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

Leonardo Calegari

TREINAMENTO FÍSICO AERÓBIO MELHORA A SENSIBILIDADE BARORREFLEXA, O QUIMIORREFLEXO PERIFÉRICO E O PERFIL INFLAMATÓRIO MUSCULAR EM RATOS COM INSUFICIÊNCIA CARDÍACA

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Devo primeiramente fazer alguns experimentos antes de prosseguir, pois é minha intenção mencionar a experiência primeiro, e então demonstrar pelo raciocínio por que tal experiência é obrigada a operar de tal maneira. Essa é a regra verdadeira que aqueles que especulam sobre os efeitos da natureza devem seguir.

- Leonardo da Vinci, c. 1513

RESUMO

A insuficiência cardíaca (IC) é uma síndrome com repercussões sistêmicas que limita a oferta de suprimento energético para o organismo. Considerando a prevalência crescente e a morbimortalidade associada, representa um importante problema de saúde pública. As alterações dos reflexos cardiovasculares e o aumento de citocinas pró-inflamatórias são características dos portadores de IC e contribuem para o aumento da mortalidade. O treinamento físico aeróbio (TFA) vem sendo indicado para melhorar a aptidão cardiorrespiratória e a qualidade de vida em pacientes com IC. Seus efeitos sobre a função hemodinâmica, reflexos cardiovasculares e perfil inflamatório ainda carecem de investigações. Assim, o objetivo do presente estudo foi avaliar os efeitos do TFA sobre a função hemodinâmica, sensibilidade barorreflexa, quimiorreflexo periférico e perfil inflamatório no músculo gastrocnêmio em um modelo experimental de IC. Ratos Wistar machos foram submetidos à ligadura da artéria coronária esquerda para indução do infarto agudo do miocárdio ou cirurgia *sham*. Após seis semanas, tempo necessário para o desenvolvimento da IC, os animais foram aleatoriamente distribuídos entre quatro grupos: *sham* sedentário, *sham* treinado, IC sedentário e IC treinado. O protocolo de TFA foi realizado na esteira com velocidade de 16m/min por 60min, 5 dias por semana durante 2 meses. Nossos resultados mostraram que o TFA promoveu redução da pressão diastólica final do ventrículo esquerdo, melhora na sensibilidade barorreflexa (SBR) e atenuação da resposta pressórica ao cianeto de potássio no grupo IC treinado quando comparado ao grupo IC sedentário. Observamos que a melhora na SBR esteve associada à redução da resposta pressórica no grupo IC treinado. Além disso, o TFA foi capaz de melhorar o perfil inflamatório com aumento da interleucina-10 (IL-10) e redução do fator de necrose tumoral alfa (TNF-α) no músculo gastrocnêmio dos ratos IC treinados comparado aos ratos IC sedentários. Juntos, estes resultados mostram que o TFA promove melhora da função cardíaca, aumenta a sensibilidade dos barorreceptores, atenua a atividade dos quimiorreceptores periféricos e induz a expressão de IL-10 no músculo esquelético de ratos com IC.

Palavras-chave: Insuficiência cardíaca, Exercício físico, Barorreflexo, Citocinas, Gastrocnêmio.

ABSTRACT

Heart failure (HF) is a syndrome with systemic effects, which limits the offer of energy supply to the body. Considering the growing prevalence, morbidity and mortality involved, it represents an important public health problem. Changes of cardiovascular reflexes and increase of pro-inflammatory cytokines are characteristic of people with HF and contribute to increased mortality. The aerobic physical training (APT) has been suggested to improve cardiorespiratory fitness and quality of life in patients with HF. Its effects on the hemodynamic function, cardiovascular reflexes and inflammatory profile still require investigation. Thus, the aim of the present study was to evaluate the effects of physical training on the hemodynamic function, baroreflex sensitivity, peripheral chemoreflex and inflammatory profile in the gastrocnemius muscle in an experimental model of HF. Male Wistar rats were submitted to ligation of left coronary artery for inducing myocardial infarction or sham surgery. After six weeks, time required for the development of the HF, the animals were randomly distributed into four groups: sham sedentary, sham trained, HF sedentary and HF trained. The APT protocol was carried out on treadmill with 16m/min speed for 60 min, 5 days per week for 2 months. Our results showed that APT promoted reduction of left ventricular end diastolic pressure, improved baroreflex sensitivity (BRS) and attenuated the pressure response induced by potassium cyanide in the trained HF group when compared to the sedentary HF group. We observed that the improvement in the BRS was associated with reduced pressure response in the trained HF group. In addition, the APT improved the inflammatory profile, increasing interleukin-10 (IL-10) and decreasing the tumor necrosis factor (TNF-α) in the gastrocnemius muscle of the trained HF rats compared to sedentary HF rats. Together, these results show that APT promotes improvement of cardiac function, increases the sensitivity of the baroreceptors, reduces the activity of peripheral chemoreceptors and induces the expression of IL-10 in skeletal muscle of rats with HF.

Keywords: Heart failure, Physical exercise, Baroreflex, Cytokines, Gastrocnemius.

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ANG II: Angiotensina II

ASICs: Canais iônicos sensíveis a ácidos

AT_1: Receptor para angiotensina II

β_1: Receptor beta-adrenérgico subtipo 1

β_2: Receptor beta-adrenérgico subtipo 2

CO: Monóxido de carbono

DNA: Ácido desoxirribonucleico

ECA: Enzima conversora de angiotensina

ENaC: Canais de sódio epiteliais degenerina

EUA: Estados Unidos da América

FC: Frequência cardíaca

FOXO: *Forkhead Box O*

GMPc: Monofosfato de guanosina cíclico

GRK: Proteína cinase acoplada a proteína G

H_2S: Sulfato de hidrogênio

IAM: Infarto agudo do miocárdio

IC: Insuficiência cardíaca

IL-1β: Interleucina 1 beta

IL-6: Interleucina 6

IL-10: Interleucina 10

KCN: Cianeto de potássio

MAFbx: *Muscle-Atrophy F-box*

NF-kB: *Factor nuclear kappa B*

MuRF-1: *Muscle-Specific Ring Finger*

NO: Óxido nítrico

nNOS: Óxido nítrico sintase neuronal

NTS: Núcleo do trato solitário

NYHA: Associação do coração de Nova York

PDFVE: Pressão diastólica final do ventrículo esquerdo

PGC-1α: *Peroxisome proliferator activated receptor gamma coactivator-1 alpha*

PVN: Núcleo paraventricular do hipotálamo

RNA: Ácido ribonucleico

SNC: Sistema nervoso central

SNS: Sistema nervoso simpático

SRAA: Sistema renina angiotensina aldosterona

SBR: Sensibilidade barorreflexa

SUS: Sistema único de saúde

TFA: Treinamento físico aeróbio

TNF-α: Fator de necrose tumoral alfa

VO_{2pico}: Consumo de oxigênio no pico do esforço

VE: Ventrículo esquerdo

VE/VCO_2: Equivalente ventilatório para o gás carbônico

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SUMÁRIO

1. INTRODUÇÃO

As doenças cardiovasculares figuram entre as mais letais em diversos países, entre eles o Brasil. Considera-se a insuficiência cardíaca (IC), a via final comum de muitas doenças cardiovasculares e sua prevalência vem aumentando nos últimos anos (Albuquerque *et al.*, 2015). A hiperatividade do sistema nervoso simpático é uma característica fisiopatológica da IC, que por sua vez é modulada, entre outros fatores, pelas aferências barorreceptora e quimiorreceptora sistêmicas (Floras e Ponikowski, 2015). Pesquisas recentes vêm mostrando que as citocinas pró-inflamatórias contribuem para o agravamento da IC (Briasoulis *et al.*, 2016).

Entre as estratégias terapêuticas para a IC, o exercício físico vem sendo considerado um importante complemento ao tratamento farmacológico (Mcmurray *et al.*, 2012; Adams e Niebauer, 2015). Novas evidências científicas oriundas de modelos experimentais podem ampliar os benefícios sobre o sistema cardiovascular induzido pelo treinamento físico na IC (Nunes *et al.*, 2013; Patel *et al.*, 2013; Souza *et al.*, 2014; Marcus *et al.*, 2015; Nunes *et al.*, 2015).

A presente tese faz uma revisão atual da literatura sobre a epidemiologia, fisiopatologia e os efeitos do treinamento físico aeróbio (TFA) na IC. Logo após são listados o objetivo geral e os específicos que foram contemplados em dois artigos. O primeiro é denominado *"Exercise training attenuates the pressor response evoked by peripheral chemoreflex in rats with heart failure"* aceito para publicação no Canadian Journal of Physiology and Pharmacology. Neste artigo estudamos as adaptações induzidas pelo TFA sobre a função hemodinâmica e a sensibilidade dos barorreceptores e quimiorreceptores periféricos. Além disso, avaliamos a associação entre os reflexos inibitórios (barorreflexo) e excitatórios (quimiorreflexo) e os efeitos do TFA sobre esta interação em ratos IC acordados. O segundo artigo intitulado "*Exercise training improves the IL-10/TNF-α cytokine balance in the gastrocnemius of rats with heart failure*" foi desenvolvido para avaliar os efeitos do TFA sobre os mediadores pró e anti-inflamatórios no músculo esquelético com predomínio de fibras tipo II. Os estudos são apresentados a seguir, em formato de artigo científico, de acordo com as normas das revistas para os quais foram submetidos. Ao final da

tese são listadas as principais conclusões dos estudos e relatadas as perspectivas para os próximos estudos.

1.1 Epidemiologia

A insuficiência cardíaca (IC) é um dos principais problemas de saúde pública mundial, com uma prevalência de 5 milhões e 700 mil casos nos Estados Unidos da América (EUA) e mais de 26 milhões em todo o mundo (Ambrosy *et al.*, 2014). A IC vem sendo considerada uma epidemia e projeções mostram que sua prevalência aumentará 46% de 2012 a 2030, resultando em mais de 8 milhões de pessoas com IC nos EUA (Roger, 2013). Embora os resultados dos tratamentos ambulatoriais tenham melhorado, pacientes com IC continuam apresentado alta ocorrência de hospitalização e mortalidade (Ambrosy *et al.*, 2014). Dados do estudo de *Framingham* (Levy *et al.,* 2002) e *Olmsted County* (Roger *et al.*, 2004), realizados na década de 90, indicam altas taxas de mortalidade ao longo do tempo. A ocorrência de mortalidade estimada para 5 anos foi de 59% nos homens e 45% nas mulheres no estudo de *Framingham* e 50% nos homens e 46% nas mulheres, no estudo de *Olmsted County*. A sobrevida após o diagnóstico de IC não é animadora, mas tem aumentado substancialmente nos últimos anos (Dunlay e Roger, 2014). Em estudos mais recentes, a mortalidade após um ano de tratamento otimizado, incluindo o uso rotineiro de inibidores da enzima conversora de angiotensina (ECA) e betabloqueadores, foi de aproximadamente 10 a 15% (Packer *et al.*, 2001). Em 2006, os custos financeiros diretos e indiretos da IC atingiram 27 bilhões de dólares nos EUA. Estes valores elevados se devem as internações hospitalares recorrentes. Em muitas nações desenvolvidas cerca de 1 a 2% dos gastos totais com saúde estão relacionadas com internações hospitalares por IC descompensada (Baliga, Dec e Narula, 2013).

No Brasil, segundo os dados do DATA-SUS, de janeiro a setembro de 2015 houve 836.919 internações por doenças do aparelho circulatório e aproximadamente 19,4% foram relacionadas à IC. Neste mesmo período, houve 17.045 mortes relacionadas à IC no Brasil (Ministério da Saúde, 2015). No Rio Grande do Sul, de

janeiro a setembro de 2015 houve 71.570 internações por doenças do aparelho circulatório e aproximadamente 17,9% foram relacionadas à IC. Neste mesmo período, houve 1.213 mortes relacionadas à IC em nosso estado (Ministério da Saúde, 2015). A principal etiologia da IC é a cardiopatia isquêmica crônica associada à hipertensão arterial. Em determinadas regiões geográficas do país ainda existem formas de IC associadas à doença de Chagas, endomiocardiofibrose e a cardiopatia valvular reumática crônica (Bocchi *et al.*, 2009). Em um recente estudo brasileiro que acompanhou os pacientes hospitalizados por IC aguda, os autores relataram alta taxa de mortalidade intra-hospitalar, representando 12,6% dos casos (Albuquerque *et al.*, 2015). Nos EUA a mortalidade intra-hospitalar foi de 4% e o tempo médio de permanência hospitalar foi de 4,3 dias (Adams *et al.*, 2005). Semelhante ao estudo norte americano, o *Euro Heart Survey* apresentou uma taxa de mortalidade de 3,8% (Maggioni *et al.*, 2013). Albuquerque *et al*. (2015) relataram que houve pouca prescrição de medicamentos baseado em evidências clínicas para a IC, explicando em parte os altos índices de mortalidade de pacientes com IC no Brasil.

2. FISIOPATOLOGIA DA INSUFICIÊNCIA CARDÍACA

De acordo com as Diretrizes do “Colégio Americano de Cardiologia”, a IC é definida uma “síndrome clínica complexa que pode resultar de disfunção estrutural ou funcional que prejudica a capacidade do ventrículo esquerdo de ser preenchido com sangue ou de ejetar o sangue” (Jessup *et al.*, 2009). A IC é uma síndrome, não uma doença, e seu diagnóstico é baseado no exame clínico. Para avaliar a incidência na população e estudar sua epidemiologia, são necessários critérios padronizados que podem ser utilizados em grande escala. Vários critérios vêm sendo propostos para o diagnóstico da IC. Entre eles, destacam-se os Critérios de *Framingham*, de *Bostom*, *Gothenburg* e da Sociedade Europeia de Cardiologia. Todos têm em comum indicadores de dispneia e intolerância ao esforço além de elevadas pressões de enchimento ventricular, combinado com dados da história médica, exame físico e radiografia do tórax (Roger, 2013). A IC tem sido classicamente categorizada com base na intensidade dos sintomas em 4 classes

propostas pela *New York Heart Association* (NYHA). Estas classes estratificam o grau de limitação imposto pela doença para atividades cotidianas do indivíduo. As quatro classes propostas são: I) ausência de sintomas (dispneia) durante atividades cotidianas. A limitação para esforços é semelhante à esperada em indivíduos normais. II) sintomas desencadeados por atividades cotidianas; III) sintomas desencadeados em atividades menos intensas que as cotidianas ou pequenos esforços; IV) sintomas em repouso (Bocchi *et al.*, 2009).

A IC é uma condição que está associada à incapacidade e à morbimortalidade do paciente. Os resultados clínicos adversos e a natureza progressiva da síndrome tem levado à investigação sistemática de múltiplos mecanismos que contribuem para o início e progressão da IC. Os principais fatores envolvidos nos mecanismos fisiopatológicos da IC incluem a ação de neurohormônios, a inflamação, o estresse oxidativo, fatores de crescimento e anormalidades na homeostase do cálcio (Lee e Vasan, 2008). O entendimento sobre a fisiopatologia da IC progrediu muito nos últimos anos. O modelo hemodinâmico de "bomba cardíaca insuficiente" tem avançado para um modelo mais complexo, que envolve vias de ativação neuroendócrina, imunológica e metabólica. Um grande número de sistemas hormonais são ativados na IC, e virtualmente todas as substâncias vasoconstritoras estão aumentadas, incluindo angiotensina II (ANG II), endotelina-1, vasopressina e noradrenalina. Atualmente acredita-se que os potentes sistemas vasodilatadores estão dessensibilizados na IC (Zucker, Patel e Schultz, 2012). O óxido nítrico (NO) e seus alvos intracelulares, como o GMP cíclico, estão reduzidos e contribuem para o quadro generalizado de vasoconstrição (Zucker, Patel e Schultz, 2012). Além disso, a IC vem sendo considerada um estado pró-inflamatório e uma condição caracterizada por altos níveis de estresse oxidativo (Zucker, Patel e Schultz, 2012). Entre estes mecanismos neuro-hormonais, a hiperatividade do sistema nervoso simpático (SNS) e do sistema renina-angiotensina-aldosterona (SRAA) desempenham papel fundamental na fisiopatologia da IC (Floras, 2009). Baseado em técnicas como a microneurografia e a mensuração de liberação de noradrenalina (*spillover*), demonstra-se que a atividade nervosa simpática está claramente elevada na IC (Groehs *et al.*, 2015). Inicialmente, estes sistemas são capazes de compensar a disfunção miocárdica e preservar a homeostase cardiovascular. No longo prazo, entretanto, a hiperativação

dos sistemas simpático e SRAA exerce efeitos deletérios sobre as estruturas e funções do miocárdio, levando a descompensação e progressiva piora da função cardíaca (Zucker, Patel e Schultz, 2012; Piepoli e Crisafulli, 2014; Floras e Ponikowski, 2015).

A integração central, para a qual chegam informações dos metaborreceptores, barorreceptores e quimiorreceptores em vários níveis do sistema nervoso central (SNC), pode modular o tônus simpático e parassimpático (Schmidt *et al.*, 2005). As alterações promovidas pela pobre perfusão tecidual, característica da IC, promove um aumento na ativação dos quimiorreceptores, que por sua vez, participa, em parte, da exacerbação da atividade eferente simpática (Schmidt *et al.*, 2005). A sensibilidade do quimiorreflexo periférico está exagerada, facilitando a elevação da atividade simpática e a disfunção barorreflexa na IC (Sun *et al.*, 1999). Alguns investigadores relacionam a hipersensibilidade dos quimiorreceptores ao aumento nas concentrações de ANG II e à diminuição de NO no sistema nervoso central (Zucker, Patel e Schultz, 2012).

2.1 Hiperatividade Simpática e sua Repercussão Sistêmica

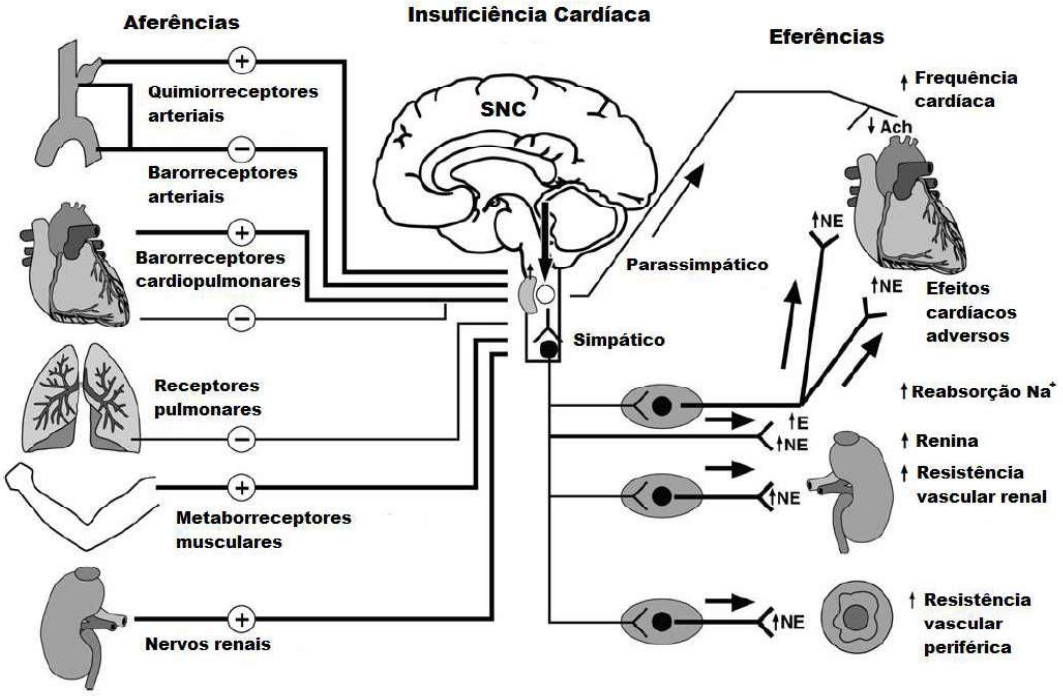
O sistema nervoso simpático possui uma atividade tônica basal que é ajustada em resposta a uma variedade de estímulos aferentes, por exemplo, barorreceptores arteriais, quimiorreceptores e receptores cardiopulmonares, dentre outros (Malpas, 2010). Estes ajustes ocorrem rapidamente, dentro de 1 segundo, em resposta a alterações na pressão arterial; em alguns minutos ou horas, em resposta as alterações no volume sanguíneo; e, em longos períodos de tempo, em resposta às alterações nos níveis hormonais ou estímulos crônicos como o estresse. O início da disfunção ventricular sistólica promove aumento da pressão diastólica final ou redução da fração de ejeção, que por sua vez, altera o equilíbrio entre o SNS e parassimpático sobre o controle cardiovascular (Floras e Ponikowski, 2015). Na IC, o desequilíbrio do sistema nervoso homeostático é caracterizado pela hiperatividade simpática com consequências clínicas relevantes. Estas incluem progressão da IC,

desenvolvimento de intolerância ao esforço, remodelamento ventricular, arritmias e morte prematura (Floras e Ponikowski, 2015).

A compreensão dos mecanismos que explicam esses processos foi ampliada nas últimas décadas por meio de diversos experimentos em animais e pelo avanço tecnológico (Marcus *et al.*, 2015; Pügge *et al.*, 2016). Atualmente, os estudos sugerem que o ponto de equilíbrio da atividade eferente do sistema nervoso simpático e parassimpático está alterado dentro do próprio sistema nervoso central, induzidos por vários mecanismos, incluindo inflamação, alteração da sinalização encefálica do SRAA, geração das espécies reativas de oxigênio e sensibilização dos quimiorreceptores (Lymperopoulos, Rengo e Koch, 2013). Além disso, a interação entre os reflexos neurais periféricos favorecem o desequilíbrio simpático/parassimpático na IC (Floras e Ponikowski, 2015). Acredita-se que há um deslocamento para cima do ponto de equilíbrio homeostático dos eferentes simpáticos; que a alteração da neurotransmissão ganglionar prejudique a modulação vagal da frequência cardíaca; que os reflexos inibitórios cardiopulmonares estejam atenuados; e, que as respostas mecanorreflexas e metaborreflexas simpatoexcitatórias estejam exacerbadas (Abboud, 2010; Floras e Ponikowski, 2015), como mostra a Figura 1.

O SNS exerce uma variedade de efeitos cardiovasculares, incluindo aumento da frequência cardíaca (FC) (cronotropismo positivo, predispondo a arritmias), aumento da contratilidade cardíaca (inotropismo positivo), relaxamento cardíaco aumentado (lusitropismo positivo), redução na capacitância venosa e vasoconstrição arterial (Lymperopoulos, Rengo e Koch, 2013). Embora a FC possa ser controlada por ambos, sistema nervoso parassimpático e SNS, a contração e o relaxamento ventricular são controlados quase que exclusivamente pelo SNS (Pierpont *et al.*, 1985). A estimulação simpática exacerbada promove aumento da FC e da contratilidade do miocárdio, entretanto, no longo prazo parece contribuir com a piora da função cardíaca, evidenciada pela redução da fração de encurtamento, alargamento dos cardiomiócitos e redução da capacidade máxima ao exercício (Brum *et al.*, 2010).

Figura 1. Fisiopatologia da IC. Aferências convergem ao SNC para elevar o ponto de equilíbrio e modular as eferências do sistema nervoso simpático. Com a progressão da disfunção sistólica, as aferências simpatoinibitórias (linhas finas) estão reduzidas. Ademais, a resposta da frequência cardíaca (eferente vagal) ao barorreflexo está atenuada (linha fina). As aferências excitatórias estão aumentadas (linhas grossas). Fonte: Adaptado de *Floras e Ponikowski*, 2015.



Como o coração torna-se progressivamente ineficiente ao efeito estimulatório das catecolaminas, a estimulação crônica do SNS promove aumento na liberação de noradrenalina para o coração (Lymperopoulos, Rengo e Koch, 2013). Em pacientes com IC, o esperado efeito inibitório dos receptores adrenérgicos α_2 pré-sinápticos sobre a liberação da noradrenalina está significativamente atenuado, contribuindo para o aumento da noradrenalina plasmática observada na IC crônica (Aggarwal *et al.*, 2001; Philipp e Hein, 2004).

No coração saudável, os receptores β_1-adrenérgicos estão expressos em maior quantidade quando comparados aos receptores β_2-adrenérgicos (80:20%, respectivamente), prevalecendo desta forma a ação dos receptores β_1-adrenérgicos (Brodde, 1993). Na IC a relação β_1:β_2 modifica-se, pois ocorre dessensibilização dos receptores β_1-adrenergicos na membrana, fazendo com que a razão β_1:β_2 chegue a valores de aproximadamente (60:40%, respectivamente) (Lamba e Abraham, 2000). Como os receptores β_2-adrenérgicos tem menor ação inotrópica positiva, há uma diminuição da função contrátil cardíaca. No meio intracelular, a fosforilação dos receptores alfa e beta adrenérgicos pelas proteínas cinases acopladas à proteína G (GRK) causam a internalização dos receptores adrenérgicos. Deve-se considerar que os níveis das GRK_2 e GRK_5 estão elevados nos homens e nos modelos animais com IC (Rengo *et al.*, 2011). Atualmente, é consenso que na IC crônica a excessiva estimulação do SNS reduza os receptores beta adrenérgicos, desencadeado pelo aumento da GRK_2 dentro do cardiomiócito, levando a uma redução da reserva inotrópica do coração (Rengo, Lymperopoulos e Koch, 2009).

Além da influência dos receptores adrenérgicos, outros fatores que agem dentro do SNC e controlam a atividade do SNS cardíaco. Os neurônios monoaminérgicos subcorticais suprabulbares e a ANG II do tronco encefálico tem atraído o interesse dos pesquisadores por sua capacidade em regular a atividade eferente do SNS em pacientes com IC (Lymperopoulos, Rengo e Koch, 2013). A velocidade de metabolização e de renovação (*turnover*) da noradrenalina em regiões subcorticais é significantemente maior nos pacientes com IC do que em sujeitos saudáveis (Aggarwal *et al.*, 2002). Além disso, a liberação da noradrenalina subcortical se correlaciona com a atividade nervosa simpática (Aggarwal *et al.*, 2002). Foi sugerido que a atividade do SNS sofre influências da ANG II que por sua vez desempenha um papel fundamental nas respostas hemodinâmicas adversas e no remodelamento do ventrículo esquerdo, possivelmente pela formação de superóxido (Wang *et al.*, 2004).

2.2 Barorreflexo na IC

Estudos prévios sugerem que a IC provoca redução na sensibilidade barorreflexa (SBR) (Rondon *et al.*, 2006; Groehs *et al.*, 2015). Um estudo relatou que a mortalidade após infarto agudo do miocárdio (IAM) em pacientes com fração de ejeção do ventrículo esquerdo < 35% foi duas vezes maior quando a sensibilidade dos barorreceptores estava atenuada < 3 ms/mmHg; (La Rovere *et al.*, 1998). Acredita-se que a sensibilidade atenuada dos barorreceptores poderia contribuir com a hiperatividade do SNS (Abboud, 2010). Além disso, a atividade parassimpática reduzida e a hiperatividade simpática sustentada se correlacionam fortemente com o aumento na mortalidade (Cohn *et al.*, 1984). Embora os mecanismos fisiológicos não sejam plenamente conhecidos, há evidências de que as concentrações plasmáticas elevadas de ANG II e espécies reativas de oxigênio contribuem para a diminuição da SBR (Negrão e Middlekauff, 2008).

Além da atenuação dos barorreceptores, estudos em animais e humanos demonstraram que existe uma interação antagônica direta entre o controle quimiorreflexo e o controle barorreflexo (Abboud, 2010). Na IC, a sensibilidade do quimiorreflexo periférico está aumentada, provocando um balanço positivo na ativação simpática (Sun *et al.*, 1999; Schmidt *et al.*, 2005). A ativação dos barorreceptores promovem a supressão da vasoconstrição simpática e aumento da bradicardia parassimpática que tem um efeito benéfico durante crises de hipertensão, mas provoca mal estar quando causa perda da consciência na síncope neurogênica (Abboud, 2010). Por outro lado, a supressão dos barorreceptores e a ativação dos quimiorreceptores são excitatórios (Abboud, 2010). As modulações recíprocas destes dois aferentes sensoriais resultam em um poderoso efeito adicional sobre a hiperatividade simpática. Esta modulação sensorial recíproca pode ser benéfica em estados de choque circulatório, quando a hipotensão e a hipóxia coexistem e ambas elevam o tônus simpático e a ventilação para superar o choque (Abboud, 2010). Entretanto, quando este desequilíbrio entre os sistemas simpático e parassimpático ocorre em doenças crônicas, como na IC, representa um ajuste inadequado do sistema nervoso homeostático (Floras, 2009; Malpas, 2010; Floras e Ponikowski, 2015).

Os componentes moleculares da mecanotransdução nos barorreceptores vêm sendo estudados nos últimos anos (Abboud, 2010; Abboud e Benson, 2015). Os mecanorreceptores estão ligados ao citoesqueleto e as moléculas da matriz extracelular e permitem que sejam identificados deslocamentos físicos relativos à superfície celular (Drummond *et al.*, 1998). A superfamília dos canais de sódio epiteliais degenerina (ENaC), especialmente as subunidades beta e gama, desempenham um papel fundamental na homeostase hidroeletrolítica e são expressos na via neuronal aferente dos barorreceptores aórticos e carotídeos (Drummond *et al.*, 1998). O bloqueio farmacológico dos ENaC com amilorida impede o influxo de cálcio e consequente despolarização dos neurônios barorreceptores aórticos e carotídeos em coelhos anestesiados (Drummond *et al.*, 1998; Snitsarev *et al.*, 2002). Além dos ENaC, os canais iônicos sensíveis a ácido (ASICs) também estão envolvidos na mecanotransdução dos barorreceptores (Lu *et al.*, 2009; Abboud e Benson, 2015). Estudos mostraram que o canal iônico ASICs tipo 2, expresso em abundância no gânglio nodoso de camundongos, participa do braço aferente do arco reflexo (Abboud e Benson, 2015). Camundongos *knockout* $ASIC_2$ apresentam atenuação do controle barorreflexo e aumento da atividade nervosa simpática (Lu *et al.*, 2009), desempenhando um importante papel na sensibilidade dos barorreceptores (Abboud, 2010).

2.3 Quimiorreflexo na IC

Os quimiorreceptores periféricos em conjunto com os quimiorreceptores centrais são responsáveis por manter adequadas as pressões parciais de oxigênio e gás carbônico na corrente sanguínea. Enquanto os quimiorreceptores centrais são sensíveis às concentrações de dióxido de carbono, os quimiorreceptores periféricos reagem principalmente à hipóxia (Schmidt *et al.*, 2005; Schultz, 2011; Kumar e Prabhakar, 2012). Entretanto, outras condições patológicas são reconhecidas como potenciais ativadores dos quimiorreceptores periféricos, incluindo acidose, hipercapnia, hipertermia, hiperosmolaridade e hiperglicemia (Kumar e Prabhakar, 2012). O interesse nos quimiorreceptores periféricos no contexto da IC se originou pelos estudos desenvolvidos na metade dos anos 90 (Chua, *et al.*, 1996; Ponikowski

et al., 1997). A sensibilidade dos quimiorreceptores carotídeos está elevada na IC e contribui para a hiperatividade simpática que está associada com a progressão desta síndrome e a mortalidade decorrente (Zucker, Patel e Schultz, 2012). Atualmente há evidências que as aferências dos quimiorreceptores arteriais, particularmente os do corpo carotídeo, contribuem para instabilidade ventilatória e desequilíbrio do sistema nervoso homeostático (Schultz *et al.*, 2012). Além disso, a diminuição do fluxo sanguíneo na carótida aumenta a resposta do quimiorreflexo periférico em portadores de IC, contribuindo para o desenvolvimento de sintomas como a intolerância ao exercício (Chua, *et al.*, 1996; Ponikowski *et al.*, 2001). Os mecanismos responsáveis pela sensibilidade elevada dos quimiorreceptores podem estar relacionados com perfusão inadequada e alterações na atividade dos neurotransmissores gasosos, NO, monóxido de carbono (CO) e sulfato de hidrogênio (H_2S) nos corpos carotídeos (Schultz *et al.*, 2012). A dessensibilização na produção de NO e CO nos corpos carotídeos reduz seus efeitos inibitórios sobre a atividade dos quimiorreceptores em condições de normoxia e hipóxia, enquanto os efeitos excitatórios do H_2S permanecem intactos na IC (Schultz e Li, 2007; Schultz *et al.*, 2012). Essas alterações fisiopatológicas mudam a excitabilidade dos quimiorreceptores carotídeos para um estado mais ativo (Schultz e Li, 2007; Schultz *et al.*, 2012). Além destas alterações nos neurotransmissores gasosos, o estresse oxidativo mediado pela ANG II e outros efeitos pró-oxidantes também desempenham um papel relevante da resposta ao oxigênio dos corpos carotídeos na IC (Schultz, 2011).

A transdução do sinal nos quimiorreceptores ocorre pelos canais de potássio existentes nas células glômicas que são sensíveis a alterações da pressão parcial de oxigênio (Tan *et al.*, 2007; Abboud, 2010). Quando a pressão parcial de oxigênio é reduzida, poucos canais de potássio ficam combinados com o oxigênio, e um maior número desses canais se fecha, aumentando a concentração de potássio intracelular e ativando os canais de cálcio dependentes de voltagem (Tan *et al.*, 2007; Abboud, 2010). Essa resposta aumenta a concentração citosólica de cálcio que, por sua vez, induz a exocitose das vesículas e liberação de seus neurotransmissores (Schultz e Li, 2007).

Considerando que os corpos carotídeos expressam as enzimas para formação local da ANG II (Lam e Leung, 2002), e possuem o RNA mensageiro para

os receptores AT_1 elevados nos corpos carotídeos (Li *et al.*, 2006), acredita-se que a sinalização ANG II/AT_1 aumente a resposta do oxigênio pelos canais de potássio nas células glômicas tipo I, contribuindo para o aumento da sensibilidade à hipóxia nos corpos carotídeos de coelhos com IC (Li *et al.*, 2006).

As ações dos quimiorreceptores exercem importantes efeitos modulatórios sobre os sistemas respiratório e cardiovascular. O aumento da ventilação minuto é a reação dominante à estimulação dos quimiorreceptores periféricos e foi demonstrado que a resposta ventilatória foi abolida completamente após desnervação bilateral dos corpos carotídeos em pacientes com IC (Niewinski *et al.*, 2014). Porém, os efeitos da estimulação dos quimiorreceptores periféricos sobre o sistema cardiovascular dependem da interação de vários fatores e as respostas cardiovasculares são secundárias ao aumento da ventilação (Kumar, 2009). Nas repostas do fluxo sanguíneo do antebraço durante hipóxia e hipercapnia em pacientes com IC e sua relação com a atividade nervosa simpática muscular, não houve aumento do fluxo sanguíneo durante hipóxia e hipercapnia, sugerindo que a hiperatividade simpática observada nos pacientes IC possa suprimir o estímulo de vasodilatação induzido pela hipóxia (Di Vanna *et al.*, 2007). Em outros estudos experimentais, a estimulação dos quimiorreceptores periféricos pode ser realizada com cianeto de potássio (KCN) (Franchini e Krieger, 1993). Os efeitos do KCN sobre os corpúsculos carotídeos promovem taquipneia, bradicardia, diminuição do débito cardíaco, vasoconstrição periférica e aumento da pressão arterial em ratos (Franchini, Oliveira e Krieger, 1997). Em estudo que comparou as respostas hemodinâmicas desencadeadas pelo KCN ou pela redução da fração inspirada de O_2, ambos os estímulos produzem efeitos similares sobre o sistema cardiovascular; e, as ligaduras das artérias dos corpos carotídeos aboliram o aumento da pressão arterial e a bradicardia, sugerindo que os corpos carotídeos modulam as respostas hemodinâmicas (Barros *et al.*, 2002). Entretanto, poucos estudos investigaram os efeitos do KCN sobre o sistema cardiovascular em ratos com IC.

2.4 Mediadores inflamatórios na IC

Durante o processo de desenvolvimento da IC há um desequilíbrio entre mediadores pró-inflamatórios e anti-inflamatórios (Adams e Niebauer, 2015). O

protótipo dentre as citocinas pró-inflamatórias na IC é o fator de necrose tumoral-α (TNF-α) (Westman *et al.*, 2016). Adicionalmente, outras citocinas inflamatórias como a interleucina (IL)-6 e IL-1β tem sido descritas por estarem elevadas em pacientes com IC (Gullestad *et al.*, 2012). Ao menos três hipóteses foram propostas para descrever a origem das citocinas inflamatórias no plasma: 1) produção e secreção pelas células mononucleares como macrófagos (Batista *et al.*, 2006); 2) secreção pelos cardiomiócitos lesados ou pelas células dos tecidos periféricos, principalmente o músculo esquelético (Meador *et al.*, 2008); e 3) aumento do edema da parede intestinal e consequente indução do TNF-α a partir de lipossacarídeos (Sandek *et al.*, 2012; Adams e Niebauer, 2015).

As citocinas agem como fatores catabólicos envolvidos na patogênese da diminuição da força muscular esquelética e cardíaca (Zizola e Schulze, 2013). Níveis plasmáticos elevados de TNF-α foram encontrados em pacientes com reduzida área de secção transversa dos músculos esqueléticos (Anker e Von Haehling, 2004). Além disso, há associação entre aumento das concentrações plasmáticas de citocinas pró-inflamatórias com a classe funcional da NYHA e parâmetros de intolerância ao esforço (Gielen *et al.*, 2003; Westman *et al.*, 2016).

A atrofia muscular, em geral, pode resultar da diminuição da síntese de proteína, aumento da degradação de proteína, ou ambas (Zizola e Schulze, 2013). Em outras palavras, o catabolismo está predominante sobre o anabolismo nos músculos esqueléticos de pacientes com IC e podem ocorrer devido a mudanças nos níveis de citocinas inflamatórias, disponibilidade de nutrientes, níveis de atividade física e fatores de crescimento (Zizola e Schulze, 2013). O aumento da ativação do sistema ubiquitina proteossoma exerce um papel importante na indução da fraqueza muscular em diversas condições como neoplasias, sepse e IC (Torre-Amione *et al.*, 1996). A elevada atividade da via proteossoma na caquexia parece ser mediada pela ativação dos fatores de transcrição FOXO (*forkhead Box O)* e NF-kB (*factor nuclear kappa B*) que induzem a formação de duas enzimas MuRF-1 (*Muscle-Specific Ring Finger)* e a MAFbx (*Muscle-Atrophy F-box)* (Bowen, Schuler e Adams, 2015). Em camundongos, TNF-α ativa o sistema ubiquitina proteossoma e prejudica a função muscular (Bowen *et al.*, 2015). Portanto, o aumento das citocinas pró-inflamatórias na IC parece contribuir com a fraqueza muscular pela ativação de múltiplas vias de sinalização intracelular.

3. TREINAMENTO FÍSICO NA INSUFICIÊNCIA CARDÍACA

A intolerância ao exercício é uma característica marcante na IC (Roveda *et al.*, 2003; Hunt *et al.*, 2005; Negrão e Middlekauff, 2008; Piepoli e Crisafulli, 2014; Groehs *et al.*, 2015). Nos últimos anos, a Biologia Molecular ajudou a compreender os mecanismos responsáveis pela intolerância ao exercício em pacientes com IC crônica e os benefícios desencadeados pelo treinamento físico aeróbio (TFA); (Piepoli, 2013; Piepoli e Crisafulli, 2014; Adams e Niebauer, 2015). Exercícios aeróbios e de fortalecimento muscular produzem benefícios fisiológicos e clínicos em pacientes com IC (Belardinelli *et al.*, 1999; Hunt *et al.*, 2005; Mcmurray *et al.*, 2012). O TFA realizado regularmente em intensidades adequadas promove alterações estruturais benéficas ao coração chamadas de remodelamento cardíaco fisiológico ou remodelamento reverso (Brum *et al.*, 2010). Neste sentido, o primeiro estudo prospectivo randomizado foi realizado por (Hambrecht *et al.*, 2000) tendo-se demostrado que o treinamento aeróbio promoveu remodelamento reverso do ventrículo esquerdo (VE), com modesta melhora na fração de ejeção de 30% para 35%, e com redução no diâmetro diastólico final do VE. Os resultados deste estudo foram confirmados por duas metanálises (Haykowsky *et al.*, 2007; Chen *et al.*, 2012) que demostraram como o treinamento aeróbio, especialmente com duração maior do que 6 meses, melhora a função do VE, enquanto o treinamento de força isolado não promove remodelamento reverso. Todos os benefícios relacionados ao treinamento físico contribuem para a melhora clínica dos pacientes e reduzem o risco de eventos cardiovasculares (Hunt *et al.*, 2005; Mcmurray *et al.*, 2012).

As recomendações para o treinamento físico em geral incluem atividades aeróbias desenvolvidas por no mínimo 30 minutos, 5 ou mais dias por semana, com parâmetros específicos que definem a intensidade do exercício, duração e frequência (Herdy *et al.*, 2014). A intensidade do exercício deve ser prescrita, preferencialmente pelos limiares ventilatórios fornecidos pela ergoespirometria (Hunt *et al.*, 2005; Negrao e Middlekauff, 2008; Herdy *et al.*, 2014). O intervalo da intensidade do exercício deve ser entre o limiar anaeróbio e 10% abaixo do ponto de compensação respiratória. Na falta de uma avaliação ergoespirométrica, a

intensidade de exercício pode ser entre 50% a 70% da FC de reserva (Roveda *et al.*, 2003; Gomes-Santos *et al.*, 2014).

As diretrizes da Sociedade Europeia de Cardiologia (Mcmurray *et al.*, 2012) e do Colégio Americano de Cardiologia (Hunt *et al.*, 2005) para o manejo e tratamento da IC recomendam o treinamento físico para melhora da tolerância ao exercício, da qualidade de vida e redução na ocorrência de hospitalização em pacientes com IC. Infelizmente, estas recomendações ainda são pobremente implementadas na prática clínica diária. Em uma investigação entre 673 hospitais em 43 países europeus, somente 63% relataram ter programas para o manejo de pacientes com IC, e somente 42% destes incorporavam o treinamento físico como forma de tratamento associado (Jaarsma *et al.*, 2006). Apesar do conhecimento dos benefícios da reabilitação cardiovascular, uma fração muito pequena, algo entre 5 a 30% dos pacientes elegíveis para participar de programas de exercícios físicos, são encaminhados para os mesmos (Piepoli, 2013). É provável que uma fração menor do que essa reflita a realidade brasileira (Herdy *et al.*, 2014).

Neste contexto, a investigação de intervenções terapêuticas como o TFA, visto como um complemento ao tratamento clínico, pode contribuir com a redução da morbimortalidade observadas nos pacientes com IC.

3.1 Adaptações induzidas pelo treinamento físico

Nos últimos anos muitos estudos relataram adaptações fisiológicas em vários órgãos e sistemas induzidas pelo treinamento físico em modelos experimentais de IC (Zheng *et al.*, 2005; Rondon *et al.*, 2006; Lenk *et al.*, 2009; Souza *et al.*, 2014; Pügge *et al.*, 2016). O TFA normalizou a expressão dos receptores AT_1 no SNC e promoveu a melhora na sensibilidade barorreflexa em coelhos com IC (Mousa *et al.*, 2008). Essa é uma importante alteração, pois a melhora no controle barorreflexo arterial e a redução na atividade nervosa simpática renal provocada pelo treinamento físico dependem da normalização da ANG II, da expressão do RNA mensageiro correspondente e da concentração de proteínas dos receptores AT_1 (Zucker, Patel e Schultz, 2012). Kleiber *et al*. (2010) sugeriram que um dos

mecanismos pelo qual o exercício físico reduz o tônus simpático na IC seria pela normalização da expressão de receptores glutamatérgicos no núcleo paraventricular do hipotálamo (PVN). Outra possibilidade seria o restabelecimento da enzima óxido nítrico neuronal (nNOS) em neurônios do PVN (Zheng *et al.*, 2005). É interessante que haja uma forte interação funcional entre NO e ANG II neuronais (Chen *et al.*, 2015). O NO exerce um efeito inibitório sobre a expressão dos receptores AT_1 no PVN (Sharma *et al.*, 2012). Evidências recentes indicam que não são somente as aferências somatossensoriais (dos mecanorreceptores musculares, barorreceptores, quimiorreceptores e receptores cardiopulmonares), mas também as projeções do PVN que convergem para o núcleo do trato solitário (NTS), coordenam os ajustes cardiovasculares durante o exercício (Michelini e Stern, 2009). Um protocolo de exercício durante 3 meses é capaz de induzir remodelamento da inervação noradrenérgica no PVN em ratos hipertensos (Higa-Taniguchi *et al.*, 2007). Essas evidências sugerem que o treinamento físico promova adaptações funcionais no SNC, especialmente no PVN, que em conjunto participam da normalização do tônus simpático na IC.

Em pacientes portadores de IC, o treinamento físico aeróbio promoveu melhoras significativas na vasodilatação mediada pelo endotélio, na circulação periférica e coronariana, além de promover aumento no fator de crescimento do endotélio vascular (Gustafsson *et al.*, 2001). O treinamento físico também diminui significativamente a atividade nervosa simpática muscular em pacientes com IC em estágio avançado (Roveda *et al.*, 2003). Essa redução na hiperativação simpática tem relevância clínica, uma vez que a atividade nervosa simpática é um preditor independente de mortalidade em pacientes com IC (Barretto *et al.*, 2009). Ademais, o treinamento físico promove melhora no fluxo sanguíneo para o antebraço e reduz a resistência vascular periférica em homens e mulheres com IC, aumenta o consumo de oxigênio (VO_{2pico}) e redução da inclinação do VE/VCO_2, importantes marcadores de prognóstico em pacientes com IC (Antunes-Correa *et al.*, 2010). Ocorre igualmente aumento na densidade mitocondrial e da atividade de enzimas oxidativas, restabelecendo a capacidade oxidativa e, consequentemente, contribuindo para a melhora da tolerância ao esforço (Hambrecht *et al.*, 1997). Essa melhor capacidade oxidativa vem sendo atribuída ao aumento na expressão da proteína PGC-1α *(peroxisome proliferator activated receptor gamma coactivator-1*

alpha), um importante regulador da biogênese mitocondrial (Liang e Ward, 2006), que tem sua expressão muscular reduzida na IC (Vescovo *et al.*, 2005). Embora haja poucas evidências, os estudos sugerem que os benefícios do treinamento físico sobre o músculo esquelético sejam mediados em parte pela PGC-1α (Forman *et al.*, 2014). O treinamento físico preveniu a atrofia muscular induzida pela IC por aumentar a expressão de PGC-1α no músculo plantar de ratos (Souza *et al.*, 2014).

Marcadores inflamatórios na IC como o TNF-α e a proteína C reativa (PCR) também modificam-se pelo treinamento físico (Feiereisen *et al.*, 2013; Adamopoulos *et al.*, 2014). Após revisão sistemática considera-se que a realização de 5 ou mais sessões de exercícios físicos por semana seja capaz de reduzir os níveis séricos de TNF-α (Smart e Steele, 2011). Estudo adicional multicêntrico descreveu a ocorrência de redução nos níveis de PCR após protocolo de treinamento aeróbio em pacientes com IC (Adamopoulos *et al.*, 2014). Estudo prévio em nosso laboratório investigou o impacto de 8 semanas de exercício físico sobre variáveis hemodinâmicas, estresse oxidativo e níveis plasmáticos de interleucinas com propriedades anti-inflamatórias (IL-10) em um modelo experimental de IC em ratos (Nunes *et al.*, 2008). Os resultados demonstraram que a atividade física baseada em um protocolo de natação foi capaz de reduzir a PDFVE, aumentar os níveis plasmáticos de IL-10 e, melhorar os níveis de peroxidação lipídica muscular. O treinamento físico também promoveu aumento da razão IL-10/TNF-α no plasma e redução do conteúdo de colágeno no VE de animais com IC (Nunes *et al.*, 2013). São raros os estudos que investigaram os efeitos do TFA sobre a expressão de citocinas no músculo esquelético de ratos com IC.

4. MODELO EXPERIMENTAL DE IC

O presente modelo experimental de IC é produzido pela ligadura da artéria coronária esquerda e consequente infarto agudo do miocárdio (Salazar *et al.*, 2014). A ligadura da artéria coronária esquerda produz modificações hemodinâmicas, com necrose do miocárdio e disfunção ventricular esquerda (Pfeffer *et al.*, 1979; Francis *et al.*, 2001). Este modelo simula a causa mais comum de IC em humanos e permite

avaliar a progressão das respostas neuro-hormonais e da atividade nervosa simpática renal (Patel, 1997). Nesses estudos ocorre prazo de 4 a 6 semanas para que a síndrome da IC se desenvolva (Pfeffer e Braunwald, 1990; Francis *et al.*, 2001). Áreas de infarto maiores que 30% correspondem a disfunção ventricular grave com pressão diastólica final do ventrículo esquerdo (PDFVE) maiores que 20 mmHg, atenuação do barorreflexo e hiperativação simpática (Pfeffer *et al.*, 1979; Pfeffer e Braunwald, 1990; Francis *et al.*, 2001). Tal abordagem metodológica serve para avaliar os efeitos de várias estratégias farmacológicas e não farmacológicas sobre marcadores inflamatórios (Nunes *et al.*, 2008; Hentschke *et al.*, 2013; Nunes *et al.*, 2013), conteúdo de colágeno no VE (Alves *et al.*, 2014), mecânica ventilatória (Jaenisch *et al.*, 2011), sensibilidade barorreflexa (Jaenisch *et al.*, 2011; Lima *et al.*, 2015), estresse oxidativo e dano ao DNA (Biasibetti *et al.*, 2014). Neste contexto, a investigação dos efeitos do TFA sobre a sensibilidade do quimiorreflexo periférico e a expressão de citocinas no músculo esquelético pode ampliar o rol dos benefícios desta estratégia não farmacológica no modelo experimental da IC.

5. OBJETIVOS

O objetivo geral do presente estudo é avaliar o impacto de um programa de TFA de moderada intensidade em esteira, com duração de 8 semanas, sobre a função hemodinâmica, sensibilidade barorreflexa, quimiorreflexo periférico e perfil inflamatório no músculo gastrocnêmio em ratos com insuficiência cardíaca crônica após infarto agudo do miocárdio.

Os objetivos específicos deste trabalho são:

- Avaliar o impacto do TFA regular de 8 semanas sobre a função ventricular esquerda, a sensibilidade barorreflexa e a resposta hemodinâmica ao KCN em animais com IC induzida por IAM.

- Analisar a correlação entre a sensibilidade barorreflexa e a resposta pressórica ao KCN em animais com IC induzida por IAM.

- Avaliar a resposta pressórica ao KCN durante hipotensão induzida pelo nitroprussiato de sódio em animais com IC induzida por IAM.

- Avaliar o efeito do TFA regular de 8 semanas sobre as concentrações de TNF-α, IL-6 e IL-10 no músculo gastrocnêmio em animais com IC induzida por IAM.

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Dear Prof.Dr. Dal Lago (cc to all co-authors),

It is a pleasure to accept your manuscript entitled **"Exercise training attenuates the pressor response evoked by peripheral chemoreflex in rats with heart failure"** in its current form for publication in the Canadian Journal of Physiology and Pharmacology. The comments of the reviewer(s) who reviewed your manuscript are included at the foot of this letter.

The Editorial Office will contact the corresponding author about publication.

Thank you for your fine contribution. On behalf of the Editors of the Canadian Journal of Physiology and Pharmacology, we look forward to your continued contributions to the Journal.

Sincerely,

Dr. Jeffrey Wigle

Editor, Canadian Journal of Physiology and Pharmacology

Exercise training attenuates the pressor response evoked by peripheral chemoreflex in rats with heart failure

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Abstract: The effects of exercise training (ExT) on the pressor response elicited by potassium cyanide (KCN) in the rat model of ischemia-induced HF are unknown. We evaluated the effects of ExT on chemoreflex sensitivity and its interaction with baroreflex in rats with HF. Wistar rats were divided into four group: trained HF (Tr-HF), sedentary HF (Sed-HF), trained sham (Tr-Sham) and sedentary sham (Sed-Sham). Trained animals underwent to a treadmill running protocol for 8 weeks (60m/d, 5x/wk, 16m/min). After ExT, arterial pressure (AP), baroreflex sensitivity (BRS), peripheral chemoreflex [KCN 100μg · (kg body mass)$^{-1}$] and cardiac function were evaluated. The results demonstrate that ExT induces an improvement in BRS and attenuates the pressor response to KCN relative to Sed-HF ($P < 0.05$). The improvement in BRS was associated with a reduction in the pressor response following ExT in HF rats ($P < 0.05$). Moreover, ExT induced a reduction in left ventricular end-diastolic pressure and pulmonary congestion compared with the Sed-HF ($P < 0.05$). The pressor response to KCN in the hypotensive state is decreased in sedentary HF rats. These results suggest that ExT improves cardiac function and baroreflex sensitivity and attenuates the pressor response evoked by KCN in HF rats.

***Key words*:** exercise training, heart rate, blood pressure, autonomic dysfunction, baroreceptor reflex, chemoreceptor reflex, heart failure

Introduction

Beyond changes in cardiac function, the pathophysiologic state of heart failure (HF) is characterized by impaired cardiovascular reflex associated with increased sympathetic activity (Zucker et al. 2012). Inhibitory and excitatory inputs from the baroreceptors and chemoreceptors modulate sympathetic nervous system activity (Floras 2009). Experimental (Jaenisch et al. 2011; Sun et al. 1999) and clinical (Mortara et al. 1997; Ponikowski et al. 2001) studies have shown decreases in baroreflex sensitivity (BRS) and increases in peripheral chemosensitivity in HF. Furthermore, the close association between the peripheral chemoreflex and the baroreflex has been reported in HF patients (Ponikowski et al. 1997).

In addition to the elevated ventilation response during peripheral chemoreceptor stimulation, secondary cardiovascular reflexes are mediated by the sympathetic and parasympathetic nervous system (Kumar 2009). Several studies have been described hyperventilatory response to activation of peripheral chemoreceptors using lower inspired O_2 in HF state (Ponikowski et al. 2001; Ponikowski et al. 1997). However, the cardiovascular response remains under debates. Lower O_2 concentration induced increase in renal sympathetic nerve activity (RSNA) and hyperventilatory response without changes in the mean arterial pressure in a rabbit model of HF (Li et al. 2008; Sun et al. 1999). Thus, the alternative method for investigation in cardiovascular alteration induced by peripheral chemoreceptors is the utilization of potassium cyanide (KCN). The use of KCN simulates a hypoxic response via inhibition of mitochondrial oxidative respiration, is a methodological approach to test chemoreflex responses (Krylov SS and SV 1968). Previous studies have suggested that KCN administration is appropriate to produce the activation of the arterial chemoreceptor in rats (Franchini et al. 1997). Furthermore, KCN injection

produces similar effects on the cardiovascular system via activation of the carotid chemoreceptors with low inspired O_2 tension in healthy rats (Barros et al. 2002). Studies using KCN to test chemoreflex activation with a focus on the cardiovascular component responses are less frequently investigated in myocardial-infarcted rats (Wang et al. 2008).

The arterial baroreflex is the primary sympatho-inhibitory cardiovascular reflex that regulates the vascular sympathetic outflow and it has been widely studied in the HF state (Grassi et al. 1995; Negrão and Middlekauff 2008). In this respect, few reports have investigated the association between peripheral chemoreflex sensitivity and baroreflex sensitivity in HF in humans (Ponikowski et al. 1997; Schmidt et al. 2005), though an increased chemoreceptor activity seems to be linked to the impairment in baroreflex (Abboud 2010).

European and American guidelines for the treatment and management of chronic HF have incorporated recommendations for regular aerobic exercise to improve functional capacity and symptoms (Hunt et al. 2005; McMurray et al. 2012). Several peripheral physiological adaptations induced by exercise training (ExT) support these recommendations, such as increased capillary density, blood flow, mitochondrial volume density, fibre size, slow twitch fibre content and vascular resistance (Hambrecht et al. 1997). Studies from our laboratory have demonstrated that ExT improves hemodynamic function, anti-inflammatory response and cardiac remodelling in chronic HF rats (Nunes et al. 2013; Nunes et al. 2008). In addition, ExT reduces RSNA and improves the arterial baroreflex in rabbit and in rat models of HF (Liu et al. 2000; Rondon et al. 2006). However, to our knowledge, there are no studies that have assessed the effect of ExT on the hemodynamic response elicited by KCN in the rat model of ischemia-induced HF. Also, there are no reports on the

interaction between chemo- and baroreflex and the effects of ExT using awake animals in the present model of HF.

Here, we hypothesized that eight weeks of ExT would normalize baroreflex sensitivity and attenuate the pressor response to KCN in HF rats. Furthermore, we investigated the association between baroreflex and chemoreflex sensitivity and whether baroreceptor unloading could enhance the activity of the peripheral chemoreceptors in awake rats.

Methods

Animals

Experiments were performed on 32 male Wistar rats, obtained from the Animal Breeding Unit at the Federal University of Health Science of Porto Alegre (UFCSPA; Brazil), weighing 220 – 270 g. The animals were kept on a 12:12 h light-dark cycle with free access to water and pellet rodent chow diet and were maintained under standard conditions of temperature (22 ± 1 ºC). All procedures were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH Publication nº 85-23, revised 1996). All procedures outlined in this study were approved by the UFCSPA Ethics and Research Committee (protocol 39/11).

Experimental design

The method of producing a myocardial infarction (MI) and subsequent HF was similar to that previously described (Hentschke et al. 2013; Jaenisch et al. 2011; Nunes et al. 2008). Briefly, rats were anaesthetized with xylazine (12 mg/kg, ip) and ketamine (90 mg/kg, ip), intubated and artificially ventilated (SamWay VR 15) with a breathing rate of 60 breaths/min and an oxygen inspired fraction of 100%. After

thoracotomy, coronary artery ligation was performed to induce MI. The rats received a single dose of penicillin (20 000 U, ip) and subcutaneous buprenorphine (0.15 mg/kg). The Sham groups underwent the same surgical procedure without artery ligation. At week six all rats were divided into four experimental groups: sedentary sham-operated (Sed-Sham), trained sham-operated (Tr-Sham), sedentary HF (Sed-HF) and trained HF rats (Tr-HF). All groups have originally 8 animals. Due to technical conditions, some records were obtained from 7 rats, as indicated in the results and figure legends.

Aerobic exercise training protocol

Exercise training consisted of treadmill running five days per week for eight weeks (Nunes et al. 2013; Xu et al. 2008). Initially, low speed (10 m/min) and short duration (10 min per session) were used to familiarize the rats with running on the treadmill. The ExT protocol was started one week after this adaptation period. The speed and duration were gradually increased to 16 m/min and 60 min per session, corresponding to an intensity of 55% VO_{2max}, as described previously (Véras-Silva et al. 1997). This intensity was maintained constant throughout the protocol. A previous study demonstrated that this exercise protocol improved aerobic capacity and citrate synthase activity in post-MI rats (Wan et al. 2007). Only rats that ran steadily with little or no prompting were used in the study.

Cardiovascular measurements in awake rats

After the second day of the final training session, two catheters filled with saline (0.06 mL) and heparin (0.01 mL) were implanted in anesthetized rats (12 mg/kg xylazine and 90 mg/kg ketamine) into the femoral artery and vein (PE 10) for direct measurements of arterial pressure signals and drug administration,

respectively. The catheters were tunnelled subcutaneously and exteriorized through the back of the neck to be connected to the pressure transducer. On the subsequent day, the arterial catheter was connected to a strain-gauge pressure transducer (Miniature Pulse Transducer RP-155, Narco Bio-Systems, Houston, TX), coupled to a pressure amplifier (Stemtech, Houston, TX) and were delivered to a microcomputer equipped with an analog-to-digital converter board (CODAS, 1 kHz; Dataq Instruments, Akron, USA). The recorded data were analysed on a beat-to-beat basis to quantify basal MAP and heart rate (HR), as described previously (Jaenisch et al. 2011; Neckel et al. 2012; Quagliotto et al. 2008). The rats were allowed to acclimate to a Plexiglas recording box for 20 to 30 min. Cardiovascular parameters were monitored during this time and basal values were recorded during the last 15 min. All data were recorded in the morning (from 8 to 12 h) to avoid unpredictable circadian variations in the results.

Baroreflex and chemoreflex sensitivity

Baroreflex-mediated changes were measured during peak decreases or increases in MAP due to systemic venous injection of a single dose of sodium nitroprusside (SNP) (100 µg/mL; Sigma Chemical) or phenylephrine (8 µg/mL; Sigma Chemical, St. Louis, MO) dissolved in 0.1 mL saline, respectively (Jaenisch et al. 2011; Quagliotto et al. 2008). The induced changes in MAP ranged between 10 to 30 mmHg. These changes in the MAP were followed by corresponding changes in the HR and various data points served to compose sigmoidal curves to determine the baroreflex sensitivity by fitting the MAP and HR changes to a sigmoidal logistic equation, as described previously (Head and McCarty 1987).

Chemoreflex sensitivity was tested with intravenous dose of potassium cyanide (KCN: 100 μg · (kg body mass)$^{-1}$; Merck, Germany) as previously described (Dall'Ago et al. 1997; Quagliotto et al. 2008). Injected volumes ranged from 0.1 to 0.18 mL. HR and MAP were measured continuously 10 s before and 15 s after the injection of KCN. The apex of the changes in MAP and HR were used as the chemoreflex response values. The baroreflex and chemoreflex activities were tested in random order for each rat in the three experimental groups.

Chemoreflex stimulation during hypotension

This protocol was initiated thirty minutes after the last evaluation of baroreflex or chemoreflex sensitivity, when all parameters had returned to basal levels. The hypotensive state was induced by SNP (100 μg/mL; Sigma Chemical) (Al-Hesayen and Parker 2004) and 5 - 10 s later, when the MAP had reached its lowest level (nadir), a bolus injection of potassium cyanide [KCN 100 μg · (kg body mass)$^{-1}$] was administered. This experimental protocol was conducted to evaluate whether the unloading of baroreceptors via reduction of blood pressure affects responses to stimulation of the peripheral chemoreflex (Heistad et al. 1974). The magnitude of the MAP response to KCN in hypotension was compared with the MAP response to KCN in the normotensive state as mentioned above.

Final cardiac hemodynamic evaluation

One day after baroreflex and chemoreflex evaluation, animals were anesthetized with xylazine (12 mg/kg, ip) and ketamine (90 mg/kg, ip), and a small incision in the anterior cervical region was performed for the insertion of a polyethylene catheter (PE-50) into the right carotid artery. First, arterial pressure

signals were recorded for 5 min. 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. These data were used to determine LV systolic pressure (LVSP), LV maximum change in pressure over time ($+dP/dt_{max}$) and LV minimum change in pressure over time ($-dP/dt_{max}$), and LV end-diastolic pressure (LVEDP), as previously described (Alves et al. 2014; Hentschke et al. 2013). The last parameter was determined manually by detecting the point of inflection to the end of diastole via analysis of the ventricular pressure wave.

Infarct size, cardiac hypertrophy and pulmonary and hepatic congestion

The animals were euthanized with an overdose of anaesthetic (thiopental 80 mg/kg, ip), and the heart, lungs, and liver were removed and weighed. The total left ventricle area and MI scar were manually drawn on scanned images. The size of the infarct was determined by planimetry (Lindpaintner et al. 1993) The right ventricle (RV) and LV were dissected and weighed. To evaluate cardiac hypertrophy, the heart weight-to-body weight ratio (HW/BW), LV/BW and RV/BW (mg/g) were determined. The lungs and liver were dehydrated (80 ºC) for 48 h and weighed again to evaluate the percent water content.

Statistical analysis

Data are presented as means ± SD. Data were tested for normal distribution using the Kolmogorov-Smirnov test. Comparisons were made between groups using two-way ANOVA (exercise training and HF as the main factors) followed by the Newman-Keuls *post hoc* test. Pearson's correlation was performed to test the

association between chemosensitivity and baroreflex sensitivity. A *P* value <0.05 was considered statistically significant.

Results

Characterization of the model of heart failure

Mortality in MI-induced HF rats, during or after surgery, was 35%. Infarct sizes were similar in the two infarcted groups, and there were no significant differences in body weight among all experimental groups. Morphometric characteristics of the four groups are shown in Table 1. At the end of the study, the pulmonary congestion, HW/BW, LV/BW and RV/BW were higher in the Sed-HF group compared with the Sham groups ($P < 0.001$), whereas the pulmonary congestion, HW/BW and RV/BW were decreased in the trained HF group ($P < 0.01$). These results indicate that exercise is capable of reducing the compensatory hypertrophy and lung congestion present in the Sed-HF group.

Hemodynamic parameters in untrained and trained Sham and HF rats are show in Table 2. HF groups (trained and sedentary) showed higher values of left ventricular end diastolic pressure (LVEDP) compared with sham groups ($P < 0.001$). However, when the trained HF group was compared with the Sed-HF group, there was an improvement in diastolic function ($P = 0.04$). The negative derivative of LV pressure ($-dP/dt_{max}$) was significantly reduced in the HF groups compared with the Sham groups ($P = 0.02$). All hemodynamic and reflex responses were assessed while the rats were awake whereas the last cardiac hemodynamic evaluation of HF was done under anaesthesia.

Cardiovascular responses evoked by chemoreflex activation

The intravenous injection of KCN produced a marked increase in blood pressure (sympathetic effect) and a marked decrease in heart rate (parasympathetic effect) in all groups. The pressor responses evoked by KCN was attenuated in the trained HF group when compared with the Sed-HF and Sham groups (Tr-HF: 27.8 ± 8.2 mmHg; Sed-HF: 42.5 ± 5.9 mmHg; Tr-Sham: 38.7 ± 9.2 mmHg; Sed-Sham: 43.2 ± 14.2 mmHg, *P* = 0.02, Fig 1A). Furthermore, when expressed in percentage (%) of MAP this response was attenuated in the trained HF group when compared with the Sed-HF (Tr-HF: 30.2 ± 8 %; Sed-HF: 48.8 ± 6.6 %; *P* = 0.002, Fig 1B). No statistically significant difference was found in the HR changes induced by KCN injection (*P* = 0.9; Fig. 1C).

Baroreflex sensitivity

Table 3 shows the BRS of the studied groups. The maximum gain in reflex response was significantly decreased in Sed-HF rats when compared with Sham rats (*P* = 0.04), consistent with a blunted baroreflex response in the HF state. However, when the Tr-HF group was compared with the Sed-HF group, there was an improvement in BRS (*P* = 0.01), and the BRS was nearly restored to the level found in Sham rats. The Sed-HF group showed a significant decrease in the MAP_{50} when compared with Sed-Sham group (*P* = 0.009).

Effect of hypotension on the peripheral chemoreflex

The basal levels of MAP, before SNP administration, were 104.7 ± 5.2 mmHg, 107.1 ± 7.4 mmHg, 91.4 ± 5.1 mmHg and 94.5 ± 3.6 mmHg in the Sed-Sham, Tr-

Sham, Sed-HF and Tr-HF groups, respectively. The baseline MAP was lower in HF groups when compared with Sham groups ($P < 0.001$). Administration of SNP resulted in a reduction in MAP of 29.1 ± 4.8 mmHg, 27 ± 2 mmHg, 19.5 ± 7.1 mmHg and 19.6 ± 5.5 mmHg in Sed-Sham, Tr-Sham, Sed-HF and Tr-HF groups, respectively. The reduction in MAP was lower in HF groups when compared with Sham groups ($P = 0.01$). However, when expressed in percentage (%) of MAP the magnitude of change was similar ($P = 0.12$), -27.6 ± 4.9 %, -25 ± 2.8 %, -21.6 ± 8 % and -21 ± 5.7 % in Sed-Sham, Tr-Sham, Sed-HF and Tr-HF groups, respectively.

Fig. 2 shows the differences in MAP between normotensive and hypotensive responses to chemoreceptor stimulation by KCN 100 μg · (kg body mass)$^{-1}$. The hypotensive state resulted in potentiation of the pressor response to KCN of 17 ± 9.9 mmHg, 18.2 ± 15.2 mmHg, 5.6 ± 4.5 mmHg and 11 ± 5.7 mmHg in Sed-Sham, Tr-Sham, Sed-HF and Tr-HF groups, respectively. These experimental results indicate that unloading of the baroreceptors significantly increased chemoreceptor activity. However, this response was attenuated in Sed-HF when compared with Sed-Sham rats ($P = 0.03$), (Fig. 2).

Correlation between chemosensitivity and baroreflex sensitivity

A significant positive correlation was found between chemosensitivity and baroreflex sensitivity ($r = 0.56$, $P = 0.03$, Figure 3A) when the Sed-HF and Tr-HF groups were analysed. That is to say, the values of the pressor response to KCN expressed in percentage (%) of MAP as an indicator of the chemoreflex sensitivity were positively correlated with the gain in baroreflex sensitivity, calculated by the phenylephrine method and expressed in bpm · (mm Hg)$^{-1}$. However, when the Sed-

Sham and Tr-Sham groups were analysed, there were no significant correlation between chemosensitivity and baroreflex sensitivity (r = -0.16, *P* = 0.57, Figure 3B).

Discussion

In the present study, we reported that the pressor response evoked by intravenous KCN in awake HF rats was attenuated following exercise training. In addition, this study demonstrated that high values of peripheral chemosensitivity were associated with impaired baroreflex sensitivity, and the potentiation of the pressor response to KCN in hypotensive state was attenuated in Sed-HF.

The results presented here suggest that ExT attenuated the response evoked by KCN in HF rats. There was an attenuation of the pressor response (mmHg or % of MAP change) to KCN in Tr-HF rats. It is interesting to note that cardiovascular changes in response to KCN were abolished after bilateral ligature of the carotid body (CB) arteries, indicating that the pressor response is dependent on the integrity of the chemosensitive cells of the CB (Barros et al. 2002; Haibara et al. 1995). In a rabbit model of HF, ExT normalized the exaggerated ventilatory responses to hypoxia and RSNA, avoiding the elevation in CB chemoreceptor discharge observed in the HF state (Li et al. 2008). It has been shown that ExT improves the neurotransmission γ–aminobutyric acid (GABAergic) into the paraventricular nucleus (PVN) of HF rats (Patel et al. 2013) and this inhibitory neurotransmitter plays a pivotal role in the modulation of peripheral chemoreflex cardiovascular responses (Reddy et al. 2005).

In addition, our study confirms previous findings that demonstrated an improvement in baroreflex control following ExT in HF rats (Patel et al. 2013; Rondon et al. 2006). The maximum gain in baroreflex responses revealed that the average sensitivity was significantly increased in Tr-HF rats. The present data do not allow us to further discern whether the afference, the central integration, or the efference component is primarily involved in this improvement in BRS. However, Rondon and colleagues (Rondon et al. 2006) investigated the effect of ExT on the afferent portion of the arterial baroreflex and they found an increase in aortic depressor nerve sensitivity. Another important result of our study was the positive correlation between BRS and chemosensitivity in HF rats, suggesting that the improvement in BRS was associated with a reduction in the pressor response evoked by KCN. We postulated that one of several mechanisms responsible for decreasing the pressor response to KCN in Tr-HF rats was related to the increase in BRS. Although we did not find this association in sham rats, our results suggest that ExT normalized the sympatho-vagal balance, consistent with previous reports (Liu et al. 2000; Mousa et al. 2008).

Regarding the interaction between baro- and chemoreflex, Heistad and colleagues (1974) elegantly demonstrated that baroreflex unloading by haemorrhage potentiates the MAP response to peripheral chemoreflex activation. Neurophysiological studies have demonstrated that carotid baroreceptor and chemoreceptor neurons are distributed in close proximity to each other in the paramedian reticular nucleus of the medulla (Miura and Reis 1972). These findings suggest a facilitative interaction between baroreceptors and chemoreceptors, whereby the chemoreceptor response is enhanced by reduced baroreceptor activity (Abboud 2010). The reciprocal sensory modulation may be beneficial in states of circulatory collapse and shock when severe hypotension and hypoxia coexist and

mutually enhance sympathetic drive and ventilation to overcome the acute crisis (Abboud, 2010). It is possible that the chronic hypotensive condition can provoke a more effective sympathetic influence on vasoconstrictor response after chemoreceptor stimulation because the baroreceptors have their set-point for blood pressure reflex responses changed to a lower level in the HF condition. The chemoreflex would provide a strong response after stimulation to compensate the lack of a more intense effect of the baroreceptors.

To test the possibility of baro- and chemoreflex functional interaction in HF rats, we administrated sodium nitroprusside for baroreceptor unloading followed by KCN. It is conceivable that the percentage changes in the MAP following SNP were not different among groups because the absolute values in the HF groups were initially reduced (before the administration of SNP) and would not reach much lower after SNP. Previous reports have shown that endothelial dysfunction is related to the HF condition (Chong et al. 2004, Heitzer et al. 2005). The lower reduction in the MAP observed in HF rats could be associated with a decreased responsiveness of the endothelium and/or of vascular smooth muscle to SNP. This impairment in endothelium-mediated vasodilatation also occurs in patients with HF (Morgan et al. 2004). Nevertheless, our results support the concepts mentioned above (Heistad et al. 1974) and reinforce that baroreceptors modulate the pressor response evoked by peripheral chemoreceptors in the HF state. It was reported that baroreceptor unloading with SNP increase the sympathetic nerve activity (Azevedo et al. 2000; Mandel and Schreihofer 2008). The pressor response evoked by KCN was related to increase in vascular peripheral resistance which is probably associated with peripheral sympathetic activation (Franchini et al. 1997). We argue that hypotension may induce more effective sympathetic influence on vasoconstrictor response to

KCN. In other words, afferent input of baroreceptor unloading may potentiate the chemoreflex ability to modulate vasomotor sympathetic activity. This idea is supported by another work showing that the pressor response to KCN was potentiated after atropine administration (Franchini et al. 1997). However, the potentiation of the pressor response to KCN was found in Sed-Sham, Tr-Sham and Tr-HF, but not at the same magnitude in the Sed-HF group. The underlying mechanisms by which the pressor response in the hypotensive state is lower may be related to an impaired cardiac mechanic function and altered function of vascular reactivity in HF rats (Hubert et al. 2014). Recently, some studies have reported that low frequency component of blood pressure variability, an indicator of vascular sympathetic activity, is attenuated in rats HF (Dantas et al. 2013; Henze et al. 2013; Sabino et al. 2013). It was hypothesized that high catecholamine levels may produce down-regulation of adrenergic receptors and loss of the vascular sympathetic activity (van de Borne et al. 1997). Another possibility involves possible systemic effects of KCN. It was demonstrated that KCN actives the anaerobic metabolism and increase lactate concentration in skeletal muscle (Zhang et al. 2006). Currently it was demonstrated that the reflex pressor response is blunted in rats with HF when lactic acid and capsaicin is administered into the arterial blood supply hindlimb (Xing et al. 2015). Evidences suggest that both acid sensing channel subtype 3 (ASIC3) and capsaicin receptor transient receptor potential vanilloid 1 (TRPv1) are decreased in dorsal root ganglia of HF rats (Li et al. 2004; Smith et al. 2005; Xing et al. 2015). The alterations of the group IV afferent metabolic fibbers could partially explain the attenuation of pressor response to KCN during hypotension in HF rats. We do not know whether the pressor responses to KCN could be indirectly mediated by sensitive group IV afferents in HF rats, although bilateral ligature of the carotid body

arteries blunted the cardiovascular response to KCN in healthy rats (Barros et al. 2002). Taken together, our results suggest that the pressor response in the hypotensive state is more pronounced in healthy rats and ExT increased by 97% this effect in HF rats. Additional neurophysiological studies are needed to fully reveal the central mechanisms that modulate the interaction between baroreflex and chemoreflex in different health and pathological conditions.

In Sed-HF rats, we observed the development of RV hypertrophy and pulmonary congestion. In the experimental model of MI in rats, an increase in RV systolic and central venous pressures indicated that chronic HF was accompanied by pulmonary hypertension (Sakai et al. 1996). Furthermore, in pulmonary hypertension associated with chronic HF, RV afterload and LV preload are increased, leading to further myocardial dysfunction. The heart and lungs work together and dysfunction in one system may produce an indirect influence on the other. However, in the present study, we observed that the Tr-HF group decreased RV hypertrophy and pulmonary congestion found in Sed-HF rats. Interestingly, other forms of training, such as respiratory muscle (Jaenisch et al. 2011) and resistance training (Alves et al. 2014) developed in our laboratory, have demonstrated similar results for hemodynamic and cardiac adaptations.

The present study extends previous reports concerning hemodynamic and cardiac adaptations following ExT in HF rats (Alves et al. 2014; Jaenisch et al. 2011; Nunes et al. 2013). We demonstrated that eight weeks of ExT after MI provides beneficial effects on cardiac function and heart remodelling via an 18% reduction in LVEDP and a 10% reduction in the HW/BW ratio. Rengo and colleagues (Rengo et al. 2010) reported a reduction in LVEDP following 10 weeks of ExT related to a decrease in the expression of remodelling-associated genes and downregulation of

cardiac enzyme G protein-coupled receptor kinase-2 (GRK2), indicating a reduction in sympathetic activity, which is in line with increased baroreflex control and attenuation of the pressor response observed in the present study. Taken together, our data and findings from other authors (Bertagnolli et al. 2008) suggest that attenuated sympathetic modulation of the cardiovascular system following ExT likely decreases maladaptive cardiac hypertrophy.

Heart failure is characterized by enhanced ventilatory responses to hypoxia (Ponikowski et al. 2001; Ponikowski et al. 1997). However, the ventilatory component has been more extensively studied than the cardiovascular component elicited by stimulation of peripheral chemoreceptors. In the present study, there were no statistically significant differences (40.9 ± 13.2% vs. 48.8 ± 6.6%, P = 0.16) in the pressor response to KCN between the Sham and Sed-HF groups, respectively. To our knowledge, there is only one report that investigated the pressor response of KCN in HF rats (Wang et al. 2008). Our results do not confirm the exaggerated increase in the pressor response to KCN reported by other authors (Wang et al. 2008). These controversial results could be explained by methodological differences. For example, the former study utilized anesthetized rats, whereas we utilized awake rats. Also, the administration and doses of KCN were different: right intracarotid artery vs. intravenous bolus and 1 and 10 μg per rat vs. 100 μg · (kg body mass)$^{-1}$.

Although the present study indicates the importance of the ExT in improving sympatho-vagal balance via normalization of baro- and chemoreflexes, there remain several limitations. Our study provides no direct information on sympathetic nervous activity. Analyses of plasma catecholamine levels and RSNA were not performed in the present study. Such an assessment could provide more accurate data to help identify the effects of ExT on the deactivation of sympathetic nervous activity in the

HF state. In the present study, the SNP-induced unloading of arterial baroreceptors and this may result in different sympathetic outputs between sham and HF rats. The methodological approach to examine interaction between chemoreflex and baroreflex function should be claimed with caution, since that metabolic pathways of nitroprusside may release cyanide anions and influence the action of KCN administrated (Hottinger et al. 2014).

In conclusion, the present study has demonstrated that eight weeks of ExT attenuates the pressor response evoked by KCN. In addition, our findings show that ExT improves heart hypertrophy, hemodynamic function and lung congestion. Furthermore, the present data provide new insights into the interaction between the baroreflex and chemoreflex following ExT in HF awake rats. The magnitude of the pressor response in the hypotensive state is decreased in sedentary HF rats.

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Figure Legends

Figure 1.

Values are means ± SD. Parameters of chemoreceptor reflex to potassium cyanide (KCN 100 µg (kg body mass)$^{-1}$ in Sed-Sham sedentary sham group (baseline MAP, 105.9 ± 5.8 mmHg, n = 7), Tr-Sham trained sham group (baseline MAP, 108.4 ± 8.1 mmHg, n = 7), Sed-HF sedentary heart failure group (baseline MAP, 89.8 ± 6 mmHg, n = 7), and Tr-HF trained heart failure group (baseline MAP, 93.4 ± 3.9 mmHg, n = 8). Change in median arterial pressure (Δ MAP, mmHg; A), and (Δ MAP, % of baseline, B), heart rate (Δ HR, bpm; C). *P < 0.05 compared with Sed-Sham; †P < 0.05 compared with Sed-HF.

Figure 2.

Values are means ± SD. The pressor response to bolus injection of KCN 100 µg (kg body mass)$^{-1}$ was tested in two different situations (normotensive and hypotensive states). Hypotension was induced by an i.v. bolus injection of sodium nitroprusside (100 µg.mL^{-1}). Figure 2 shows the difference between the median arterial pressure (MAP) in response to KCN in the hypotensive state and the MAP response in the normotensive state (shown in Fig 1). Sed-Sham sedentary sham group (n = 7), Tr-Sham trained sham group (n = 7), Sed-HF sedentary heart failure group (n = 7), and Tr-HF trained heart failure group (n = 8). *P < 0.05 compared with Sed-Sham.

Figure 3.

Correlation between chemosensitivity (horizontal axis, % of MAP) and baroreflex sensitivity (vertical axis, bpm · (mm Hg)$^{-1}$) in Sed-HF (▲, n=7) and Tr-HF (♦, n=7). Pearson's correlation (r = 0.56, P = 0.03, Fig. 3A). Correlation between chemosensitivity (horizontal axis, % of MAP) and baroreflex sensitivity (vertical axis, bpm · (mm Hg)$^{-1}$) in Sed-Sham (• n=7) and Tr-Sham (▪, n=7). Pearson's correlation (r = - 0.16, P = 0.57, Fig. 3B).

Table 1. Body weight, morphometric cardiac characteristics, infarct area, lung and hepatic congestion of sham-operated rats and rats with HF.

Groups	Sed-Sham	Tr-Sham	Sed-HF	Tr-HF
Initial BW, g	241 ± 11	245 ± 8.6	248 ± 13	244 ± 13
Final BW, g	355 ± 28	359 ± 26.9	346 ± 33	348 ± 29
HW/BW, mg/g	2.69 ± 0.1	2.7 ± 0.1	3.85 ± 0.2*	3.47 ± 0.43*†
LV/BW, mg/g	2.12 ± 0.1	2.11 ± 0.1	2.49 ± 0.18*	2.5 ± 0.15*
RV/BW, mg/g	0.57 ± 0.06	0.58 ± 0.1	1.35 ± 0.13*	0.97 ± 0.36*†
Infarcted area, %	-	-	35.9 ± 7	34.6 ± 6
PC, %	73.6 ± 4	74.3 ± 1.1	81.4 ± 4*	77 ± 1.2†
HC, %	72.4 ± 1	72.7 ± 0.6	73.6 ± 1.1	73.1 ± 1.2

Note: Values are means ± SD; n=8 for all groups. Sed-Sham, sedentary sham group; Tr-Sham, trained sham group; Sed-HF, sedentary heart failure group; and Tr-HF, trained heart failure group. BW, body weight; HW/BW, heart weight-to-BW ratio; LV/BW, left ventricle-to-BW ratio; RV/BW, right ventricle-to-BW ratio; PC, pulmonary congestion; HC, hepatic congestion. *$P < 0.05$ compared with Sed-Sham and Tr-Sham; †$P < 0.05$ compared with Sed-HF.

Table 2. Hemodynamic characteristics of sham-operated rats and rats with HF.

Groups	Sed-Sham	Tr-Sham	Sed-HF	Tr-HF
LVEDP, mmHg	4.85 ± 1.7	4.05 ± 1	21.8 ± 5.4*	17.8 ± 1.9*†
LVSP, mmHg	108.7 ± 9.4	113.5 ± 12.5	104.4 ± 15	107.4 ± 24.9
$+dP/dt_{máx}$, mmHg/s	5.626 ± 1.569	6.234 ± 1.177	5.300 ± 1.345	5.023 ± 1.005
$-dP/dt_{máx}$, mmHg/s	-4.481 ± 1.030	-4.715 ± 1.179	-3.418 ± 662*	-3.366 ± 369*

Note: Values are means ± SD; n=8 for all groups. Sed-Sham, sedentary sham group; Tr-Sham, trained sham group; Sed-HF, sedentary heart failure group; and Tr-HF, trained heart failure group. LVEDP, LV end-diastolic pressure; LVSP, LV systolic pressure; $+dP/dt_{máx}$, maximum positive LV derivate; $-dP/dt_{máx}$, maximum negative LV derivate. *$P < 0.05$ compared with Sed-Sham and Tr-Sham; †$P < 0.05$ compared with Sed-HF.

Table 3. Effect of exercise training on baroreflex curve parameters in sham and HF rats.

Groups	Sed-Sham	Tr-Sham	Sed-HF	Tr-HF
MAP_{50} (mmHg)	95.6 ± 7.2	96.9 ± 5.8	85.3 ± 6.8*	89.7 ± 7
Maximum Gain (beats.min.$^{-1}$(mmHg)$^{-1}$)	-10.7 ± 3.8	-12 ± 6	-5.8 ± 1.9*	-13.3 ± 4.6†

Note: Values are means ± SD; n=7 for all groups. Parameters for logistic sigmoidal baroreflex curve analysis of the baroreceptor reflex responses and of the heart rate in Sed-Sham, sedentary sham group; Tr-Sham, trained sham group; Sed-HF, sedentary heart failure group; and Tr-HF, trained heart failure group. MAP, median arterial pressure; MAP_{50}, MAP that corresponds to the value found at half of the HR range evoked by the baroreflex response. *P < 0.05 compared with Sed-Sham; †P < 0.05 compared with Sed-HF.

Figure 1.

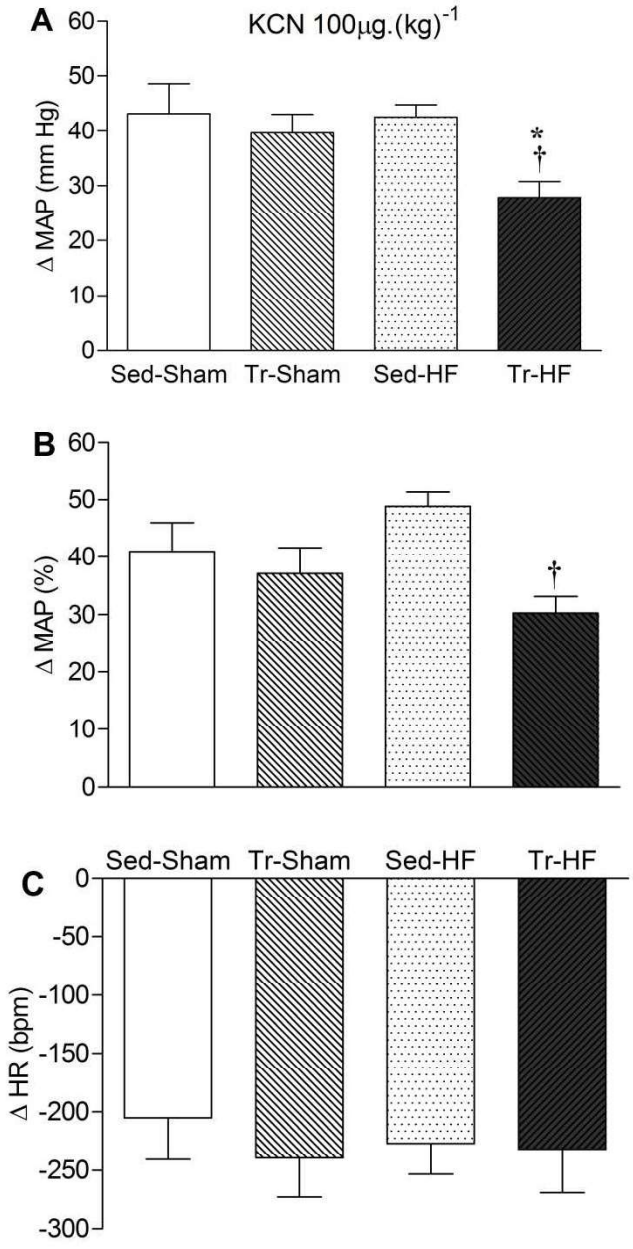

A
KCN 100μg.(kg)-1
Δ MAP (mm Hg)
60
50
40
30
20
10
0
*
†
Sed-Sham
Tr-Sham
Sed-HF
Tr-HF
B
Δ MAP (%)
60
50
40
30
20
10
0
†
C
Sed-Sham
Tr-Sham
Sed-HF
Tr-HF
Δ HR (bpm)
0
-50
-100
-150
-200
-250
-300

Figure 2.

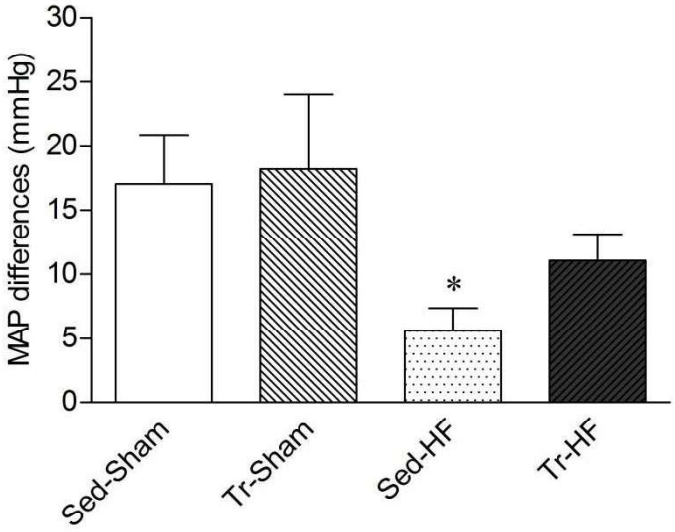
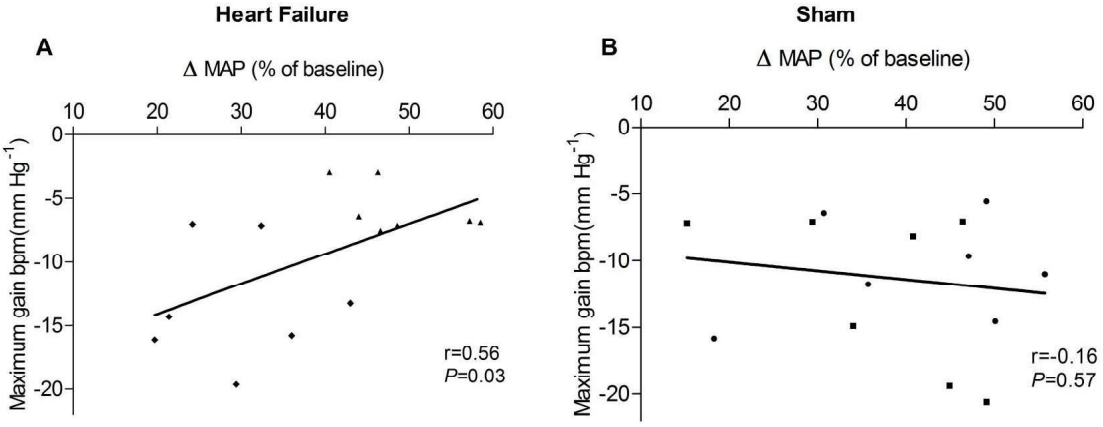

MAP differences (mmHg)
30
25
20
15
10
5
0
*
Sed-Sham
Tr-Sham
Sed-HF
Tr-HF

Figure 3.


Heart Failure
A
Δ MAP (% of baseline)
10
20
30
40
50
60
0
-5
-10
-15
-20
Maximum gain bpm(mm Hg-1)
r=0.56
P=0.03
Sham
B
Δ MAP (% of baseline)
10
20
30
40
50
60
0
-5
-10
-15
-20
Maximum gain bpm(mm Hg-1)
r=-0.16
P=0.57

ARTIGO 2

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Exercise training improves the IL-10/TNF-α cytokine balance in the gastrocnemius of rats with heart failure

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Short title: Exercise training effect on cytokine in HF rats

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Keywords: skeletal muscle, cytokine, inflammation, exercise training, heart failure

ABSTRACT:

Heart failure (HF) involves an inflammatory component that induces skeletal muscle atrophy. Despite the known benefits of exercise training (ExT) in improving the functional capacity and symptoms in HF, the anti-inflammatory benefits of ExT are still up for debate. The aim of the current study was to examine the effects of ExT upon concentration of tumor necrosis factor - alpha (TNF-α), interleukin - 6 (IL-6) and interleukin - 10 (IL-10) in the gastrocnemius of rats with HF. Adult male Wistar rats randomly fell into one of four experimental groups: trained HF (Tr-HF), sedentary HF (Sed-HF), trained sham (Tr-Sham) and sedentary sham (Sed-Sham). ExT protocol was performed on treadmill for a period of 8 weeks (60m/days, 5x/week, 16m/min), which started 6 weeks after left coronary artery ligation. After ExT, the hemodynamic variables were recorded and gastrocnemius muscle was collected. Sed-HF group presented increase of TNF-α level when compared with the Sed-Sham group ($P < 0.05$). ExT reduced by 59% TNF-α level in Tr-HF group (1.1 ± 0.5 pg/ml; 2.8 ± 0.5 pg/ml; $P < 0.05$) and increased IL-10 (26.1 ± 6.1 pg/ml; 11 ± 5.2 pg/ml; $P < 0.05$) when compared with the Sed-HF group. Thus, the gastrocnemius muscle IL-10/TNF-α ratio was increased ($P < 0.01$). Moreover, ExT reduced left ventricular end-diastolic pressure ($P < 0.05$) and increased gastrocnemius mass related to Sed-HF group. These results demonstrate that ExT not only attenuates TNF-α level but also improves the IL-10 cytokine level in skeletal muscle of HF rats. Therefore, ExT may disrupt the vicious cycle of local inflammation and skeletal muscle atrophy induced by HF.

BULLET POINTS

Pro-inflammatory cytokines in skeletal muscle of HF rats.

Exercise training reverses the atrophy of gastrocnemius induced by HF.

Exercise training increases IL-10 and decreases TNF-α level in gastrocnemius.

INTRODUCTION

Heart failure (HF) is a complex and multifactorial syndrome associated with disability, morbidity, and mortality. The adverse clinical outcomes and progressive nature of this syndrome has led to a systematic investigation of multiple mechanisms that may contribute to disease progression[1, 2]. Beyond neurohumoral activation, the inflammatory component plays an important role in the development of left ventricular dysfunction and peripheral myopathy, which in turn impair the functional capacity of patients with HF[3]. Plasma TNF-α and interleukin-6 (IL-6) levels are correlated with severity of HF symptoms and oxygen consumption capacity upon exercise[4]. The cytokine concentrations are not only elevated in the systemic circulation, but also in the skeletal muscle. In HF, tissue hypoperfusion and oxidative stress produce local inflammation, which stimulates inflammation and systemic cytokine release[4]. In this way, cytokines are not only thought to be involved in autocrine and paracrine effects of the skeletal muscle but appear to act also as an endocrine effector in the peripheral circulation. Weight loss and body wasting, commonly termed as cachexia, is an independent mortality risk. One proposed mechanism of cardiac cachexia is the development of an anabolic-catabolic imbalance, with reduced anabolism and enhanced catabolism, because of abnormalities in the neurohormonal systems and the activation of pro-inflammatory cytokines[5]. Skeletal myopathy seems to be an important factor associated with exercise intolerance, fatigue, and dyspnea in HF patients[2].

TNF-α may affect muscle metabolism and strength by stimulating expression of inducible nitric oxide synthase (iNOS) via nuclear-factor-kappa-B (NF-kB)[6]. Indeed,

contractil dysfunction can result from TNF-α overexpression that signals via nitric oxide to decrease strength of skeletal muscle[7]. Moreover, an increased expression of TNF-alpha and IL-6 was observed in skeletal muscle biopsies from patients with stable HF[8]. As previously reported, seven-day subcutaneous administration of recombinant human IL-6 to rats resulted in a dose-dependent respiratory and peripheral skeletal muscle atrophy[9]. Nevertheless, physical training decreases the expression of TNF-α in skeletal muscle that was accompanied by a reduction of atrophy in HF rats[10] and humans[8].

Exercise training (ExT) is associated with improvement of sympathetic and parasympathetic dysfunction in patients with HF[1]. Recent studies have established a critical role of the sympathetic nervous system (SNS) in mediating interactions between the nervous and immune systems[11]. Interleukin 10 (IL-10) could contribute to mediate the anti-inflammatory effects of ExT. Accumulating evidence suggests that ExT promotes anti-inflammatory benefits in HF experimental models[12, 13] and clinical studies[14, 15]. Although IL-10 is increased in plasma[16] and soleus muscle[13] in post-MI HF rats after treadmill endurance training, the effect of ExT on this cytokine in gastrocnemius muscle of HF rats is unclear.

Therefore, considering the important role of skeletal muscle in the release of cytokine, and the potential advantage of ExT in attenuating the loss of muscle mass and inflammation in chronic diseases, we have sought to evaluate the effects of ExT upon muscle mass, expression of TNF-α, IL-6 and IL-10 in the white gastrocnemius of rats with HF. In addition, we examined the balance between IL-10 and TNF-α production as an indicator anti-inflammatory following ExT.

METHODS

Animals

Experiments were performed on 28 male Wistar rats weighing 220–270 g, obtained from the Animal Breeding Unit at the *Universidade Federal de Ciências da Saúde de Porto Alegre* (UFCSPA; Brazil). The animals were allocated in groups of three to a cage with free access to water and pellet rodent chow diet and were maintained under standard conditions of temperature (22 ± 1 ºC). All procedures were performed in accordance with the *Guideline for the Care and Use of Laboratory Animals* (NIH Publication nº 85-23, revised 1996). All procedures outlined in this study were approved by the UFCSPA Ethics and Research Committee (protocol no. 39/11).

Experimental design

To induce myocardial infarction (MI), rats were anaesthetized with xylazine (12 mg/kg IP) and ketamine (90 mg/kg IP), intubated and artificially ventilated (SamWay VR 15) with a breathing rate of 60 breaths/min and oxygen inspired fraction of 100%. After thoracotomy, coronary artery ligation (CAL) was performed to induce MI as previously described[12]. During the first 48 h, the animals were treated for post-operative pain with subcutaneous buprenorphine (0.15 mg/kg) and given a single dose of penicillin (20,000 U IP). After MI, the animals were allowed 6 weeks for recovery (time necessary to develop the HF state)[17] and divided into four experimental groups: sedentary sham-operated (Sed-Sham, n = 7), trained sham-operated (Tr-Sham n = 7), sedentary HF (Sed-HF, n = 7) and trained HF rats (Tr-HF, n = 7).

Aerobic exercise training protocol

Rats in the training groups (Sham and HF) performed an aerobic exercise training (ExT) program for 8 weeks (5 x/week)[16, 18]. Initially, low speed (10 m/min) and short duration (10 min per session) were used to familiarize the rats with running on the treadmill. The ExT protocol was started 1 week after this adaptation period. The speed and duration were gradually increased to 16 m/min and 60 min per session[19]. This intensity was maintained at a constant level throughout the experiment. Previous study demonstrated that this exercise protocol improved the aerobic capacity and citrate synthase activity in post-myocardial infarction rats[20]. Only rats that ran steadily with little or no prompting were used in the study.

Cardiac hemodynamic evaluation and infarct size

After a rest period of 48 h from the last training session, the cardiac hemodynamic evaluation was performed. The animals were anesthetized with xylazine (12 mg/kg IP) and ketamine (90 mg/kg IP), and 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 (AP) was recorded first during a 5-min period by connecting the arterial cannula to a pressure transducer (Miniature Pulse Transducer RP-155, Narco Biosystems, Houston, TX, USA), coupled to a pressure amplifier (General Purpose Amplifier 4, Model 2, Stemtech, Hudson, WI, USA). 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 analogue signals were digitalized by a data acquisition system (AT/Codas, Dataq Instruments, Akron, OH, USA) at a sampling rate of 2,000 Hz. These data were used to determine LV maximum change

in pressure over time ($+dP/dt_{max}$) and LV minimum change in pressure over time ($-dP/dt_{max}$), and LV end-diastolic pressure (LVEDP)[21]. The last parameter was determined manually by detecting the point of inflection to the end of diastole via analysis of the ventricular pressure wave. The total left ventricle area and myocardial infarction scar were manually drawn on scanned images. ImageJ 1.47 software used planimetry to determine the size of the infarct. All photographs were analyzed independently by two blinded investigators.

Skeletal muscle collection

After cardiac hemodynamic evaluation, the animals were euthanized with an overdose of anesthetic (thiopental 80 mg/kg IP), soleus muscle and the superficial white gastrocnemius muscle were collected, weighed, immediately frozen in liquid nitrogen, and stored at -80ºC. The samples of fast-glycolytic muscles were obtained by dissection of gastrocnemius muscles, which were quickly excised from the left hindlimbs of the animals and dissected according to the muscle color (corresponding to myoglobin concentration) into a white portion derived from the superficial part of both lateral and medial head.

Gastrocnemius muscle sample preparation and determination of tissue TNF-α, IL-6 and IL-10 protein levels

The gastrocnemius muscles were chosen for cytokine assays because of their metabolic characteristics and tend to be more affected in disease conditions[10]. The gastrocnemius samples were homogenized in potassium phosphate buffer (KPi, pH 7,4) containing 4,08 g/L KH_2PO_4, 8,9 g/L KCl, 8,71 ug/ml phenylmethylsulfonyl fluoride (PSMF), 0,1 ug/ml aprotinin, 0,1 ug/ml leupeptin and 0,1 ug/ml pepstatin proteases inhibitors using a hand-held homogenizer. The homogenates were

centrifuged at 12,000 g (Mikro 220 R, Hettich Zentrifugen, Tuttlingen, Germany) for 60 min at 4 ºC. The supernatant was removed and TNF-α, IL-6 and IL-10 protein levels were determined by multiplex bead array using Milliplex^{TM} MAP rat cytokine kits (RCYTO-80K) (Millipore, Billerica, MA, USA). All samples were run in duplicates and the average value is reported as pg/ml.

Statistical analyses

The data are presented as the mean ± SD. The data were tested for normal distribution using the Kolmogorov-Smirnov test. Comparisons were made between groups using two-way ANOVA followed by the Newman-Keuls post hoc test. When Gaussian normality failed, a Kruskal-Wallis test on ranks and Dunn's test were performed. Unpaired student *t* test compared infarcted areas between the Sed-HF and Tr-HF groups. A P value < 0.05 was considered statistically significant.

RESULTS

Infarcted area, body weight and muscle weights

The perioperative mortality in the MI-groups was 35%. The infarct sizes of the LV were similar in two infarcted groups, (Sed-HF 37.1 ± 6.6%; Tr-HF 33.9 ± 7%; P = 0.4). Among the four groups, no differences were detected regarding the body weight at end of study (Table 1). After 14 weeks post-MI there was a significant reduction in the muscle weight and ratio of muscle weight to body weight of the gastrocnemius muscle in the Sed-HF group (P < 0.01), suggesting muscle atrophy. However, when the exercise-trained HF group was compared with the Sed-HF group, there was an increase in the weight and ratio of weight to body weight of the gastrocnemius

muscle. However, there were no differences in the weight and ratio of weight to body weight of the soleus muscle between groups.

Hemodynamic data

Table 2 shows the hemodynamic characteristics in sham and post-MI rats. As expected, left ventricular end diastolic pressure (LVEDP) was elevated in both HF groups compared with the sham groups ($P < 0.001$). However, when the trained HF group was compared with the Sed-HF group, there was an improvement in diastolic function (18.1 ± 1.8 mmHg; 22.6 ± 5.3 mmHg; $P < 0.05$), respectively. This result suggests that 8-week ExT have a beneficial hemodynamic effect in post-MI rats. The negative derivative of LV pressure ($-dP/dt_{max}$) was significantly reduced in the HF groups compared with the sham groups ($P < 0.05$). No differences were observed in mean arterial pressure (MAP) or positive derivative of LV pressure ($+dP/dt_{max}$). All hemodynamic variables were assessed while the rats were under anaesthesia.

Gastrocnemius muscle TNF-α, IL-6 and IL-10 protein levels

Muscular levels of TNF-α (Fig 1A) were elevated in the Sed-HF group when compared with the Sed-Sham group ($P < 0.05$). ExT significantly reduced by 59% TNF-α level in Tr-HF group when compared with the Sed-HF group (1.1 ± 0.5 pg/ml; 2.8 ± 0.5 pg/ml; $P < 0.05$). We observed an increase in IL-10 (Fig 1B) in trained HF group when compared with the Sed-HF and Sed-Sham groups (26.1 ± 6.1 pg/ml; 11 ± 5.2 pg/ml; 7.5 ± 4.4 pg/ml; $P < 0.05$). However, no differences were found in the IL-6 (Fig 1C) among groups. The gastrocnemius muscle IL-10/TNF-α ratio (Fig 2) was significantly higher in the Tr-HF group than in the Sed-HF and Sham groups ($P < 0.01$). Cytokine analyses were performed only with muscle samples feasible.

DISCUSSION

The main findings of the present study were that exercise training was able to reverse the muscle atrophy and improve inflammatory profile in skeletal muscle of post-MI rats. Evidence of this effect was provided by reduction of TNF-α level, as well as by the increase of IL-10 level and IL-10/TNF-α ratio in gastrocnemius muscle. In addition, the beneficial effect of ExT was accompanied by the decrease in LVEDP.

Chronic HF is associated with wasting muscle and intolerance to exercise induced by elevated pro-inflammatory cytokines, neurohumoral alterations, and reactive oxygen species (ROS) production[8, 22, 23]. Among skeletal muscle abnormalities, elevated TNF-α can modulate immune and inflammatory response, exacerbating muscle wasting, which was found upregulated in gastrocnemius muscle of HF rats. The result of HF-induced muscle atrophy matched other studies[10, 24]. It was suggested that elevated TNF-α level could stimulate expression of the myostatin via NF-kB[25]. The present work also demonstrates that after 8 weeks of ExT, there was a restoration in TNF-α level and increase of gastrocnemius mass in post-MI rats. Our results support previous studies in humans[8] and animal models[10] suggesting that ExT can be used as an effective intervention to prevent skeletal muscle wasting[22, 26].

The present study extends previous reports developed from our laboratory concerning the anti-inflammatory effects of aerobic[12, 16] and resistance[21] training in HF rats. Regarding skeletal muscle IL-10, the current study demonstrated that 8-week ExT program after MI increased IL-10 in gastrocnemius. A study reported similar improvement in soleus IL-10 levels by physical exercise in post MI-rats[13]. Likewise, ExT-induces IL-10 expression in paraventricular nucleus (PVN) and rostral ventrolateral medulla (RVLM) of spontaneously hypertensive rats[27]. Furthermore, in

healthy rats the ExT increased IL-10 in white adipose tissue of mesenteric depot[28]. All these studies suggest that ExT improves the anti-inflammatory defense mechanism by the increase of IL-10 at different tissues. A network of molecular and metabolic pathways could mediate this adaptation. Muscle contraction, which triggers intracellular signaling cascades, could activate such a network. During exercise, the contraction of skeletal muscle produces IL-6 and stimulates anti-inflammatory cytokines, such as IL-1 receptor antagonist and IL-10, and inhibits the liberation of pro-inflammatory cytokine TNF-α[14, 29]. At the molecular level, IL-10's anti-inflammatory effects are mediated through indirect actions of the signal transducer and activator of transcription 3 (STAT3) on target inflammatory genes[30]. STAT3-induced transcriptional inhibitor can selectively control transcription at inflammatory promoters, like TNF-α[30].

Although IL-6 has been most studied in the healthy skeletal muscle[29], its role in HF remains unclear, regarding both acute and chronic physical exercise responses[15]. Studies from our laboratory have shown that aerobic exercise and resistance training can reduce plasma IL-6 level in HF rats[16, 21]. The results presented here display decreasing trends in gastrocnemius IL-6 level in HF. Similarly, Gielen et al.[8] has shown that ExT may lower intramuscular IL-6 in the absence of serum changes in patients with HF. It remains to be determined if the training adaptations occur in plasma or locally within the skeletal muscle. Furthermore, it was suggested that the major role of IL-6 is to regulate the metabolism rather than acting as an inflammatory mediator[31].

We found loss in gastrocnemius muscle mass but not in the soleus muscle. Animal model of HF[32] has demonstrated that faster-twitch, more glycolytic fibers lose more vascularization and enhanced E3 ligase *MAFBx/Atrogin* gene expression. In

addition, upregulation of autophagy-related genes appeared only in plantaris muscle of infarcted rats[24]. However, the cross-sectional area of the gastrocnemius muscle was not changed after 6 months post-induced myocardial infarction[33] but reduction in the type I fibers in soleus was reported by others[10]. It is possible that muscle fiber type-specific can be differentially affected during developed of HF.

Besides the anti-inflammatory benefits, regular exercise training seems able to induce hemodynamic adaptations in HF rats[12, 16, 26, 34]. Our results showed a significant reduction in LVEDP after ExT in HF rats, indicating positive effect on cardiopulmonary function. This finding is in line with previous reports showing that low to moderate treadmill running offers beneficial effects on the left ventricles of HF rats[16]. Several mechanisms have been related to hemodynamic improvement such as enhanced antioxidant enzyme capacity, restoration of β-adrenergic signaling and attenuation of oxidative stress in myocardium[20, 26].

A limitation of the current study was the absence of histological assessment in skeletal muscle. The determination of the cross-sectional area would help identify the effects of ExT in muscle fiber adaptation. However, this adaptation following ExT has been previously described in soleus and plantaris muscle[10, 35].

In conclusion, this study demonstrates that ExT benefits inflammatory modulation, especially by increase of IL-10 level in gastrocnemius muscle. In addition, ExT attenuates TNF-α level as well as avoids gastrocnemius atrophies. The improvement in the balance of pro- and anti-inflammatory cytokine induced by ExT prevents skeletal muscle inflammation and reduces muscle wasting in HF state.

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Figure Legend

Figure 1 - Mean data showing the effects of exercise training in the gastrocnemius muscle of anti- and pro-inflammatory cytokines. A) Tumor Necrosis Factor-alpha (TNF-α); * $P < 0.05$ vs. Sed-HF; # $P < 0.05$ vs. Sed-Sham. B) Interleukin-10 (IL-10); * $P < 0.05$ vs. Sed-Sham and Sed-HF. C) interleukin-6 (IL-6). Values are the means ± SD, n = 5 for all groups.

Figure 2 - IL-10/TNF-α ratio. Values are the means ± SD, n = 5 for all groups.* $P < 0.01$ vs. all groups.

Table 1. Body weight, and muscle weights in sham and post-MI rats after 8 weeks of exercise training.

Groups	Sed-Sham	Tr-Sham	Sed-HF	Tr-HF
BW, g	347 ± 20	353 ± 24	338 ± 28	352 ± 29
Soleus, g	0.164 ± 0.01	0.173 ± 0.02	0.152 ± 0.01	0.161 ± 0.02
Soleus/BW, mg/g	0.471 ± 0.02	0.493 ± 0.06	0.45 ± 0.04	0.46 ± 0.07
Gastrocnemius, g	1.92 ± 0.16	1.99 ± 0.22	1.62 ± 0.18*	1.91 ± 0.26†
Gastrocnemius/BW, mg/g	5.54 ± 0.4	5.63 ± 0.3	4.8 ± 0.5*	5.4 ± 0.35†

Values are means ± SD; n=7 for all groups. Sed-Sham, sedentary sham group; Tr-Sham, trained sham group; Sed-HF, sedentary heart failure group; and Tr-HF, trained heart failure group. BW, body weight. *$P < 0.05$ compared with Sed-Sham and Tr-Sham; †$P < 0.05$ compared with Sed-HF.

Table 2. Hemodynamic characteristics in sham and post-MI rats after 8 weeks of exercise training.

Groups	Sed-Sham	Tr-Sham	Sed-HF	Tr-HF
MAP, mmHg	102.5 ± 9	98.6 ± 11	96.6 ± 15	95.6 ± 10.3
LVEDP, mmHg	5.32 ± 1.5	4.1 ± 1.1	22.6 ± 5.3*	18.1 ± 1.8*†
$+dP/dt_{máx}$, mmHg/s	5.537 ± 1.673	5.979 ± 1.006	5.271 ± 1.450	5.192 ± 956
$-dP/dt_{máx}$, mmHg/s	-4.351 ± 1.039	-4.553 ± 1.173	-3.446 ± 710*	-3.407 ± 379*

Values are means ± SD; n=7 for all groups. Sed-Sham, sedentary sham group; Tr-Sham, trained sham group; Sed-HF, sedentary heart failure group; and Tr-HF, trained heart failure group. MAP, mean arterial pressure, LVEDP, LV end-diastolic pressure; $+dP/dt_{máx}$, maximum positive LV derivate; $-dP/dt_{máx}$, maximum negative LV derivate. *$P < 0.05$ compared with Sed-Sham and Tr-Sham; †$P < 0.05$ compared with Sed-HF.

Figure 1.

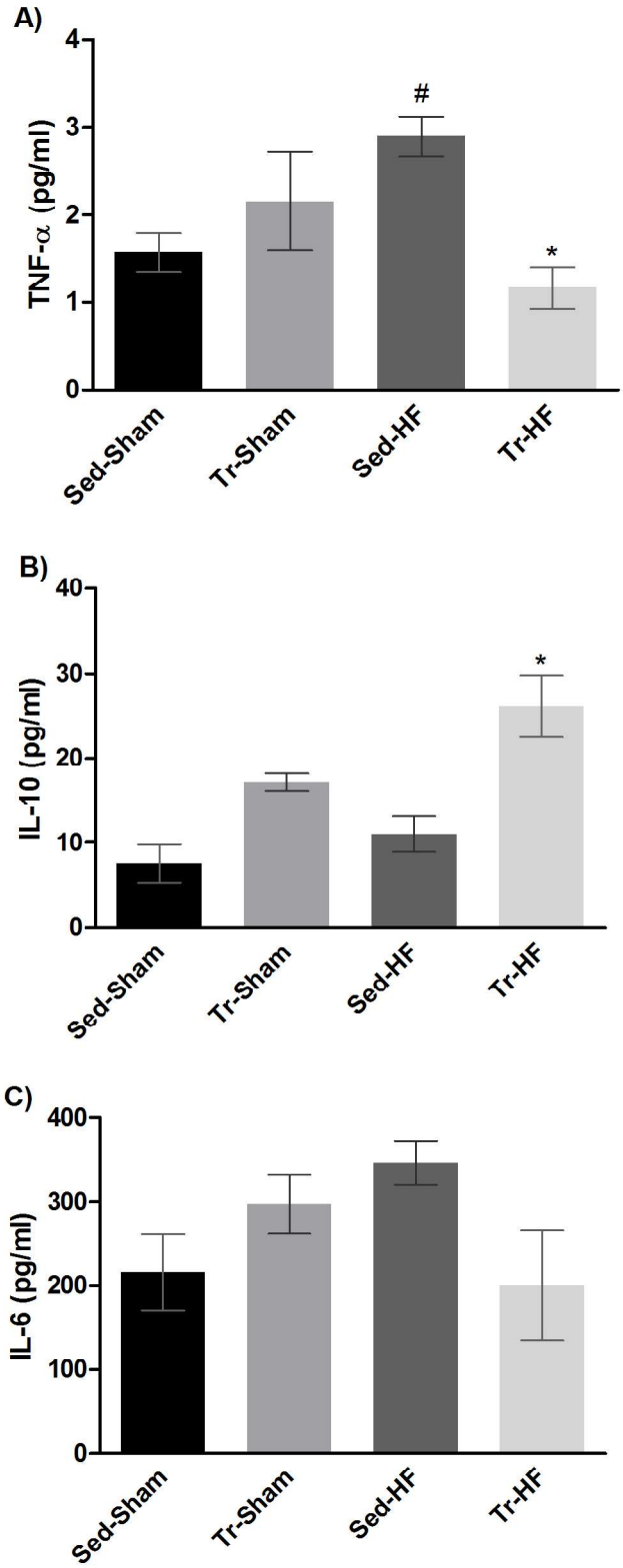
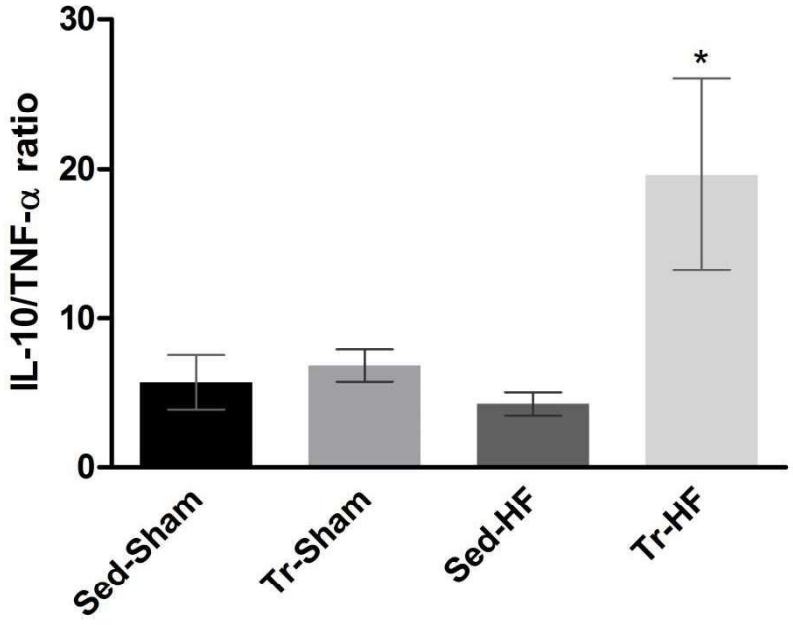

A)
TNF-α (pg/ml)
0
1
2
3
4
#
*
Sed-Sham
Tr-Sham
Sed-HF
Tr-HF
B)
IL-10 (pg/ml)
0
10
20
30
40
*
Sed-Sham
Tr-Sham
Sed-HF
Tr-HF
C)
IL-6 (pg/ml)
0
100
200
300
400
Sed-Sham
Tr-Sham
Sed-HF
Tr-HF

Figure 2.



9. CONCLUSÕES

O TFA durante 8 semanas promoveu melhora da função cardíaca dos ratos com IC, evidenciado pela diminuição da pressão diastólica final do ventrículo esquerdo, redução da congestão pulmonar e da hipertrofia cardíaca compensatória.

As adaptações ao treinamento físico foram observadas na melhora da sensibilidade barorreflexa e na atenuação da resposta pressórica ao cianeto de potássio dos ratos com IC, sugerindo um efeito modulatório do treinamento sobre o sistema nervo homeostático. Além disso, a melhora na sensibilidade barorreflexa esteve associada à redução da resposta pressórica no grupo IC treinado, indicando uma possível interação entre os barorreceptores e os quimiorreceptores.

A hipotensão induzida pela administração de nitroprussiato de sódio promoveu potencialização da resposta pressória ao KCN. Este efeito foi menor no grupo IC sedentário e sugere perda da reatividade vascular simpática.

O TFA melhorou o perfil inflamatório com aumento da interleucina-10 (IL-10) e redução do fator de necrose tumoral (TNF-α) no músculo gastrocnêmio dos ratos IC treinados. O aumento da relação IL-10/TNF-α sugere um efeito anti-inflamatório do TFA sobre o músculo esquelético de ratos IC.

10. PERSPECTIVAS

Introduzimos na literatura evidências sobre as adaptações do TFA nas respostas pressóricas evocadas pelo cianeto de potássio em ratos acordados com IC. Devido a redundância do controle cardiovascular, a fisiopatologia multissitêmica da IC e os inúmeros benefícios do TFA sobre o organismo, surgem novas questões para explorar os mecanismos fisiológicos envolvidos na adaptação da resposta pressórica relatada no artigo 1. Investigações sobre a adaptação induzida pelo TFA dentro do sistema nervoso central, especialmente no núcleo paraventricular do hipotálamo e na amígdala medial póstero-dorsal, áreas que modulam o sistema nervoso simpático e função cardiovascular. Outros aspectos a serem investigados estão relacionados às adaptações do TFA sobre a resistência vascular periférica e no endotélio de ratos com IC. Além disso, determinar se o cianeto de potássio pode elevar as concentrações de lactato sanguíneo nos ratos com IC, poderia esclarecer a influência ou não do cianeto sobre os receptores metabólicos (aferências do grupo IV).

Ao final da década de 90 e início dos anos 2000, as diretrizes norte-americanas e europeias utilizadas no manejo de pacientes com IC incluíram a recomendação do treinamento físico para melhora da aptidão cardiorrespiratória e da qualidade de vida destes pacientes. Este e outros estudos sugerem que o TFA pode induzir adaptações sobre os reflexos cardiovasculares (barorreflexo e quimiorreflexo). Além disso, os resultados do segundo estudo mostraram melhora no perfil inflamatório no músculo esquelético dos ratos IC treinados. Espera-se que em um futuro próximo as diretrizes recomendem o TFA como uma estratégia para atenuar a exacerbada atividade simpática bem como para melhorar o perfil inflamatório em pacientes com IC.

ANEXO 1 – Aprovação do Comitê de Ética no Uso de Animais

UFCSPA

COMISSÃO CIENTÍFICA E COMISSÃO DE PESQUISA E ÉTICA EM SAÚDE

COMISSÃO DE ÉTICA NO USO DE ANIMAIS - CEUA
UFCSPA

A Comissão de Ética no uso de Animais, analisou o Projeto:

Projeto: 11-039 **Versão do Projeto:** **Versão do TCLE:**

Pesquisadores:
PEDRO DALL'AGO
LEONARDO CALEGARI

Título: EFEITOS DO TREINAMENTO FÍSICO SOBRE REFLEXOS CARDIOVASCULARES NA INSUFICIÊNCIA CARDÍACA EM RATOS: ADAPTAÇÕES FISIOLÓGICAS NA AMÍGDALAS MEDIAL.

Este projeto foi aprovado em seus aspectos éticos e metodológicos. Todo e qualquer alteração do projeto, assim com eventos adversos graves, deverão ser comunicados a esta CEUA.

Porto Alegre, 13 de junho de 2011

Pedro R. T. Romão
UFCSPA

ANEXO 2 – Instruções para autores - Brazilian Journal of Physical Therapy

The Brazilian Journal of Physical Therapy (BJPT) publishes original research articles, reviews, and brief communications on topics related to the professional activity of physical therapy and rehabilitation, including clinical, basic or applied studies on the assessment, prevention, and treatment of movement disorders. Our Editorial Board is committed to disseminating quality scientific investigations from many areas of expertise.

The BJPT follows the principles of publication ethics included in the code of conduct of the Committee on Publication Ethics (COPE).

The BJPT accepts the following types of study, which must be directly related to the journal's scope and expertise areas:

a) Experimental studies: studies that investigate the effect(s) of one or more interventions on outcomes directly related to the BJPT's scope and expertise areas.

The World Health Organization defines a clinical trial as “any research study that prospectively allocates human participants or groups of humans to one or more health-related interventions to evaluate the effect(s) on health outcome(s)”. Clinical trials include single-case experimental studies, case series, nonrandomized clinical trials, and randomized clinical trials. Randomized controlled trials (RCTs) must follow the CONSORT (Consolidated Standards of Reporting Trials) recommendations, which are available at: http://www.consort-statement.org/consort-statement/overview0/.

The CONSORT checklist and Statement Flow Diagram, available at http://www.consortstatement.org/downloads/translations, must be completed and submitted with the manuscript.

Clinical trials must provide registration that satisfies the requirements of the International Committee of Medical Journal Editors (ICMJE), e.g. ttp://clinicaltrials.gov/ and/or http://www.anzctr.org.au. The complete list of all clinical trial registries can be found at: http://www.who.int/ictrp/network/primary/en/index.html

b) **Observational studies:** studies that investigate the relationship(s) between variables of interest related to the BJPT's scope and expertise areas without direct manipulation (e.g. intervention). Observational studies include cross-sectional studies, cohort studies, and case-control studies.

c) **Qualitative studies:** studies that focus on understanding needs, motivations, and human behavior. The object of a qualitative study is guided by in-depth analysis of a topic, including opinions, attitudes, motivations, and behavioral patterns without quantification. Qualitative studies include documentary and ethnographic analysis.

d) **Systematic reviews:** studies that analyze and/or synthesize the literature on a topic related to the scope and expertise areas of the BJPT. Systematic reviews that include meta-analysis will have priority over other systematic reviews. Those that have an insufficient number of articles or articles with low quality in the Methods

section and do not include an assertive and valid conclusion about the topic will not be considered for peer-review analysis. The authors must follow the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist to format their systematic reviews. The checklist is available at http://prisma-statement.org/statement.htm and must be filled in and submitted with the manuscript. Potential authors are encouraged to read the paper Mancini MC, Cardoso JR, Sampaio RF, Costa LCM, Cabral CMN, Costa LOP. Tutorial for writing systematic reviews for the Brazilian Journal of Physical Therapy (BJPT). Braz J Phys Ther. 2014 Nov-Dec; 18(6):471-480. http://dx.doi.org/10.1590/bjpt-rbf.2014.0077.

e) **Studies on the translation and cross-cultural adaptation of questionnaires or assessment tools:** studies that aim to translate into and/or cross-culturally adapt foreign questionnaires to a language other than that of the original version of existing assessment instruments. The authors must use the checklist (Appendix) to format this type of paper and adhere to the other recommendations of the BJPT. The answers to the checklist must be submitted with the manuscript. At the time of submission, the authors must also include written permission from the authors of the original instrument that was translated and/or cross-culturally adapted.

f) Methodological studies: studies centered on the development and/or evaluation of clinimetric properties and characteristics of assessment instruments. The authors are encouraged to use the Guidelines for Reporting Reliability and Agreement Studies (GRRAS) to format methodological papers, in addition to following BJPT instructions.

Important: Studies that report electromyographic results must follow the Standards for Reporting EMG Data recommended by ISEK (International Society of Electrophysiology and Kinesiology), available at http://www.isek-online.org/standards_emg.html.

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Studies in humans must be in agreement with COPE ethical standards and must be approved by the institution's ethics committee.

Animal experiments must comply with international guidelines (such as those of the Committee for Research and Ethical Issues of the International Association for the Study of Pain, published in Pain, 16:109110, 1983).

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The BJPT accepts submissions of manuscripts with up to six (6) authors. The BJPT's authorship policy follows ICMJE requirements for Manuscripts Submitted to Biomedical Journals (www.icmje.org), which state that "authorship credit should be based on 1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to be published." Conditions 1, 2, and 3 should all be met simultaneously. Grant acquisition, data collection, and/or general supervision of a research group do not justify authorship and must be recognized in the acknowledgements.

In exceptional cases, the editors may consider a request for submission of a manuscript with more than six (6) authors. The criteria for analysis include the type of study, potential for citation, quality, and methodological complexity, among other things. In these exceptional cases, each author's contribution must be described at the end of the text, after the Acknowledgements and right before the References as recommended by the ICMJE and the Guidelines for Integrity of Scientific Activity widely publicized by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (http://www.cnpq.br/web/guest/diretrizes).

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Manuscript form and presentation

Original manuscripts

The BJPT accepts the submission of manuscripts with up to 3,500 words (excluding title page, abstract, references, tables, figures, and legends). The information contained in appendices will be included in the total number of words allowed.

The manuscript must be written preferably in English. Whenever the quality of the English writing hinders the analysis and assessment of the content, the authors will be informed.

It is recommended that manuscripts submitted in/translated into English be accompanied by certification of revision by a professional editing and proofreading service. This certification must be included in the submission. We recommend the following services, not excluding others:

- American Journal Experts (www.journalexperts.com);
- Scribendi (www.scribendi.com);
- Nature Publishing Groups Language Editing (https://languageediting.nature.com/login).

The manuscript must include a title and identification page, abstract, and keywords before the body of the manuscript. References, tables, figures, and appendices should be inserted at the end of the manuscript.

Title and identification page

The title of the manuscript must not exceed 25 words and must include as much information about the study as possible. Ideally, the terms used in the title should not appear in the list of keywords. The identification page must also contain the following details:

Full title and short title of up to 45 characters to be used as a legend on the printed pages;

Author: author's first and last name in capital letters without title followed by a superscript number (exponent) identifying the institutional affiliation (department, institution, city, state, country). For more than one author, separate using commas;

Corresponding author: name, full address, email, and telephone number of the corresponding author who is authorized to approve editorial revisions and provide additional information if needed.

Keywords: up to six indexing terms or keywords in Portuguese and English.

Abstract

The abstract must be concise, not exceeding 250 words in a single paragraph in English, and must be inserted immediately after the title page. Do not include references, footnotes or undefined abbreviations in the abstract. It must be written in a structured format.

Bullet points

On a separate page, the manuscript must identify three to five phrases that capture the essence of the topic under investigation and the main conclusions of the paper. Each bullet point must be written in a summarized fashion and provide the main contributions of the study to the current literature, as well as the clinical implications (i.e., how the results can influence clinical practice or scientific research in the area of physical therapy and rehabilitation). These points must be presented in a text box in the beginning of the article, after the abstract. Each bullet point must have no more than 80 characters (with spaces).

Introduction

This part of the manuscript should describe and define the topic under investigation, explain the relationships with to other studies in the same field, justify the need for the study, and specify the objective(s) of the study and hypotheses, if applicable.

Methods

This section consists in describing the methodological design of the study and presenting a clear and detailed report of the study participants and data collection procedures, transformation/reduction, and analysis in order to allow reproducibility of the study. For clinical trials, the participant selection and allocation process must be organized in a flowchart containing the number of participants in each phase as well as their main characteristics (see model of CONSORT flow diagram).

Whenever relevant to the type of study, the author should include the calculation that adequately justifies the sample size for investigation of the intervention effects. All of the information needed to estimate and justify the sample size used in the study must be clearly stated.

The authors must describe the dependent and independent variables; whether the parametric assumptions were met; specify the software used in the data analysis and the level of significance; and specify the statistical tests and their purpose.

Results

The results should be presented briefly and concisely. Pertinent results must be reported with the use of text and/or tables and/or figures. Data included in tables and figures must not be duplicated in the text.

The results must be summarized into self-explanatory graphs or tables using measures of central tendency and variability (e.g. mean (SD) instead of mean±SD); must include measures of magnitude of effect (e.g. effect size) and/or indicators of the precision of the estimates (e.g. confidence intervals); must report the power of the non-significant statistical tests.

Discussion

The purpose of the discussion is to interpret the results and to relate them to existing and available knowledge, especially the knowledge already presented in the Introduction. Be cautious when emphasizing recent findings. The data presented in

the Methods and/or in the Results sections should not be repeated. Study limitations, implications, and clinical application to the areas of physical therapy and rehabilitation sciences must be described.

References

The recommended number of references is 30, except for systematic reviews of the literature. Avoid references that are not available internationally, such as theses and dissertations, unpublished results and articles, and personal communication. References should be organized in numerical order of first appearance in the text, following the Uniform Requirements for Manuscripts Submitted to Biomedical Journals prepared by the ICMJE.

Journal titles should be written in abbreviated form, according to the List of Journals of Index Medicus. Citations should be included in the text as superscript (exponent) numbers without dates. The accuracy of the references appearing in the manuscript and their correct citation in the text are the responsibility of the author(s).

Examples: http://www.nlm.nih.gov/bsd/uniform_requirements.html.

Tables, Figures, and Appendices

An overall total of five (5) tables and figures is allowed. Appendices must be included in the number of words allowed in the manuscript. In the case of previously published tables, figures, and appendices, the authors must provide a signed permission from the author or editor at the time of submission.

For articles submitted in Portuguese, the English version of the tables, figures, and appendices and their respective legends must be attached in the system as a supplementary document.

-Tables: these must include only indispensable data and must not be excessively long (maximum allowed: one A4 page with double spacing). They should be numbered consecutively using Arabic numerals and should be inserted at the end of the text. Small tables that can be described in the text are not recommended. Simple results are best presented in a phrase rather than a table.

- Figures: these must be cited and numbered consecutively using Arabic numerals in the order in which they appear in the text. The information in the figures must not repeat data described in tables or in the text. The title and legend(s) should explain the tables and figures without the need to refer to the text. All legends must be double-spaced, and all symbols and abbreviations must be defined. Use uppercase letters (A, B, C, etc.) to identify the individual parts of multiple figures.

Whenever possible, all symbols should be placed in the legends. However, symbols identifying curves in a graph can be included in the body of the figure, provided this does not hinder the analysis of the data. Figures in color will only be published in the online version. With regard to the final artwork, all figures must be in high resolution or in its original version. Low-quality figures will not be accepted and may result in delays in the process of review and publication.

- Acknowledgements: these must include statements of important contributions specifying their nature. The authors are responsible for obtaining the authorization of individuals/institutions named in the acknowledgements.

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It is the authors' responsibility to remove all information (except on the title and identification page) that may identify the article's source or authorship.

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