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Efeitos da suplementação de L-Arginina e do treinamento aeróbico contínuo ou intervalado na tolerância ao exercício, função hemodinâmica, perfil inflamatório e nos parâmetros de estresse oxidativo na insuficiência cardíaca experimental.

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Orientador: Dr.Ramiro Barcos Nunes
Co-orientador: Dr. Pedro Dal Lago

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“A lei da mente é implacável.
O que você pensa, você cria;
O que você sente, você atrai;
O que você acredita
Torna-se realidade.”

Buda

RESUMO

Introdução: A insuficiência cardíaca (IC) é uma síndrome multissistêmica. Disfunções hemodinâmicas, vasculares e, além disso, o quadro inflamatório e de estresse oxidativo caracterizam esta patologia. O conjunto de disfunções contribui para o principal sintoma da síndrome: a intolerância ao exercício. Ainda, anormalidades na biodisponibilidade de L-arginina (Arg) podem agravar a função vascular destes pacientes. Objetivos: Avaliar a capacidade funcional, os parâmetros hemodinâmicos, a massa muscular, o estresse oxidativo e o perfil inflamatório após oito semanas de suplementação oral de Arg em associação ou não com o treinamento aeróbico contínuo (TAC) ou treinamento aeróbico intervalado (TAI) em ratos com IC. Metodologia: 38 Wistar machos, após 6 semanas da cirurgia de infarto agudo do miocárdio (IAM), foram randomizados em 6 grupos: sedentários (SED), sedentários suplementados com Arg (SED+Arg), treinamento aeróbico contínuo (TAC), treinamento aeróbico contínuo e suplementados com Arg (TAC+Arg), treinamento aeróbico intervalado (TAI) e treinamento aeróbico intervalado e suplementados com Arg (TAI+Arg). Todos os animais foram submetidos ao teste de tolerância máxima ao exercício (TTE) no período pré e pós protocolos. Os grupos suplementados recebiam diariamente L-Arginina (1g/kg) por gavagem e o treinamento aeróbico era realizado cinco vezes por semana em esteira elétrica. O TAI consistiu de 7 séries de 3 min a 85% da velocidade máxima atingida no TTE intercalada com 4 min de recuperação ativa a 60% da velocidade máxima do TTE. O TAC era realizado a 60% da velocidade máxima do TTE até atingir a mesma distância realizada no TAI. Ao término de oito semanas de protocolos foi realizada a coleta de sangue e tecidos e, posteriormente, as avaliações de hemodinâmica, perfil inflamatório e de estresse oxidativo. Resultados: Todos os grupos treinados foram capazes de aumentar a distância percorrida e a duração do TTE em comparação aos grupos sedentários e aos testes pré-protocolos intragrupo. A melhora da função hemodinâmica foi observada principalmente nos grupos que associaram o treinamento com a suplementação. Houve redução da pressão diastólica final do ventrículo esquerdo nos grupos TAC+Arg, TAI e TAI+Arg em relação ao SED. Além disso, o grupo TAI+Arg apresentou tendência de normalização da pressão sistólica do ventrículo esquerdo, da pressão arterial sistólica, além do índice de contratilidade e de relaxamento. Os grupos TAI+Arg e TAC+Arg apresentaram maior atividade de superóxido dismutase (SOD) no gastrocnêmio e maior atividade de catalase (CAT) e menor lipoperoxidação nos rins. No gastrocnêmio, além do TAI+Arg e TAC+Arg, o grupo SED+Arg também apresentou redução da lipoperoxidação. Os grupos submetidos ao TAI apresentaram menores valores plasmáticos de TNF-α, além de uma maior razão IL-10/TNF-α em relação aos demais grupos. Já os grupos que associaram o exercício aeróbico com a suplementação de Arg (TAC+Arg e TAI+Arg) apresentaram uma maior massa do gastrocnêmio em relação aos SED. Conclusão: TAC e TAI foram capazes de aumentar a tolerância ao exercício de ratos com IC e a associação de ambos com a suplementação de Arg atenuou a perda muscular. Associação do TAI+Arg promoveu melhoras expressivas na hemodinâmica, perfil inflamatório e nos parâmetros de estresse oxidativo nos ratos IC.

Palavras-chave: Insuficiência cardíaca, Arginina, Estresse Oxidativo, Terapia por Exercício, Hemodinâmica, Citocinas.

ABSTRACT

Introduction: Heart failure (HF) is a multifactorial syndrome, which reduced exercise tolerance is the major symptom. Diminished bioavailability of L-Arginine (Arg) in HF could be related to dysfunction in vascular system. Hence, all dysfunctions together lead to an inflammatory and oxidative stress state. Objective: To investigate the effects of 8 weeks of L-arginine (Arg) supplementation associated with aerobic continuous training (ACT) or aerobic interval training (AIT) on maximal exercise capacity, pulmonary and hepatic congestion, muscle mass, and hemodynamic, inflammatory, and oxidative stress parameters in rats with chronic heart failure (CHF).Methods: Thirty-eight male Wistar rats post 6 weeks of myocardial infarction (MI) surgery were randomly assigned into 6 CHF groups: sedentary (SED, *n* = 6); SED+Arg (*n* = 7); ACT (*n* = 8); ACT+Arg (*n* = 5); AIT (*n* = 7); AIT+Arg (*n* = 5). Exercise test capacity (ETC) was performed pre and post 8 weeks of intervention. Supplemented rats received Arg (1g/kg) by oral gavage (7x/week). Exercise training was performed on a rat treadmill (5x/week). The AIT consisted of 7 bouts of 3 min at 85% of maximum ETC velocity with 4-min active recovery at 60% of maximum ETC velocity. The ACT was performed at 60% of maximum ETC velocity until reaching the same distance of AIT. Hemodynamic variables, tissue collection, congestion, inflammatory cytokines, and oxidative parameters were evaluated at the end of protocols. Results: All trained groups showed a superior exercise capacity compared to SED groups on the post-intervention test. Pulmonary congestion was attenuated in AIT and AIT+Arg compared with the SED group. Left ventricular end-diastolic pressure was lower in continuous ACT+Arg, AIT, and AIT+Arg groups than SED group. Association of AIT with Arg supplementation was able to improve hemodynamic responses (left ventricular systolic pressure, systolic blood pressure, dP/dt_{max}, and $-dP/dt_{max}$, likewise, decrease muscular and renal lipid peroxidation and tumor necrosis factor (TNF)-α, and increase interleukin (IL)-10/TNF-α plasmatic levels. Groups associated with aerobic exercise and Arg supplementation (ACT+Arg and AIT+Arg) revealed higher gastrocnemius mass compared to the SED group. Conclusions: Both aerobic training protocols were capable to improve aerobic capacity, and the association with Arg supplementation was important to attenuate muscle loss. Moreover, interval training associated with Arg supplementation elicits greater improvements in hemodynamic parameters, contributing to reduction in pulmonary congestion, and demonstrated particular responses in the inflammatory profile and in the antioxidant status.

Key words: Heart failure, Arginine, Oxidative Stress, Exercise Therapy,

Hemodynamics, Cytokines

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ADMA	Dimetilarginina Assimétrica
ANG II	Angiotensina II
BH4	Tetrahidropterina
CAT	Catalase
cGMP	Guanosina Monofosfato Cíclica
DM	Diabetes Mellitus
DNA	Ácido Desoxirribonucléico
eNOS	Óxido Nítrico Sintase Endotelial
ERN	Espécie Reativa de Nitrogênio
ERO	Espécie Reativa de Oxigênio
FAD	Flavina Adenil Dinucleotídeo
FEVE	Fração de Ejeção do Ventrículo Esquerdo
FMN	Flavina Mononucleotídeo
GP_x	Glutationa Peroxidase
GTP	Guanosina Trifosfato
H_20_2	Peróxido de Hidrogênio
IAM	Infarto Agudo do Miocárdio
IC	Insuficiência Cardíaca
IL-1	Interleucina-1
IL-10	Interleucina-10
IL-6	Interleucina-6
iNOS	Óxido Nítrico Sintase Induzível
MDA	Malondialdeído
NADPH	Nicotinamida Adenina Dinucleotídeo fosfato
nNOS	Óxido Nítrico Sintase Neuronal
NO	Óxido Nítrico
NO_2^-	Nitrito
NO_3^-	Nitrato
NOS	Óxido Nítrico Sintase
O_2	Oxigênio
$O_2^{\cdot -}$	Ânion Radical Superóxido

OH^-	Radical Hidroxila
$ONOO^-$	Peróxinitrito
PAS	Pressão Arterial Sistólica
PDFVE	Pressão Diastólica Final do Ventrículo Esquerdo
PSVE	Pressão Sistólica do Ventrículo Esquerdo
SOD	Superóxido Dismutase
SRAA	Sistema Renina-Angiotensina-Aldosterona
TAC	Treinamento Aeróbico Contínuo
TAI	Treinamento Aeróbico Intervalado
TTE	Teste de Tolerância ao Exercício
TNF-α	Fator de Necrose Tumoral alpha
VE	Ventrículo Esquerdo
VO_2	Consumo de Oxigênio
$VO_{2máx}$	Consumo de Oxigênio máximo

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1 INTRODUÇÃO

A insuficiência cardíaca (IC) crônica é caracterizada pela incapacidade do coração em manter o fluxo sanguíneo sistêmico adequado, prejudicando a função da maioria dos órgãos e sistemas corporais. Essa condição é considerada uma síndrome complexa e multifatorial, que está diretamente relacionada à redução da qualidade de vida e sobrevida dos pacientes. Dentre os diversos marcadores prognósticos dessa síndrome, a intolerância ao exercício tem demonstrado ser um forte preditor de mortalidade nessa condição (1, 2). As alterações cardiovasculares e ventilatórias observadas nesta síndrome contribuem significativamente para a progressão e agravamento da IC. Contudo, fatores neuro-humorais, tais como maior ativação do sistema simpático e do sistema renina-angiotensina-aldosterona (SRAA) (3, 4), além de disfunções vasculares e musculoesqueléticas, estão diretamente relacionados à fadiga precoce e intolerância ao exercício em pacientes com IC (5, 6).

A função vascular depende diretamente da biodisponibilidade de óxido nítrico (NO), que é um importante fator envolvido no relaxamento do músculo liso dos vasos sanguíneos. Anormalidades na sua função podem estar relacionadas a baixas concentrações de L-Arginina (Arg), ou ainda, à reduzida expressão da óxido nítrico sintase endotelial (eNOS) (7, 8). Além disso, o conjunto de alterações características dessa síndrome, contam com um quadro inflamatório e de estresse oxidativo sistêmicos (9) que agravam o sintoma de intolerância ao exercício e culminam também em atrofia da musculatura esquelética.

O óxido nítrico exerce funções fundamentais para a saúde vascular, sendo responsável pela vasodilatação, inibição da adesão plaquetária e fatores antiateroscleróticos (10, 11). Assim, a inadequada produção de NO, devido à baixa biodisponibilidade de L-Arginina tem sido associada a distúrbios cardiovasculares (12). Alguns estudos ao longo dos últimos anos têm investigado o uso da suplementação de L-Arginina em doenças cardiovasculares e sua ação sobre a função endotelial, perfil inflamatório e até mesmo sua ação antioxidante (13, 14).

O exercício físico é outra intervenção não farmacológica que sem dúvida tem um notável papel na reabilitação de pacientes com insuficiência cardíaca. O treinamento aeróbico tem sido amplamente estudado e já é bem estabelecido que,

quando realizado em pacientes estáveis com IC e com supervisão profissional, é uma ferramenta segura. Além disso, esta prática está diretamente relacionada a menor taxa de internações hospitalares e a um menor risco de mortalidade (15, 16).

Nos últimos anos, o treinamento aeróbico intervalado (TAI) tem sido alvo de investigações, tanto em pacientes com insuficiência cardíaca, quanto em outras patologias ou em indivíduos saudáveis. Quando esse treinamento baseia-se na alternância de períodos de baixa a moderada intensidade com períodos de alta intensidade, permite que o indivíduo consiga totalizar um maior tempo em alta intensidade e, ainda, realizar um treino mais curto do que os de intensidade contínua (17). Há evidências sugerindo superioridade do TAI de alta intensidade em relação ao treinamento aeróbico contínuo de moderada intensidade, principalmente em relação ao consumo máximo de oxigênio ($VO_{2máx}$), tanto em indivíduos saudáveis, quanto em pacientes com IC (18, 19). Todavia, a superioridade dos benefícios do treinamento intervalado nos parâmetros hemodinâmicos, inflamatórios e de estresse oxidativo ainda não é clara (19-21).

Tendo em vista a necessidade de inovações nas intervenções não farmacológicas para a reabilitação na insuficiência cardíaca, o presente estudo objetivou verificar os efeitos da suplementação de L-Arginina e do treinamento aeróbico, tanto contínuo de intensidade moderada, quanto o intervalado de alta intensidade, sobre a capacidade funcional, estresse oxidativo e parâmetros hemodinâmicos e inflamatórios. O estudo foi conduzido em modelo experimental de ratos Wistar com IC induzida por ligação da artéria coronária descendente esquerda. A pesquisa experimental nos proporciona uma abordagem mais completa dos aspectos fisiopatológicos que contribuem para progressão da insuficiência cardíaca, uma vez que alguns parâmetros não seriam possíveis de ser analisados em estudos clínicos. Por fim, nossa hipótese é que o treinamento intervalado de alta intensidade possa promover ganhos mais expressivos comparado ao treinamento contínuo de intensidade moderada e, ainda, que a associação do treinamento aeróbico com a suplementação oral de L-Arginina – pelo método da gavagem - possa promover efeitos ainda mais benéficos comparados à utilização de apenas os protocolos de exercício físico.

2 REVISÃO DA LITERATURA

2.1 FISIOPATOLOGIA DA INSUFICIÊNCIA CARDÍACA

A insuficiência cardíaca tem como principal característica a incapacidade do coração em manter um adequado suprimento sanguíneo para as necessidades metabólicas do organismo (1). Atualmente, é considerada uma síndrome clínica complexa, pois há o envolvimento de diversos sistemas orgânicos. Não só o sistema cardiovascular é prejudicado, mas anormalidades musculoesqueléticas, renais, neuroendócrinas e imunológicas contribuem sobremaneira para o agravamento desta síndrome (2, 22).

A IC pode decorrer de uma alteração estrutural ou funcional ao músculo cardíaco, seja pelo uso contínuo de álcool ou drogas, infecção viral, hipertensão ou o infarto agudo do miocárdio (3). Após a lesão das células miocárdicas e consequente disfunção ventricular, mecanismos compensatórios são ativados. Estes mecanismos, em um primeiro momento, estabilizam a função cardíaca, porém em longo prazo levam à piora progressiva da falência cardíaca (23).

Quando ocorre redução da capacidade funcional do miocárdio pode ser observado um aumento na ativação do sistema nervoso simpático, e, consequentemente, estimulação dos receptores β-adrenérgicos que, inicialmente, ajudam a aumentar a força, a contratilidade e a frequência das contrações no miocárdio (4). Além disso, a hiperativação do SRAA auxilia a hemodinâmica pelo aumento da retenção de sódio e do volume sanguíneo (3). Essa complexa modulação de mecanismos neuro-humorais está exemplificada na Figura 1.

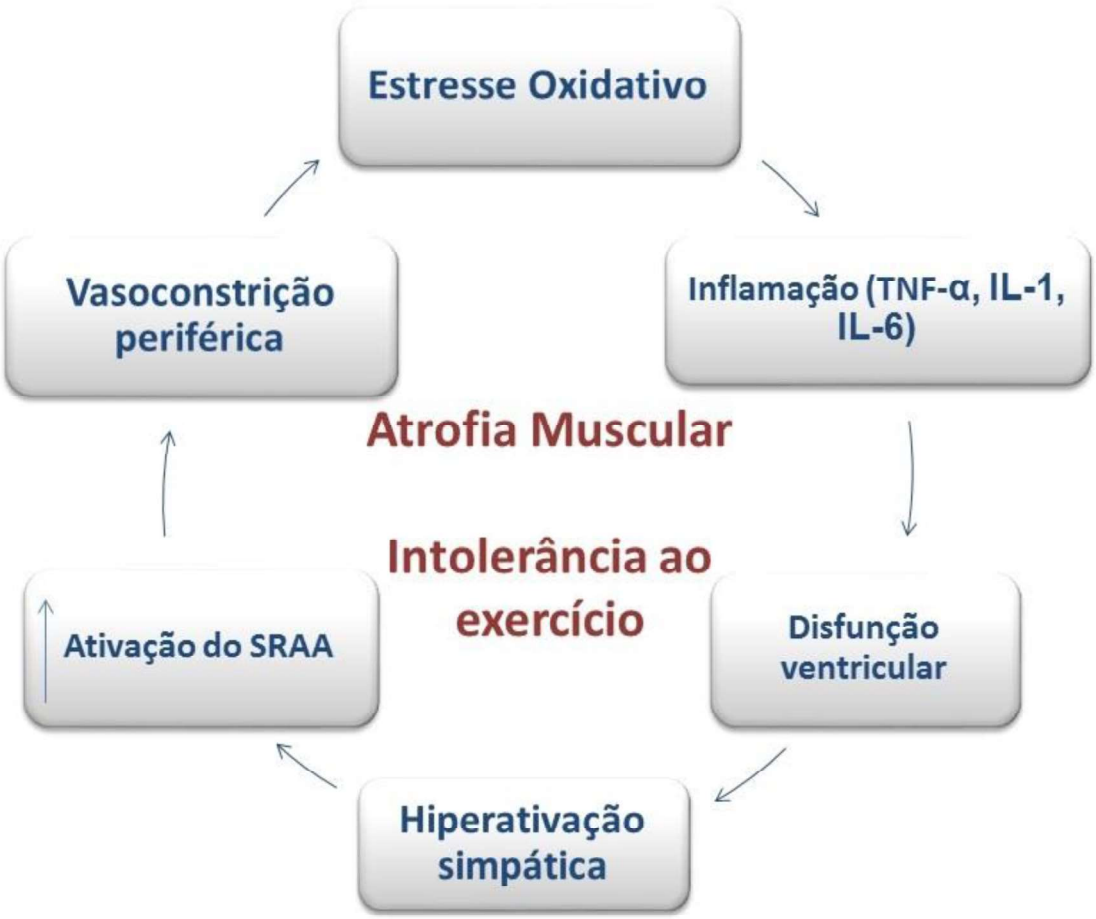

Figura 1. Rede de mecanismos compensatórios após o dano agudo ao miocárdio. Adaptado de Packer (3). TNF-α: Fator de necrose tumoral alpha; IL-1: Interleucina 1; IL-6: Interleucina 6; SRAA: Sistema renina angiotensina aldosterona

Os principais sintomas relacionados à redução da qualidade de vida nesta síndrome são a dispnéia, a fadiga muscular precoce e a intolerância ao exercício físico (6). Sabe-se que os sintomas de fadiga muscular pouco se relacionam com a disfunção hemodinâmica central da IC. Os fatores periféricos relacionados, como anormalidades no músculo esquelético e na função vascular, têm sido investigados. Observa-se uma redução do número de fibras do tipo I, de contração lenta (oxidativas), que são compensadas por fibras do tipo II – fibras de contração rápida (glicolíticas), culminando em prejuízo do metabolismo oxidativo. Entretanto, apesar da maior quantidade de fibras do tipo II, estas sofrem atrofia (5). Ocorrem também outras anormalidades, tais como diminuição no volume de mitocôndrias, ou ainda, alterações na dinâmica e na homeostase de cálcio intracelular (1, 24, 25).

A redução na capacidade funcional também está relacionada com mediadores inflamatórios (25). As citocinas pró-inflamatórias mais envolvidas na patologia são o TNF-α, a IL-1 e a IL-6 (22). Estas contribuem para o estado de anorexia e caquexia, que caracterizam a falta de apetite, perda de massa muscular e de tecido adiposo nos pacientes com IC (26). Uma vez instalados os sintomas de caquexia, estes podem, ainda, contribuir para a formação de radicais livres e levar ao estado de estresse oxidativo (3). O conjunto destes fatores culmina na piora progressiva dos indivíduos com IC.

2.2 ALTERAÇÕES HEMODINÂMICAS

O dano ao miocárdio e os decorrentes prejuízos estruturais incitam diretamente a prejuízos hemodinâmicos. Desde 1937, Fishberg *et al.* já reconheciam duas formas de insuficiência cardíaca. A insuficiência cardíaca diastólica ou IC com fração de ejeção preservada e, a mais comum, a insuficiência cardíaca sistólica – de fração de ejeção reduzida.

A perda de tecido muscular cardíaco íntegro - característico de situações pós IAM - leva à queda no volume de ejeção. As reduções da capacidade de contratilidade e de dilatação do ventrículo não permitem que o coração expulse todo o sangue da câmara cardíaca. Desta maneira, a IC sistólica engloba redução na fração de ejeção, no trabalho e volume sistólicos e, portanto, na diminuição do débito cardíaco (27, 28).

Já a IC diastólica, a qual pode ocorrer concomitantemente à IC sistólica, caracteriza-se pela fração de ejeção preservada. As alterações mais comuns são anormalidades no padrão de enchimento e maior pressão diastólica final do ventrículo esquerdo (PDFVE) (29). No ventrículo íntegro, a maior taxa de enchimento diastólico exigida durante o exercício é suprida pela capacidade de distensibilidade da câmara, que resulta num maior volume diastólico final no VE, sem que ocorra aumento de pressão. Entretanto, no portador de IC com disfunção diastólica, há comprometimento da distensibilidade do ventrículo, resultando no rápido aumento de pressão diastólica, bem como na pressão venosa pulmonar (30).

A reduzida velocidade de relaxamento ventricular é outra anormalidade característica da disfunção diastólica. Dentre os fatores envolvidos no relaxamento ventricular, pode-se citar a ligação entre as pontes cruzadas, a recaptação de cálcio

pelo retículo e, ainda, a formação de óxido nítrico (NO) (31). Todavia, na insuficiência cardíaca há baixa biodisponibilidade de NO, uma vez que o quadro de estresse oxidativo e inflamação dessa síndrome favorecem a metilação de compostos de seu aminoácido precursor – a L-Arginina. Ocorre também inibição da enzima que catalisa a formação de NO – a óxido nítrico sintase (NOS). A principal isoforma relacionada a esta inibição é a dimetilarginina (ADMA), a qual também está relacionada tanto com a disfunção sistólica, quanto à disfunção diastólica e, inclusive, pode servir como marcador de eventos adversos (32).

Alguns pacientes com fração de ejeção preservada, apesar de não apresentarem aumento na deposição de colágeno, também demonstram, entretanto, alterações hemodinâmicas similares àqueles com maior fração de colágeno. Com base nisso, verificou-se a importância da rigidez de cardiomiócitos na disfunção hemodinâmica. O principal fator molecular que tem sido ligado à rigidez do ventrículo é o aumento da expressão de titina – proteína elástica do citoesqueleto. A hiperfosforilação de resíduos da proteína, ou ainda, o estresse oxidativo gerado pelas pontes dissulfeto na molécula de titina poderiam contribuir para a rigidez passiva na diástole, mesmo sem haver deposição de colágeno (31).

2.3 L-Arginina na Insuficiência Cardíaca

2.3.1 Metabolismo da L-Arginina

A L-arginina é um aminoácido condicionalmente essencial, sendo essencial ou não essencial de acordo com a idade biológica ou estado de saúde do indivíduo (33, 34). Aminoácido essencial é todo aquele que não é produzido endogenamente e deve ser consumido a partir da dieta para suprir as necessidades fisiológicas. As fontes dietéticas de L-Arginina são, principalmente, fontes proteicas, tais como carne de gado, porco, peixe, nozes, sementes e concentrados proteicos de arroz e soja (35). De forma contrária, aminoácidos não essenciais são capazes de ser sintetizados a partir de outros compostos pelo metabolismo, não sendo necessária a ingestão.

A síntese endógena da L-Arginina ocorre pelo catabolismo proteico ou através da citrulina. Os rins são os maiores responsáveis por manter os níveis plasmáticos de L-Arginina, junto com a ingestão dietética. A síntese ocorre entre o eixo intestinal

e renal. A citrulina é sintetizada na mitocôndria dos enterócitos através da glutamina, glutamato e prolina e, após, é liberada na corrente sanguínea e captada pelos rins para sua conversão em arginina (35, 36). A L-arginina exógena, por sua vez, é absorvida no intestino e, em mamíferos adultos – humanos, roedores e porcos –, boa parte da ingestão (40%) é degradada no intestino delgado logo no início do metabolismo do aminoácido. O aminoácido também pode ser transportado para o fígado no ciclo da uréia e ser degradado a ornitina e uréia (37). O metabolismo da L-Arginina está melhor exemplificado na imagem a seguir.

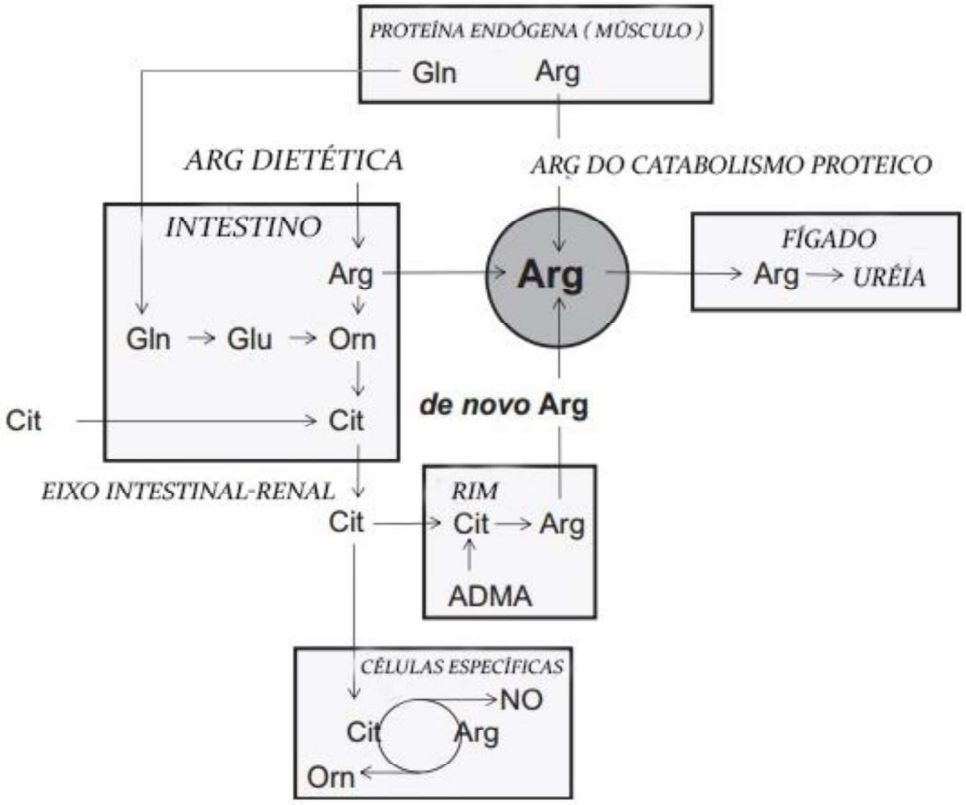

Figura 2. Metabolismo extra-hepático da L-Arginina em humanos saudáveis. Arg: Arginina, Cit: Citrulina, Gln: Glutamina, Glu: Glutamato, Orn: Ornitina, ADMA: Dimetilarginina assimétrica, NO: óxido nítrico. Adaptado de Luiking, et al. (38).

O transporte da L-Arginina, assim como de outros aminoácidos catiônicos, ocorre principalmente pelo sistema y^+. O sistema y^+ é caracterizado por ser independente de sódio, nesse sistema o aminoácido catiônico liga-se com a membrana plasmática da célula alvo e, após, é internalizado por proteínas carregadoras específicas (13).

A L-Arginina quando não convertida em ornitina, pode gerar outras substâncias com notáveis funções fisiológicas. Dentre elas, as poliamidas, a creatina e o óxido nítrico. As enzimas envolvidas na conversão de arginina nessas substâncias são, respectivamente, a arginil-RNAt sintetase, a arginina-glicina amidinotransferase e, por fim, a óxido nítrico sintase (NOS) (39). São reconhecidas três diferentes isoformas de NOS, a NOS endotelial (eNOS ou NOS3), NOS neuronal (nNOS ou NOS1) e a NOS induzível (iNOS ou NOS2) (40). Essa última é expressa em estados inflamatórios através de citocinas e endotoxinas bacterianas. Já a nNOS pode ser expressa tanto em nervos, quanto no músculo esquelético e a eNOS em células endoteliais e em miócitos (13, 41). Ademais, a atividade das NOS depende de cofatores, tais como o NADPH, a flavina mononucleotídeo (FMN), a tetrahidropterina (BH_4), a flavina adenil dinucleotídeo (FAD) e também da disponibilidade de seu substrato – a L-Arginina (42). Na figura 3 está ilustrada a estrutura molecular da NOS. Para a formação de NO, há a transferência de elétrons do NADPH para o domínio oxidase, via FAD e FMN. Estes elétrons, por sua vez, são transportados da enzima NOS para a L-Arginina, fazendo com que o aminoácido se reduza à L-Citrulina e libere o NO (43).

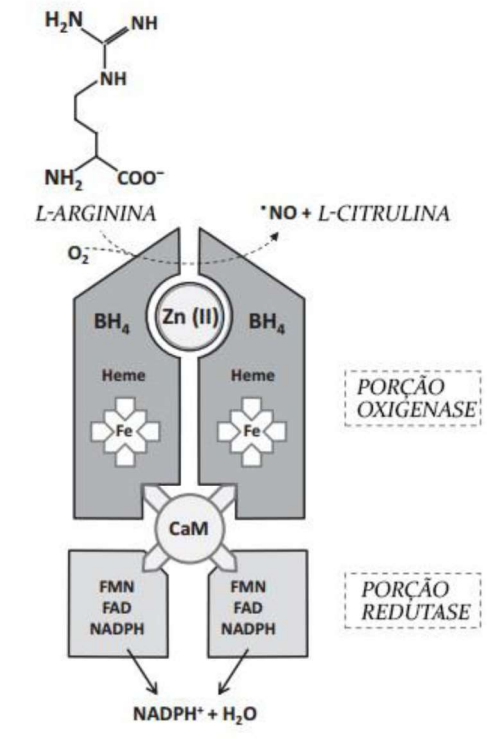

Figura 3. Estrutura molecular da óxido nítrico sintase (NOS). FMN: flavina mononucleotídeo, FAD: flavina adenil dinucleotídeo. Adaptado de Lorin, et al. (13).

2.3.2 Suplementação de L-Arginina na Insuficiência Cardíaca

A média populacional de ingestão de L-Arginina através da dieta, de acordo com o estudo de King, et al. (44), é de 4,4g/dia e em torno de 25% dos indivíduos apresentam uma ingestão menor do que 2,6g/dia. Além disso, sabe-se que aproximadamente metade do que é ingerido é transformado em ornitina e uréia pela arginase (45). No entanto, ainda que pouca parte do catabolismo da L-arginina seja direcionado para a síntese do óxido nítrico, esse é o único substrato para a formação de NO, o que a torna chave essencial desta via metabólica (46).

O óxido nítrico é essencial para a regulação do funcionamento cardíaco, é o principal mediador da vasodilatação endotelial, capaz de reduzir a resistência periférica, aumentar a perfusão, bem como modular a função contrátil do coração. Além disso, age inibindo a adesão plaquetária e tem sido denominado como uma molécula antiaterosclerótica (10, 11, 47). Em condições normais o NO é produzido no endotélio em presença de oxigênio (O_2) pela catalisação da L-Arginina através da enzima eNOS. Na musculatura lisa adjacente o NO é modulado pela guanilato ciclase, que tem a função de converter a guanosina trifosfato (GTP) em guanosina monofosfato cíclica (cGMP), gerando, por fim, o relaxamento da parede vascular (48).

Anormalidades na produção e transporte de NO podem agravar diversas doenças cardiovasculares, além de gerar disfunção endotelial. Na insuficiência cardíaca e em outras condições patológicas os níveis plasmáticos de L-arginina podem estar reduzidos (7, 8, 49). Além disso, pode haver maior expressão de substâncias que competem pelo substrato L-Arginina. Como já descrito anteriormente, a arginase utiliza a L-Arginina como substrato para a formação de uréia e ornitina. Desta maneira, a arginase e a NOS competem pelo mesmo substrato. Existem duas formas de arginase, a Arginase I, a qual é citosólica e se expressa no fígado como componente do ciclo da uréia e a Arginase II que é mitocondrial e está expressa nos rins. Em células endoteliais a presença da arginase pode limitar a síntese de NO. Outro inibidor endógeno da NOS tem sido estudado nos últimos anos, a dimetilarginina assimétrica (ADMA) (45). A ação da ADMA e da suplementação de L-arginina na liberação de óxido nítrico estão exemplificadas na Figura 4.

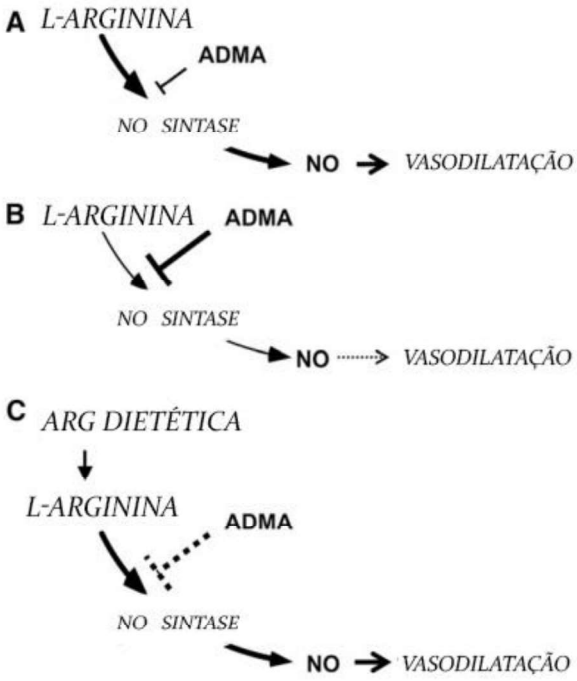

Figura 4. Interação entre as concentrações de L-Arginina e ADMA no funcionamento da enzima NOS.
Concentrações fisiológicas normais de L-Arginina permitem o funcionamento total da enzima NOS, na presença de concentrações normais (baixos níveis) de ADMA (A). Entretanto, na presença de concentrações elevadas de ADMA, a conversão de L-Arginina em NO está bloqueada, resultando na deficiência das ações biológicas do NO (B). Nessas condições, pequenas doses de suplementação de L-Arginina através da dieta podem restaurar a produção de NO aos níveis normais (C). Adaptado de *Boger RH. (45)*.

Na insuficiência cardíaca e em outras patologias há menor concentração dos níveis plasmáticos de L-Arginina, maiores concentrações de ADMA e, portanto, reduzida síntese de NO. Desta maneira, a suplementação de L-Arginina nessas condições tem sido investigada (8). O primeiro estudo com o objetivo de investigar os efeitos da suplementação de L-Arginina na função vascular foi conduzido em 1991, por *Drexler et al. (50)*. Deste então, os estudos têm demonstrado resultados distintos, porém os estudos realizados especificamente em pacientes com insuficiência cardíaca, ainda são escassos.

Estudo realizado em 15 pacientes com IC que receberam de forma randomizada, de 5,6 a 12,6g/dia de L-arginina ou placebo, por seis semanas,

constatou que aqueles que receberam a suplementação do aminoácido aumentaram não só o fluxo sanguíneo do antebraço durante exercícios para esse local, como também a distância percorrida no teste de caminhada de seis minutos (51). Outro delineamento utilizou a dosagem de 5g/3x ao dia e verificou uma melhora na função renal de pacientes com insuficiência cardíaca crônica (52). Além disso, a suplementação de L-Arginina (8g/dia) associada a protocolos de exercício durante quatro semanas mostrou-se benéfica e as intervenções, quando associadas, foram capazes de produzir efeitos aditivos (53).

No entanto, alguns estudos não foram capazes de demonstrar efeitos positivos da suplementação de L-Arginina em pacientes com insuficiência cardíaca. A dosagem de 20g/dia suplementada por 28 dias não apresentou nenhuma influencia na função endotelial em estudo de Chin-Dusting, et al. (54). Da mesma forma, a administração de 3g/3x/dia no período pós infarto do miocárdio fracassou em demonstrar melhora na complacência ou na fração de ejeção (55).

Outras linhas de pesquisa têm descrito a L-Arginina como um importante antioxidante, mecanismo pelo qual a sua suplementação poderia beneficiar condições patológicas específicas (56). Ao reestabelecer as quantidades fisiológicas necessárias circulantes do substrato para a formação de NO, os danos oxidativos vasculares poderiam ser minimizados, uma vez que em condições de baixa disponibilidade do aminoácido L-arginina, o óxido nítrico torna-se mais susceptível a reagir com ânions superóxidos e formar o potente oxidante peróxinitrito (7).

2.3.3 Estresse Oxidativo e L-Arginina

Na insuficiência cardíaca, o aumento de agentes oxidantes pode decorrer de anormalidades da própria doença e, associados à baixa atividade de antioxidantes, são capazes de agravar ainda mais determinados sistemas orgânicos na patologia. Por fim, o estresse oxidativo - estado que pode ser definido como o desequilíbrio entre a formação de agentes oxidantes e a capacidade de defesa antioxidante – contribui para o agravamento do quadro de pacientes com IC (9).

As Espécies Reativas de Oxigênio (ERO) englobam a maioria dos agentes oxidantes, porém também há o grupo das Espécies Reativas de Nitrogênio (ERN). Entre as principais EROs, pode-se citar o ânion radical superóxido ($O_2^{\cdot -}$), o peróxido de hidrogênio (H_2O_2) e o radical hidroxila ($OH^{\cdot}$). Já entre as ERNs destacam-se o

óxido nítrico (NO), nitritos (NO_2^-), nitratos (NO_3^-) e o peróxinitrito ($ONOO^-$) (57). Ressalta-se que em condições normais as espécies reativas são necessárias a diversos processos fisiológicos (Ray PD, 2012), todavia quando em excesso geram danos oxidativos a macromoléculas celulares, atingindo proteínas (carbonilação de proteínas), lipídeos (lipoperoxidação) ou o DNA, contribuindo para disfunção celular e para a progressão de doenças cardiovasculares (58, 59).

Na doença cardiovascular, sabe-se que há uma produção excessiva de superóxido e de peróxido de hidrogênio – este último sendo o produto da reação entre o superóxido e a enzima superóxido dismutase (SOD). Há, ainda, maior expressão e atividade da enzima xantina oxidase – a qual doa elétrons para moléculas de oxigênio, contribuindo, portanto, para o aumento do $O_2^{\cdot-}$ e do H_2O_2. O superóxido em excesso reage com o óxido nítrico, formando o peróxinitrito - composto altamente citotóxico (57, 60). Esse potente oxidante é capaz de inativar o NO, além de gerar o desacoplamento da eNOS (61, 62). A produção deficiente de óxido nítrico, por sua vez, promove aumento do metabolismo da L-Arginina como um mecanismo compensatório para formar mais NO. Ocorrendo, portanto, depleção dos estoques de L-Arginina (63). As reações envolvidas na geração e degradação de EROs estão ilustradas na Figura 5, a seguir.

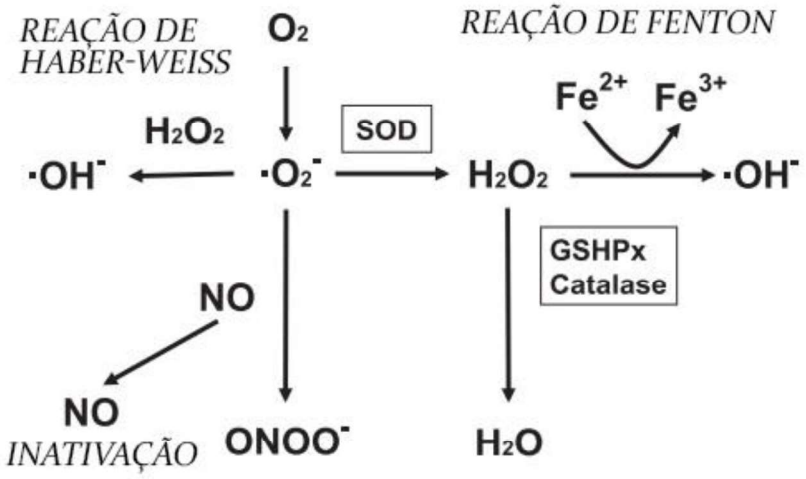

Figura 5. Reações envolvidas na geração e degradação de espécies reativas de oxigênio. SOD: Superóxido dismutase, NO: Óxido nítrico, GSHPx: Glutationa peroxidase, OH^-: Radical hidroxila, H_2O_2: Peróxido de hidrogênio, O_2^-: Superóxido, $ONOO^-$: Peróxinitrito, Fe: Ferro, H_2O: água. Adaptado de Tsutsui, et al. (58).

Células com depleção de L-Arginina tendem a favorecer ainda mais a formação de oxidantes. De acordo com alguns estudos, em células com deficiência de L-Arginina a iNOS tende a produzir mais $O_2^{\cdot-}$ e NO, favorecendo a formação de peróxinitrito. Ademais, durante o exercício físico a iNOS também estaria envolvida com a maior formação de $O_2^{\cdot-}$ (64, 65).

Outro mecanismo associado ao aumento de espécies reativas de oxigênio e, consequentemente, do estresse oxidativo na insuficiência cardíaca é a hiperativação do sistema nervoso simpático e do SRAA. A angiotensina II é capaz de ativar a NADPH oxidase através dos receptores AT1 que, por sua vez, irá favorecer a formação de superóxido. A partir disso, é desencadeado um complexo mecanismo de formação de espécies reativas de oxigênio e o desacoplamento da NO sintase (9).

Diversos mecanismos antioxidantes agem a fim de combater o excesso de agentes oxidantes e estabelecer um equilíbrio no estado redox. Os antioxidantes podem transformar as espécies reativas em outra molécula menos reativa ("*scavenger*"), como também neutralizar completamente a molécula ("*quencher*") (66). Os mecanismos de defesa antioxidante também podem ser divididos em dois grupos, os antioxidantes enzimáticos, tais como a SOD, a catalase e a glutationa peroxidase, e os antioxidantes não enzimáticos, que incluem a glutationa, vitamina C, vitamina E, entre outros. Da mesma forma que vitaminas e minerais, outros antioxidantes podem ser adquiridos de forma exógena pela dieta, com base nisso diversas pesquisas têm sido realizadas na área de suplementação de nutracêuticos – especificamente suplementação de antioxidantes (58).

Apesar de ainda ser uma área nova e em ascendência, alguns estudos tem investigado o papel da L-Arginina como antioxidante. Estudo de Shan, et al. (56) estudou os efeitos da suplementação de L-Arginina em ratos submetidos a protocolos de natação (6x/semana). Os animais suplementados apresentaram maiores concentrações séricas de NO, além disso, o conjunto de resultados encontrados sugeriram menor produção de superóxido do que aqueles que apenas treinaram. Da mesma forma, estudos em Diabetes *mellitus* têm demonstrado resultados promissores. A L-Arginina nessa condição patológica foi capaz de aumentar a atividade de enzimas antioxidantes, tais como a glutationa peroxidase, SOD e catalase (67-69). Estudos testando o papel antioxidante da L-Arginina na

insuficiência cardíaca ainda são escassos, dessa maneira deve ocorrer a realização de mais investigações na área para que futuramente seja possível a prescrição deste suplemento com embasamento científico.

2.4 O Exercício Físico na Reabilitação Cardíaca da IC

A redução na qualidade de vida dos pacientes com insuficiência cardíaca está estreitamente relacionada com a intolerância ao exercício, e à fadiga precoce. Esses fatores são determinantes para que o indivíduo consiga realizar suas tarefas cotidianas com independência. Nesse âmbito, o exercício físico surge como uma terapia eficaz para a melhora da capacidade funcional e reinserção à vida social dos pacientes com IC (16).

Figura 6. Figura esquemática dos principais mecanismos que levam à intolerância ao exercício na insuficiência cardíaca. eNOS: Óxido nítrico sintase endotelial, EROs: Espécies reativas ao oxigênio, GH=Hormônio do crescimento, IGF-1: Fator de crescimento semelhante à insulina. Adaptado de Okita, et al. (5).

Apesar de na década de 50 já existirem evidências de que o exercício físico poderia ser um fator protetor para doenças cardiovasculares (70), até o início da década de 90 não era recomendado que o indivíduo com IC realizasse exercícios físicos, pois se acreditava que àqueles com fração de ejeção reduzida teriam risco aumentado de morbimortalidade relacionado ao exercício físico (17). O principal marco para essa mudança de paradigma foi o estudo de Sullivan, et al. (71), o qual comprovou que o treinamento aeróbico crônico não gerou prejuízos na fração de ejeção do ventrículo esquerdo (FEVE).

Desde então, diversos estudos randomizados tem sido conduzidos em pacientes com insuficiência cardíaca (72-76), confirmando que o exercício físico quando realizado em pacientes estáveis e com supervisão profissional é uma ferramenta segura e capaz de reduzir, tanto internações hospitalares, quanto o risco de mortalidade (77-79). Atualmente sabe-se que o exercício e a reabilitação cardíaca são capazes de modular as disfunções presentes em diferentes sistemas orgânicos nessa patologia e classificam-se como nível A de evidência e classe I de recomendação em diretrizes das sociedades de cardiologia (80, 81).

O aumento da capacidade máxima ao exercício, associado a modificações na função metabólica, tônus vascular, produção de citocinas e modulação neuro-hormonal, sugere um importante e positivo papel do exercício físico (16, 80, 82). Atenuação da atividade simpática, melhora do tônus parassimpático e a consequente melhora na variabilidade da frequência cardíaca durante o exercício foram verificadas em ensaios randomizados e, recentemente, compilados em uma revisão sistemática (83-85). A atenuação da hiperativação da angiotensina II, aldosterona e norepinefrina também são fatores associados ao treinamento físico que é capaz de atuar na regulação da disfunção autonômica (86-88).

O desequilíbrio pró/anti-inflamatório, por sua vez, também parece estar envolvido com a disfunção autonômica, através da influência da interleucina-6 no eixo hipotálamo-pituitária-adrenal (89). O exercício poderia não só promover a redução das citocinas pró-inflamatórias (IL-6, TNF-α, IL-1), mas também aumentar os níveis de citocinas anti-inflamatórias (IL-10) (90-92). Em estudo experimental os ratos com IC que realizaram treinamento aeróbico contínuo por oito semanas (5x/sem) apresentaram menores valores plasmáticos de IL-6 e TNF-α e maior

expressão da IL-10. Além disso, a melhora do perfil inflamatório na IC parece estar associada à melhora da função cardíaca e do padrão de remodelamento (93).

Alguns autores têm atribuído a capacidade de reversão do remodelamento cardíaco diretamente ao exercício físico. Hambrecht, et al. (94) verificaram um aumento em torno de 30% na fração de ejeção e redução no diâmetro do ventrículo esquerdo ao final da diástole. Principalmente o exercício aeróbico (EA) e com duração de treinamento maior do que seis meses tem se mostrado eficaz na reversão do remodelamento (95, 96). Em estudo do nosso grupo de pesquisa, citado anteriormente, o exercício aeróbico contínuo foi capaz de atenuar o remodelamento cardíaco, principalmente devido a menor deposição de colágeno na matriz extracelular (93).

Apesar de o treinamento físico ser capaz de melhorar o percentual de fração de ejeção, com o avanço das pesquisas nessa área, verificou-se que a intolerância ao exercício observada na IC pouco se correlaciona com fatores hemodinâmicos. Assim, possivelmente, os prejuízos periféricos da musculatura esquelética tem uma importante participação nas queixas de fadiga precoce (97, 98). Uma série de alterações pode levar à miopatia esquelética, a qual está associada a disfunções metabólicas, tais como mudança do tipo de fibra (99, 100), menor expressão da PGC-1α e, consequentemente, diminuição na densidade de mitocôndrias (101, 102). A resposta da musculatura esquelética ao exercício aeróbico é maior do que terapias farmacológicas isoladas. O EA, portanto, é capaz de reverter a maioria dos distúrbios relacionados à miopatia e contornar a deficiência na produção e utilização de energia, resultando principalmente num aumento no VO_2 de pico (15, 103, 104).

Além do miocárdio e da musculatura esquelética, o sistema vascular está igualmente prejudicado na insuficiência cardíaca e também pode sofrer influências positivas do exercício físico. A função vascular é regulada principalmente pelo NO, todavia na IC há o desacoplamento da eNOS (68, 105). O exercício ou o estresse de cisalhamento são capazes de ativar a eNOS, sendo assim, os principais fatores de estímulo para vasodilatação endotélio-dependente (68, 106, 107). Em contrapartida, a reduzida disponibilidade do substrato L-Arginina e o excesso de agentes oxidantes contribuem para a reduzida biodisponibilidade de NO (7, 33). O exercício aeróbico crônico já se mostrou capaz de estimular enzimas antioxidantes, atenuando, portanto o estresse oxidativo. Dessa maneira, a aplicação mútua do exercício aeróbico em conjunto com a suplementação de L-Arginina, além de poder atuar mais

expressivamente na estabilidade do estado redox, seria capaz de promover melhora do tônus vascular e da função endotelial (108).

2.4.1 Diferentes Programas de Treinamento Aeróbico e suas Particularidades na IC

Exercícios aeróbicos como caminhada, corrida ou exercício em bicicleta ergométrica são amplamente estudados na população com insuficiência cardíaca. O uso de protocolos com velocidade contínua e com intensidade e duração bem definidas são amplamente utilizados em protocolos de reabilitação cardíaca (109). A utilização mais frequente de intensidades baixas a moderadas e velocidade contínua se deve ao fato de que se acreditava que a intensidade máxima que os indivíduos com IC poderiam atingir seria dentro do primeiro limiar ventilatório, entre 50-60% do pico de VO_2 (17).

Os pacientes com IC, todavia, em comparação a indivíduos saudáveis, precisam de um maior percentual do seu VO_2 de pico para exercer suas funções cotidianas (17). Nesse contexto, atualmente tem se discutido o uso de intensidades acima do primeiro limiar ventilatório. Utilizando-se de intensidades entre 65-90% do $VO_{2máx}$, no limite entre alta intensidade e intensidade severa, no treinamento de indivíduos com IC sem que haja o risco de situações adversas (110, 111). Dessa maneira tem surgido o interesse por diferentes estratégias de treinamento para essa população, como por exemplo, o treinamento aeróbico intervalado.

O treinamento aeróbico intervalado (TAI) pode ser descrito pela alternância de curtos períodos de alta intensidade (>80-85%$VO_{2máx}$) com períodos de baixa intensidade ativa ou passiva. Diferentes protocolos podem ser definidos, temos como exemplo um protocolo de 4 minutos de alta intensidade (90-95%$VO_{2máx}$) intercalados com 3 minutos de baixa intensidade (50% $VO_{2máx}$) (20, 112, 113).

Diversos estudos têm surgido a fim de determinar se o exercício aeróbico intervalado é superior ao exercício aeróbico contínuo, tanto em populações saudáveis, quanto em populações especiais. Alguns estudos tem demonstrado resultados promissores, tais como reversão do remodelamento cardíaco, melhora da função endotelial e melhor capacidade aeróbica, com aumento do $VO_{2máx}$ (113, 114). Entretanto outros delineamentos não verificaram diferenças entre os dois protocolos de treinamento, porém com efeitos positivos de ambos em relação ao grupo controle ou ao *baseline* (20, 115). Vale ressaltar que o grande número de possibilidades de

protocolos pode estar envolvido com a heterogeneidade observada nos ensaios clínicos.

Em 2013, uma meta-análise, ao avaliar diferentes intensidades de treinamento em indivíduos com IC, concluiu que quanto maior a intensidade do exercício maior são os ganhos em capacidade cardiorrespiratória, principalmente devido a maior adesão dos pacientes nesse tipo de protocolo, visto que o aumento de intensidade possibilita a utilização de treinos de curta duração (116). Em contrapartida, outro estudo verificou que as principais variáveis para melhora da capacidade funcional, em ordem decrescente, são: gasto energético da sessão, frequência de sessões, duração do treino e, por último, a intensidade do exercício (117). Em relação aos benefícios cardiovasculares, estudos meta-analíticos têm demonstrado que o TAI é mais efetivo em gerar aumento no VO_2 máximo, tanto em pacientes com doenças cardiovasculares, quanto em indivíduos saudáveis, porém falha em se mostrar superior no aumento da FEVE na população com IC (18, 19). Sendo uma área de investigação ainda muito recente, mais investigações devem ser realizadas.

2.5 Modelos Experimentais de IC e Exercício Aeróbico

O uso de modelos animais é essencial para entender mecanismos moleculares, fisiológicos e testar diferentes terapias. Há a facilidade de desenvolver delineamentos bem controlados e testar diferentes tratamentos comparando com grupos placebo. Nesse contexto, modelos experimentais de insuficiência cardíaca têm sido utilizados com o objetivo de melhor compreender os mecanismos fisiopatológicos envolvidos nessa síndrome. Em virtude do grande número de investigações e novas condutas de tratamento na área, nos últimos 40 anos, foi possível reduzir a mortalidade da IC (118, 119).

O modelo de IC através da cirurgia de infarto agudo do miocárdio é amplamente utilizado. No grupo de Pesquisa em Interação Cardiopulmonar (GPIC), diversos trabalhos já foram conduzidos a partir desse modelo (90, 93, 120-122). Uma vez que o IAM é uma das principais causas de IC em humanos (23), esse modelo animal é capaz de mimetizar disfunções importantes observadas em humanos (123).

O procedimento utilizado pelo grupo de pesquisa foi, pela primeira vez, descrito por Pfeffer et al. (124). A cirurgia de IAM se baseia na ligadura da artéria coronária (LAC) descendente esquerda. Para isso, o animal é anestesiado e intubado, após é realizada a toracotomia esquerda, o coração é exposto, o átrio esquerdo afastado e, então, é realizada a LAC, provocando a isquemia miocárdica. Após a cirurgia, os animais levam em torno de quatro a seis semanas para desenvolver características da fisiopatologia da IC. Nesse modelo, infelizmente, tanto a área de infarto, quanto a mortalidade são dependentes da experiência do cirurgião. A área de infarto pode variar de 4-59%, já a mortalidade de 16 a 60% (124-126).

Apesar da alta mortalidade, os animais que sobrevivem e desenvolvem uma área de infarto acima de 30% mimetizam alterações importantes típicas de pacientes com IC. Observa-se a disfunção tanto sistólica, quanto diastólica, uma vez que os animais apresentam queda no volume sistólico, nas derivadas positivas e negativas de pressão e na pressão sistólica ventricular esquerda (PSVE), bem como aumento da pressão diastólica final do ventrículo esquerdo - valores acima de 20mmHg (125).

Além da indução de patologias, nosso laboratório tem desenvolvido protocolos de exercício especialmente para ratos Wistar (21, 90, 93, 127, 128). Já foram realizados trabalhos com exercício aeróbico, tais como natação e corrida, como também exercício de força, realizado em aparelho de agachamento próprio para ratos. O exercício aeróbico de corrida ou caminhada é realizado em esteira elétrica adaptada para ratos. O aparelho consiste de oito divisórias – podendo treinar oito animais por vez-, porém todas são submetidas à mesma velocidade, a qual será estipulada pelo pesquisador. É possível desenvolver diferentes protocolos de exercício em esteira, tais como treinamento contínuo ou intervalado com distintas intensidades, inclinação e duração.

De acordo com o supracitado, o presente trabalho foi realizado com o intuito de avaliar os efeitos crônicos da suplementação de L-Arginina e do treinamento aeróbico, tanto o contínuo de intensidade moderada, quanto o intervalado de alta intensidade, isolados ou combinados sobre a capacidade funcional, massa muscular, parâmetros hemodinâmicos, perfil inflamatório plasmático e parâmetros de estresse oxidativo no gastrocnêmio e rim de ratos com insuficiência cardíaca.

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4 ARTIGO

L-Arginine supplementation associated with continuous or interval aerobic training improves hemodynamic parameters, inflammatory profile, oxidative stress and attenuates muscle loss in chronic heart failure rats

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Abstract

Objective: To investigate the effects of 8 weeks of L-arginine (Arg) supplementation associated with aerobic continuous training (ACT) or aerobic interval training (AIT) on maximal exercise capacity, pulmonary and hepatic congestion, muscle mass, and hemodynamic, inflammatory, and oxidative stress parameters in rats with chronic heart failure (CHF).

Methods: Thirty-eight male Wistar rats post 6 weeks of myocardial infarction (MI) surgery were randomly assigned into 6 CHF groups: sedentary (SED, $n = 6$); SED+Arg ($n = 7$); ACT ($n = 8$); ACT+Arg ($n = 5$); AIT ($n = 7$); AIT+Arg ($n = 5$). Exercise test capacity (ETC) was performed pre and post 8 weeks of intervention. Supplemented rats received Arg (1g/kg) by oral gavage (7x/week). Exercise training was performed on a rat treadmill (5x/week). The AIT consisted of 7 bouts of 3 min at 85% of maximum ETC velocity with 4-min active recovery at 60% of maximum ETC velocity. The ACT was performed at 60% of maximum ETC velocity until reaching the same distance of AIT. Hemodynamic variables, tissue collection, congestion, inflammatory cytokines, and oxidative parameters were evaluated at the end of protocols.

Results: All trained groups showed a superior exercise capacity compared to SED groups on the post-intervention test ($p<0.0001$). Pulmonary congestion was attenuated in AIT and AIT+Arg compared with the SED group ($p<0.05$). Left ventricular end-diastolic pressure (LVEDP) was lower in continuous ACT+Arg, AIT, and AIT+Arg groups than SED group ($p<0.05$). Association of AIT with Arg supplementation was able to improve hemodynamic responses (left ventricular systolic pressure (LVSP), systolic blood pressure (SBP), dP/dt_{max}, and $-dP/dt_{max}$ ($p<0.05$), likewise, decrease muscular and renal lipid peroxidation and tumor necrosis factor (TNF)-α, and increase interleukin (IL)-10/TNF-α plasmatic levels ($p<0.01$). Groups associated with aerobic exercise and Arg supplementation (ACT+Arg and AIT+Arg) revealed higher gastrocnemius mass compared to the SED group ($p<0.01$).

Conclusions: Both aerobic training protocols were capable to improve aerobic capacity, and the association with Arg supplementation was important to attenuate muscle loss. Moreover, interval training associated with Arg supplementation elicits greater improvements in hemodynamic parameters, contributing to reduction in pulmonary congestion, and demonstrated particular responses in the inflammatory profile and in the antioxidant status.

Keywords: L-Arginine, Chronic heart failure, exercise, interval training, oxidative stress, inflammation

Abbreviation list: +dP/dtmax, maximum positive derivative of LV pressure; ACT, aerobic continuous training; AIT, aerobic interval training; Arg, L-Arginine; CAT, catalase; CHF, chronic heart failure; DBP, diastolic blood pressure; –dP/dtmax, maximum negative derivative of LV pressure; eNOS, endothelial nitric oxide synthase; ETC, exercise test capacity; GM:BM, gastrocnemius mass:body mass; HF, heart failure; HIIT, high intensity interval training; HR, heart rate; IA, infarcted area; IL, interleukin; LV, left ventricle; LVEDP, left ventricular end-diastolic pressure; LVM:BM, left ventricle mass:body mass; LVSP, left ventricular systolic pressure; MDA, malondialdehyde; MI, myocardial infarction; NO, nitric oxide; NOS, nitric oxide synthase; RNS, reactive nitrogen species; ROS, reactive oxygen species; SBP, systolic blood pressure; SED, sedentary; SOD, superoxide dismutase; TNF, tumor necrosis factor.

1. INTRODUCTION

Chronic heart failure (CHF) is a multifactorial syndrome, which leads to important injuries in several organs and tissues [1]. Reduced exercise tolerance is the leading contributor to a poor quality of life in those with CHF. Cardiovascular and ventilatory abnormalities associated with increased activation of neurohumoral systems (i.e., renin-angiotensin-aldosterone and sympathetic systems) are known as the major predictors for early exercise fatigue [2, 3]. In addition, peripheral factors, such as vascular and intrinsic skeletal muscle dysfunctions, inflammation, and increased oxidative stress, are apparently correlated with skeletal muscle wasting and exercise intolerance [4]. Abnormalities of the vascular system in CHF are directly related to impaired nitric oxide (NO) production. Defects in NO bioavailability might be explained by the low plasmatic levels of its amino acid precursor, L-arginine (Arg) or by reduced endothelial NO synthase expression (eNOS) [5].

Since NO plays an important role in endothelial vasodilatation and vascular structure and in inhibition of platelet adhesion [5], diminished bioavailability of Arg in those with CHF was associated with advanced myocardial disturbances [6, 7]. Thereby, some studies have been investigating the restoration of Arg by supplementation. Enhanced blood flow through Arg supplementation may be crucial for increasing exercise tolerance or even for increasing nutrient supply to the skeletal muscle [8, 9]. Moreover, in cardiovascular dysfunctions, Arg might have an important role in inflammation and oxidative stress reduction [10, 11]. In that view, Arg may be a potential supplement for preventing muscle atrophy.

Moreover, since exercise intolerance is the main symptom in those with CHF, there are no doubts that exercise training is an indispensable therapeutic approach [12]. Several studies have been conducted in patients with CHF, confirming that physical exercise when

performed in stable patients and under professional supervision is a safe tool for reducing hospital admissions and mortality risk. Aerobic exercise training is able to improve functional capacity elicited by molecular changes in multiple organs [13]. Furthermore, moderate-intensity continuous training was previously related to an anti-inflammatory effect in those with CHF. Studies conducted in rats verified reductions in both tumor necrosis factor (TNF)-α and interleukin (IL)-6 levels and augmented IL-10 and IL-10/TNF-α ratio levels in plasma or skeletal muscle [14, 15]. Improvements in the inflammatory profile were also related to preserved cardiac function and muscle mass [16].

Currently, training prescription for cardiac rehabilitation has been discussed, considering intensity, duration, frequency, and caloric expenditure. In this way, aerobic interval training (AIT) has emerged. When the main goal is to achieve high intensities, the training is based in interspersed high-intensity intervals with recovery periods of low to moderate intensities or passive recovery. Consequently, AIT allows heart failure (HF) patients to achieve longer periods in high-intensity zones, compared to aerobic continuous training (ACT) [17]. The AIT protocols have demonstrated to be more effective than ACT for improvements on VO_2 peak either in healthy individuals or clinically stable patients with HF [18, 19]. On the other hand, the beneficial effects of AIT on hemodynamic parameters, myocardial hypertrophy attenuation, immune function, and redox status in those with CHF are not well established [18, 20, 21].

Considering the beneficial effects of aerobic exercise training and a possible superiority of AIT associated with Arg supplementation on HF disturbances, the present study was designed to test the effects of 8 weeks of Arg supplementation associated with ACT or AIT on maximal exercise capacity, pulmonary and hepatic congestion, muscle mass, and hemodynamic, inflammatory and oxidative stress parameters in rats with CHF.

2. METHODS

2.1 Animals

Experiments were performed on 38 male Wistar rats weighing between 240 and 290 g (~90 days of age) obtained from the Animal Breeding Unit of the Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA-Brazil). Animals were housed in groups of 3 per cage and received water and a standard chow ad libitum in a controlled room maintained at 22ºC under 12:12 hours light-dark cycle. The investigation obeyed law nº 11,794 of 10/08/2008, law nº 6,899 of 07/15/2009, and Resolution nº 879 of 02/15/2008 (CFMV). All

procedures in this study were approved by UFCSPA Ethics Committee and Institution of Animal Use, under the protocol 14-151.

2.2 Induction of Myocardial Infarction

Rats ($n = 90$) were anesthetized by inhalation of 2% isoflurane (isoforine, 100 ml-Cristália) 100% oxygen [22], endotracheally intubated, and artificially ventilated (Sam Way VR 15). The animals were kept in anesthetic level III plan Guedel during the surgery [23]. The myocardial infarction (MI) induction was based on the method previously described by Pfeffer et al [24]. The heart was exposed through the left thoracotomy between the fourth and fifth ribs and then a 6-O mononylon suture (Ethilon, Ethicon, São Paulo, Brazil) was passed into the main left descending coronary artery, at a point between 1 and 2 mm distal to the edge of the left atrium, and the left coronary artery was ligated. The thorax was closed; the skin was sutured, and the pneumothorax was drained by a continuous aspiration system. After the surgeries, the animals were placed in a heated environment for recovery and received an injection of ketoprofen (5.4 mg/kg/ml, i.p.) every 6 hours completing 48 hours and a single dose of penicillin (20,000 U, i.p.).

2.3 Exercise Test Capacity (ETC)

Five weeks after MI surgery, all rats were submitted to a progressive treadmill running test to measure maximal running capacity. First, animals were submitted to an adaptation period (5 days) and ran 10 to 20 min/day at 10 to 15 m/min. The test protocol was performed at 15º angulation and started at 5 m/min and then the speed was incrementally increased 5 m/min every 3 min until exhaustion. The same test was also performed at the end of the experiments.

2.4 Experimental Design

Animals were allowed 6 weeks for recovery before MI (time necessary to develop the CHF state). Rats were randomized to one of 6 experimental groups: sedentary group (SED, $n = 6$), sedentary supplemented with Arg group (SED+Arg, $n = 7$), aerobic continuous training group (ACT, $n = 8$), aerobic continuous training and supplemented with Arg group (ACT+Arg, $n = 5$), aerobic interval training group (AIT, $n = 7$), and aerobic interval training supplemented with Arg group (AIT+Arg, $n = 5$).

The mortality rate post-surgery of the animals was ~45% and 9 animals were excluded because they lacked the minimum infarcted area to characterize HF (infarct size <35%) (see Figure 1).

2.5 L-Arginine Supplementation Protocol

Supplemented groups were given a daily treatment of Arg (presentation form: powder, 99.9% purity, Sigma-Aldrich, Brazil). The supplementation dose was 1 g/kg/ml per day; solutions were made with distilled water and given by oral gavage to ensure that the desired dose was achieved. Animals were weighed every 15 days to adjust the dose if necessary.

2.6 Training Protocol

After the adaptation period, the animals allocated to the trained groups began the aerobic exercise training. It was performed on a motorized treadmill 5 days/week for 8 weeks. Previous to the specific protocol, both AIT and ACT groups performed 8-minute warm-up periods at 10 m/min. The AIT consisted of 7 periods of 7 minutes of exercise, consisting of intervals of 3 minutes at 85% of maximum ETC velocity (~20 m/min) and 4 minutes of active recovery at 60% of maximum ETC velocity (~14 m/min). In addition, ACT was performed at 60% maximum ETC velocity (~14 m/min) until completing the same distance reached at AIT.

2.7 *Cardiac Hemodynamic, Infarct size, Lung and Liver Edema, Blood and Tissue Collection*

At the end of the 8-week training and supplementation protocols, in order to rule out any interference of acute exercise or supplementation, assessments were performed 2 days after the final ETC. Afterward, rats were anesthetized with xylazine (12mg/kg, i.p.) and ketamine (90mg/kg, i.p.) for hemodynamic evaluation. A catheter was connected to a pressure transducer (Strain – Gage – Narco Biosystem Miniature Pulse Transducer RP-155, Houston, TX, USA) and coupled to a pressure amplifier (Stoelting, Wood Dale, IL, USA) and was placed into the right carotid artery to measure arterial and ventricular pressures. Arterial pressure was first recorded and then the catheter was positioned inside the left ventricle (LV); both arterial and ventricular pressures were recorded during a 5-minute period. Signals were digitized with a data acquisition system (CODAS – Date Acquisition System). The collected data were used to determine heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), left ventricular systolic pressure (LVSP), maximum positive (+dP/dtmax)

and negative (–dP/dtmax) derivatives of left ventricular pressure, and left ventricular end-diastolic pressure (LVEDP).

Arterial blood was collected, stored in 2 ml Eppendorf microtubes containing ethylenediaminetetraacetic acid (EDTA), and subsequently centrifuged (3,000 rpm for 10 minutes at 4ºC). Supernatant plasma was collected and stored at -20ºC. Following the blood collection, tissues (heart, lungs, kidney, liver, and gastrocnemius) were collected and weighed. The heart was removed and the LVs were dissected and weighed. The LV and gastrocnemius weight were adjusted for animal body mass and were expressed in milligrams of tissue per gram of body mass (mg/g BM). The MI area was evaluated by planimetry using Image J 1.47 software. The lungs and liver were dehydrated (80ºC) for 48 hours and then weighed again to assess water percentage. The other tissues were frozen at -80ºC.

2.8 Oxidative Stress Evaluation

2.8.1 Tissue homogenization

Gastrocnemius muscle and kidney samples were homogenized in a KPi buffer (30 mM KCl + KH_2PO_4), pH 7.4, with a proportion of 9 mL/g of tissue for gastrocnemius and 5 mL/g for the kidneys. Homogenized samples were centrifuged at 3,000 rpm for 10 minutes at 6ºC to remove cell debris, and the supernatant was used as a sample.

2.8.2 Total protein by Bradford method

The Bradford method was used to assess the dosage of protein in the tissue [25]. A homogenized sample (10 µl) was diluted in distilled water (190 µl). Sixty microliters of this solution were placed in plastic cuvettes (optical path: 10 mm) containing 2,900 uL of Bradford reagent, previously prepared, and 40 uL of distilled water. Absorbance readings were taken at 595 nm, in a Lambda 35 spectrophotometer (PerkinElmer of Brazil, SP, Brazil). The protein standard curve was obtained from known concentrations of bovine serum albumin solutions. Results are expressed in milligrams of protein per milliliter of sample.

2.8.3 Catalase activity

Catalase activity (CAT) was determined through the decomposition of hydrogen peroxide (H_2O_2). In a quartz cuvette, 2,865 µl of phosphate buffer (50 mM; pH 7.0) and 30 µl of homogenized tissue were added. Then, 105 µl of 0.02 M hydrogen peroxide was added to the solution. Sample absorbance was determined in a Lambda 35 spectrophotometer

(PerkinElmer of Brazil, SP, Brazil), at 240 nm per 120 s, and the results are expressed in CAT units per milligram of total protein.

2.8.4 Superoxide dismutase activity

Superoxide dismutase (SOD) activity consists of the reaction of inhibition of pyrogallol auto-oxidation by SOD activity. One unit of SOD is defined as the enzyme quantity capable of inhibiting 50% of the reaction. A total of 970 µl of tris buffer (TRIS 50 mM/EDTA 1 mM; pH 8.2), 4 µl of 30 µM catalase and 10 µl of homogenized tissue supernatant was placed into cuvettes. Then, 16 µl of 24 mM pyrogallol in 10 mM HCl was added to the solution. Absorbance measures were determined in a Lambda 35 spectrophotometer (PerkinElmer of Brazil, SP, Brazil), at 420 nm after 60 and 120 s. Results were expressed in SOD units per milligram of total protein.

2.8.5 Malondialdehyde through thiobarbituric acid reactive substances test

To measure lipid peroxidation, malondialdehyde (MDA) concentration was determined by the technique of thiobarbituric acid reactive species (TBARS). To promote the precipitation of proteins, 1 ml of tissue homogenate was added to 1 ml of 10% trichloroacetic acid solution (TCA). After standing on ice for 30 min, samples were centrifuged (3,000 rpm for 10 min at 6ºC). Supernatant was collected (~1.8 ml) and 1.8 ml of 0.670% thiobarbituric acid was added (1:1 proportion). Next, the mixture was heated at 100ºC in a water-bath for 1 hour. After cooling, 2.5 ml of butanol was added and samples were once again centrifuged (2,000 rpm for 5 min at 6ºC), the supernatant was collected and placed in glass cuvettes to determine the absorbance at 532 nm in a Lambda 35 spectrophotometer (PerkinElmer of Brazil, SP, Brazil). The MDA standard curve was obtained from known concentrations of 1, 1, 3, 3 tetramethoxypropane (TMP). Results were expressed in nanomoles per milligram of total proteins.

2.9 Determination of Plasma TNF-α and IL-10 Levels

Plasma TNF-α and IL-10 protein levels were determined by multiplex bead array using Milliplex MAP rat cytokine kits (RCYTO-80 K) (Millipore, Billerica, MA, USA). Cytokines results are reported in picograms per milliliter.

2.10 Statistical Analysis

All data are presented as the mean ± SD. The Kolmogorov-Smirnov test was used to test normal distribution. One-way analysis of variance (ANOVA) and the Student Newman-Keuls post-hoc test were used to compare the groups. The two-way ANOVA followed by the Bonferroni post-hoc test was used to test groups at different times. The accepted significance level was 5% ($p<0.05$). Data were evaluated using the software GraphPad Prism 5 for Windows (GraphPad Software, San Diego, CA).

3. RESULTS

3.1 Infarct Size, Body Mass, Heart and Gastrocnemius Mass, Pulmonary and Hepatic Congestion

Infarct size, initial and final body mass, heart and gastrocnemius mass, and congestion measures are shown in Table 1. No statistical differences were observed among groups on the myocardial infarcted area, suggesting a uniform disease condition in all groups. The LVM:BM ratio and initial and final body mass were similar between groups. Groups associated with aerobic exercise and Arg supplementation (ACT+Arg and AIT+Arg) revealed higher gastrocnemius mass compared to the SED group. Lung and liver wet-to-dry ratios were taken to determine the water percentage in tissues, as an indication of congestion. Hepatic congestion did not show statistical differences among groups. By contrast, pulmonary congestion was attenuated in both AIT groups (AIT and AIT+Arg) compared with the SED group.

3.2 Maximum Exercise Test

At the beginning of the study, all groups presented similar performance on pre ETC ($p>0.05$). After 8 weeks of training, AIT and ACT protocols were able to improve maximal exercise capacity when comparing pre and post measurements within each trained group and compared to SED groups on the post-intervention test (Figure 2.A and 2.B) ($p<0.001$).

3.3 Hemodynamic Variables

Hemodynamic variables are listed in Table 2. There were no statistical differences in resting HR or DBP among groups. The LVEDP was lower in the continuous training group supplemented with Arg (ACT+Arg) and in both interval training groups (AIT and AIT+Arg) than in the SED group. Animals in the AIT+Arg group also presented higher LVSP compared

to the sedentary animals (SED and SED+Arg) and higher SBP versus the SED group only. Similar results were observed on positive and negative dP/dt, on which the AIT+Arg group presented superior values when compared to SED, SED+Arg, ACT, and SED, respectively.

3.4 Oxidative Stress and Antioxidant Enzymes Activity

Figure 3 shows the antioxidant enzymes activity and lipoperoxidation levels in gastrocnemius. The CAT activity in gastrocnemius was similar among groups (Figure 3.A), while activity of SOD was higher in trained supplemented groups (ACT+Arg and AIT+Arg) compared to other groups (Figure 3.B). Lower MDA concentrations were observed in supplemented groups when compared to SED and ACT groups (Figure 3.C).

Figure 4 shows the antioxidant enzyme activity and lipoperoxidation levels in the kidney. Renal CAT activity was greater in ACT+Arg and AIT+Arg groups compared to SED and also ACT+Arg compared to SED+Arg (Figure 4.A). No differences were observed among groups in relation to SOD activity in this tissue (Figure 4.B). Lower MDA concentrations were observed in both trained and supplemented groups (ACT+Arg and AIT+Arg) in relation to all other groups (Figure 4.C).

3.5 Plasma TNF-α and IL-10

Figure 5 shows the cytokine plasmatic levels after 8 weeks of intervention. Plasma levels of TNF-α were lower in both aerobic interval training groups (AIT and AIT+Arg) compared with all other groups (Figure 5.A). When IL-10 plasma levels were analyzed there were no significant differences among groups (Figure 5.B); however, the IL-10/TNF-α ratio was greater in the AIT groups versus all other groups (p<0.0001) (Figure 5.C).

4. DISCUSSION

To our knowledge, this is the first study that investigated the effects of Arg supplementation associated with distinct aerobic training protocols (ACT and AIT) in experimental CHF. According to our aims, the major findings of the present study were 1) association of aerobic training and Arg supplementation (ACT+Arg and AIT+Arg groups) improved muscle mass, 2) pulmonary congestion was reduced only in AIT and AIT+Arg groups, 3) both training protocols were able to increase maximal exercise tolerance, and 4) association of AIT with Arg supplementation was able to improve hemodynamic responses (LVSP, SBP, dP/dt_{max} and $-dP/dt_{max}$), likewise, decrease muscular and renal lipid peroxidation and TNF-α, and increase IL-10/TNF-plasmatic levels.

Induction of HF was based on MI through descendent coronary left artery ligation, which is the most frequent experimental model for CHF studies [24]. Our model mimics a severe CHF. An inflammatory profile and higher lipoperoxidation levels were previously demonstrated by this HF model when compared to sham-operated groups [14, 16].

In the present study, there was no difference in the infarcted area size between groups and the average infarcted area was 40%, which is bigger than preceding studies (~35%), which is in relation to several systemic complications [14, 16, 26]. The increase left ventricular mass was present in all groups. Pulmonary and hepatic congestion is a common symptom, although interval training groups showed lower pulmonary congestion compared to the SED group. Gastrocnemius mass was higher in the ACT+Arg and AIT+Arg groups compared to the SED group. In this view, our experimental model probably induces muscle wasting in non-trained animals. Moreover, functional capacity was impaired and the LVEDP was significantly increased (>20 mmHg) in the SED group.

Exercise tolerance capacity was improved in all trained groups. In contrast to the previous study of our group, AIT was not superior to ACT for improving functional capacity. In the present report, 85% of maximum ETC speed was used in the higher intensity zone, while Nunes et al. used 92% of maximum ETC speed [21]. In that view, exercise intensity possibly has an important role in improving exercise tolerance in CHF. Conversely, a recent meta-regression analysis demonstrated that, in order of importance, the major contributors for exercise capacity are total energy expenditure, session frequency, session duration, and session intensity [27]. Thus, possible explanations for equal improvements in ACT and AIT in the present study are that, in order to correct disparities, both groups had matched session frequency and distance roamed at the end of each training session.

On the other hand, AIT seems to be superior for improving hemodynamic parameters, primarily when associated with Arg supplementation. Association of AIT and Arg was able to increase positive and negative dP/dtmax as well as normalize LVSP and SBP. Moreover, LVEDP was significantly reduced in both AIT along with ACT+Arg. On AIT groups, hemodynamic responses went along with improvements on pulmonary congestion. Diminished lung water percentage can be explained by lower LVEDP, since higher afterload will possibly culminate in severe pulmonary congestion [28].

Improvements on the hemodynamic aspects by Arg supplementation in conditions of endothelial dysfunction had already been described. The Arg actions on hemodynamic are primarily related to improvements in blood flow [29], controlled mainly by endothelium-dependent relaxation via NO [30, 31]. In healthy subjects, Arg supplementation in association

with resistance training did not change hemodynamic or vascular parameters [32]. In our study, Arg supplementation by itself was not able to significantly improve hemodynamic parameters. However, Arg apparently enhanced aerobic interval training effects, suggesting a synergic effect between AIT and Arg therapies.

The Arg effects may not be exclusively centered on NO production [30]. It was previously described that Arg also acts as an angiotensin-converting enzyme (ACE) inhibitor [33]. Angiotensin II (ANG II) modulates sympathetic nerve activity promoting sympatho-excitation. In cardiovascular diseases, an excess of ANG II stimulates removal of mitochondrial ROS to cytosol, leading to more ROS production and, thus, eNOS uncoupling [34]. At the same time, ANG II is able to arouse NO synthase (NOS) to turn into a source of $O_2^{\bullet-}$ via protein kinase C (PKC) [35]. Moreover, aerobic exercise training has the potential to diminish sympathetic activation through ANG II [36]. These data complement the theory for antioxidant actions of Arg and, apart from that, support the synergic effects on hemodynamic parameters among Arg supplementation and AIT in our study.

The present results demonstrated that groups with Arg supplementation combined with aerobic exercise, either ACT or AIT, significantly diminished MDA levels. These results are in concordance with a study by Huang et al., which demonstrated Arg was able to blunt lipoperoxidation caused by an exhaustive exercise in both skeletal muscles and kidneys [10]. In our study, gastrocnemius MDA levels were also lower in SED+Arg versus SED and ACT groups. However, SOD and CAT were not increased in the gastrocnemius of the SED+Arg group. Accordingly to this, Arg might have acted as an ROS scavenger, capable of reducing lipid damage itself.

In classical situations of imbalance between ROS formation and ROS degradation, like cardiovascular diseases and exhaustive exercise, the SOD enzyme was decreased in both skeletal muscle and plasma [37-39]. This is probably because, in stress conditions, an excess of H_2O_2 could inhibit SOD activity [40]. Nevertheless, as shown in previous studies, Arg supplementation was able to restore SOD activity [38, 40]. Thus, a longer supplementation period results in greater SOD activity [40]. In concordance with those previous trials, we found augments in gastrocnemius SOD activity in both trained groups after chronic Arg supplementation. No differences were seen in gastrocnemius CAT activity. Conversely, kidney SOD activity was similar among groups, and CAT activity was increased in both trained and supplemented groups (ACT+Arg and AIT+Arg).

Excess of reactive oxygen species (ROS) and reactive nitrogen species (RNS) could intensify the inflammatory process that occurs after muscle damage or in disease conditions,

such as HF [41, 42]. Despite this, several studies have reported that regular aerobic exercise training has an anti-inflammatory effect in CHF [14, 43]. In the present study, anti-inflammatory responses were seen in AIT groups; both had lower plasma TNF-α expression and increased IL-10/TNF-α ratio in relation to other groups. Increased IL-10/TNF-α ratio and decreased TNF-α expression in CHF have been reported by previous experimental studies, although those improvements were seen after continuous moderate-intensity protocols [16, 44]. Similar to the present report, Souza et al. observed an increase in serum TNF-α levels in the HF SED group compared to the SED control group [45]. However, moderate-intensity continuous exercise only attenuated TNF-α, with no significant decreases in relation to the HF sedentary group. In other chronic diseases, it has already been proposed that high-intensity interval training (HIIT) may elicit greater benefits. In addition, HIIT was proposed to reduce oxidation and inflammation by up-regulating specific mRNA expression [46, 47].

Gastrocnemius mass was higher in trained groups associated with Arg supplementation. It has already been reported that Arg has the potential to stimulate anabolism and also to improve immune function [48]. In HF, a balance between catabolic and anabolic function is directly related to oxidative stress and the immune system [49]. Thereby, aerobic exercise training and Arg supplementation may act synergistically in preserving skeletal muscle mass. Since gastrocnemius MDA levels were reduced in both ACT+Arg and AIT+Arg, those groups might have increased anti-inflammatory cytokines or decreased TNF-α in skeletal muscle, leading to a reduced skeletal muscle atrophy.

Our study has some limitations. Training stimulus was the same during the 8 weeks without ETC during protocols for progressive intensity adjustment. In addition, echocardiography to assess cardiac function or changes in ventricular diameter and histological technique for LV mass evaluation could have improved the accuracy of our results. Finally, future studies with ANG II, NO, IL-6, and eNOS analysis may contribute for more robust conclusions about the mechanisms underlying those CHF therapies.

In conclusion, our data indicated that aerobic physical training is important to improve exercise tolerance in CHF-induced rats, despite intensity. In association with Arg supplementation, aerobic exercise was able to attenuate muscle loss. Moreover, AIT associated with Arg supplementation elicits greater improvements in hemodynamic parameters, contributing to a reduction in pulmonary congestion, and was shown to have particular responses in the inflammatory profile and antioxidant state.

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Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

Barcelos GT and Nunes RB: study design, conduct of the study, molecular biological analysis, data collection and statistical analysis, data interpretation, manuscript writing and critical review. Dal Lago P: study design, statistical analysis, data interpretation, manuscript writing and critical review. Rossato DD, Jaenisch RB, Pinheiro LP, Perini JL: conduct of the study and data collection. Carvalho C: conduct of the study, molecular biological analysis and data collection. Rodhen CR: molecular biological analysis.

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Tables

Table 1: Infarct Size, body mass, pulmonary and hepatic congestion, heart and gastrocnemius mass of the 6 studied groups

	SED	SED+Arg	ACT	ACT+Arg	AIT	AIT+Arg
IBM (g)	274±10	263±18	272±18	263±18	276±10	256±12
FBM (g)	356±45	342±41	338±30	344±34	334±22	344±38
IA (%)	38±3	45±2	39±4	40±1	41±6	42±2
PC (%)	83±2	80±1	78±3	79±5	76±3*	76±3*
HC (%)	71±1	71±1	70±2	71±1	71±1	71±1
LVM:BM (mg/g)	2.5±0.1	2.7±0.2	2.5±0.2	2.5±0.2	2.6±0.2	2.5±0.1
GM:BM (mg/g)	4.5±0.6	5.4±0.7	5.2±0.8	6.2±0.7*	5.6±0.2	6.0±1*

Values are mean±SD. SED: sedentary; ACT: aerobic continuous training; AIT: aerobic interval training; IBM: Initial body mass; FBM: final body mass; IA: infarcted area; PC: pulmonary congestion; HC: hepatic congestion; LVM:BW: left ventricle mass:body mass; GM:BM: gastrocnemius mass: body mass. One-way ANOVA followed by the Student-Newman-Keuls post-hoc test was used for statistical analysis.
*$p<0.05$ vs. SED

Table 2: Hemodynamic of the 6 studied groups after 8 weeks of intervention

	SED	SED+Arg	ACT	ACT+Arg	AIT	AIT+Arg
HR (bpm)	223±21	223±20	208±11	207±21	220±11	230±19
LVEDP (mmHg)	30±6†	22±5	22±8	15±9*	14±6*	16±9*
LVSP (mmHg)	84±12	97±13	103±9	103±12	101±6	125±28‡
dP/dt_{max}(mmHg/s)	3597±773	3944±829	4146±399	4748±704	4456±567	5792±1300†
$-dP/dt_{max}$ (mmHg/s)	2401±489	2701±544	2647±170	2965±717	3040±227	3100±645*
SBP (mmHg)	84±11	95±12	97±14	100±10	96±8	109±11*
DBP (mmHg)	77±8	82±13	80±8	77±6	79±3	86±5

Values are mean±SD. SED: sedentary; ACT: aerobic continuous training; AIT: aerobic interval training; HR: heart rate; LVEDP: left ventricular end-diastolic pressure; LVSP: left ventricular systolic pressure; +dP/dtmax: maximum positive derivative of LV pressure; – dP/dtmax : maximum negative derivative of LV pressure; SBP: systolic blood pressure; DBP: diastolic blood pressure. One-way ANOVA followed by the Student-Newman-Keuls post-hoc test was used for statistical analysis.
*P<0.05 vs. SED; †P<0.05 vs. SED, SED+Arg and ACT; ‡P<0.05 vs. SED and SED-Arg

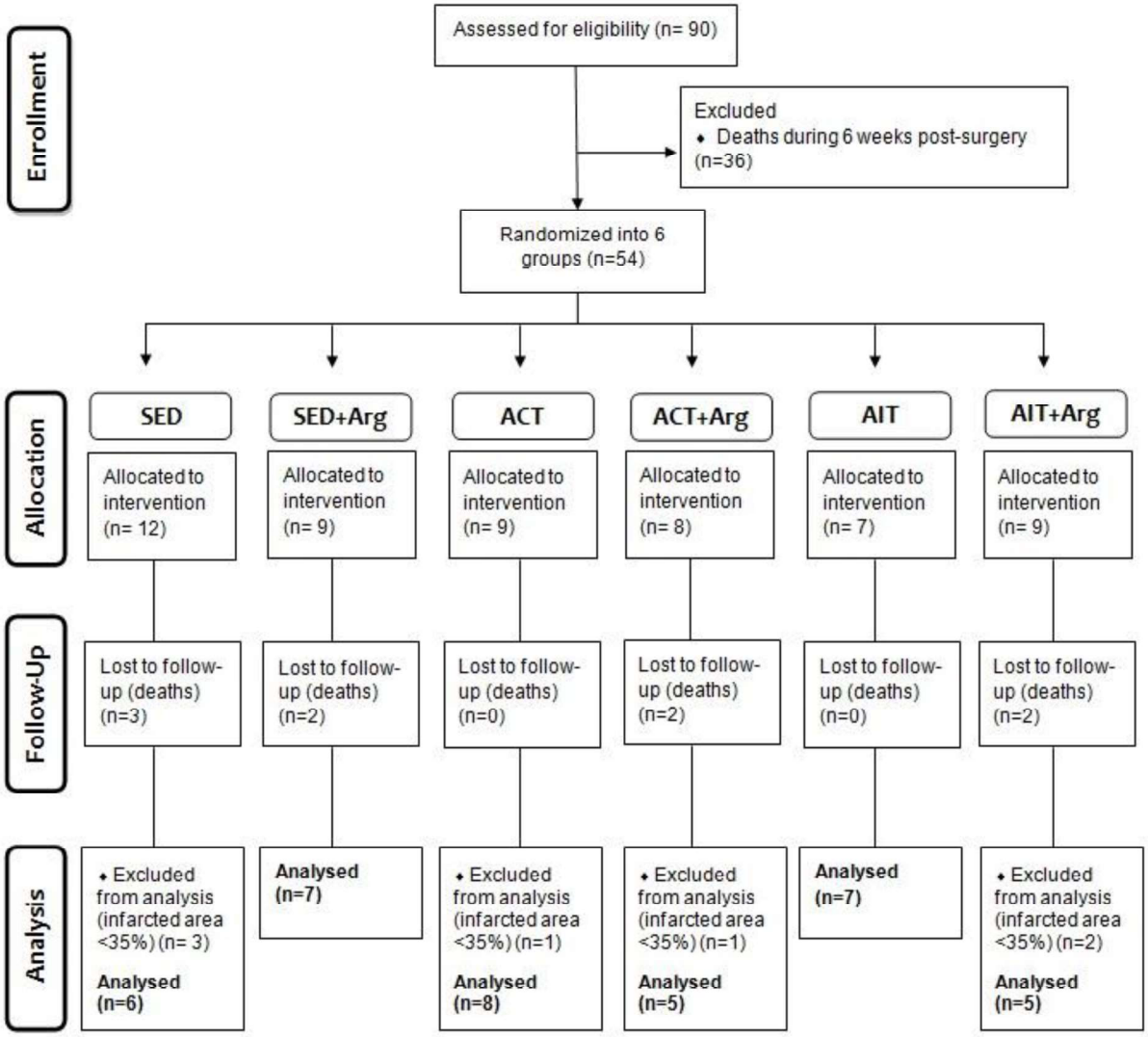

Enrollment
Assessed for eligibility (n= 90)
Excluded
• Deaths during 6 weeks post-surgery (n=36)
Randomized into 6 groups (n=54)
Allocation
SED
SED+Arg
ACT
ACT+Arg
AIT
AIT+Arg
Allocated to intervention (n= 12)
Allocated to intervention (n= 9)
Allocated to intervention (n= 9)
Allocated to intervention (n= 8)
Allocated to intervention (n= 7)
Allocated to intervention (n= 9)
Follow-Up
Lost to follow-up (deaths) (n=3)
Lost to follow-up (deaths) (n=2)
Lost to follow-up (deaths) (n=0)
Lost to follow-up (deaths) (n=2)
Lost to follow-up (deaths) (n=0)
Lost to follow-up (deaths) (n=2)
Analysis
• Excluded from analysis (infarcted area <35%) (n= 3)
Analysed (n=6)
Analysed (n=7)
• Excluded from analysis (infarcted area <35%) (n=1)
Analysed (n=8)
• Excluded from analysis (infarcted area <35%) (n=1)
Analysed (n=5)
Analysed (n=7)
• Excluded from analysis (infarcted area <35%) (n=2)
Analysed (n=5)

Fig. 1

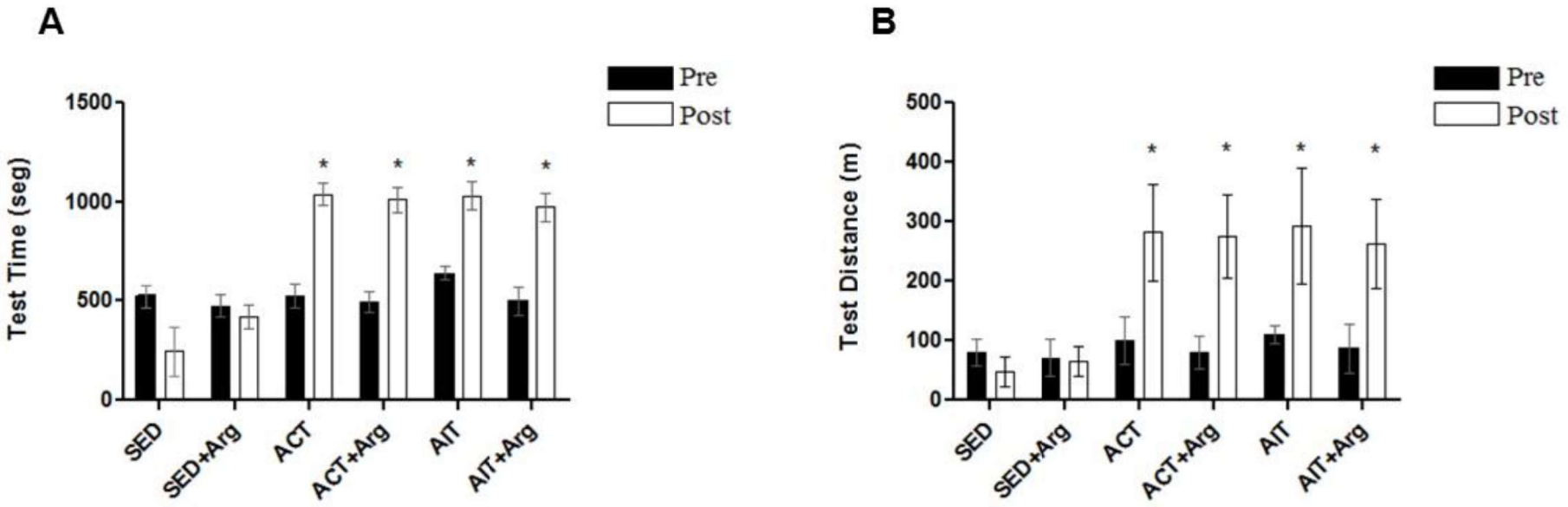

A
Test Time (seg)
1500
1000
500
0
Pre
Post
*
SED
SED+Arg
ACT
ACT+Arg
AIT
AIT+Arg
B
Test Distance (m)
500
400
300
200
100
0
Pre
Post
*
SED
SED+Arg
ACT
ACT+Arg
AIT
AIT+Arg

Fig. 2

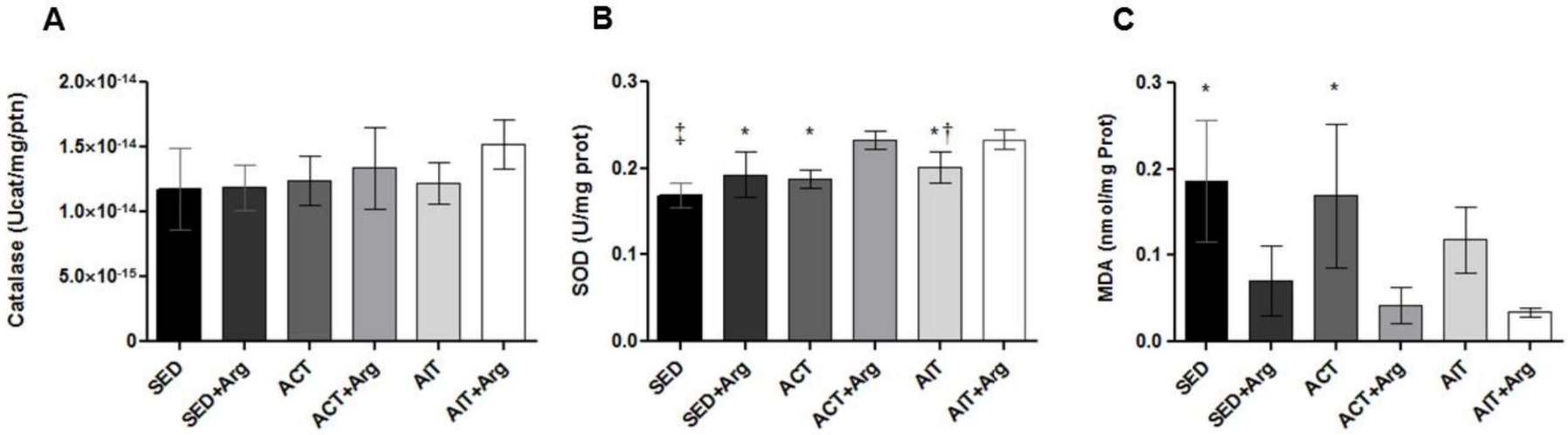

A
Catalase (Ucat/mg/ptn)
2.0×10-14
1.5×10-14
1.0×10-14
5.0×10-15
0
SED
SED+Arg
ACT
ACT+Arg
AIT
AIT+Arg
B
SOD (U/mg prot)
0.3
0.2
0.1
0.0
C
MDA (nmol/mg Prot)

Fig. 3

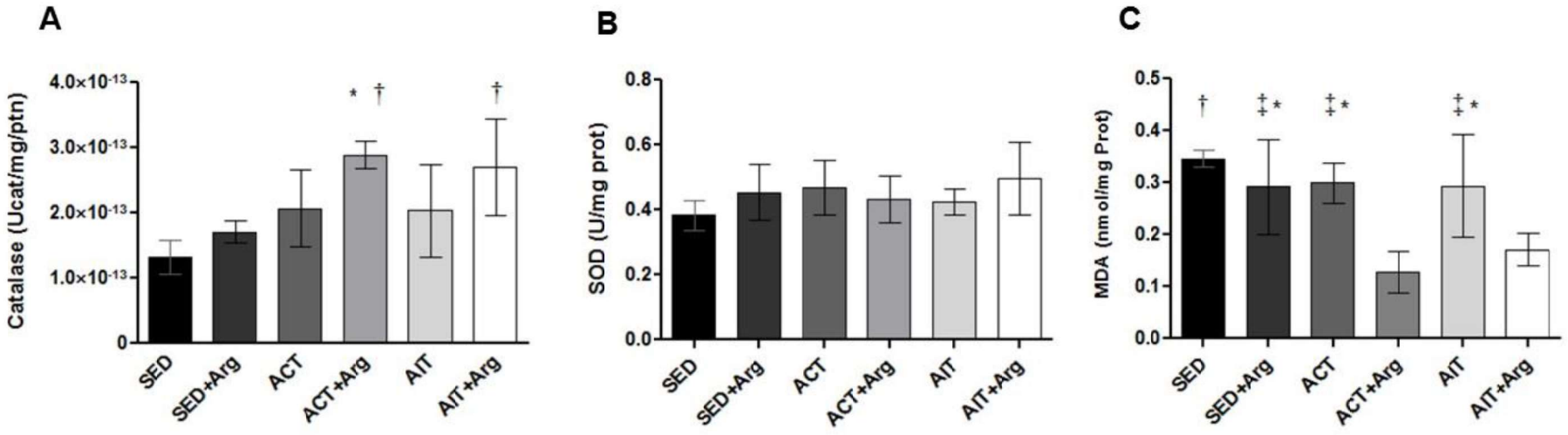

A
Catalase (Ucat/mg/ptn)
4.0×10-13
3.0×10-13
2.0×10-13
1.0×10-13
0
SED
SED+Arg
ACT
ACT+Arg
AIT
AIT+Arg
B
SOD (U/mg prot)
0.8
0.6
0.4
0.2
0.0
C
MDA (nmol/mg Prot)
0.5
0.4
0.3
0.2
0.1
0.0

Fig. 4

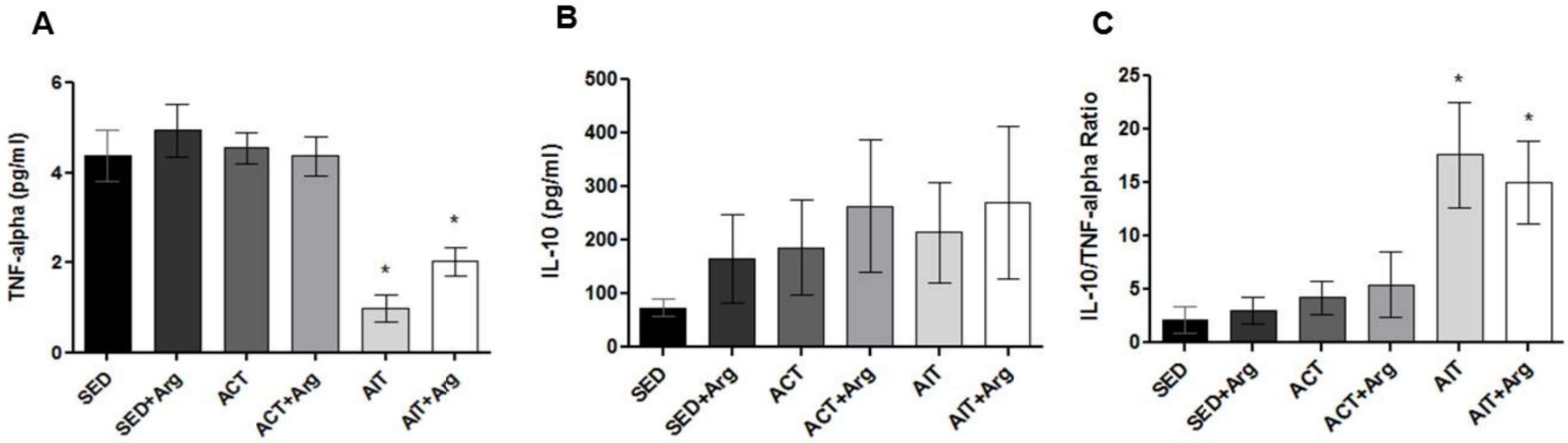

A
TNF-alpha (pg/ml)
0
2
4
6
*
*
SED
SED+Arg
ACT
ACT+Arg
AIT
AIT+Arg
B
IL-10 (pg/ml)
0
100
200
300
400
500
SED
SED+Arg
ACT
ACT+Arg
AIT
AIT+Arg
C
IL-10/TNF-alpha Ratio
0
5
10
15
20
25
*
*
SED
SED+Arg
ACT
ACT+Arg
AIT
AIT+Arg

Fig. 5

Figures Legends

Figure 1 – Fluxogram
SED: sedentary; ACT: aerobic continuous training; AIT: aerobic interval training;

Figure 2- Exercise tolerance test pre and post intervention
Values are the means ± SD. SED: sedentary; ACT: aerobic continuous training; AIT: aerobic interval training. Two-way ANOVA followed by Bonferroni post-hoc test was used for the statistical analysis.
*P<0.01 vs. SED and SED+Arg in pre and post moment; ACT, ACT+Arg, AIT, AIT+Arg in pre moment.

Figure 3 - Oxidative stress in gastrocnemius after 8 weeks of intervention. Concentrations of A) Catalase (CAT) in gastrocnemius; B) Superoxide dismutase (SOD) activity in gastrocnemius; and C) Malondialdehyde (MDA) activity in gastrocnemius. Values are the means ± SD. SED: sedentary; ACT: aerobic continuous training; AIT: aerobic interval training. One-way ANOVA followed by Student-Newman-Keuls post-hoc test was used for the statistical analysis.
B) * P<0.001 vs. ACT+Arg, AIT+Arg ; † P<0.01 vs. AIT+Arg; ‡ P<0.0001 vs. ACT+Arg, AIT+Arg
C) * P<0,001 vs. SED+Arg, ACT+Arg, AIT+Arg

Figure 4 - Oxidative stress in kidney after 8 weeks of intervention.
Concentrations of A) Catalase (CAT) in kidney; B) Superoxide dismutase (SOD) activity in kidney; and C) Malondialdehyde (MDA) activity in kidney. Values are the means ± SD. SED: sedentary; ACT: aerobic continuous training; AIT: aerobic interval training. One-way ANOVA followed by Student-Newman-Keuls post-hoc test was used for the statistical analysis.
A) * P< 0.01 vs. SED+Arg; † P<0.01 vs. SED
C) * P<0.01 vs. AIT+Arg; † P<0.01 vs. AIT+Arg, ACT+Arg; ‡ P<0.001 vs. ACT+Arg

Figure 5 - Plasmatic levels of anti and pro-inflammatory cytokines after 8 weeks of intervention.
Plasmatic concentrations of A) Tumor Necrosis Factor-alpha (TNF-α); B) Interleukin-10 (IL-10); and C) IL-10:TNF-α ratio. Values are the means ± SD. SED: sedentary; ACT: aerobic continuous training; AIT: aerobic interval training. One-way ANOVA followed by Student-Newman-Keuls post-hoc test was used for the statistical analysis.
*P<0.001 vs. all SED, SED+Arg, ACT, ACT+Arg.

5 CONCLUSÃO GERAL

Ao final de nosso estudo concluímos que os protocolos de exercício físico, aeróbico contínuo ou intervalado, foram capazes de aumentar a capacidade máxima ao exercício. Ainda, tanto o protocolo de exercício contínuo, quanto o intervalado, em associação à suplementação de L-Arginina foi eficaz em atenuar a perda de massa muscular no modelo experimental estudado.

O treinamento intervalado principalmente quando associado à suplementação de L-arginina promoveu efeitos mais expressivos na melhora da função hemodinâmica, na diminuição da congestão pulmonar, no perfil inflamatório plasmático e nos parâmetros de estresse oxidativo no músculo gastrocnêmio e nos rins.

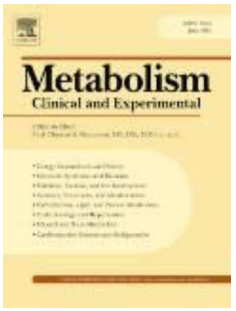
Este trabalho ressalta que estratégias multidisciplinares, tais como a combinação da nutrição e do exercício físico, podem ter um ganho adicional na terapêutica da insuficiência cardíaca crônica. Assim, o presente trabalho espera abrir novas perspectivas de estudo clínicos na reabilitação cardiovascular.

ANEXOS

ANEXO A

METABOLISM
Clinical and Experimental

AUTHOR INFORMATION PACK



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ISSN: 0026-0495

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ANEXO B

UFCSPA

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COMISSÃO DE ÉTICA NO USO DE ANIMAIS - CEUA
UFCSPA

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

Projeto: 14-151 **Versão do Projeto:** **Versão do TCLE:**

Pesquisadores:
PEDRO DALL'AGO
RAMIRO BARCOS NUNES
GIOVANA BARCELOS

Título: TREINAMENTO AERÓBIO CONTÍNUO OU INTERVALADO E SUPLEMENTAÇÃO DE L-ARGININA NA INSUFICIÊNCIA CARDÍACA EXPERIMENTAL

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, 12 de novembro de 2014.

Katya V. Rigatto
Coordenadora do CEUA
UFCSPA