01$ for training, and $p< .05$ for interaction effects) and OTM ($p< .01$ for group, $p< .001$ for training, and $p< .01$ for interaction effects) compared with the Sed-HF group. All of these data are summarized in Figures 1 and 2.

We also examined the association between DNA damage in the diaphragm and hemodynamics parameters and pulmonary congestion. Positive correlations were found between % DNA tail and pulmonary congestion ($r = 0.78$, $p< .0001$) and LVEDP ($r = 0.92$, $p< .0001$); TM and pulmonary congestion ($r = 0.74$, $p< .001$) and LVEDP ($r = 0.78$, $p< .0001$); and OTM and LVEDP ($r = 0.84$, $p< .0001$).

Discussion

In the present article, it was demonstrated that: *1*) RMT in rats with HF had improved

cardiovascular function, as demonstrated by decreased LVEDP, RV hypertrophy, and lung congestion; 2) DNA damage was increased in the diaphragm of the HF rats, and; *3*) RMT in rats with HF had reduced DNA damage on diaphragm, which was verified by the decrease in the % Tail DNA, TM and OTM. There were no differences between the RMT-Sham and Sed-Sham groups in any of the studied parameters. In addition, the intensity of RMT may have been insufficient to produce improvements in normal rats.

Studies have shown that there was an increase in the inspiratory muscle strength and resistance, which lead to improved peak VO_2, dyspnea, and quality of life after RMT in patients with HF [20, 21]. It is possible that the RMT reduces the sympathetic nervous activity increasing the peripheral skeletal muscle blood flow resulting in higher VO_2 and cardiac and peripheral autonomic control improvement as previously showed [27]. In normal rats, RMT increased diaphragmatic mass and lead to hypertrophy in IIa and IIb fiber types [24, 28]. Even if a previous study showed that RMT promotes better cardiopulmonary function [22] of HF rats, there is no consensus regarding the responses to RMT in the diaphragm oxidative stress in HF.

The experimental model of MI in rats results in HF [22, 23] and the MI areas > 30% represent a severe myocardial loss, which results in sustained hemodynamic dysfunction. In the present study, the infarcted area was ~ 38%, which was associated with impairment of the hemodynamic function, demonstrated by higher on LVEDP, HW/BW, RV/BW and pulmonary congestion. Recently, our group demonstrated that [22] rats with MI submitted a 6-wk RMT protocol showed a reduction of 33% in LVEDP, 32% in RV compensatory hypertrophy [22]. It was also demonstrated that RMT decreased lung congestion, airway resistance, improved hemodynamic function, and lung compliance in rats with HF [22]. Similarly, the present study showed a reduced LVEDP by 57%, 26% in RV compensatory hypertrophy, and decreased lung congestion.

Skeletal muscle weakness during HF is part of a generalized myopathy,

respiratory and limb skeletal muscles are not necessarily affected in the same way, or in the same extent [17]. Considering that the diaphragm muscle contracts continuously during life, and in HF there are an increase in breathing effort caused by pulmonary congestion, reduced lung compliance and increased airway resistance [16] generating muscle overload and dyspnea, new strategies to treat this symptoms are important in HF. A clinical study that investigated the ultrastructure of skeletal muscle by ultrastructural morphometry and cytochrome oxidase activity in patients with HF shows that a decrease in oxidative capacity is involved in the skeletal muscle abnormality, which may be associated with exercise intolerance [29]. Additionally, skeletal muscle exposure to hypoxia result in increased levels of ROS, leading to cell dysfunction and/or injury [30]. In the diaphragm muscle of rats, during hypoxia, there is an increase of ROS [31, 32]. In this way, inability of the heart to supply adequate amounts of blood to meet the metabolic needs of peripheral tissues [33, 34], including the diaphragm muscle, can be enhanced by RMT that could improve metabolic and morphological characteristics of diaphragm. This probably is associated with improvements in muscle dysfunction and in DNA damage as here demonstrated.

Oxidative stress has been implicated in clinical and experimental HF, and has been correlated with myocardial dysfunction and overall severity of HF [4, 5]. Supinski and Callahan [18] demonstrated that HF rats presented a raise in free radical, mitochondrial H_2O_2 generation, protein carbonyl and 8-isoprostane levels, beside force generated reduced in the diaphragm [18]. After treatment with polyethylene glycol- superoxide dismutase (PEG-SOD), the authors found that force generated in the diaphragm was restored to control levels, indicating that cardiac dysfunction increases diaphragm free radical generation and that evoked reductions in diaphragm force generation [18].

High concentration levels of oxidative products in mitochondrial DNA (mtDNA)

indicate a bigger extent of genotoxic damage [35]. Chronic increases in oxygen radical production in the mitochondria can lead to a catastrophic cycle of mtDNA damage as well as functional decline, further oxygen radical generation, and cellular injury [35]. The findings from the current study demonstrated that there was a higher DNA damage, in diaphragm cells from sedentary rats with HF. This probably is associated with low skeletal muscle perfusion, including the diaphragm, in the HF. RMT was able to reduced DNA damage in diaphragm and these results support the idea that RMT promoted protection against genotoxic damage in the diaphragm of HF rats. The mechanisms associated with these responses still needs to be investigated.

To the best of our knowledge, the present report is the first to show in a HF rat model that there is DNA damage in diaphragm muscle and the beneficial effects of RMT in reducing diaphragm DNA damage and various cardiovascular parameters. Moreover, we examined association between diaphragm DNA damage and hemodynamics parameters. Positive correlations were found between genotoxic parameters (% DNA tail, TM, and OTM) and pulmonary congestion and LVEDP. These associations represents that the impairment in the hemodynamic function in rats with HF increased the DNA damage in the diaphragm. We speculate that the HF decreases the oxidative capacity of skeletal muscles. These findings show that the use of RMT is able to improve oxidative capacity by lowering oxidative stress and diaphragm DNA damage.

There are some limitations regarding the present study. First, there is no histological evaluation of the adaptations in the lungs or diaphragm. Second, there were no measurements of respiratory muscle performance. Both of them would have assisted in the identification of the effects of RMT in muscle fiber and changes in lung parenchyma. However, the effects of RMT on diaphragmatic characteristics using this model in normal rats have been previously described [24, 28].

In summary, RMT is believed to produce lung vascular adaptations, which are translated into a better cardiopulmonary function as showed here by a decreased in LVEDP, lung congestion, and RV hypertrophy. Additionally, the decreased genotoxic damage in diaphragm DNA is probably associated with a better balance between production of ROS and endogenous antioxidants, which are perhaps related with a better diaphragm oxidative capacity after respiratory muscle training in rats with HF.

Acknowledgements

We are grateful for the excellent technical assistance provided by Terezinha Stein, and Rosalva Theresa Meurer.

Funding

Support for this work was provided by grants from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (Brazil) and the Universidade Federal de Ciências da Saúde de Porto Alegre (Brazil).

Conflict of interest: The authors have no conflicts of interest to declare.

References

[1] Meyer FJ, Borst MM, Zugck C, Kirschke A, Schellberg D, Kubler W, et al. Respiratory muscle dysfunction in congestive heart failure: clinical correlation and prognostic significance. Circulation. 2001;103:2153-8.

[2] van Hees HW, Ottenheijm CA, Granzier HL, Dekhuijzen PN, Heunks LM. Heart failure decreases passive tension generation of rat diaphragm fibers. International journal of cardiology. 2010;141:275-83.

[3] Empinado HM, Deevska GM, Nikolova-Karakashian M, Yoo JK, Christou DD, Ferreira LF. Diaphragm dysfunction in heart failure is accompanied by increases in neutral sphingomyelinase activity and ceramide content. European journal of heart failure. 2014;16:519-25.

[4] Grieve DJ, Shah AM. Oxidative stress in heart failure. More than just damage. European heart journal. 2003;24:2161-3.

[5] Keith M, Geranmayegan A, Sole MJ, Kurian R, Robinson A, Omran AS, et al. Increased oxidative stress in patients with congestive heart failure. Journal of the American College of Cardiology. 1998;31:1352-6.

[6] Tsutsui H, Kinugawa S, Matsushima S. Oxidative stress and heart failure. Am J Physiol Heart Circ Physiol. 2011;301:H2181-90.

[7] Botto N, Masetti S, Petrozzi L, Vassalle C, Manfredi S, Biagini A, et al. Elevated levels of oxidative DNA damage in patients with coronary artery disease. Coron Artery Dis. 2002;13:269-74.

[8] Kobayashi S, Susa T, Tanaka T, Wada Y, Okuda S, Doi M, et al. Urinary 8-hydroxy-2'-deoxyguanosine reflects symptomatic status and severity of systolic dysfunction in patients with chronic heart failure. European journal of heart failure. 2011;13:29-36.

[9] Fortini P, Pascucci B, Parlanti E, D'Errico M, Simonelli V, Dogliotti E. 8-Oxoguanine

DNA damage: at the crossroad of alternative repair pathways. Mutat Res. 2003;531:127-39.

[10] Dizdaroglu M. Oxidatively induced DNA damage and its repair in cancer. Mutat Res Rev Mutat Res. 2015;763:212-45.

[11] Collins AR. Measuring oxidative damage to DNA and its repair with the comet assay. Biochim Biophys Acta. 2014;1840:794-800.

[12] Collins AR. The comet assay: a heavenly method! Mutagenesis. 2015;30:1-4.

[13] Tsutsui H, Ide T, Hayashidani S, Suematsu N, Shiomi T, Wen J, et al. Enhanced generation of reactive oxygen species in the limb skeletal muscles from a murine infarct model of heart failure. Circulation. 2001;104:134-6.

[14] Linke A, Adams V, Schulze PC, Erbs S, Gielen S, Fiehn E, et al. Antioxidative effects of exercise training in patients with chronic heart failure: increase in radical scavenger enzyme activity in skeletal muscle. Circulation. 2005;111:1763-70.

[15] Poole-Wilson PA, Ferrari R. Role of skeletal muscle in the syndrome of chronic heart failure. Journal of molecular and cellular cardiology. 1996;28:2275-85.

[16] McParland C, Resch EF, Krishnan B, Wang Y, Cujec B, Gallagher CG. Inspiratory muscle weakness in chronic heart failure: role of nutrition and electrolyte status and systemic myopathy. Am J Respir Crit Care Med. 1995;151:1101-7.

[17] Coirault C, Guellich A, Barbry T, Samuel JL, Riou B, Lecarpentier Y. Oxidative stress of myosin contributes to skeletal muscle dysfunction in rats with chronic heart failure. Am J Physiol Heart Circ Physiol. 2007;292:H1009-17.

[18] Supinski GS, Callahan LA. Diaphragmatic free radical generation increases in an animal model of heart failure. J Appl Physiol (1985). 2005;99:1078-84.

[19] Silva FL, Mazzotti NG, Picoral M, Nascimento DM, Martins MIM, Klein AB. Infarto do miocárdio experimental e aumento do estresse oxidativo em diafragma de ratos. Jornal Brasileiro de Pneumologia. 2005;31:506-10.

[20] Dall'Ago P, Chiappa GR, Guths H, Stein R, Ribeiro JP. Inspiratory muscle training in patients with heart failure and inspiratory muscle weakness: a randomized trial. Journal of the American College of Cardiology. 2006;47:757-63.

[21] Adamopoulos S, Schmid JP, Dendale P, Poerschke D, Hansen D, Dritsas A, et al. Combined aerobic/inspiratory muscle training vs. aerobic training in patients with chronic heart failure: The Vent-HeFT trial: a European prospective multicentre randomized trial. European journal of heart failure. 2014;16:574-82.

[22] Jaenisch RB, Hentschke VS, Quagliotto E, Cavinato PR, Schmeing LA, Xavier LL, et al. Respiratory muscle training improves hemodynamics, autonomic function, baroreceptor sensitivity, and respiratory mechanics in rats with heart failure. J Appl Physiol (1985). 2011;111:1664-70.

[23] Pfeffer MA, Pfeffer JM, Fishbein MC, Fletcher PJ, Spadaro J, Kloner RA, et al. Myocardial infarct size and ventricular function in rats. Circulation research. 1979;44:503-12.

[24] Bisschop A, Gayan-Ramirez G, Rollier H, Gosselink R, Dom R, de Bock V, et al. Intermittent inspiratory muscle training induces fiber hypertrophy in rat diaphragm. Am J Respir Crit Care Med. 1997;155:1583-9.

[25] Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Experimental cell research. 1988;175:184-91.

[26] Konca K, Lankoff A, Banasik A, Lisowska H, Kuszewski T, Gozdz S, et al. A cross-platform public domain PC image-analysis program for the comet assay. Mutat Res. 2003;534:15-20.

[27] Mello PR, Guerra GM, Borile S, Rondon MU, Alves MJ, Negrao CE, et al. Inspiratory muscle training reduces sympathetic nervous activity and improves inspiratory muscle weakness and quality of life in patients with chronic heart failure: a clinical trial. Journal of cardiopulmonary rehabilitation and prevention. 2012;32:255-61.

[28] Rollier H, Bisschop A, Gayan-Ramirez G, Gosselink R, Decramer M. Low load inspiratory muscle training increases diaphragmatic fiber dimensions in rats. Am J Respir Crit Care Med. 1998;157:833-9.

[29] Drexler H, Riede U, Munzel T, Konig H, Funke E, Just H. Alterations of skeletal muscle in chronic heart failure. Circulation. 1992;85:1751-9.

[30] Zuo L, Best TM, Roberts WJ, Diaz PT, Wagner PD. Characterization of reactive oxygen species in diaphragm. Acta Physiol (Oxf). 2015;213:700-10.

[31] Mohanraj P, Merola AJ, Wright VP, Clanton TL. Antioxidants protect rat diaphragmatic muscle function under hypoxic conditions. J Appl Physiol (1985). 1998;84:1960-6.

[32] Heunks LM, Machiels HA, de Abreu R, Zhu XP, van der Heijden HF, Dekhuijzen PN. Free radicals in hypoxic rat diaphragm contractility: no role for xanthine oxidase. Am J Physiol Lung Cell Mol Physiol. 2001;281:L1402-12.

[33] Rosca MG, Hoppel CL. Mitochondrial dysfunction in heart failure. Heart failure reviews. 2013;18:607-22.

[34] Ventura-Clapier R, De Sousa E, Veksler V. Metabolic myopathy in heart failure. News Physiol Sci. 2002;17:191-6.

[35] Tsutsui H, Ide T, Kinugawa S. Mitochondrial oxidative stress, DNA damage, and heart failure. Antioxid Redox Signal. 2006;8:1737-44.

Figures

Figure 1

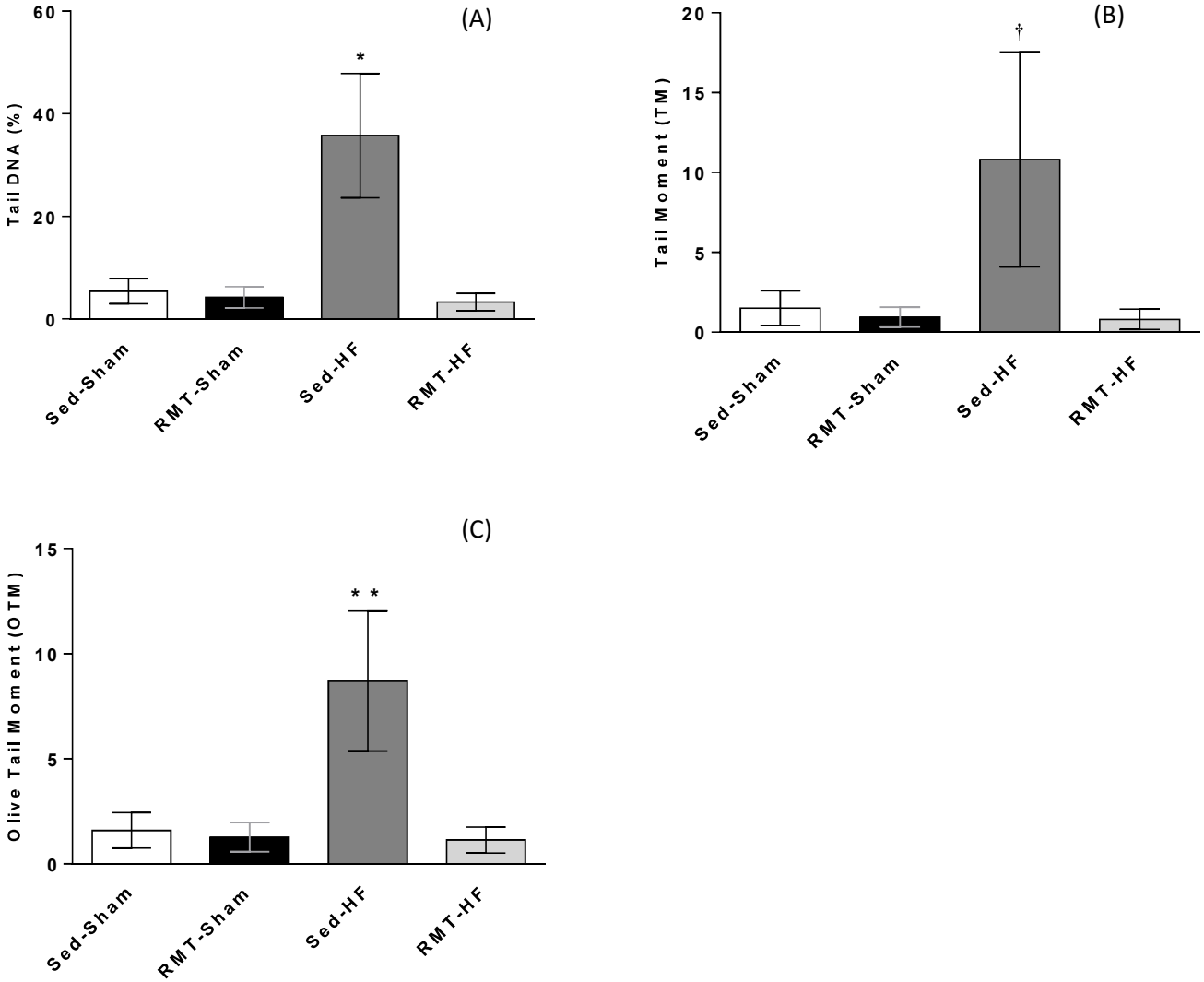

Figure 1: DNA damage in the diaphragm of the studied groups. Values are means ± SD. Groups were compared by the two-way ANOVA and Tukey post hoc tests. Sed- Sham, sedentary sham rats (n=4); RMT-Sham (n=5), respiratory muscle training sham rats; Sed-HF, sedentary heart failure rats (n=5); RMT-HF, respiratory muscle training heart failure rats (n=4). (A): % Tail DNA, percentage tail DNA. *p< .0001 compared with Sed-Sham, RMT-Sham and RMT-HF. (B): TM, tail moment. †p< .01 compared with Sed-Sham, RMT-Sham and RMT-HF. (C): OTM, olive tail moment. **p< .001 compared with Sed-Sham, RMT-Sham and RMT-HF.

Figure 2

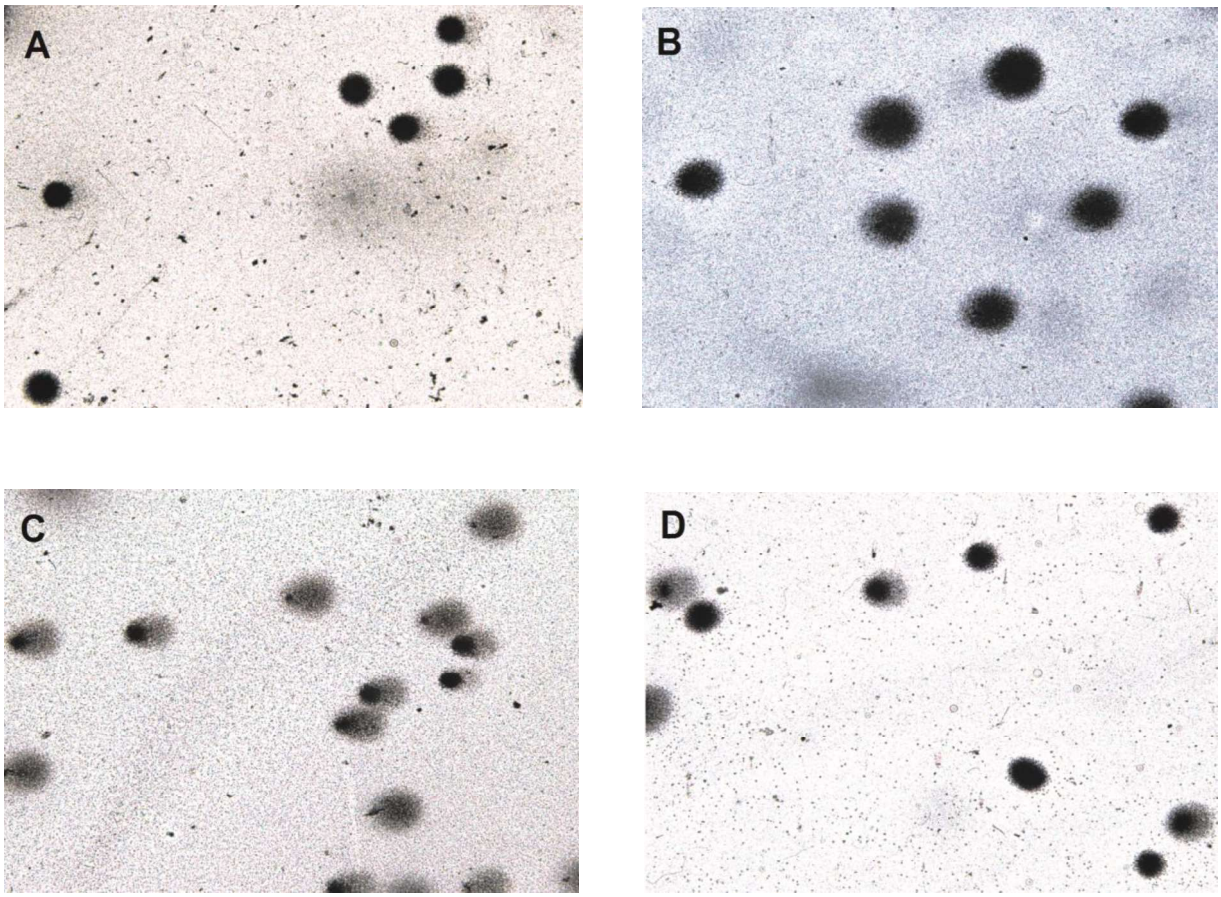

Figure 2: Image of cells submitted to SCGE (comet assay) of diaphragm of sham-operated rats and rats with left ventricular dysfunction. Panel A, B, C and D: Isolated diaphragm cells of Sed-Sham (A); RMT-Sham (B); Sed-HF (C); and RMT-HF (D) rats. Slides stained with silver nitrate. Panel A and B: cells without comets (magnification of 20x, scale bar of 20 µm). Panel C and D: cells showing comets (magnification of 20x, scale bar of 20 µm), however, in lower magnitude in D.

Tables

Table 1. *Morphological characteristics, infarct area and lung and hepatic congestion of sham-operated groups and rats with left ventricular dysfunction.*

Groups	Initial Body Weight, g	Final Body Weight, g	Infarcted Area, %	H/BW, mg/g	LV/BW, mg/g	RV/BW, mg/g	Pulmonary Congestion, %	Hepatic Congestion, %
Sed-Sham	278±12	347±9	------	2.96±0.25*	2.19±0.27	0.67±0.17	70.12±4.66	70.13±1.09
RMT-Sham	268±16	320±21	------	2.80±0.13†	2.15±0.15	0.65±0.13	68.58±2.35	70.26±1.57
Sed-HF	280±9	321±25	37.77±8	3.61±0.52	2.60±0.50	1.37±0.5‡	84.73±5.44§	71.08±0.83
RMT-HF	268±18	345±37	39.12±6	3.58±0.60	2.57±0.31	1.01±0.43	72.69±3.44	71.14±1.05

Values are means ± SD; n= 8 for each groups. Groups were compared by the two-way ANOVA and Tukey post hoc tests. Sed-Sham, sedentary sham rats; RMT-Sham, respiratory muscle training sham rats; Sed-HF, sedentary heart failure rats; RMT-HF, respiratory muscle training heart failure rats. HW/BW, Heart weight-to-body weight ratio; LV/BW, left ventricle-to-body weight ratio and RV/BW, right ventricle-to-body weight ratio. *p< .05 compared with Sed-HF and RMT-HF. †p< .01 compared with Sed-HF and RMT-HF. ‡p< .01 compared with Sed-Sham and RMT- Sham. §p< .0001 compared with Sed-Sham, RMT-Sham and RMT-HF.

Table 2. *Hemodynamics variables of sham-operated groups and rats with left ventricular dysfunction.*

Groups	LVEDP (mmHg)	LVSP (mmHg)	$+dP/dt_{max}$ (mmHg/s)	$-dP/dt_{max}$ (mmHg/s)	HR, bpm	MAP, mmHg	SAP, mmHg	DAP, mmHg
Sed-Sham	5.2±2.3‡	105.3±5.5	6190±1218	-4797±1192‡	255±70	93±15	99±21	74±16
RMT-Sham	4.2±1.8	92±18.4	6122±924	-4336±498	262±65	88±17	99±19	75±16
Sed-HF	31.1±5.7*	86.9±5.1§	4317±654#	-2865±505#	242±54	75±5	84±5	64±6
RMT-HF	13.4±5.1†	92.6±15	4936±1128	-3323±677	224±23	83±19	94±23	73±15

Values are means ± SD; n= 8 for each groups. Groups were compared by the two-way ANOVA and Tukey post hoc tests. Sed-Sham, sedentary sham rats; RMT-Sham, respiratory muscle training sham rats; Sed-HF, sedentary heart failure rats; RMT-HF, respiratory muscle training heart failure rats. LVEDP, LV end-diastolic pressure; LVSP, LV systolic pressure; $+dP/dt_{max}$, LV maximum change in pressure over time; $-dP/dt_{max}$, LV minimum change in pressure over time. *p< .0001 compared with RMT-HF, RMT-Sham and Sed-Sham. †p< .001 compared with RMT-Sham. ‡p< .01 compared with RMT-HF. §p< .05 compared with Sed-Sham. #p< .01 compared with Sed-Sham and RMT-Sham.

ESTUDO 2

Respiratory muscle training improves hemodynamic, chemoreflex, heart rate variability and respiratory mechanics in heart failure rats

Artigo submetido ao periódico ***European Journal of Heart Failure***

Respiratory muscle training improves hemodynamic, chemoreflex, heart rate variability and respiratory mechanics in heart failure rats

Running title: Respiratory muscle training in heart failure

Article for submission to **European Journal of Heart Failure**

Rodrigo B. Jaenisch[1], Edson Quagliotto[1], Chalyne Chechi[1], Leonardo Calegari[1], Fernando dos Santos[4], Pedro Dal Lago[1,2,3*]

[1] Laboratory of Experimental Physiology, Post Graduation Program in Health Sciences, Universidade Federal de Ciências da Saúde de Porto Alegre, Brazil

[2] Post Graduation Program in Rehabilitation Sciences, Universidade Federal de Ciências da Saúde de Porto Alegre, Brazil

[3] Physical Therapy Department, Universidade Federal de Ciências da Saúde de Porto Alegre, Brazil

[4] Laboratory of Experimental Hypertension, Universidade de São Paulo, Brazil

* Corresponding author: Universidade Federal de Ciências da Saúde de Porto Alegre, Sarmento Leite, 245, 90050-170, Porto Alegre, RS, Brazil. Fax: +55 51 3303-8751.

E-mail address: pdallago@ufcspa.edu.br (P. Dal Lago, PT, ScD)

Abstract

Aims: The increase of respiratory muscles strength in patients with heart failure (HF) improves many clinical outcomes related to the pathophysiological manifestations of HF. Also, in rats with HF, respiratory muscle training (RMT) is associated with beneficial effects in cardiopulmonary variables, however, some physiological response remains unclear. Therefore, the aim of the present report was to evaluate the RMT effects on hemodynamic function, chemoreflex activation, heart rate variability and respiratory mechanics in HF rats.

Methods and Results: Wistar rats were allocated into four groups: sedentary sham (Sed-Sham, *n*=8), trained sham (RMT-Sham, *n*=8), sedentary HF (Sed-HF, *n*=8) and trained HF (RMT-HF, *n*=8). The animals were submitted to a RMT protocol performed a 30 min a day, 5 days/week, for 6 weeks, while the sedentary animals did not exercise. Groups were compared by a two-way ANOVA and Tukey´s post hoc test. In rats with HF, RMT promoted the reduction of pulmonary congestion, the left ventricular end-diastolic pressure, as well as reduced right ventricular hypertrophy. Moreover, RMT produced reduction in pressure response during chemoreflex activation and improvement in autonomic function, as verified by the reduced sympathetic modulation, reduction in sympathetic-vagal balance and increase in parasympathetic modulation. After RMT, the respiratory mechanics was enhanced in HF rats, demonstrated by reduction in the respiratory system resistance, tissue resistance, respiratory system elastance, tissue elastance and quasistatic elastance.

Conclusion: These findings show that a 6-week RMT in HF rats promotes an improvement on hemodynamic and autonomic function, reduction in pressure response evoked by chemoreflex and enhances respiratory mechanics in HF rats.

Keywords: heart failure, inspiratory muscle training, chemoreflex, autonomic function, exercise.

Introduction

Sympathetic overactivity (1) and reduced vagal drive to the heart (2) are hallmarks of heart failure (HF) at the clinical and experimental settings, presumably as a consequence of hemodynamic changes associated with alteration in cardiac function (3). Autonomic dysfunction can lead to a disturbed chemoreflex function (4). In particular, the increase in chemoreflex-mediated activation of sympathetic outflow occurs in patients and experimental animals with HF (5) and correlates significantly with the higher ventilatory response to exercise and dyspnea observed in these patients (6).

Disorders of the respiratory muscles can contribute to the activation of cardiovascular reflexes, which aggravates exercise limitation in HF. Weakness of the respiratory muscles in patients with HF contributes to marked fatigue and dyspnea (7), and represents an independent predictor of poor prognosis (8). Specifically, respiratory muscle weakness is linked to increased peripheral chemoreflex (9) and is associated with abnormal cardiac autonomic control (10).

In patients with HF, respiratory muscle training (RMT) increased the inspiratory muscle strength and endurance, which leads to improvements in peakVO_2, dyspnea and quality of life (11, 12). In a study developed in our laboratory (13), RMT in rats with HF showed improvements in cardiovascular function, sympathetic and parasympathetic modulation, baroreflex gain and in respiratory mechanics. However, the effects of RMT on the chemoreflex sensitivity and heart rate variability in rats with HF had not been evaluated.

Therefore, we conducted the present study to test the hypothesis that a 6-week protocol of RMT could improve the hemodynamic function, respiratory mechanics, chemoreflex function and heart rate variability of HF rats.

Methods

Animals

The investigation followed the ethical rules established by the Guide for the Care and Use of Experimental Animals published by the National Institute of Health (NIH publication no. 85-23, revised in 1996). All of the procedures outlined in this study were approved by the Ethics Committee Research of the Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA) (protocol 070/11). Thirty-two male Wistar rats (250 to 290 g; 90 days of age) obtained from the Animal Breeding Unit of the UFCSPA were housed under standard conditions (food and water ad libitum, 12:12-h light-dark cycle; 22uC).

Surgery to induce myocardial infarction

Rats were anesthetized with xylazine (12 mg/kg ip) and ketamine (90 mg/kg ip), intubated and artificially ventilated. Ligation of the left coronary artery and sham operations were performed as previously described (13, 14). After the surgery, the animals received a single injection of monofenew (0.05 ml/100 g) and gentamicin (0.05 ml/100 g).

Experimental design

After myocardial infarction (MI), rats were allowed to recover for 4 week (the time required for the development of HF state) (14) and were assigned into four experimental groups: sedentary sham rats (Sed-Sham; n=8), trained sham rats (RMT-Sham; n=8), sedentary HF rats (Sed-HF; n=8), or trained HF rats (RMT-HF; n=8).

RMT protocol

Four weeks after the MI or sham surgery, the rats assigned to the RMT groups were submitted to a 5-day adaptation protocol, as previously described (13, 15). The RMT protocol began in the 5th week. It comprised 30 min/day, 5 days/week, for 6 weeks. The progress of the protocol was achieved by a progressive increase in resistance, by reducing the internal diameter of the hole through which the animal breathed, as previously described (13, 15).

Implantation of the vascular catheters for cardiovascular measurements in awake rats

Animals were anesthetized as described earlier and had a polyethylene catheter [PE-10 conected to a PE-50, 0.28 mm inner diameter (ID), Biocorp, Australia, filled with sterile 0.9% NaCl (pH 7.4) and heparin] placed into the abdominal aorta and another one into the inferior vena cava through the left femoral artery and vein, respectively. Each catheter was tunneled subcutaneously and exteriorized at the back of the rat neck. Gentamicin (2 mg/rat, im) was injected at the end of this procedure (16).

The next day, the arterial catheter was attached to a 40-cm polyethylene tube (PE-50, 0.5 mm ID, Biocorp), and a strain-gauge pressure transducer (P23 Db, Gould Statham, USA) was used for direct hemodynamic measurements. Signals were passed through a preamplifier (Hewlett-Packard 8805, Puerto Rico) and were delivered to a microcomputer equipped with an analog-to-digital converter board (CODAS, 1 kHz, Dataq Instruments, USA). The recorded data were analyzed on a beat-to-beat basis (16).

All rats were submitted to the same recording protocol. That is, after the catheter was connected to the transducer, rats were allowed to aclimate to a Plexiglas recording

box (25 x 15 x 10 cm) over 20 to 30 min, while cardiovascular parameters were being continuously monitored. Values of heart rate (HR) and arterial pressure (AP) were obtained during the last 15 min of this period to provide basal data.

Chemoreflex stimulation

Immediately after these initial recordings, chemoreflex sensitivity was tested, by increasing intravenous injection doses of potassium cyanide (KCN; 60, 100, 140, and 180 μg/kg; Merck, Germany). The volumes injected ranged from 0.06 to 0.18 mL. Mean HR and mean arterial pressure (MAP) were measured continuously 10 s before and 15 s after each injection of these KCN doses (16).

Power spectral analysis

Spectral analysis of systolic arterial blood pressure (SBP) and pulse interval (PI) to evaluate the sympathetic and parasympathetic cardiovascular modulation were performed by an autoregressive method. From the original recordings, samples were exported to create a database for analysis, according to the heart rate variability (HRV) guidelines (17). Succinctly, systogram was created through the beat-to-beat SBP derived from blood pressure (BP) signals. The "Low Frequencies" (LF, 0.2–0.75 Hz) and "High Frequencies" (HF, 0.75–3.0 Hz) spectral components of PI and SBP were explicited in absolute values (ms^2 and mmHg, respectively) and in normalized units (NU). The NU were obtained after calculating the power of low and high frequency components and correlating these components to the total power excluding the very low frequency components (frequencies ≤ 0.2 Hz). The means of a cross-spectral analysis were used to calculate the coherence between the PI interval and the SBP signal variability. Following the coherence calculation, alpha index was achieved through the

square root of the ratio between PI and SBP variability in the LF two major bands (17). Furthermore, the alpha index was obtained only if the magnitude of the squared coherence considering the PI and SBP signals exceeded 0.5, in an interval between 0 and 1, in the LF band.

Assessment of respiratory mechanics

Twenty-four hours after chemoreflex stimulation, the rats were anesthetized again and tracheostomized. A rigid-type cannula (2-mm ID) was inserted into the trachea and tied firmly in place. The cannula was connected to a small animal ventilator (flexiVent, Scireq, Montreal, QC, Canada). Rats were mechanically ventilated at a breath rate of 90 breaths/min, with a tidal volume of 10 ml/kg using 5 cmH_2O positive end-expiratory pressure established by a water column (13, 18). The constant-phase model described by Hantos et al. (19) was used to partition impedance into components representing the mechanical properties of the airway and parenchyma. The constant-phase model was fitted as: $Zrs = R + j\omega I + (G-jH)/\omega\alpha$, where R is the Newtonian resistance (primarily located in the airways but containing a contribution from the chest wall), I is the inertance, G is the coefficient of tissue damping, H is the co-efficient of tissue elastance, ω is the angular frequency and α represents the reciprocal frequency-dependent behaviour of G & H. Briefly, the parameters Raw and Iaw, respectively, include the Newtonian components of tissue resistance and tissue inertance. Quasistatic Est reflects the static elastic recoil pressure of the lungs at a given lung volume and was measured by applying the pressure-volume curve technique using the Salazar-Knowles equation, as described by the flexiVent manufacturer (19).

Cardiac hemodynamic evaluation

After evaluating the respiratory mechanics, while under anesthesia, as described previously, a polyethylene catheter (PE-50) was inserted into the right carotid artery for hemodynamic evaluation. The AP was recorded first during a 5-min period. Then, the catheter was positioned inside the left ventricle (LV), and the pulse wave was monitored using the typical graphic registration of ventricular pressure and recorded for 5 min. Pressure analogical signals were digitalized by a data-acquisition system (Windaq - AT/CODAS, Dataq Instruments Inc., OH, USA) with sampling rate of 2.000 Hz. These data were used to determine mean arterial pressure (MAP), heart rate (HR), left ventricular systolic pressure (LVSP), left ventricular maximum change in pressure over time ($+dP/dt_{max}$) and left ventricular minimum change in pressure over time ($-dP/dt_{max}$), and left ventricular end-diastolic pressure (LVEDP). This last parameter was determined manually by the detection of the point of inflection to the end of diastoles from the analysis of the wave of ventricular pressure (13).

Infarct size, heart hypertrophy, and pulmonary and hepatic congestion

Rats were euthanized with an overdose of anesthetic (thiopental 80 mg/kg ip), and the heart, lungs, and liver were removed and weighed. The right ventricle (RV) and LV were dissected and weighed. The LVs were filled with an insufflating latex balloon and placed in 10% formaldehyde for a minimum of 3 days before being cut into two equal transverse sections. These sections were embedded in paraffin for subsequent analysis of the infarct size. The percentage of the infarcted area was determined as described previously (14). The heart weight-to-body weight ratio (HW/BW), LV/BW, and RV/BW values were determined. Lungs and liver were dehydrated (80°C) for 48 h and then weighed again to evaluate the water percentage (20).

Statistical analysis

The mean values and the standard deviation (± SD) were calculated for all the analysed data. The Kolmogorov-Smirnov normality test was performed. To compare the effects between the groups (HF or Sham) and intervention (RMT or Sed), it was used the two-way ANOVA, followed by the Tukey post hoc test. A $p < .05$ was considered statistically significant. The GraphPad Prism 6 program (GraphPad Software, CA, USA) was used in the data analysis.

Results

It was not observed behavioral changes associated with stress or adverse effects in the rats that participated of the RMT protocol.

Mortality, morphological characteristics, pulmonary and hepatic congestion

In HF rats submitted to the MI, during or after surgery, the mortality was 31%. In sham groups, there were no deaths during the study. The initial body weights and final body weights were similar among the four groups in both the pre- and post-training period. There were no differences in the infarcted area between the HF groups. The Sed-HF group presented pulmonary congestion compared with sham groups; RMT lower the pulmonary congestion ($p < .01$ for group, $p < .001$ for training and interaction effects). There was no difference in hepatic congestion among groups.

The HW/BW and RV/BW were higher in the Sed-HF group compared with sham groups. The RV hypertrophy was lower in the RMT-HF ($p < .001$ for group, $p < .05$ for training and interaction effects) when compared to the Sed-HF group.

Furthermore, the RMT-HF group showed no difference in RV hypertrophy when compared to the sham groups. All of these data are summarized in Table 1.

Hemodynamic variables

All of these data are summarized in table 2. In sham rats, RMT had no effects on LVEDP, LVSP, $+dP/dt_{max}$, or $-dP/dt_{max}$. The LVEDP was higher in the sedentary HF rats, however, the LVSP, $+dP/dt_{max}$ and $-dP/dt_{max}$ was lower. RMT in HF rats lower LVEDP ($p < .0001$ for group and training, and $p < .001$ for interaction effects). In sedentary HF rats, the MAP, systolic arterial pressure (SAP) and diastolic arterial pressure (DAP) was lower.

Cardiovascular responses evoked by chemoreflex activation

The intravenous injection of KCN (60, 100, 140, and 180 µg/kg) produced a marked increase in blood pressure (sympathetic effect) and a marked decrease in heart rate (parasympathetic effect) in all groups. No statistically significant difference was found in the HR changes induced by KCN injections between experimental groups (Fig. 1A). On the other hand, the pressor responses evoked by KCN injection at 60 was lower in the RMT-HF group when compared Sed-Sham group *(*$p < .01$). The pressor responses evoked by KCN injection at 100 µg/kg was lower in the RMT-HF group when compared Sed-Sham, RMT-Sham and Sed-HF groups ($p < .001$; $p < .001$ and $p < .05$, respectively). Similarly, after KCN injection at 140 µg/kg, RMT was able a lower in the HF rats when compared Sed-Sham and RMT-Sham groups ($p < .001$). Responses by KCN injection at 180 was lower in the RMT-HF group when compared Sed-Sham and RMT-Sham groups *(*$p < .01$ and $p < .05$, respectively). All of these data are summarized in Fig 1B.

Heart rate variability

All of these data are summarized in table 3. In sham rats, RMT had no effects on heart rate or blood pressure variability parameters. The sympathetic modulation by absolute LF band spectrum ($p < .01$ for training effect), LF (n.u.) ($p < .001$ for group, and $p < .01$ for interaction effects) and peripheral sympathetic modulation, as can be seen in LF band of systolic blood pressure ($p < .05$ for training effect), were higher in the sedentary HF rats when compared to RMT-HF group. On the other hand, the RMT was able to higher parasympathetic modulation by absolute HF band spectrum ($p < .0001$ for group, and $p < .01$ for interaction effects), HF (n.u.) ($p < .001$ for group, and $p < .01$ for interaction effects) and rMSSD ($p < .001$ for group, and $p < .01$ for interaction effects). Associated with this, the sympathetic-vagal balance (LF/HF) was lower in RMT-HF when compared to other groups ($p < .0001$ for group, and $p < .05$ for training and interaction effects).

Respiratory mechanics

The respiratory system resistance (Rrs) and Gti (tissue resistance) were higher in the Sed-HF group compared with sham groups (Fig. 2A) (Fig. 2B). RMT lower Rrs ($p < .001$ for group, and $p < .05$ for training and interaction effects) and Gti ($p < .001$ for group, and $p < .05$ for training effects) in HF rats compared with sham groups.

The respiratory system Est (Ers) was higher in Sed-HF rats compared with sham groups; RMT lower the Ers ($p < .0001$ for group, and $p < .05$ for training and interaction effects; Fig. 3A). Tissue Est (Hti), which has a main role in Ers, was higher in HF rats; RMT lower Hti ($p < .0001$ for group, and $p < .001$ for training effects; Fig.

3B). Additionally, quasistatic Est was higher in the Sed-HF group; RMT lower Est in HF rats ($p < .05$ for interaction effect; Fig. 3C).

Discussion

In the present study, we have demonstrated that RMT in rats with HF induced: *1*) improvement in cardiovascular function, as demonstrated by the decrease in LVEDP, RV hypertrophy and lung congestion; *2*) Changes in chemoreflex sensitivity, as verified by lower in the pressor responses evoked by KCN injection at 100 µg/kg, 140 µg/kg and 180 µg/kg; *3*) improvement in autonomic function, as verified by the decrease in the absolute LF band spectrum, LF (n.u.) and peripheral sympathetic modulation, as well as increase in the parasympathetic modulation by absolute HF band spectrum, HF (n.u.) and rMSSD, associated with the decrease in the sympathetic-vagal balance (LF/HF); *4*) decrease in the Rrs and Gti, as well as Ers, Hti, and Est, respiratory mechanics parameters.

The RMT has been observed to improve many clinical outcomes related to the pathophysiological manifestations of HF (11). On the other hand, there is no consensus regarding the responses to RMT on the mechanisms that explain an improvement in these patients. Even if a previous study showed that RMT promotes better cardiopulmonary function (13) of HF rats, however, it was not evaluated the effect of RMT on cardiovascular function, respiratory mechanics, chemoreflex activity and heart rate variability in rats with HF in the same study. Therefore, these new mechanisms are important to best explain the effects of RMT in HF syndrome.

MI areas > 30% in rats represent a severe myocardial loss, and results in HF with sustained hemodynamic dysfunction (13, 14). In the present study, the infarcted area was ~39%, which is associated with impairment of the hemodynamic function,

demonstrated by higher on LVEDP, H/BW, LV/BW, RV/BW, pulmonary congestion, and lower LVSP. Here we showed a lower in the LVEDP by 56%, 31% in RV compensatory hypertrophy and 18% in the lung congestion after RMT. The improves of cardiac function observed in the RMT-HF group may have been produced by improvement in autonomic function, analyzed by heart rate variability (spectral analysis), which likely promoted peripheral vascular adaptations. Here we found that RMT in HF rats showed a reduction in the peripheral sympathetic activity. The reduction of the peripheral sympathetic activity and consequently, peripheral vascular resistance, promotes greater perfusion of peripheral muscles (21). If these peripheral adaptations are present, the decrease lung congestion occurs minimizing the RV hypertrophy.

The abnormal ventilatory response at rest and during exercise, that caracterizes advanced HF, has been associated with pulmonary congestion due to impaired hemodynamic function, also it can be associated with abnormal chemoreflex and autonomic responses (22, 23). Hyperactivation of the sympathetic nervous system (SNS) have a pivotal role in the progression of morbidity and ultimate in mortality of HF (24). It is known that multiple cardiovascular reflexes and central adjustments contribute to the hyperactivity of SNS in HF (25). In particular, enhanced chemoreflex-mediated activation of sympathetic outflow occurs in patients and experimental animals with HF (5), which results in reduced blood supply to the tissues resulting in the increase of peripheral chemoreflex sensitivity in this syndrome (25).

Respiratory muscle weakness in patients with HF shows a greater peripheral chemoreflex response than those with preserved respiratory muscle strength (9). It is noteworthy that, respiratory muscle weakness represents an independent predictor of poor prognosis (8) in patients with HF, and is also present in HF-rats (8, 26). In this

study, RMT in rats with HF demonstrated a reduced in the chemoreflex activation, verified by lower increases in blood pressure (sympathetic effect) evoked by KCN injection at 100 and 140 μg/kg when compared sedentary HF-rats.

Activation of the SNS and inhibition of the parasympathetic system have long been recognized as manifestations of the clinical syndrome of HF, presumably as a consequence of hemodynamic changes associated with the alteration in cardiac function (3). In agreement with this, Mello et al. (2012) (12) that evaluated the effect of RMT on cardiac autonomic modulation and on peripheral nerve sympathetic activity in patients with HF (12). They found an increased in parasympathetic component and decreased in the sympathetic component, besides decreased in muscle sympathetic nerve activity (12). To our knowledge, neither study evaluated the effects of RMT in sympathetic and parasympathetic balance and chemoreflex response, which can clarify the other mechanisms of cardiovascular regulation.

In the present report, RMT in rats with HF showed lower in the sympathetic modulation by absolute LF, LF (n.u.) and peripheral sympathetic modulation (LF band of systolic blood pressure). On the other hand, the RMT was able to increase parasympathetic modulation by absolute HF, HF (n.u.) and rMSSD together a reduction of the sympathetic-vagal balance (LF/HF). The reduction of the sympathetic nervous activity and consequently, peripheral vascular resistance, promotes greater perfusion of peripheral muscles (21), which can produce peripheral vascular adaptations.

Regarding to alterations in respiratory function, HF is associated with a reduced carbon monoxide diffusion capacity (27), reduced respiratory muscle strength and oxygenation (8), and increase in breathing effort caused by pulmonary congestion, reduced lung compliance and increased airway resistance (13, 27, 28). Hydrostatic pulmonary edema is caused by an increase in microvascular pulmonary pressure after

increased LVEDP (29), which promotes an increase in the tissue component of the resistance in the respiratory system and decreases the airway cross-sectional area (30), leading to fatigue and dyspnea.

In a previous study, we found that RMT in rats with HF improves respiratory mechanics (13). Similarly, in the present report, RMT in HF rats lower in tissue and Rrs. Furthermore, we also found a lower in static, tissue and static Ers. Here we found that the improvement of hemodynamic function, (lower in: LVEDP, RV hypertrophy and pulmonary congestion) after RMT, are associated with the results observed in the respiratory mechanics.

There are some limitations regarding the present report. First, there is no histological evaluation of the adaptations in the lungs or diaphragm. Second, it was not measured the respiratory muscle performance. Both of them would help to identify the effects of RMT in muscle fiber and lung parenchyma adaptations.

In conclusion, RMT is believed to produce lung and peripheral vascular adaptations, which are translated into a better hemodynamic function and respiratory mechanics parameters. Additionally, the changes observed in pressor response during chemoreflex activation are probably associated with a better sympathetic-vagal balance, demonstrated by a reduction in the sympathetic and increase in parasympathetic modulation in rats with HF and RMT.

Acknowledgements

We are grateful for the excellent technical assistance provided by Terezinha Stein, and Rosalva Theresa Meurer.

Funding

Support for this work was provided by grants from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (Brazil) and the Universidade Federal de Ciências da Saúde de Porto Alegre (Brazil).

Conflict of interest: The authors have no conflicts of interest to declare.

References

1. Zucker IH, Patel KP, Schultz HD. Neurohumoral stimulation. *Heart Fail Clin*. 2012 Jan;**8**(1):87-99.
2. Bibevski S, Dunlap ME. Evidence for impaired vagus nerve activity in heart failure. *Heart Fail Rev*. 2011 Mar;**16**(2):129-135.
3. Florea VG, Cohn JN. The autonomic nervous system and heart failure. *Circ Res*. 2014 May 23;**114**(11):1815-1826.
4. Hennersdorf MG, Hillebrand S, Perings C, Strauer BE. Chemoreflexsensitivity in chronic heart failure patients. *European journal of heart failure*. 2001 Dec;**3**(6):679-684.
5. Schultz HD, Li YL. Carotid body function in heart failure. *Respir Physiol Neurobiol*. 2007 Jul 1;**157**(1):171-185.
6. Ciarka A, Cuylits N, Vachiery JL, Lamotte M, Degaute JP, Naeije R, van de Borne P. Increased peripheral chemoreceptors sensitivity and exercise ventilation in heart transplant recipients. *Circulation*. 2006 Jan 17;**113**(2):252-257.
7. Mancini DM. Pulmonary factors limiting exercise capacity in patients with heart failure. *Prog Cardiovasc Dis*. 1995 May-Jun;**37**(6):347-370.
8. Meyer FJ, Borst MM, Zugck C, Kirschke A, Schellberg D, Kubler W, Haass M. Respiratory muscle dysfunction in congestive heart failure: clinical correlation and prognostic significance. *Circulation*. 2001 May 1;**103**(17):2153-2158.
9. Callegaro CC, Martinez D, Ribeiro PA, Brod M, Ribeiro JP. Augmented peripheral chemoreflex in patients with heart failure and inspiratory muscle weakness. *Respir Physiol Neurobiol*. 2010 Apr 15;**171**(1):31-35.
10. Reis MS, Arena R, Archiza B, de Toledo CF, Catai AM, Borghi-Silva A. Deep breathing heart rate variability is associated with inspiratory muscle weakness in chronic heart failure. *Physiother Res Int*. 2014 Mar;**19**(1):16-24.
11. Cahalin LP, Arena R, Guazzi M, Myers J, Cipriano G, Chiappa G, Lavie CJ, Forman DE. Inspiratory muscle training in heart disease and heart failure: a review of the literature with a focus on method of training and outcomes. *Expert Rev Cardiovasc Ther*. 2013 Feb;**11**(2):161-177.
12. Mello PR, Guerra GM, Borile S, Rondon MU, Alves MJ, Negrao CE, Dal Lago P, Mostarda C, Irigoyen MC, Consolim-Colombo FM. Inspiratory muscle training reduces sympathetic nervous activity and improves inspiratory muscle weakness and quality of life in patients with chronic heart failure: a clinical trial. *Journal of cardiopulmonary rehabilitation and prevention*. 2012 Sep-Oct;**32**(5):255-261.
13. Jaenisch RB, Hentschke VS, Quagliotto E, Cavinato PR, Schmeing LA, Xavier LL, Dal Lago P. Respiratory muscle training improves hemodynamics, autonomic function, baroreceptor sensitivity, and respiratory mechanics in rats with heart failure. *J Appl Physiol (1985)*. 2011 Dec;**111**(6):1664-1670.
14. Pfeffer MA, Pfeffer JM, Fishbein MC, Fletcher PJ, Spadaro J, Kloner RA, Braunwald E. Myocardial infarct size and ventricular function in rats. *Circ Res*. 1979 Apr;**44**(4):503-512.
15. Bisschop A, Gayan-Ramirez G, Rollier H, Gosselink R, Dom R, de Bock V, Decramer M. Intermittent inspiratory muscle training induces fiber hypertrophy in rat diaphragm. *Am J Respir Crit Care Med*. 1997 May;**155**(5):1583-1589.
16. Quagliotto E, Casali KR, Dal Lago P, Rasia-Filho AA. Neuropeptides in the posterodorsal medial amygdala modulate central cardiovascular reflex responses in awake male rats. *Braz J Med Biol Res*. 2015 Feb;**48**(2):128-139.

17. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation*. 1996 Mar 1;**93**(5):1043-1065.
18. Collins RA, Gualano RC, Zosky GR, Atkins CL, Turner DJ, Colasurdo GN, Sly PD. Hyperresponsiveness to inhaled but not intravenous methacholine during acute respiratory syncytial virus infection in mice. *Respir Res*. 2005;**6**:142.
19. Hantos Z, Daroczy B, Suki B, Nagy S, Fredberg JJ. Input impedance and peripheral inhomogeneity of dog lungs. *J Appl Physiol (1985)*. 1992 Jan;**72**(1):168-178.
20. Alves JP, Nunes RB, Stefani GP, Dal Lago P. Resistance training improves hemodynamic function, collagen deposition and inflammatory profiles: experimental model of heart failure. *PLoS One*. 2014;**9**(10):e110317.
21. Fraga R, Franco FG, Roveda F, de Matos LN, Braga AM, Rondon MU, Rotta DR, Brum PC, Barretto AC, Middlekauff HR, Negrao CE. Exercise training reduces sympathetic nerve activity in heart failure patients treated with carvedilol. *Eur J Heart Fail*. 2007 Jun-Jul;**9**(6-7):630-636.
22. Passino C, Poletti R, Bramanti F, Prontera C, Clerico A, Emdin M. Neuro-hormonal activation predicts ventilatory response to exercise and functional capacity in patients with heart failure. *Eur J Heart Fail*. 2006 Jan;**8**(1):46-53.
23. Malfatto G, Caravita S, Giglio A, Rossi J, Perego GB, Facchini M, Parati G. Pulmonary congestion at rest and abnormal ventilation during exercise in chronic systolic heart failure. *J Am Heart Assoc*. 2015 May;**4**(5).
24. Esler M, Kaye D, Lambert G, Esler D, Jennings G. Adrenergic nervous system in heart failure. *Am J Cardiol*. 1997 Dec 4;**80**(11A):7L-14L.
25. Ding Y, Li YL, Schultz HD. Role of blood flow in carotid body chemoreflex function in heart failure. *J Physiol*. 2011 Jan 1;**589**(Pt 1):245-258.
26. van Hees HW, Ottenheijm CA, Granzier HL, Dekhuijzen PN, Heunks LM. Heart failure decreases passive tension generation of rat diaphragm fibers. *Int J Cardiol*. 2010 Jun 11;**141**(3):275-283.
27. Gehlbach BK, Geppert E. The pulmonary manifestations of left heart failure. *Chest*. 2004 Feb;**125**(2):669-682.
28. McParland C, Resch EF, Krishnan B, Wang Y, Cujec B, Gallagher CG. Inspiratory muscle weakness in chronic heart failure: role of nutrition and electrolyte status and systemic myopathy. *Am J Respir Crit Care Med*. 1995 Apr;**151**(4):1101-1107.
29. Dixon DL, De Pasquale CG, De Smet HR, Klebe S, Orgeig S, Bersten AD. Reduced surface tension normalizes static lung mechanics in a rodent chronic heart failure model. *Am J Respir Crit Care Med*. 2009 Jul 15;**180**(2):181-187.
30. Cabanes LR, Weber SN, Matran R, Regnard J, Richard MO, Degeorges ME, Lockhart A. Bronchial hyperresponsiveness to methacholine in patients with impaired left ventricular function. *N Engl J Med*. 1989 May 18;**320**(20):1317-1322.

Figures

Figure 1

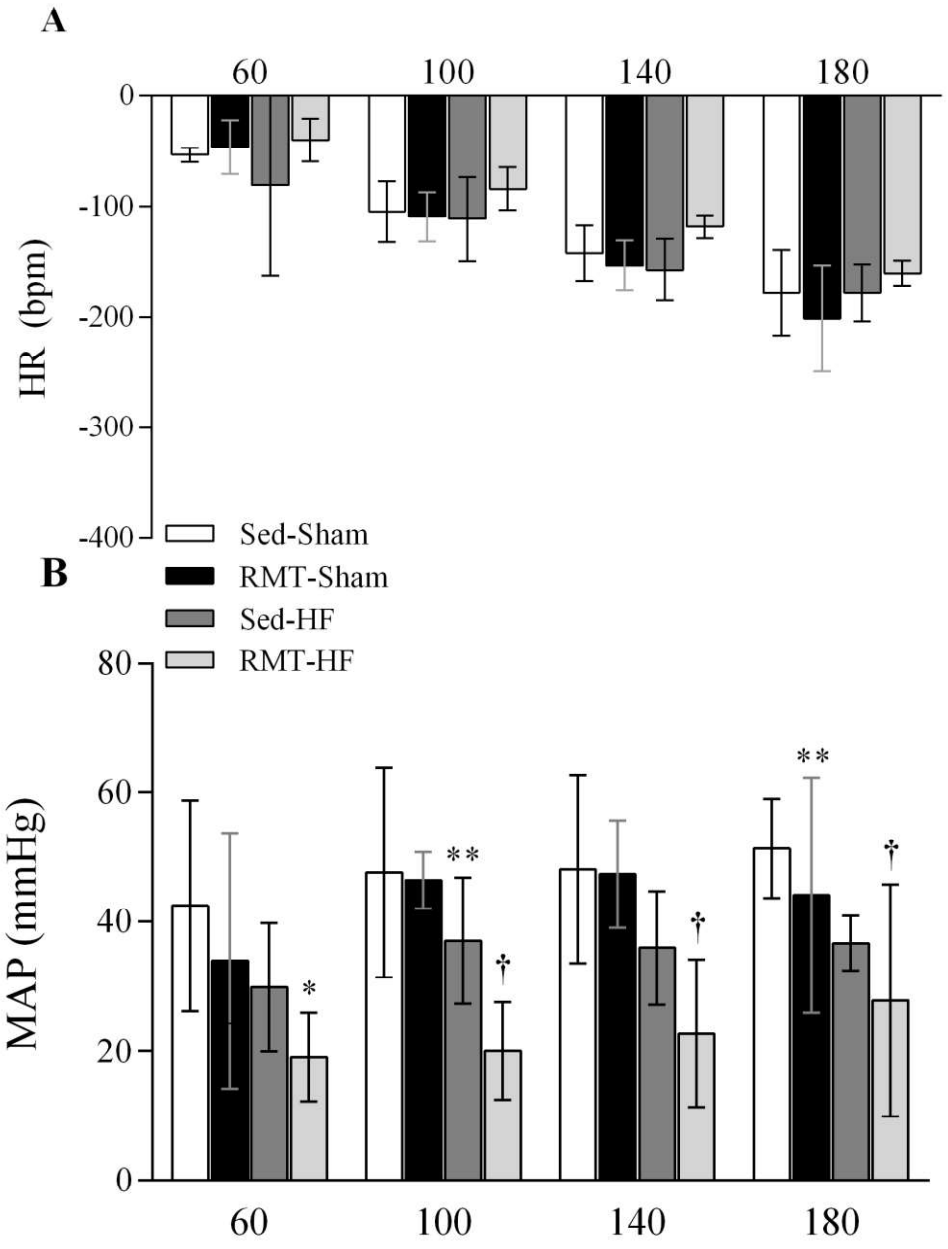

Figure 1: A, heart rate (HR) and B, mean arterial blood pressure (MAP) of the chemoreceptor reflex response induced by increasing doses of potassium cyanide (KCN, from 60 to 180 mg/kg) in studied groups. Values are means ± SD. Groups were compared by the two-way ANOVA and Tukey post hoc tests. Sed-Sham, sedentary sham rats (n=5); RMT-Sham (n=5), respiratory muscle training sham rats; Sed-HF, sedentary heart failure rats (n=5); RMT-HF, respiratory muscle training heart failure rats (n=5). *P

< .01 compared with 60 μg/kg KCN in the group RMT-HF with Sed-Sham; †*P* < .001 compared with 100 μg/kg KCN in the group RMT-HF with RMT-Sham and Sed-Sham; ***P* < .05 compared with 100 μg/kg KCN in the group Sed-HF with RMT-HF; †*P* < .001 compared with 140 μg/kg KCN in the group RMT-HF with RMT-Sham and Sed-Sham; †*P* < .001 compared with 180 μg/kg KCN in the group RMT-HF with Sed-Sham; ***P* < .05 compared with 180 μg/kg KCN in the group RMT-Sham with RMT-HF.

Figure 2

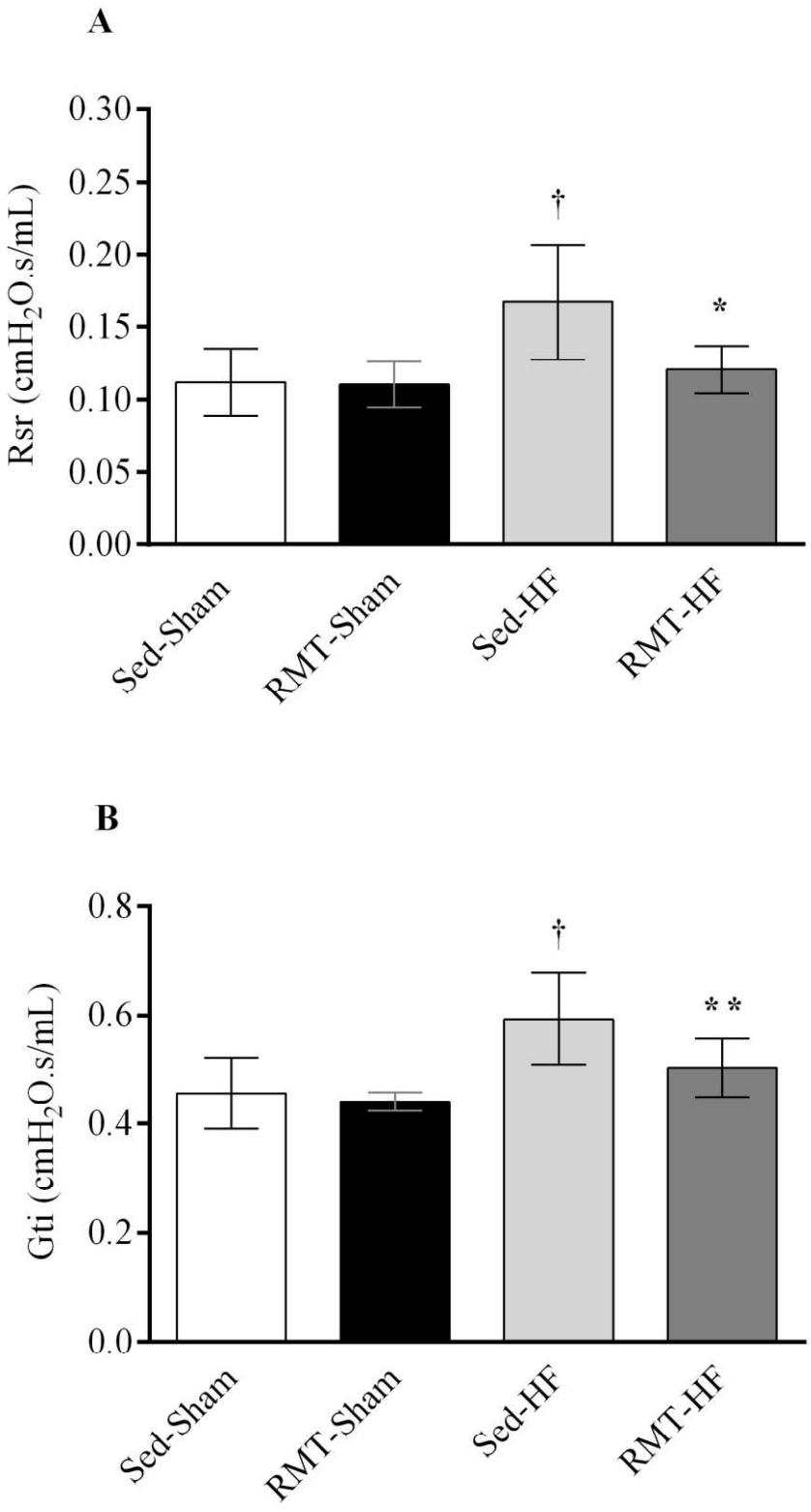
A
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Rsr (cmH2O.s/mL)
†
*
Sed-Sham
RMT-Sham
Sed-HF
RMT-HF
B
0.8
0.6
0.4
0.2
0.0
Gti (cmH2O.s/mL)
†
**
Sed-Sham
RMT-Sham
Sed-HF
RMT-HF

Figure 2: A, respiratory system resistance (Rrs) and B, tissue resistance (Gti) in studied groups. Values are means ± SD. Groups were compared by the two-way ANOVA and Tukey post hoc tests. Sed-Sham, sedentary sham rats (n=8); RMT-Sham (n=8), respiratory muscle training sham rats; Sed-HF, sedentary heart failure rats (n=8); RMT-HF, respiratory muscle training heart failure rats (n=8). *$P < .01$ compared with Sed-HF; †$P < .001$ compared with Sed-Sham and RMT-Sham; **$P < .05$ compared with Sed-HF.

Figure 3

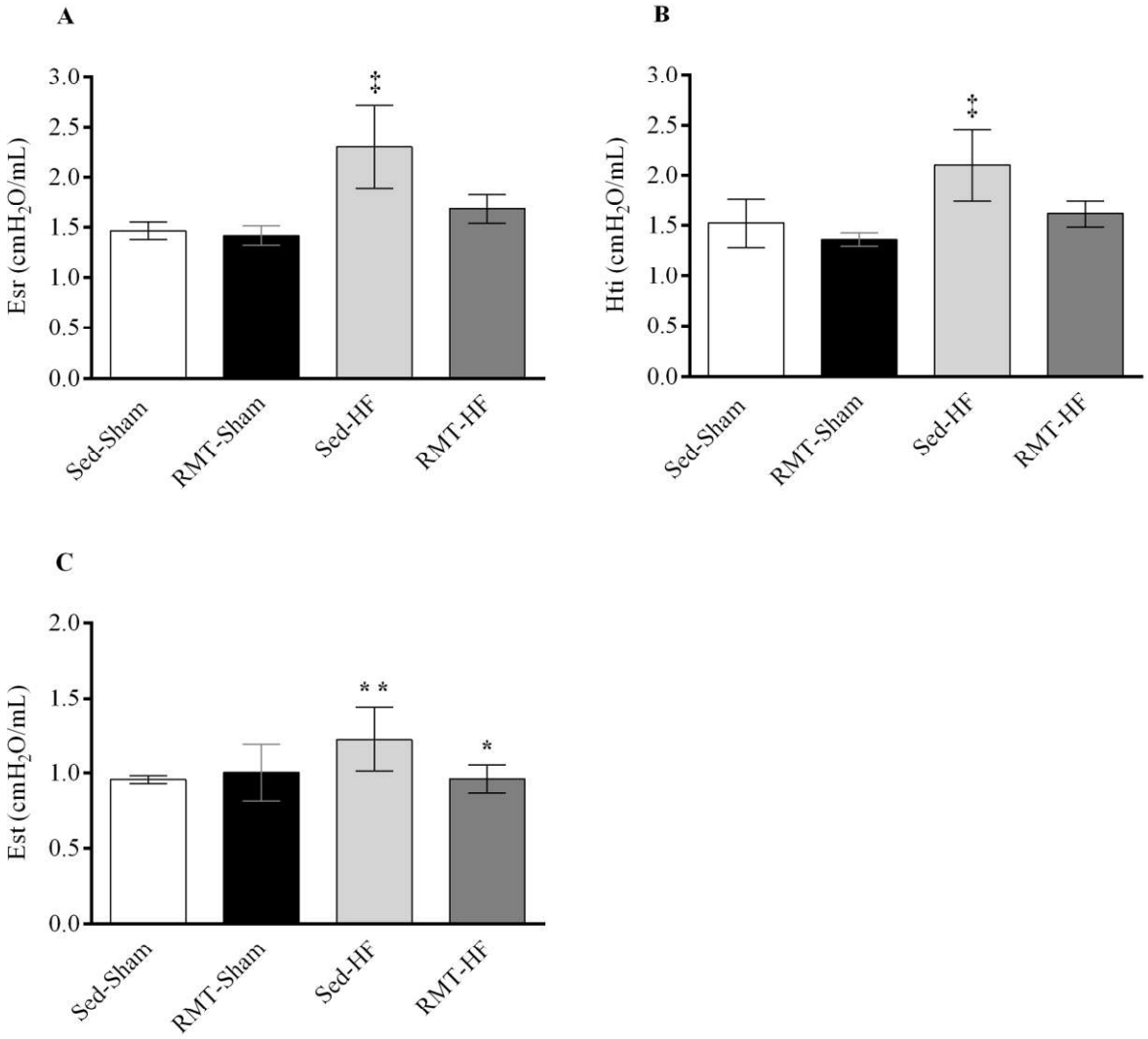

Figure 3: A, respiratory system elastance (Ers); B, tissue elastance (Hti) and C, quasistatic elastance (Est) in studied groups. Values are means ± SD. Groups were compared by the two-way ANOVA and Tukey post hoc tests. Sed-Sham, sedentary sham rats (n=8); RMT-Sham (n=8), respiratory muscle training sham rats; Sed-HF, sedentary heart failure rats (n=8); RMT-HF, respiratory muscle training heart failure rats (n=8). ‡P < .0001 compared with Sed-Sham, RMT-Sham and RMT-HF; **P < .05 compared with Sed-Sham and RMT-Sham; *P < .01 compared with Sed-HF.

Tables

Table 1. *Morphological characteristics, infarct area and lung and hepatic congestion of sham-operated groups and rats with left ventricular dysfunction.*

Groups	Initial Body Weight, g	Final Body Weight, g	Infarcted Area, %	H/BW, mg/g	LV/BW, mg/g	RV/BW, mg/g	Pulmonary Congestion, %	Hepatic Congestion, %
Sed-Sham	276±14	333±22	------	2.78±0.24	2.28±0.14	0.66±0.15	69.10±4.59	71.16±1.71
RMT-Sham	274±17	323±29	------	2.73±0.17	2.12±0.15†	0.62±0.14	70.16±2.57	72.41±0.70
Sed-HF	279±14	330±44	39.67±7	3.39±0.55*	2.52±0.46	1.23±0.22**	81.28±6.24§	71.46±1.18
RMT-HF	266±12	304±20	38.74±8	3.41±0.62*	2.55±0.22	0.85±0.4‡	68.44±2.96	71.53±1.58

Values are means ± SD; n= 8 for each groups. Groups were compared by the two-way ANOVA and Tukey post hoc tests. Sed-Sham, sedentary sham rats; RMT-Sham, respiratory muscle training sham rats; Sed-HF, sedentary heart failure rats; RMT-HF, respiratory muscle training heart failure rats. HW/BW, Heart weight-to-body weight ratio; LV/BW, left ventricle-to-body weight ratio and RV/BW, right ventricle-to-body weight ratio. *p< .05 compared with Sed-Sham and RMT-Sham. †p< .05 compared with Sed-HF and RMT-HF. **p< .001 compared with Sed-Sham and RMT-Sham. ‡p< .05 compared with Sed-HF. §p< .0001 compared with Sed-Sham, RMT-Sham and RMT-HF.

Table 2. *Hemodynamics variables of sham-operated groups and rats with left ventricular dysfunction*

Groups	LVEDP (mmHg)	LVSP (mmHg)	$+dP/dt_{max}$ (mmHg/s)	$-dP/dt_{max}$ (mmHg/s)	HR, bpm	MAP, mmHg	SAP, mmHg	DAP, mmHg
Sed-Sham	5.8±1.7	106.3±12.6	6641±739	-4526±705	262±77	88±15	97±14	75±11
RMT-Sham	4±1.3	111.2±19**	7711±2865**	-4681±1687**	309±61	103±16**	116±17**	88±15**
Sed-HF	28.9±8.3§	86.2±5.3*	4181±761*	-2796±554*	246±54	76±8*	86±8*	64±8*
RMT-HF	12.5±3.8#	97.9±16.5	5136±1639	-3420±1075	272±63	90±18	101±21	77±16

Values are means ± SD; n= 8 for each groups. Groups were compared by the two-way ANOVA and Tukey post hoc tests. Sed-Sham, sedentary sham rats; RMT-Sham, respiratory muscle training sham rats; Sed-HF, sedentary heart failure rats; RMT-HF, respiratory muscle training heart failure rats. LVEDP, LV end-diastolic pressure; LVSP, LV systolic pressure; $+dP/dt_{max}$, LV maximum change in pressure over time; $-dP/dt_{max}$, LV minimum change in pressure over time; HR, heart rate; MAP, mean arterial pressure; SAP, systolic arterial pressure; DAP, diastolic arterial pressure. §p< .0001 compared with Sed-Sham, RMT-Sham and RMT-HF. #p< .01 compared with RMT-Sham. *p< .05 compared with Sed-Sham. **p< .001 compared with Sed-HF.

Table 3. *Heart rate variability and blood pressure variability of sham-operated rats and rats with left ventricular dysfunction*

	Heart rate components							Blood pressure components	
Groups	HRV (ms^2)	rMSSD (ms^2)	LF (ms^2)	HF (ms^2)	LF (n.u.)	HF (n.u.)	LF/HF	BPV ($mmHg^2$)	LF ($mmHg^2$)
Sed-Sham	90.9±69.9	6.8±2.3	5.8±1.4	14.1±5.1	29.5±9	70.5±9	0.5±0.1	23.4±11.4	5.6±3
RMT-Sham	71±40.6	5.4±1.5	4±1.4	8.4±5.5	32.2±3.9	67.8±3.9	0.5±0.1	26.9±7.9	4.4±2.6
Sed-HF	105.2±31.6	7.7±0.8*	6±1.7*	17.8±4.6*	25.3±6.9*	74.7±6.9*	0.4±0.1*	25.6±17.2	5.7±3.6*
RMT-HF	93±34.5	10.1±0.7**	3.5±1.5	26±4.8**	12.8±5.2**	87.2±5.2**	0.2±0.1†	11.3±2.9	1.3±0.7

Values are means ± SD; n= 6 for each groups. Groups were compared by the two-way ANOVA and Tukey post hoc tests. Sed-Sham, sedentary sham rats; RMT-Sham, respiratory muscle training sham rats; Sed-HF, sedentary heart failure rats; RMT-HF, respiratory muscle training heart failure rats. HRV: Heart rate variability; rMSSD: Root Mean Square of the Successive Differences; LF: Low Frequency component, HF: High Frequency Component; LF/HF: Sympathovagal Balance; BPV: Blood Pressure Variability. *p< .05 compared with RMT-HF. **p< .01 compared with Sed-Sham and RMT-Sham. †p< .001 compared with Sed-Sham and RMT-Sham.

CONSIDERAÇÕES FINAIS

4. CONSIDERAÇÕES FINAIS

Em pacientes com IC, a diminuição do débito cardíaco promove a inadequada entrega de suprimento sanguíneo para os tecidos, com consequente prejuízo na produção energética (Jessup e Brozena, 2003; Braunwald, 2013). Resultante dessa incapacidade, a disfunção muscular esquelética periférica contribui para a fadiga precoce e limitação ao exercício desses indivíduos (Fulster *et al.*, 2013). Durante o aumento da taxa metabólica, os músculos ventilatórios e periféricos de pacientes com IC passam a competir pelo aporte sanguíneo, na tentativa de manter uma adequada ventilação (Powers *et al.*, 1997). O direcionamento de fluxo sanguíneo para o diafragma deve-se, em parte, ao metaborreflexo muscular inspiratório, que está hiperativado em pacientes com IC (Dempsey *et al.*, 2006; Floras, 2009). O aumento da resposta promovida pelo metaborreflexo inspiratório, que ocorre frente à fraqueza da musculatura ventilatória frequente em pacientes com IC, associa-se diretamente com o aumento da resposta ventilatória, a redução do fluxo sanguíneo periférico e, consequentemente, intolerância ao exercício (Clark *et al.*, 1996; Floras, 2009) . Por sua vez, a fraqueza muscular ventilatória em pacientes com IC está intimamente correlacionada com a capacidade funcional (Nishimura *et al.*, 1994), e é considerada um preditor independente de mau prognóstico em pacientes com IC (Meyer *et al.*, 2001).

Assim, por meio dos achados que relacionaram os músculos ventilatórios e sua influência na síndrome IC, alguns grupos de pesquisa direcionaram a sua atenção em verificar os efeitos do treinamento dos músculos ventilatórios em pacientes com IC.

Até o presente momento, seis revisões sistemáticas verificaram os efeitos do TMI na IC, sendo favoráveis sobre muitas manifestações fisiopatológicas da IC (Chen e Yin, 2012; Lin *et al.*, 2012; Plentz *et al.*, 2012; Sbruzzi *et al.*, 2012; Smart *et al.*, 2013; Montemezzo *et al.*, 2014). Embora o TMI tenha promovido benefícios sobre manifestações fisiopatológicas e desfechos clínicos em pacientes com IC, pouco é conhecido sobre a influência dessa modalidade terapêutica na função hemodinâmica. Ainda, até o nosso conhecimento, nenhum estudo verificou a possível influência do TMI na genotoxicidade do músculo diafragma de ratos com IC.

Dessa forma, com a possibilidade de utilizarmos o modelo experimental de IC e verificarmos os efeitos do TMV em ratos, o estudo 1 foi desenvolvido, com a

finalidade de analisar os efeitos do TMV na função hemodinâmica e no dano em DNA, no músculo diafragma de ratos com IC.

Os resultados encontrados indicaram que, em primeiro lugar, o músculo diafragma de ratos com IC apresentou dano em DNA. Após o protocolo de TMV, os animais com IC exibiram valores menores de dano em DNA no diafragma. Ainda, os animais com IC que realizaram o TMV demonstraram melhora da função hemodinâmica.

Devido às alterações cardíacas e hemodinâmicas presentes na IC, a hiperexcitação neurohumoral ocorre, inicialmente apropriada com a finalidade de preservar o adequado funcionamento cardiovascular, mas cronicamente é deletéria (Floras, 2009). A estimulação dos quimiorreceptores periféricos, localizados nos corpúsculos carotídeos e aórticos, sensíveis principalmente à queda da PO_2, provoca um aumento exagerado na ventilação pulmonar e na atividade simpática em pacientes com IC (Kara *et al.*, 2003; Di Vanna *et al.*, 2007). Estudos têm demonstrado que a atividade simpática e parassimpática estão relacionadas à doença cardiovascular, e associadas a um prognóstico desfavorável. Especificamente, tanto o aumento do sistema simpático como a diminuição da atividade parassimpática têm sido associados com um risco aumentado de morte súbita e/ou suscetibilidade a arritmias ventriculares (Lahiri *et al.*, 2008). Dentre os métodos utilizados no meio científico para mensurar a atividade do controle autônomo cardiovascular, a análise da variabilidade da frequência cardíaca vem sendo largamente empregada (Parati e Esler, 2012).

A fraqueza muscular ventilatória, em pacientes com IC, está associada ao aumento da resposta quimiorreflexa periférica (Callegaro *et al.*, 2010) e à disfunção do controle autônomo cardíaco (Reis *et al.*, 2010). Mello *et* al. (2012) (Mello *et al.*, 2012) avaliaram o efeito do TMI na modulação autônoma cardíaca e na atividade nervosa simpática muscular em pacientes com IC. Os autores verificaram um aumento no componente parassimpático e uma diminuição, tanto no componente simpático quanto na atividade nervosa simpática muscular após TMI (Mello *et al.*, 2012).

Na IC, a disfunção simpática e parassimpática que presumivelmente ocorre em associação à disfunção cardíaca (Florea e Cohn, 2014), pode conduzir o aumento na atividade dos quimiorreceptores periféricos (Hennersdorf *et al.*, 2001), principalmente pelo aumento da atividade simpática (Schultz e Li, 2007), correlacionando-se significativamente com a resposta ventilatória exacerbada ao exercício e dispneia (Ciarka *et al.*, 2006).

Até o presente momento, não foi verificado o efeito no TMI na resposta quimiorreflexa e na variabilidade da frequência cardíaca em pacientes ou animais com IC no mesmo estudo. Dessa forma, o estudo 2 da tese em questão foi desenvolvido com a finalidade de investigar a influência do TMV na função hemodinâmica, mecânica respiratória, na resposta dos quimiorreceptores periféricos e na variabilidade da frequência cardíaca de ratos com IC. Verificou-se que, após o protocolo de TMV, os ratos com IC apresentaram melhora da função hemodinâmica e da mecânica respiratória. Além disso, o TMV demonstrou melhorar o quimiorreflexo periférico, reduzir a atividade simpática e aumentar a atividade parassimpática.

Os estudos provenientes desta tese foram elaborados com o objetivo de verificar algumas respostas fisiológicas e de genotoxicidade em ratos com IC, utilizando como ferramenta terapêutica o TMV. Em conjunto com outros achados publicados pelo nosso grupo (Jaenisch *et al.*, 2011), os resultados encontrados indicam que:

1) O modelo experimental de IC, desenvolvido em nosso laboratório, mimetiza as alterações multissistêmicas encontradas na síndrome IC, o que o torna ferramenta para o desenvolvimento de inúmeros estudos;

2) O protocolo de TMV em ratos com IC, como demonstrado nos estudos da tese e previamente, oportuniza uma possibilidade ímpar no desenvolvimento de outros estudos, assim como a investigação de outras doenças ou síndromes no modelo experimental;

3) O TMV em ratos com IC promoveu a melhora da função hemodinâmica e da mecânica respiratória, a redução da resposta pressórica quimiorreflexa e da atividade simpática, assim como o aumento da parassimpática. Ainda, reduziu o dano ao DNA no músculo diafragma de ratos com IC.

Dessa forma, a contribuição para o maior conhecimento sobre as respostas do TMV na síndrome IC torna-se evidente, o que possibilita novas investigações sobre o assunto.

Com base nos achados e, em informações prévias de investigações que agregaram o TMV em pacientes com IC, a possibilidade de utilizar o TMV como estratégia adicional ao tratamento medicamentoso parece apropriada e justificável.

4.1. Perspectivas futuras

Do ponto de vista clínico, o desenvolvimento de novos estudos com um maior rigor metodológico, como ensaios clínicos randomizados e controlados por placebo, faz-se necessário. Além disso, estudos com maior tamanho amostral e protocolos de intervenção mais longos poderão determinar resultados ainda mais satisfatórios, com a perspectiva da redução da mortalidade em um período de acompanhamento. Ainda, o desenvolvimento de estudos multicêntricos poderá proporcionar maior validade externa dos resultados, com a realização de protocolos similares em diferentes centros de pesquisa.

Estudos experimentais utilizando o modelo de IC e o TMV poderão esclarecer algumas respostas ainda pouco elucidadas. Por exemplo, o conhecimento sobre a influência do TMV na capacidade oxidativa, marcadores de estresse oxidativo e o metaborreflexo de músculos periféricos poderá contribuir para o melhor entendimento dos possíveis benefícios não somente locais, mas sistêmicos, que o TMV pode exercer na síndrome IC.

Ainda, a possibilidade de agregar o TMV com outras ferramentas terapêuticas não farmacológicas como o exercício contínuo, o exercício intervalado e o exercício de força poderão, sobretudo, determinar a magnitude do incremento de diferentes modalidades, tanto em pacientes quanto em animais com IC, em análises funcionais, fisiológicas e biomoleculares.

Referências

BRAUNWALD, E. Heart failure. **JACC Heart Fail,** v. 1, n. 1, p. 1-20, Feb 2013. ISSN 2213-1787 (Electronic) 2213-1779 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24621794 >.

CALLEGARO, C. C. et al. Augmented peripheral chemoreflex in patients with heart failure and inspiratory muscle weakness. **Respir Physiol Neurobiol,** v. 171, n. 1, p. 31-5, Apr 15 2010. ISSN 1878-1519 (Electronic) 1569-9048 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20096381 >.

CHEN, Y. M.; YIN, T. Inspiratory muscle training improves submaximal exercise capacity in patients with heart failure: a systematic review of randomized controlled trials. **Int J Cardiol,** v. 158, n. 2, p. 294-6, Jul 12 2012. ISSN 1874-1754 (Electronic) 0167-5273 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22592034 >.

CIARKA, A. et al. Increased peripheral chemoreceptors sensitivity and exercise ventilation in heart transplant recipients. **Circulation,** v. 113, n. 2, p. 252-7, Jan 17 2006. ISSN 1524-4539 (Electronic) 0009-7322 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16401774 >.

CLARK, A. L.; POOLE-WILSON, P. A.; COATS, A. J. Exercise limitation in chronic heart failure: central role of the periphery. **J Am Coll Cardiol,** v. 28, n. 5, p. 1092-102, Nov 1 1996. ISSN 0735-1097 (Print) 0735-1097 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/8890800 >.

DEMPSEY, J. A. et al. Consequences of exercise-induced respiratory muscle work. **Respir Physiol Neurobiol,** v. 151, n. 2-3, p. 242-50, Apr 28 2006. ISSN 1569-9048 (Print) 1569-9048 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16616716 >.

DI VANNA, A. et al. Blunted muscle vasodilatation during chemoreceptor stimulation in patients with heart failure. **Am J Physiol Heart Circ Physiol,** v. 293, n. 1, p. H846-52, Jul 2007. ISSN 0363-6135 (Print) 0363-6135 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17434973 >.

FLORAS, J. S. Sympathetic nervous system activation in human heart failure: clinical implications of an updated model. **J Am Coll Cardiol,** v. 54, n. 5, p. 375-85, Jul 28 2009. ISSN 1558-3597 (Electronic) 0735-1097 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19628111 >.

FLOREA, V. G.; COHN, J. N. The autonomic nervous system and heart failure. **Circ Res,** v. 114, n. 11, p. 1815-26, May 23 2014. ISSN 1524-4571 (Electronic) 0009-7330 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24855204 >.

FULSTER, S. et al. Muscle wasting in patients with chronic heart failure: results from the studies investigating co-morbidities aggravating heart failure (SICA-HF). **Eur Heart J,** v. 34, n. 7, p. 512-9, Feb 2013. ISSN 1522-9645 (Electronic) 0195-668X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23178647 >.

HENNERSDORF, M. G. et al. Chemoreflexsensitivity in chronic heart failure patients. **Eur J Heart Fail,** v. 3, n. 6, p. 679-84, Dec 2001. ISSN 1388-9842 (Print) 1388-9842 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/11738219 >.

JAENISCH, R. B. et al. Respiratory muscle training improves hemodynamics, autonomic function, baroreceptor sensitivity, and respiratory mechanics in rats with heart failure. **J Appl Physiol (1985),** v. 111, n. 6, p. 1664-70, Dec 2011. ISSN 1522-1601 (Electronic) 0161-7567 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21903877 >.

JESSUP, M.; BROZENA, S. Heart failure. **N Engl J Med,** v. 348, n. 20, p. 2007-18, May 15 2003. ISSN 1533-4406 (Electronic) 0028-4793 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12748317 >.

KARA, T.; NARKIEWICZ, K.; SOMERS, V. K. Chemoreflexes--physiology and clinical implications. **Acta Physiol Scand,** v. 177, n. 3, p. 377-84, Mar 2003. ISSN 0001-6772 (Print) 0001-6772 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12609009 >.

LAHIRI, M. K.; KANNANKERIL, P. J.; GOLDBERGER, J. J. Assessment of autonomic function in cardiovascular disease: physiological basis and prognostic implications. **J Am Coll Cardiol,** v. 51, n. 18, p. 1725-33, May 6 2008. ISSN 1558-3597 (Electronic) 0735-1097 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18452777 >.

LIN, S. J. et al. Inspiratory muscle training in patients with heart failure: a systematic review. **Cardiopulm Phys Ther J,** v. 23, n. 3, p. 29-36, Sep 2012. ISSN 2374-8907 (Electronic) 1541-7891 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22993500 >.

MELLO, P. R. et al. Inspiratory muscle training reduces sympathetic nervous activity and improves inspiratory muscle weakness and quality of life in patients with chronic heart failure: a clinical trial. **J Cardiopulm Rehabil Prev,** v. 32, n. 5, p. 255-61, Sep-Oct 2012. ISSN 1932-751X (Electronic) 1932-7501 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22785143 >.

MEYER, F. J. et al. Respiratory muscle dysfunction in congestive heart failure: clinical correlation and prognostic significance. **Circulation,** v. 103, n. 17, p. 2153-8, May 1 2001. ISSN 1524-4539 (Electronic) 0009-7322 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/11331255 >.

MONTEMEZZO, D. et al. Influence of inspiratory muscle weakness on inspiratory muscle training responses in chronic heart failure patients: a systematic review and meta-analysis. **Arch Phys Med Rehabil,** v. 95, n. 7, p. 1398-407, Jul 2014. ISSN 1532-821X (Electronic) 0003-9993 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24631801 >.

NISHIMURA, Y. et al. Respiratory muscle strength and hemodynamics in chronic heart failure. **Chest,** v. 105, n. 2, p. 355-9, Feb 1994. ISSN 0012-3692 (Print) 0012-3692 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/8306727 >.

PARATI, G.; ESLER, M. The human sympathetic nervous system: its relevance in hypertension and heart failure. **Eur Heart J,** v. 33, n. 9, p. 1058-66, May 2012. ISSN 1522-9645 (Electronic) 0195-668X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22507981 >.

PLENTZ, R. D. et al. Inspiratory muscle training in patients with heart failure: meta-analysis of randomized trials. **Arq Bras Cardiol,** v. 99, n. 2, p. 762-71, Aug 2012. ISSN 1678-4170 (Electronic) 0066-782X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22964897 >.

POWERS, S. K.; COOMBES, J.; DEMIREL, H. Exercise training-induced changes in respiratory muscles. **Sports Med,** v. 24, n. 2, p. 120-31, Aug 1997. ISSN 0112-1642 (Print) 0112-1642 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/9291552 >.

REIS, M. S. et al. Deep breathing heart rate variability is associated with respiratory muscle weakness in patients with chronic obstructive pulmonary disease. **Clinics (Sao Paulo),** v. 65, n. 4, p. 369-75, Apr 2010. ISSN 1980-5322 (Electronic) 1807-5932 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20454493 >.

SBRUZZI, G. et al. Inspiratory muscle training and quality of life in patients with heart failure: systematic review of randomized trials. **Int J Cardiol,** v. 156, n. 1, p. 120-1, Apr 5 2012. ISSN 1874-1754 (Electronic) 0167-5273 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22284971 >.

SCHULTZ, H. D.; LI, Y. L. Carotid body function in heart failure. **Respir Physiol Neurobiol,** v. 157, n. 1, p. 171-85, Jul 1 2007. ISSN 1569-9048 (Print) 1569-9048 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17374517 >.

SMART, N. A.; GIALLAURIA, F.; DIEBERG, G. Efficacy of inspiratory muscle training in chronic heart failure patients: a systematic review and meta-analysis. **Int J Cardiol,** v. 167, n. 4, p. 1502-7, Aug 20 2013. ISSN 1874-1754 (Electronic) 0167-5273 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22560934 >.

5. ATIVIDADES REALIZADAS DURANTE O DOUTORADO

Durante o período da realização do doutorado (2011-2015), tive oportunidade de participar e colaborar direta ou indiretamente de atividades acadêmico-científicas, vinculadas ou não ao projeto de doutorado.

Participação em projetos de pesquisa

- Colaborador do projeto de mestrado em andamento do aluna do PPG em Ciências da Reabilitação da UFCSPA Giovanna Tedesco Barcelos intitulado de: TREINAMENTO AERÓBIO CONTÍNUO OU INTERVALADO E SUPLEMENTAÇÃO DE L-ARGININA NA INSUFICIÊNCIA CARDÍACA EXPERIMENTAL.

- Colaborador do projeto de doutorado em andamento do aluno do PPG em Ciências da Saúde da UFCSPA Jadson Pereira Alves intitulado de: EFEITOS DO TREINAMENTO DE FORÇA ISOLADO E COMBINADO AO TREINAMENTO AERÓBICO SOBRE A FUNÇÃO HEMODINÂMICA, REMODELAMENTO CARDÍACO E O PERFIL BIOQUÍMICO DE RATOS COM INSUFICIÊNCIA CARDÍACA.

Coorientações de iniciação científica e trabalhos de conclusão de curso

- Coorientação da aluna do curso de Fisioterapia da UFCSPA Camila Durante nas atividades de iniciação científica e no trabalho de conclusão de curso com projeto: EFEITOS DO TREINAMENTO MUSCULAR VENTILATÓRIO SOBRE O DANO EM DNA NO MÚSCULO DIAFRAGMA DE RATOS COM INSUFICIÊNCIA CARDÍACA, 2014.

- Coorientação do aluno do curso de Fisioterapia da UFCSPA Lucas Krolikowski nas atividades de iniciação científica.

- Coorientação da aluna do curso de Fisioterapia da UFCSPA Betina Foscarina nas atividades de iniciação científica.

- Coorientação da aluna do curso de Fisioterapia da UFCSPA Chalyne Chechi nas atividades de iniciação científica.

Produção técnico-científica vinculada ao doutorado

- Trabalhos publicados em anais de eventos (resumo)

1.JAENISCH, R. B.; STEFANI, G. P.; DURANTE, C.; CHECHI, C.; HENTSCHKE, V.S.; ROSSATO, D. D.; DAL LAGO, P. Efeitos do treinamento muscular ventilatório sobre a função hemodinâmica e o dano em DNA no músculo diafragma de ratos com insuficiência cardíaca. In: 5º Simpósio de Fisioterapia em Cirurgia Cardiovascular - 42º Congresso da SBCCV, 2015, Curitiba - PR. Brazilian Journal of Cardiovascular Surgery. SP: Associação Paulista de Bibliotecários, 2015. v. 30. p. 146-146.

2.JAENISCH, R. B.; CHECHI, C.; QUAGLIOTTO, E.; BERTAGNOLLI, M.; DAL LAGO, P. O treinamento muscular ventilatório aumenta a atividade da citrato sintase no músculo diafragma de ratos com insuficiência cardíaca. In: 5º Simpósio de Fisioterapia em Cirurgia Cardiovascular - 42º Congresso da SBCCV, 2015, Curitiba - PR. Brazilian Journal of Cardiovascular Surgery. SP: Associação Paulista de Bibliotecários, 2015. v. 30. p. 153-153.

3.JAENISCH, R. B.; STEFANI, G. P.; DURANTE, C.; CHECHI, C.; HENTSCHKE, V.S.; ROSSATO, D. D.; RHODEN, C. R.; DAL LAGO, P. Efeitos do treinamento muscular ventilatório sobre a função hemodinâmica e o dano em DNA no músculo diafragma de ratos com insuficiência cardíaca. In: SOCESP, 2015, São Paulo - SP. Suplemento Especial da Revista da Sociedade de Cardiologia do Estado de São Paulo. São Paulo -SP: Diretoria de publicações da SOCESP, 2015. v. 25. p. 229-229.

4.JAENISCH, R. B.; QUAGLIOTTO, E.; CHECHI, C.; BERTAGNOLLI, M.; DAL LAGO, P. O treinamento muscular ventilatório aumenta a atividade da citrato sintase no músculo diafragma de ratos com insuficiência cardíaca. In: SOCESP, 2015, São Paulo - SP. Suplemento Especial da Revista da Sociedade de Cardiologia do Estado de São Paulo. São Paulo - SP: Diretoria de Publicações da SOCESP, 2015. v. 25. p. 289-289.

5.BONETTO, J.; RUCATTI, A. L.; ROSSATO, D. D.; **JAENISCH, R. B.**; DALLAGO, P. Efeitos da estimulação elétrica muscular sobre a sensibilidade de barorreceptores em ratos com insuficiência cardíaca. In: VII Salão de Iniciação Científica e Extensão, I Mostra da Pós-Graduação, 2012, Porto Alegre-RS. VII Salão de Iniciação Científica e Extensão, I Mostra da Pós-Graduação, 2012.

6.BONETTO, J.; RUCATTI, A. L.; **JAENISCH, R. B.**; ROSSATO, D. D.; DAL LAGO, P. D. Efeitos da estimulação elétrica muscular sobre a sensibilidade de barorreceptores em ratos com insuficiência cardíaca. In: XIII Salão de Iniciação Científica - PUCRS, 2012, Porto Alegre-RS. XIII Salão de Iniciação Científica - PUCRS, 2012.

7.RUCATTI, A. L.; ROSSATO, D. D.; BONETTO, J.; **JAENISCH, R. B.**; DAL LAGO, P. D. Efeitos da estimulação elétrica muscular sobre a sensibilidade de barorreceptores em ratos com insuficiência cardíaca. In: XLVII Congresso Anual da SBFis e III Encontro Científico do Programa Multicêntrico do Pós-Graduação em Ciências Fisiológicas, 2012, Gramado-RS. XLVII Congresso Anual da SBFis e III Encontro Científico do Programa Multicêntrico do Pós-Graduação em Ciências Fisiológicas, 2012.

8.RUCATTI, A. L.; SILVEIRA, M. N.; ROSSATO, D. D.; BONETTO, J.; **JAENISCH, R. B.**; DAL LAGO, P. D. Estimulação elétrica melhora a sensibilidade de barorreceptores em ratos com insuficiência cardíaca. In: Congresso da Sociedade de Cardiologia do Rio Grande do Sul - SOCERGS, 2012, Gramado-RS. Congresso da Sociedade de Cardiologia do Rio Grande do Sul - SOCERGS, 2012.

9.CUNHA, MAIRA J.; CUNHA, ALINE A.; SCHERER, E. B. S.; MACHADO, F. R.; **JAENISCH, R. B.**; DAL LAGO, P. D.; NETTO, C. A.; WYSE, ANGELA T. S. Lesão pulmonar aguda experimental promove alterações no metabolismo energético e na mecânica ventilatória do pulmão de ratos: prevenção pelo exercício físico. In: 32ª Semana Científica do Hospital de Clínicas de Porto Alegre, 2012, Porto Alegre-RS. 32ª Semana Científica do Hospital de Clínicas de Porto Alegre, 2012.

10.JAENISCH, R. B.; HENTSCHKE, V. S.; QUAGLIOTTO, E.; BERTAGNOLLI, M.; DAL LAGO, P. D. Respiratory muscle training increase citrate synthase activity on diaphragm muscle in rats with heart failure. In: Sociedade Brasileira de Fisiologia - SBFis, 2012, Gramado-RS. Congresso Anual da SBFis e III Encontro Científico do Programa Multicêntrico de Pós-Graduação em Ciências Fisiológicas, 2012.

11.HENTSCHKE, V.S., **JAENISCH, R. B.**, SCHMEING, L., CAVINATO, P., DAL LAGO, P. D. Low level laser therapy decreased TNF-alfa and IL-6 and increased IL-10 levels and IL-10/TNF-alpha ratio in skeletal muscle of rats with heart failure. In: European Society of Cardiology Congress, 2011, Paris-FRA. European Heart Journal (Abstract Supplement). 2011. v.32. p.976 - 976

12.JAENISCH, R. B., HENTSCHKE, V. S., QUAGLIOTTO, E., CAVINATO, P., SCHMEING, L., DAL LAGO, P. D. Respiratory muscle training improves baroreceptor sensitivity, decrease sympathetic tonus and increase vagal effect in rats with heart failure In: European Society of Cardiology Congress, 2011, Paris-FRA. European Heart Journal (Abstract Supplement). , 2011. v.32. p.976 - 976

Produção técnico-científica não vinculada ao doutorado

- Capítulos de livros publicados

1.JAENISCH, R. B.; MONTEMEZZO, D. Treinamento muscular respiratório em doenças cardiovasculares. In: Marlus Karsten; Simone Dal Corso. (Org.). Programa de Atualização em Fisioterapia Cardiovascular e Respiratória / PROFISIO CARDIO. 2ed.Porto Alegre: Artmed, 2015, v. 2, p. 100-115.

2.JAENISCH, R. B.; DAL LAGO, P. Treinamento Muscular Inspiratório na Insuficiência Cardíaca. Fisioterapia em Cardiologia. 2ªed.: Atheneu, 2014, v. , p. 101-112.

Participação em bancas

- Cursos de graduação

1.JAENISCH, R.B; BERNARDI, C. Participação em banca de Bruna Mozaquatro. EFEITO DA HIPOTENSÃO SOBRE O QUIMIORREFLEXO PERIFÉRICO EM RATOS COM INSUFICIÊNCIA CARDÍACA. 2014. Trabalho de Conclusão de Curso (Graduação em Fisioterapia) - Universidade Federal de Ciências da Saúde de Porto Alegre.

2.JAENISCH, R.B; SILVA, E.; GUTHS, H. Participação em banca de Poline Timm. SEGURANÇA E EFICÁCIA DO EXERCÍCIO FÍSICO EM INDIVÍDUOS COM HIPERTENSÃO PULMONAR. 2012. Trabalho de Conclusão de Curso (Graduação em Fisioterapia) - Centro Universitário La Salle - Canoas.

3.JAENISCH, R.B.; SILVA, E.; GUTHS, H. Participação em banca de Paulo Ricardo Marques Filho. CONHECIMENTO SOBRE HIPERTENSÃO ARTERIAL SISTÊMICA EM COLABORADORES DE UMA INSTITUIÇÃO DE ENSINO DA REGIÃO METROPOLITANA DE PORTO ALEGRE/RS. 2012. Trabalho de Conclusão de Curso (Graduação em Fisioterapia) - Centro Universitário La Salle - Canoas.

4.JAENISCH, R.B.; ZANOTTA, R.; GUTHS, H. Participação em banca de Luciane Ritter. OS EFEITOS DA AURICULOTERAPIA NO CONTROLE DO TABAGISMO. 2012. Trabalho de Conclusão de Curso (Graduação em Fisioterapia) - Centro Universitário La Salle - Canoas.

5.JAENISCH, R.B.; PILZ, M.; SILVA, E. Participação em banca de Carina Hofsetz. EFEITO DA REABILITAÇÃO COGNITIVA NA FUNCIONALIDADE DE PACIENTE COM DOENÇA DE ALZHEIMER. 2012. Trabalho de Conclusão de Curso (Graduação em Fisioterapia) - Centro Universitário La Salle - Canoas.

6.JAENISCH, R.B.; PILZ, M.; SILVA, E. Participação em banca de Andressa Franzen. EFICÁCIA DE UM PROTOCOLO CINESIOTERAPÊUTICO DE EQUILÍBRIO APLICADO EM PACIENTES COM DOENÇA DE ALZHEMIER. 2012. Trabalho de Conclusão de Curso (Graduação em Fisioterapia) - Centro Universitário La Salle - Canoas.

7.JAENISCH, R.B. Participação em banca de Carlos Vinicius Aguirre Pereira. PROGRAMAS DE EDUCAÇÃO SOBRE ASMA PARA CRIANÇAS. 2011. Trabalho de Conclusão de Curso (Graduação em Fisioterapia) - Centro Universitário La Salle - Canoas.

8.JAENISCH, R.B.; GUTHS, H. Participação em banca de Sabrina Borges. AVALIAR O PERFIL DE CUIDADORES DE CRIANÇAS COM DOENÇAS NEUROLÓGICAS DAS CLÍNICAS INTEGRADAS DO UNILASALLE EM CANOAS. 2011. Trabalho de Conclusão de Curso (Graduação em Fisioterapia) - Centro Universitário La Salle - Canoas.

9.JAENISCH, R.B.; SILVA, E. Participação em banca de Marisa Favero. O EFEITO DA REFLEXOTERAPIA PODAL NA PRESSÃO ARTERIAL E NA QUALIDADE DE VIDA DO PACIENTE HIPERTENSO. 2011. Trabalho de Conclusão de Curso (Graduação em Fisioterapia) - Centro Universitário La Salle - Canoas.

- Cursos de aperfeiçoamento/especialização

1.JAENISCH, R.B. Participação em banca de Daianne Cristina Rocha. ASSOCIATION OF HELICOBACTER PYLORI INFECTION WITH NUTRITIONAL STATUS AND FOOD INTAKE. 2015. Monografia (Aperfeiçoamento/Especialização em Nutrição Clínica e Esportiva) - Instituto de Pesquisas e Gestão em Saúde.

2.JAENISCH, R.B. Participação em banca de Fernanda Donner Alves. FADIGA INDUZIDA PELO EXERCÍCIO DE ALTA INTENSIDADE E MECANISMOS FISIOLÓGICOS DE AÇÃO DA BETA-ALANINA E CARNOSINA: UMA REVISÃO. 2015. Monografia (Aperfeiçoamento/Especialização em Nutrição Clínica e Esportiva) - Instituto de Pesquisas e Gestão em Saúde.

3.JAENISCH, R.B. Participação em banca de Patrícia Ferrarini. VITAMINA D NO ESPORTE. 2015. Monografia (Aperfeiçoamento/Especialização em Nutrição Clínica e Esportiva) - Instituto de Pesquisas e Gestão em Saúde.

4.JAENISCH, R.B. Participação em banca de Luan Benigno Lisboa Souza. EFICÁCIA DO USO DE WHEY PROTEIN ASSOCIADO AO EXERCÍCIO, COMPARADA A OUTRAS FONTES PROTEICAS SOBRE A MASSA MUSCULAR DE INDIVÍDUOS JOVENS E SAUDÁVEIS. 2015. Monografia (Aperfeiçoamento/Especialização em Nutrição Clínica e Esportiva) - Instituto de Pesquisas e Gestão em Saúde.

5.JAENISCH, R.B. Participação em banca de Isabel Maria Ventura Rodrigues de Seabra. EFEITO DA SUPLEMENTAÇÃO DE ÁCIDO FOSFATÍDICO SOBRE AS ADAPTAÇÕES MUSCULOESQUELÉTICAS. 2015. Monografia (Aperfeiçoamento/Especialização em Nutrição Clínica e Esportiva) - Instituto Superior de Ensino, Pesquisas e Extensão.

6.JAENISCH, R.B. Participação em banca de Tássio de Sant Anna Machado. WAXY MAIZE: POSSÍVEL RECURSO NUTRICIONAL NO ESPORTE. 2015. Monografia (Aperfeiçoamento/Especialização em Nutrição Clínica e Esportiva) - Instituto Superior de Ensino, Pesquisas e Extensão.

7.JAENISCH, R.B. Participação em banca de Pedro Bruno Correa Pereira. GLUTAMINE SUPPLEMENTATION IN SPORT: A LITERARY REVIEW ON APPLICATION RESEARCH BASED PRACTICE IN BRAZIL. 2015. Monografia (Aperfeiçoamento/Especialização em Nutrição Clínica e Esportiva) - Instituto Superior de Ensino, Pesquisas e Extensão.

Prêmios e títulos

Melhor Trabalho Científico do Simpósio de Fisioterapia - Efeitos do treinamento muscular ventilatório sobre a função hemodinâmica e o dano em DNA no músculo diafragma de ratos com insuficiência cardíaca. XXXVI Congresso da Sociedade de Cardiologia do Estado de São Paulo, 2015.

Prêmio Mérito Interdisciplinar - Efeitos do treinamento muscular ventilatório sobre a função hemodinâmica e o dano em DNA no músculo diafragma de ratos com insuficiência cardíaca. XXXVI Congresso da Sociedade de Cardiologia do Estado de São Paulo, 2015.

1º Lugar - Temas Livres - Efeitos do treinamento muscular ventilatório sobre a função hemodinâmica e o dano em DNA no músculo diafragma de ratos com insuficiência cardíaca., 5º Simpósio de Fisioterapia Cardiovascular - 42º Congresso da SBCCV, 2015.

Menção honrosa - Efeito do treinamento muscular inspiratório em pacientes com síndrome coronariana aguda submetidos a um programa de reabilitação cardíaca na fase III. IX Semana Científica do Unilasalle, 2013.

3º Lugar – Temas Livres - Grupo de estudos em interação cardiopulmonar: uma nova abordagem de conhecimento. VIII Semana Científica do Unilasalle, 2012.

3º Lugar – Temas Livres - Lesão pulmonar aguda experimental promove alterações no metabolismo energético e na mecânica ventilatória no pulmão de ratos: prevenção pelo exercício físico. 32ª Semana Científica do Hospital de Clínicas de Porto Alegre, 2012.

Produção bibliográfica

1.AMARO, F. S., FENELON, G. D., GUIZILINI, S., ALVES, V. L. S., **JAENISCH, R. B.**, CATAI, A. M., SILVA, A. B. E., UMEDA, I., REIS, M. S., TRIMER, R., QUITERIO, R. J., PAPA, V. I manual SOCESP de condutas multidisciplinares no paciente grave. Revista da Sociedade de Cardiologia do Estado de São Paulo. v.25, p.13 - 58, 2015.

2.CUNHA, MAIRA J., CUNHA, ALINE A., SCHERER, EMILENE B. S., MACHADO, FERNANDA ROSSATO, LOUREIRO, SAMANTA O., **JAENISCH, RODRIGO B.**, GUMA, FÁTIMA, LAGO, PEDRO DAL, WYSE, ANGELA T. S. Experimental lung injury promotes alterations in energy metabolism and respiratory mechanics in the lungs of rats: prevention by exercise. Molecular and Cellular Biochemistry. , v.V, p.100 - 110, 2013.

3.HENTSCHKE, VÍTOR S., **JAENISCH, RODRIGO B.**, SCHMEING, LETIANE A., CAVINATO, PAULO R., XAVIER, LEDER L., DAL LAGO, PEDRO. Low-level laser therapy improves the inflammatory profile of rats with heart failure. Lasers in Medical Science. v.28, p.1007 - 1016, 2012.

Anexo A - Parecer do Comitê de Ética no Uso de Animais da Universidade Federal de Ciências da Saúde de Porto Alegre

PARECER CONSUBSTANCIADO DE PROJETO DE PESQUISA E ENSINO

1) PROTOCOLO Nº: 070/11 PARECER: 122/12

2) DATA DO PARECER: 18/04/2012

3) TÍTULO DO PROJETO:

Efeito do treinamento muscular ventilatório combinado com o exercício físico sobre o perfil inflamatório, o metaboreflexo muscular e a mecânica respiratória em ratos com insuficiência cardíaca

4) PESQUISADOR RESPONSÁVEL:

Pedro Dall'Ago

5) RESUMO DO PROJETO:

6) OBJETIVOS DO PROJETO:

GERAL:

Avaliar o efeito do treinamento muscular ventilatório e do exercício físico regular de moderada intensidade sobre o perfil inflamatório, o metaboreflexo muscular e a mecânica respiratória em ratos com insuficiência cardíaca induzida por IAM.

ESPECÍFICOS:

Avaliar o impacto do treinamento muscular ventilatório de 6 semanas sobre o metaboreflexo muscular, a mecânica respiratória e níveis plasmáticos de TNF- alfa , IL-6 e IL-10 em animais saudáveis e com IC induzida por IAM.

Avaliar o impacto do exercício físico regular de 8 semanas sobre o metaboreflexo muscular, a mecânica respiratória e níveis plasmáticos de TNF-alfa, IL-6 e IL-10 em animais saudáveis e com IC induzida por IAM.

Avaliar a associação do treinamento muscular ventilatório de 6 semanas e o exercício físico regular de 8 semanas sobre o metaboreflexo muscular, a mecânica respiratória e níveis plasmáticos de TNF-alfa, IL-6 e IL-10 em animais saudáveis e com IC induzida por IAM.

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

8) ITENS METODOLÓGICOS E ÉTICOS DO PROJETO:

Título	[X] Adequado	[ ] Comentários
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
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: 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:

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

Se achar inadequada cite abaixo as melhorias necessárias com metodologia substituta:

Local de Realização (Biotério/Labotarório): Laboratório de Fisiologia (UFCSPA) salas 10,12,e14.

Outra instituição. Qual?

11) CRONOGRAMA DE UTILIZAÇÃO DE ANIMAIS

Data	Espécie	Sexo	Quantidade

Experimentos com animais serão iniciados em agosto, dezembro de 2012 e abril de 2013.

12) RECOMENDAÇÃO:

[X] Aprovado

[ ] Com Pendência

[ ] Não aprovado

Anexo B - Normas para a publicação da revista *Journal of Cardiac Failure*

JOURNAL OF CARDIAC FAILURE

DESCRIPTION

Journal of Cardiac Failure publishes original, peereviewed communications of scientific exc ellenceand review articles on clinical research, basic human studies, animal studies, and bench researchwith potential clinical applications to **heart failure** - **pathogenesis**, **etio logy**, **epidemiology**,**pathophysiological mechanisms**, assessment, prevention, and treat ment.

Benefits to authors
We also provide many author benefits, such as free PDFs, a liberal copyright policy, special d iscounts
on Elsevier publications and much more. Please click here for more information on our author services.

Please see our Guide for Authors for information on article submission. If you require an y further
information or help, please visit our support pages: http://support.elsevier.com

IMPACT FACTOR

2014: 3.051 © Thomson Reuters Journal Citation Reports 2015

ABSTRACTING AND INDEXING

CINAHL
Chemical Abstracts
Cumulative Index to Nursing and Allied Health Literature
Current Contents / Clinical Medicine
Index Medicus
Science Citation Index
Scopus
ISI Alerting Services

EDITORIAL BOARD

Editor-in-Chief:
Paul J. Hauptman, MD, Saint Louis University, Saint Louis, MO

GUIDE FOR AUTHORS

Your Paper Your Way

We now differentiate between the requirements for new and revised submissions. You may c hoose tosubmit your manuscript as a single Word or PDF file to be used in the refereeing pr ocess.

Only whenyour paper is at the revision stage, will you be requested to put your paper in t o a 'correct format'for acceptance and provide the items required for the publication of you r article.**To find out more, please visit the Preparation section below.**

Introduction

The *Journal of Cardiac Failure* publishes peerReviewed manuscripts of interest to clinici ans andresearchers in the field of heart failure and related disciplines. These include original communicationsof scientific importance and review articles involving clinical research, healt h services and outcomesresearch, animal studies, and bench research with potential clini cal applications to heart failure.The Journal also publishes manuscripts that report the de sign of ongoing clinical trials and editorialperspectives that comment on new developments pertinent to the field of heart failure or manuscripts published in other journals.

Contact details

Authors may send queries concerning the submission process, manuscript status, or journal procedures to the Editorial Office, aleong@hfsa.org.

BEFORE YOU BEGIN

Ethics in publishing

For information on ethics in publishing and ethical guidelines for journal publicat ion see http://www.elsevier.com/publishingethics and http://www.elsevier.com/journal-authors/ethics.

Human and animal rights

Published research must be in compliance with human studies guidelines and animal welfareregulations.

Authors should indicate in the manuscript that human subjects have given informed consent and that the institutional committee on human research has approved the study protocol.

Similarly, they should indicate that studies involving experimental animals conform to inst itutional standards.

Conflict of interest

If a potential conflict exists, its nature should be stated for each author and the informatio n should be outlined in the Disclosure section of the manuscript.

When there is a stated potential conflict ofinterest and the editors consider that it may have relevance to the accompanying paper, a footnote willbe added indicating the author(s)' equity interest in or other affiliation with the identified commercial firms.

All potential conflicts of interest must be identified within the text of the manuscript, u nder theconflicts with interest heading. This includes relationships with pharmaceutical and biomedicaldevice companies or other corporations whose products or services are related to the subjectmatter of the article. Such relationships include, but are not li mited to, employment by anindustrial concern, equity or stock ownership by authors or family member, membership on astanding advisory council or committee, being ont he board of directors or publicly associatedwith the company or its products. Other areas of real or perceived conflict of interest couldinclude receipt of honoraria or cons ulting fees or receiving grants or funds from such corporationsor individuals representing such corporations. See also http://www.elsevier.com/conflictsofinterest.

Further information and an example of a Conflict of Interest form can be founat:http://service.elsevier.com/app/answers/detail/a_id/286/supporthub/publishing. If t here are "none"this should be stated in the Disclosure section.

Submission declaration

Submission of an article implies that the work described has not been published previously (exceptin the form of an abstract or as part of a published lecture or academic thesis or as an electronicpreprint, see http://www.elsevier.com/postingpolicy), that it is not under consideration for publicationelsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsibleauthorities where the work was carried out, and that, if accepted, it will not be published elsewhere,including electronically, in the same form, in English or in any other language, without the written consent of the publisher.

Submission form

Original works will be accepted with the understanding that they are contributed solely to the *Journalof Cardiac Failure*, are not under review by another publication, and have not previously beenpublished except in abstract form. Accepted manuscripts become the sole property of the journal andmay not be published elsewhere without the consent of the *Journal of Cardiac Failure*. A form statingthat the authors transfer all copyright ownership to the journal will be sent from the publisher whenthe manuscript is accepted; this form must be signed by all authors of the article.
The submission form includes three statements: (1) that there has been no duplicate publication orsubmission elsewhere of any part of the work (excluding abstracts), (2) that all authors have readand approved the manuscript, and (3) that there are no financial or other relations that could lead toa conflict of interest. This form must be downloaded, signed by all authors, and faxed to the editorialoffice.

Contributors

Each author is required to declare his or her individual contribution to the article: all authors musthave materially participated in the research and/or article preparation, so roles for all authors shouldbe described. The statement that all authors have approved the final article should be included in the disclosure.

Authorship

All authors should have made substantial contributions to all of the following: (1) the conception anddesign of the study, or acquisition of data, or analysis and interpretation of data, (2) drafting thearticle or revising it critically for important intellectual content, (3) final approval of the version to be submitted.
This policy concerns the addition, deletion, or rearrangement of author names in the authorship of accepted manuscripts:
Before the accepted manuscript is published in an online issue: Requests to add or remove an author,or to rearrange the author names, must be sent to the Journal Manager from the corresponding authorof the accepted manuscript and must include: (a) the reason the name should be added or removed,or the author names rearranged and (b) written confirmation (e-mail, fax, letter) from all authorsthat they agree with the addition, removal, or rearrangement. In the case of addition or removalof authors, this includes confirmation from the author being added or removed. Requests that arenot sent by the corresponding author will be forwarded by the Journal Manager to the correspondingauthor, who must follow the procedure as described above. Note that: (1) Journal Managers will informthe editors of any such requests and (2) publication of the accepted manuscript in an online issue issuspended until authorship has been agreed.*After the accepted manuscript is published in an online issue*: Any requests to add, delete, or rearrangeauthor names in an article published in an online issue will follow the same policies as noted above and result in a corrigendum.

Copyright

Upon acceptance of an article, authors will be asked to complete a 'Journal Publishing Agreement' (formore information on this and copyright, see http://www.elsevier.com/copyright).
An email will besent to the corresponding author confirming receipt of the manuscript

Anexo C - Normas para a publicação da revista *European Journal of Heart Failure*

Author Guidelines

REQUIRED FORMS

European Journal of Heart Failure requests that all authors complete:
An ICMJE Conflicts of Interest disclosure form
An Author Contribution form
The Corresponding author should also complete a ColourWork Agreement form, if the article contains colour figures.

INTRODUCTION

Thank you for your interest in *European Journal of Heart Failure*. Please consult the following instructions for help in preparing your manuscript, and feel free to contact us with any questions. To ensure fast peer review and publication, manuscripts that do not adhere to the following instructions will be returned to the corresponding author for technical revision before undergoing peer review. We look forward to your submission.

AIMS AND SCOPE

The *European Journal of Heart Failure* is the international journal of the Heart Failure Association of the European Society of Cardiology dedicated to the advancement of knowledge in the field of heart failure. The journal publishes reviews and editorials in order to improve the understanding, prevention, investigation and treatment of heart failure. Molecular and cellular biology, pathology, physiology, electrophysiology, pharmacology, as well as the clinical, social and population sciences all form part of the discipline that is heart failure. Accordingly, submission of manuscripts on basic, clinical and population sciences is invited. Original contributions on nursing, care of the elderly, primary care, health economics and other specialist fields related to heart failure are also welcome.

HEART NETWORK

The *European Journal of Heart Failure* participates in the HEART Network which is a network of Editors from most cardiovascular journals. Information is exchanged between Editors on a regular basis. The network has recently approved a common ethics standard.

Its purpose is to ensure transparency and honesty in the scientific process that promotes ethical conduct in performance and publication of research.

The following will be considered as parts of this process:

a. Disclosure of potential conflicts of interest for all involved in the performance of research and in the evaluation and publication process of a manuscript. Relevant relationships with commercial interests should be disclosed according to the guidelines of the journal's sponsoring society, or, when no such guidelines exist, according to those of the AHA, ACC, or ESC.

b. Establish thorough review processes particularly alert to discovering scientific fraud and data falsification, redundant or duplicate publication, and plagiarism, and to adopt a uniform standard of dealing with authors guilty of fraudulent practices.

c. To maintain confidentiality and embargos where appropriate.

d. To create uniform criteria to establish authorship. To qualify for authorship, individuals must have made substantial contributions to the intellectual content of the paper in at least one of the following areas: conceived and designed the research, acquired the data, analysed and interpreted the data, performed statistical analysis, handled funding and supervision, drafted the manuscript, or made critical revision of the manuscript for important intellectual content. Authors must give final approval of the version to be submitted and any revised version to be published. For multi-centre trials, individuals who accept direct responsibility for the manuscript should fully meet the criteria for authorship defined above and contributors not meeting these criteria should be acknowledged.

e. Avoidance of false claims of ownership, priority, by attention to previous publications.

f. Avoidance of excessive claims of benefits of a product/technique, in the publication as well as with news media.

g. Noting compliance with institutional review board requirements and, when appropriate, approved laboratory procedures for animal research, and that the research conforms to the ethical standards of the *Declaration of Helsinki*, the *Geneva Declaration*, the *Belmont Report*, and *Good Clinical Practices* from the FDA, and the submission conforms to the *International Committee of Medical Journal Editors (ICMJE): Uniform Requirements for Manuscripts Submitted to Biomedical Journals: writing and editing for biomedical publication* (*Haematologica* 2005; **89:**264).

PRE-SUBMISSION

1. Editorial Review and Acceptance

The acceptance criteria for all papers are the quality and originality of the research and its significance to our readership. Except where otherwise stated, manuscripts are double-blind peer reviewed by two anonymous reviewers and the Editor. Final acceptance or rejection rests with the Editorial Board, who reserves the right to refuse any material for publication.

Manuscripts should be in a clear, concise and direct style. Where contributions are judged as acceptable for publication on the basis of content, the Editor and the Publisher reserve the right to modify typescripts to eliminate ambiguity and repetition and improve communication between author and reader. If extensive alterations are required, the manuscript will be returned to the author for revision.

2. Pre-submission Resources

2.1. Author Services

Prior to submission, we encourage you to browse Wiley's Author Resources site, which provides useful information on topics such as preparing your article and digital artwork; publishing ethics; copyright and open access; and how to promote your published work.

2.2. Pre-submission English-language Editing

Authors for whom English is a second language are advised to consider having their manuscript professionally edited before submission to improve the English, and to ensure the paper is clearly written in standard, scientific English language appropriate to the discipline. This can be undertaken by a service such as the Wiley English Language Editing Service, at http://wileyeditingservices.com. Please note that using the Wiley English Language Editing Service does not guarantee that your paper will be accepted by this journal, and all services are paid for and arranged by the author.

3. Manuscript Preparation

3.1. Manuscript Categories and Criteria

The *European Journal of Heart Failure* accepts the following categories of articles:

Full Length Articles

These should not exceed 3500 words (excluding references, tables and figures) and may include up to a maximum of 6 figures and/or tables and up to 30 references. Full length articles should be divided into the following sections: (1) Title page, (2) Abstract and up to six Keywords, (3) Introduction, (4) Methods, (5) Results, (6) Discussion, (7) Acknowledgements, (8) Funding, (9) Conflict of interest, (10) References, (11) Figure legends, (12) Appendices, (13) Tables, (14) Figures. The Abstract should be divided into the following sections 'Aims', 'Methods and results' and 'Conclusion'; it should not exceed 250 words.

Reviews

The *European Journal of Heart Failure* publishes a limited number of scholarly, comprehensive review papers. Reviews should not exceed 3500 words. They should summarise and critically evaluate research in the subject area, and should discuss implications for the future. Reviews have unstructured abstracts with no headings, which should not exceed 250 words and may include up to 45–50 references. Please see below for systematic reviews.

Systematic Reviews

These reviews should follow the format of full length articles (refer to the section, 'Full Length Articles'). These should be submitted as a full length article during the submission process.

Editorials

All editorials should be limited to 1500 words (excluding references), with a maximum of 15 references. They do not require an abstract.

Short Reports

These reports should not exceed 1500 words and should comprise a Background section (≈100 words), Aims (≈50 words), Methods (≈300 words), Results (300 words) and Conclusion (250 words). The editorial team reserves the right to decide which of the tables/figures submitted are necessary. A structured abstract not exceeding 250 words is also required for Internet purposes.

Letters to the Editor

Relevant correspondence will be considered. This should not exceed 400 words in length excluding references, and a maximum of three authors.

Case Reports

These reports should not exceed 1200 words. Case reports should include an unstructured Abstract with no subheadings (not exceeding 100 words), an Introduction, a Description of the case(s) under the heading, 'Case Report' and a Discussion of the findings in the context of current practice.

Study Design

These should not exceed 3500 words (excluding references, tables, and figures) and may include up to a maximum of 6 figures and/or tables and up to 30 references. Study design papers should be divided into the following sections: (1) Title page, (2) Abstract and up to six Keywords, (3) Introduction, (4) Study Design, (5) Discussion, (6) Acknowledgements, (7) Funding, (8) Conflict of Interest, (9) References, (10) Figure legends, (11) Appendices, (12) Tables, (13) Figures. The Abstract should be divided into the following sections 'Aims', 'Methods', and 'Conclusion'; it should not exceed 250 words.

3.2. Manuscript Format and Structure

General Format

Prepare your manuscript text using a Word processing package (save in .doc or .rtf format). Submissions of text in the form of PDF files are not permitted. Manuscripts should be double-spaced, including text, tables, legends and references.

Number each page. Please avoid footnotes; use instead, and as sparingly as possible, notes within brackets. Enter text in the style and order of the journal. Type references in the correct order and style of the journal. Type unjustified, without hyphenation, except for compound words (where two words are joined to form a new word e.g. end-systolic, non-infarcted). Type headings in the style of the journal. Use the TAB key once for paragraph indents. Where possible use Times New Roman for the text font and Symbol for Greek and special characters. Use the word processing formatting features to indicate bold, italic, Greek, maths, superscript and subscript characters. Clearly identify unusual symbols and Greek letters. Differentiate between the letter O and zero; the letters l and I; and the number 1.

Check the final copy of your paper carefully, as any spelling mistakes and errors may be translated into the typeset version.

Style and Spelling

Oxford English spelling should be used. Authors whose first language is not English are requested to have their manuscripts checked carefully before submission. This will help expedite the review process and avoid confusion.

Abbreviations

Abbreviations of standard SI units of measurement only should be used.

3.3. Parts of the Manuscript

Title Page

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

Abstract and Keywords

All abstracts may not contain more than 250 words and should be submitted as a separate file. The abstract should be formatted with the following heading: (1) Aims, (2) Methods and Results, (3) Conclusion.

A maximum of six keywords may be submitted.

Introduction

This section should provide a rationale for conducting the study within the context of previous work by other authors.

Methods

This section should be sufficiently detailed to enable repetition of the study by other investigators. If pertinent, the section may be divided into headed subsections. For animal studies, this section should contain a statement that, "The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1985)". Human studies should contain a statement that, "The investigation conforms with the principles outlined in the *Declaration of Helsinki*" (*Br Med J* 1964; **ii:** 177). In addition details of the ethics committee approval procedures and a statement that all subjects gave written informed consent to participate in the study should be included.

Results

If pertinent, the section may be divided into headed subsections. For presentation of data, figures are preferred to tables. Data should not be duplicated in both figures and tables. Extensive numerical data should be presented in legends to the figures rather than in the main body of text. SI units should be used throughout.

Discussion

Four manuscript pages should in general be enough to compare and interpret the findings of the study with regard to previous work by (other) authors. This section should also contain 1–4 paragraphs dealing with topics that are beyond the scope of the study. Limitations to the study should also be discussed.

Figures

All illustrations (line drawings and photographs) are classified as figures. The review process will not begin until all figures are received. Figures should be limited to the number necessary for clarity and must not duplicate data given in tables or in the text. They must be suitable for high quality reproduction and should be submitted in the desired final printed size so that reduction can be avoided. Figures should be no larger than 125 (height) x 180 (width) mm (5 x 7 inches) and each figure should be submitted in a separate file from that of the main manuscript text. More information about figure requirements and advice on preparation is available on our Author Resources Digital Artwork page.

Line Drawings: Please provide these as clear, sharp illustrations, suitable for reproduction as submitted. All labelling should be on the original. Faint and grey shading or stippling will be lost upon reproduction and should be avoided. Where various shadings are used within one figure please ensure that it is easy to differentiate between them, using standard shadings (see the hard copy of the journal for examples). There should be sufficient white space between lines and dots to ensure the areas will not fill in and look grey. If stippling is used, this should be made up of clear black dots with visible white space between them.

Ensure that the size of the lettering is in proportion with the overall dimensions of the drawing. Ideally, the drawings should be submitted in the desired final printed size to avoid reduction. If submitting line drawings which require reduction, please check that the lettering will be clearly legible after the drawing has been reduced to the size at which it will be printed. After reduction, letters should not be smaller than 2 mm in height.

Photographs: Photographs should be of sufficiently high quality with respect to detail, contrast and fineness of grain to withstand the inevitable loss of contrast and detail inherent in the printing process. Please indicate the magnification by a rule on the photograph.

Wiley's Figure Preparation service can provide publication ready figure files, set to the correct size, resolution and layout to suit this journal. Authors just need to upload their existing figures, along with the name of the journal, to the site. Visit our site for more details of the service and charges.

Figure Legends

These should be on a separate, numbered page, and grouped under the heading "Legends". Define all symbols and abbreviations used in the figure. Common abbreviations and others in the preceding text should not be redefined in the legend.

Colour Figures

The European Journal of Heart Failure does not charge for colour figures.

Tables

Tables should be typed with double spacing, but minimising redundant space, and each should be placed on a separate sheet. Tables should be submitted, wherever possible, in a portrait, as opposed to landscape, layout. Each Table should be numbered in sequence using Arabic numerals. Tables should also have a title above and an explanatory footnote below. All abbreviations used should be defined in the footnote. **NB: tables must be submitted in an editable format, such as Excel or Word, and not embedded as an image or presented as an image file.**

Permissions

If any figures are to be duplicated from previously published work, written permission must be obtained both from the publisher and the author, and a credit line giving the source added to the relevant figure legend. If text material (250 to 300 words) is to be reproduced from published sources, written permission is required from both publisher and author. For shorter quotations, it is sufficient to add a bibliographic credit. The Letters containing the permission for the reproduction of either text or illustrations must be uploaded with the manuscript files. If you have been unable to obtain permission, please indicate this.

Permission Note
This should list any material (Figures, Tables and/or large amounts of text) that has been previously published. Permissions from the previous publisher/copyright holder should be provided, or alternatively, if all material is original to this submission, then a word document stating this fact should be uploaded with your submission.

Acknowledgements

Substantive contributions of individuals should be noted in the Acknowledgements, positioned before the Conflict of Interest statement.

Conflicts of Interest

All authors must make a formal statement indicating any potential conflicts of interest that might constitute an embarrassment to any of the authors if it were not to be declared and were to emerge after publication. Such conflicts might include, but are not limited to, shareholding in or receipt of a grant or consultancy fee from a company whose product features in the submitted manuscript or which manufactures a competing product. The statement should be positioned before the list of references. If there are no conflicts of interest, please insert the wording, 'Conflicts of Interest: none declared'. *European Journal of Heart Failure* uses the ICMJE Conflicts of Interest disclosure form, and requests that each author submits a completed form along with the submission.

References

References should be identified in the text by Arabic numerals and numbered in the order cited. All references should be compiled at the end of the article in the Vancouver style, except that ALL authors should be listed.

Complete information should be given for each reference including the title of the article, abbreviated journal title and page numbers.

Personal communications, manuscripts in preparation and other unpublished data should not be cited in the reference list but may be mentioned in parentheses in the text. Authors should get permission from the source to cite unpublished data. Titles of journals should be abbreviated in accordance with *Index Medicus* (see list printed annually in the January issue of *Index Medicus*). If a journal is not listed in *Index Medicus* then its name should be written out in full.

We recommend the use of a tool such as EndNote or Reference Manager for reference management and formatting. EndNote reference styles can be searched for here: http://www.endnote.com/support/enstyles.asp. For Reference Manager we recommend using the *European Heart Journal* reference style which can be searched for here: http://www.refman.com/support/rmstyles.asp.

Article Citation Example:

1. Lainchbury JG, Troughton RW, Frampton CM, Yandle TG, Hamid A, Nicholls MG, Richards AM. NTproBNP-guided drug treatment for chronic heart failure: design and methods in the "BATTLESCARRED" trial. *Eur J Heart Fail* 2006; **8:**532-538.

If an article has been published online but has not yet been given issue or page numbers please use the Digital Object Identifier (doi) number when referencing the article as in the example below:

2. Asger A, Møller JM, Daugaard PC, Kjær SU, Erik S. Effects of phosphodiesterase-5 inhibition by sildenafil in the pressure overloaded right heart. *Eur J Heart Fail*;doi:10.1016/j.eheart.2008.09.016. Published online ahead of print 12 March 2008.