“HEMODINÂMICA NÃO-INVASIVA, BIOLOGIA MOLECULAR E MARCADORES INFLAMATÓRIOS NA PERSPECTIVA DO EXERCÍCIO FÍSICO: DA INSUFICIÊNCIA CARDÍACA AO TRANSPLANTE CARDÍACO”

Tese

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SUMÁRIO

RESUMO

As doenças cardiovasculares (DCVs) apresentam uma grande prevalência no Brasil. A taxa de pessoas com algum tipo de DCVs é de 6025 para cada 100 mil habitantes. O estado do Rio Grande do Sul está acima da média nacional, com um valor de 6182 para cada 100 mil habitantes (valores padronizados por idade). A incidência também é alta, com um número de 687 casos por 100 mil habitantes no Brasil. Dentre os inúmeros tipos de DCVs, podemos destacar as três mais prevalentes, sendo elas a doença isquêmica do coração (DIC), acidente vascular cerebral (AVC) e a insuficiência cardíaca (IC). A IC em especial pode ser considerada uma síndrome complexa, de diagnóstico clínico, a qual pode estar relacionada com a maioria das DCVs, por exemplo um infarto agudo do miocárdio (IAM) que leva a um remodelamento cardíaco causando insuficiência na contratilidade do miocárdio. Abordaremos em especial a insuficiência cardíaca com fração de ejeção reduzida (ICFEr), a qual possui prognóstico preservado quando o paciente adere ao tratamento farmacológico. No entanto, em alguns casos, o prognóstico é ruim e há risco de óbito, e em casos de doença terminal, o transplante cardíaco (TxC) pode ser indicado. O paciente com ICFEr apresenta uma redução na fração de ejeção, ou seja, o débito cardíaco (DC) pode estar comprometido contribuindo para a intolerância ao esforço. Com avanço tecnológico, diversas ferramentas surgiram com o intuito de auxiliar no diagnóstico e no tratamento. Avaliar o DC em tempo real é uma tecnologia disponível e acessível, por meio da cardioimpedânicia de sinal morfológico (SM-ICG). A vantagem desse método é que por não ser invasivo, pode ser associado a outros testes com a intenção de aumentar quantitativamente e qualitativamente as informações para o acompanhamento do paciente com ICFEr. Alguns estudos avaliam indivíduos com ICFEr utilizando o SM-ICG durante o teste de caminhada de 6 minutos (TC6M), no entanto, para nosso conhecimento, ainda não foi demonstrado a resposta da aceleração e desaceleração do DC ao TC6M, duas importantes variáveis que estão diretamente relacionadas com a intolerância ao esforço. Portanto, o objetivo principal da tese foi avaliar a resposta da aceleração e desaceleração do DC ao TC6M, comparando os resultados entre indivíduos saudáveis e com ICFEr. Por sua vez, desenvolvemos três estudos: 1º - Signal-morphology impedance cardiography responses during the six-minute walk test in heart failure with reduced ejection fraction: a case-control study; 2º - Aerobic exercise and telomere length in patients with systolic heart failure: protocol study for a randomized controlled trial; 3º - Cytokines responses after aerobic training in heart transplant recipients: a systematic review and meta-analysis. Traremos os principais

resultados dos três artigos. Para o primeiro artigo, o qual tange o objetivo principal da presente tese, encontramos valores semelhantes para a idade na comparação entre os grupos (ICFEr, 64±8 anos; controle saudável, 65±5 anos; P=0,66). A aceleração do DC diferiu significativamente entre os grupos (P<0,01), em favor dos controles. Em contraste, a desaceleração do CO não diferiu entre os grupos (P=0,07). Houve diferenças significativas no volume sistólico (VS), DC e índice cardíaco entre os grupos, especialmente para os valores de pico (P<0,01). A regressão linear mostrou prejuízo na contribuição do VS (22,9% versus 57,4% no controle) para a alteração do DC na ICFEr, como esperado. O segundo artigo tem como temática um estudo de protocolo para condução de um ensaio clínico randomizado (ECR), o qual apresenta todo racional teórico e metodológico do ECR. O estudo de protocolo é sobre todos métodos por trás de um treinamento aeróbio e seus possíveis efeitos no comprimento dos telômeros em indivíduos com ICFEr. Para a condução do ECR, homens e mulheres entre 50-80 anos serão recrutados e alocados em dois diferentes grupos: um que fará treinamento aeróbio de intensidade moderada (TAIM) e um grupo controle sham. Comprimento dos telômeros, capacidade funcional, variáveis ecocardiográficas, função endotelial e velocidade autosselecionada serão avaliados antes e após 16 semanas de intervenção (32 sessões). Esse estudo de protocolo é fundamental para o prosseguimento do ECR, onde será possível discutir com a literatura e por meio de peer-review sobre todo racional teórico antes de iniciar a intervenção. Compreender o papel do exercício físico no envelhecimento biológico em pacientes com ICFEr é relevante. Devido à senescência celular, esses indivíduos apresentam um menor comprimento de telômero. TAIM pode retardar o envelhecimento biológico de acordo com um equilíbrio no estresse oxidativo por meio da ação antioxidante. Resultados positivos para o comprimento dos telômeros são esperados para o grupo de TAIM. O terceiro artigo aborda um outro cenário de paciente: o que sofre TxC, o qual está diretamente relacionado com a ICFEr. Nesse último artigo, abordamos a relação entre o treinamento físico e a modulação no sistema inflamatório em indivíduos pós-TxC, por meio de uma revisão sistemática com metanálise. Nesse estudo encontramos que o grupo de realizou treinamento aeróbio apresentou uma redução na interleucina-6 (IL-6) em comparação com o grupo controle (Effect size - ES: -0,53; Intervalo de confiança – IC95%: 0,99 a -0,06 pg/ml; P = 0,026). No entanto, o treinamento físico não mostrou efeito significativo nos níveis de fator tumoral de necrose-alfa (TNF-alfa) e na adiponectina (adpN) (ES: -0,33; IC95%: -0,79 a 0,13; P = 0,16 e ES: -0,20; IC95%: -0,70 a 0,30 pg/ ml; P = 0,444, respectivamente). Por

fim, todos artigos tem uma temática central: cardiologia e exercício físico. Os dados apresentados são parte dos resultados encontrados nos artigos desenvolvidos no presente projeto de doutorado para apresentação da tese. É importante salientar que o exercício físico tem papel central na melhora de diferentes parâmetros do paciente cardiopata. Portanto, a nós profissionais da saúde que trabalham com exercício físico, é importante que reforcemos a sua relevância visto que essa ferramenta possui baixo custo e alta efetividade para diferentes desfechos.

1. INTRODUÇÃO

As doenças cardiovasculares (DCVs) corroboram para um cenário de grande frequência no Brasil. Em 2017, a taxa de prevalência padronizada por idade por 100 mil habitantes foi de 6025 (IC95%: 5785; 6274) para ambos os sexos, sendo mais frequente em homens (6536 – IC95%: 6282; 6806).[1] Nesse quesito, o estado do Rio Grande do Sul encontra-se acima da prevalência nacional, apresentando uma taxa de 6182 (IC95%: 5906; 6490) para 100 mil habitantes para ambos os sexos.[2] Tal estatística, cuja última avaliação em nível nacional foi realizada em 2017, evidenciava que as DCVs se consolidavam como a principal causa de morte no país.[1] A incidência também é alta, com 687 (IC95: 663; 712) casos por 100 mil habitantes, no entanto, menor que há duas décadas, com cifras em torno de 755 (IC95%: 731; 783) casos por 100 mil habitantes.[1]

Sabemos que as DCVs possuem associação com sexo feminino (OR= 1,1; IC95%: 1,1-1,1), hipertensão (OR= 2,4; IC95%: 2,2-2,7), elevação de colesterol (OR= 1,6; IC95%: 1,5; 1,8), sobrepeso (OR= 1,5; IC95%: 1,4; 1,8) ou obesidade (OR= 2,0; IC95%: 1,7; 2,2), sedentarismo (OR= 1,5; IC95%: 1,02; 2,1) e com tabagismo (OR= 1,2; IC95%: 1,03; 1,3), ou seja, inúmeros fatores que também estão relacionados com o estilo de vida.[3] Dentre os diferentes tipos de DCVs, podemos destacar as três mais prevalentes, sendo elas a doença arterial coronária isquêmica (DAC), o acidente vascular cerebral (AVC) e a insuficiência cardíaca (IC).[4] A IC em especial é uma síndrome complexa, de diagnóstico clínico, e que pode ser a consequência final da maioria das DCVs, por exemplo, um infarto agudo do miocárdio (IAM), o qual pode promover um remodelamento cardíaco, causando prejuízo na contratilidade miocárdica.[5]

Na atualidade, a síndrome da IC tem sido dividida em IC com disfunção diastólica, no caso, a fração de ejeção (FE) é preservada, mais relacionada com sobrepeso ou obesidade, diabetes, sexo feminino e principalmente hipertensão arterial sistêmica (HAS).[6] O outro tipo de IC é conhecido como insuficiência cardíaca com fração de ejeção reduzida (ICFEr).[7] Nela ocorre um déficit na contratilidade do músculo cardíaco reduzindo a FE. Um dos principais prejuízos observados na ICFEr é o comprometimento do débito cardíaco (DC), o qual resulta em hipoperfusão sistêmica.[5, 7] Esse cenário hemodinâmico hostil, quando associado a alterações pulmonares, periféricas e neuro-humorais, contribuem para uma baixa tolerância ao esforço.[8] A ICFEr possui quatro classificações funcionais por meio da *New York Heart Association* (NYHA) e também possui quatro classificações para categorias de consumo de oxigênio de pico (VO_2pico), denominada de classes funcionais de Weber (Tabela 1).[9]

Tabela 1. Classificações funcionais NYHA e Weber para insuficiência cardíaca.

	Classe de Weber		**Classe Ventilatória**		
Severidade Doença		**VO_2pico**	**NYHA**	**VE/VCO_2*Slope***	**Prejuízo Funcional**
Leve / Nenhum	A	> 20	I	≤ 29,9	AVDs normais
Leve / Moderada	B	16-20	II	30-35,9	AVDs com pequeno prejuízo
Moderada / Severa	C	10-16	III	36-44,9	Redução nas AVDs
Severo	D	< 10	IV	≥ 45	Grande prejuízo nas AVDs

Nota: *New York Heart Association* (NYHA); Eficiência Ventilatória (VE/VCO_2*Slope*); Atividades da Vida Diária (AVDs); VO_2pico ($ml.kg^{-1}.min^{-1}$). Adaptado de Arena & Sietsema, 2011.

O tratamento da IC é clínico, com utilização de fármacos específicos para reduzir sintomas, retardar a evolução da doença, promovendo mais qualidade de vida e melhorando o prognóstico a longo prazo.[7] O exercício físico pode ser uma ferramenta utilizada para melhorar o prognóstico da doença, visto que o treinamento físico regular é capaz de promover aumento no VO_2pico, o qual está diretamente relacionado com prognóstico da IC.[10, 11] Em estudo clássico de Kavanagh et al., 2002, 2003, foi evidenciado que cada 1 $ml.kg^{-1}.min^{-1}$ de aumento no VO_2pico está diretamente associado com uma redução de 10% no risco de mortalidade por DCVs.[12, 13] No entanto, quando o paciente não responde ao tratamento clínico, assim como às opções não farmacológicas, a gravidade é maior e o desfecho pode ser de arritmias malignas e/ou falha de bomba. Nesses casos, o transplante cardíaco (TxC) é o tratamento recomendado para indivíduos com IC terminal.[14] O TxC é capaz de melhorar o prognóstico e reduzir a morbidade a médio prazo. Entretanto, mesmo com aumento na sobrevida, um estado inflamatório elevado permanece, parte herdado pela IC e parte pelo uso de imunossupressores.[15, 16]

Pensando em TxC, precisamos abordar os instrumentos que levam a decisão da equipe médica para realização do procedimento. A estratificação de risco na IC, bem como o conhecimento da gravidade da doença e decisão final para TxC é também guiada pelo teste cardiopulmonar de exercício (TCPE). Pacientes com VO_2pico < 10 $ml.kg^{-1}.min^{-1}$, assim como quadro clínico complexo e desfavorável, estarão elegíveis para o TxC.[14] Além disso, o TCPE é utilizado para otimizar o tratamento clínico, conhecendo a capacidade funcional (CF) do indivíduo, assim com sua eficiência ventilatória (EV), entre

outras variáveis prognósticas.[17] Ele também pode ser utilizado para diagnosticar arritmias malignas, as quais inviabilizam a prática de exercício físico, visto que o TCPE é a principal ferramenta para liberação da prática de exercício físico no cenário da IC. Por fim, o TCPE também é utilizado para prescrição do treinamento aeróbico, onde é possível conhecer precisamente a frequência cardíaca (FC) correspondente aos limiares ventilatórios (LV), fazendo com que a intensidade de exercício seja corretamente exercida durante a prática de exercício físico.[17] Apesar do TCPE ser o padrão ouro para prescrição de exercício físico, ele não é a realidade de muitos locais, não sendo acessível para todos hospitais ou clínicas de saúde. Uma alternativa ao TCPE, é o teste de caminhada de 6 minutos (TC6M).[18] Este exame é um teste submáximo barato, seguro e eficaz para se conhecer a CF de indivíduos com IC.

O TC6M é amplamente usado para avaliar as respostas agudas ao exercício físico. A distância total percorrida tem valor prognóstico, comprovado por diferentes autores. Arslan et al., 2007, demonstraram que uma distância ≤ 300m possue~~m~~ uma taxa de mortalidade de 76%, sendo que uma distância > 300m apresenta apenas 7% de mortalidade em um seguimento de 30 meses.[19] Em estudo clássico, Ingle et al., 2014 apresentaram que o TC6M é um preditor independente de mortalidade por todas as causas em um seguimento de 5 anos. Indivíduos que percorrem uma distância <46m apresentam uma taxa de sobrevida menor (24%), enquanto que os que percorrem uma distância > 360m apresentam 70% de taxa de sobrevida.[20] Embora o TC6M tendo valor prognóstico, ele é um teste submáximo e consequentemente não fornece informações suficientes para a prescrição de exercício físico. Entretanto, a utilização de instrumentos tecnológicos que agreguem informações durante o TC6M pode ser uma alternativa para aumentar o valor qualitativo do teste. A cardioimpedância de sinal morfológico (SM-ICG) pode ser uma alternativa viável na utilização durante o TC6M.[21]

O SM-ICG é um método não invasivo que fornece medidas precisas do DC, volume sistólico (VS), FC e índice cardíaco (IxC).[22] Esse instrumento pode ser utilizado tanto em indivíduos saudáveis quanto em indivíduos com ICFEr. Ele é acurado em avaliar o DC quando comparado aos métodos de Fick e de termodiluição, tanto em repouso, quanto em exercício.[22] O SM-ICG possui capacidade de fornecer dados hemodinâmicos durante um TC6M, podendo ser útil em indivíduos com ICFEr, visto que eles apresentam redução no VS durante o esforço e também apresentam prejuízo no DC.[23] Alguns estudos avaliam indivíduos com ICFEr utilizando o ICG durante o TC6M, no entanto, até onde temos conhecimento, ainda não foi demonstrado a resposta da aceleração e desaceleração

do DC ao TC6M, duas importantes variáveis que estão diretamente relacionadas com a intolerância ao esforço. Portanto, o objetivo principal desta tese foi avaliar a resposta da aceleração e desaceleração do DC ao TC6M, comparando os resultados entre indivíduos saudáveis e aqueles com ICFEr. No entanto, cabe salientar, que esta tese será dividida em três artigos, com assuntos interligados que versam sobre o trinômio cardiologia, IC e exercício, sendo eles:

- 1º: *Cardiodynamic noninvasive measured during a 6-minute walk test in heart failure patients using impedance cardiography*;
- 2º: *Aerobic exercise and telomere length in patients with systolic heart failure: protocol study for a randomized controlled trial*;

2. REVISÃO DA LITERATURA

2.1. Impedância Cardiográfica e Teste de Caminhada de 6 Minutos

A impedância cardiográfica é constituída por sensores (eletrodos) que conectam o aparelho (ICG) ao tórax do paciente.[22] A técnica é baseada na Lei de Ohm, cuja equação é: R=V/I onde R=resistência (Ohm, Ω), V=diferença de potencial, voltagem (Volt) e I=corrente elétrica (À=ampere). A resistência alternada é chamada impedância (Z) e pode ser calculada como Z=V/I. Nesse método, dois eletrodos transmitem uma corrente de alta frequência e baixa amplitude e outros dois eletrodos detectam alterações instantâneas na voltagem. A impedância de base (Zo) é refletida por alterações no volume e velocidade do sangue nos grandes vasos durante a sístole e diástole. A variação da impedância (ΔZ) é filtrada para não sofrer variação do volume respiratório. Essa ΔZ em função do tempo (Δt) pode determinar o fechamento da válvula aórtica, abertura das válvulas pulmonar e aórtica, fechamento da artéria pulmonar, abertura da válvula mitral/enchimento ventricular. Em pacientes com IC esse método se torno importante devido as alterações hemodinâmicas ocasionadas pela doença.[24, 25]

Esse tipo de instrumento pode ser utilizado em conjunto ao TC6M, adicionando informações qualitativas e quantitativas sobre o perfil hemodinâmico durante os 6 minutos de teste.[23] Por sua vez, o TC6M é amplamente utilizado na prática clínica, seja para fins prognósticos ou para pesquisas científicas.[18] É um teste seguro, válido e confiável, sem requerer um complexo preparo ou equipamentos de difícil acesso. Ele é capaz de avaliar a distância percorrida em uma superfície plana, preferencialmente um

corredor com 30m, durante 6 minutos. Além disso, o TC6M possui boa reprodutibilidade, com forte correlação intra-testes. Como foi descrito anteriormente, apesar do TC6M não servir para prescrição de exercício propriamente dito, ele tem valor prognóstico, ou seja, a distância percorrida é preditor independente de morbimortalidade, e as alterações na distância percorrida se correlacionam com a piora do quadro da IC.[19]

Diferentes estudos avaliam a ICG durante o TC6M em indivíduos com ICFEr. No entanto, o que baseou a definição do tema da presente tese é o interesse em aprofundar o conhecimento sobre duas variáveis fundamentais para o entendimento da intolerância ao esforço nesses indivíduos: a aceleração e a desaceleração do débito cardíaco.[23] A primeira representa a diferença entre o valor em repouso e a média de todos os valores obtidos durante o primeiro minuto do TC6M, enquanto que a desaceleração é caracterizada pela diferença entre o valor medido ao final do teste e a média de todos os valores obtidos durante o primeiro minuto de recuperação. O estudo pioneiro a avaliar essas duas variáveis no cenário da ICG e TC6M foi conduzido por Tonelli et al., 2013, no entanto, em população diferente da avaliada nesta.[23] No estudo supracitado os autores investigaram indivíduos com hipertensão pulmonar. Como principal achado, pacientes com hipertensão pulmonar apresentam prejuízo significativo tanto na aceleração quanto na desaceleração do DC quando comparados a saudáveis pareados por sexo e idade ($P= 0,001$; $P<0,001$). Portanto, diante da lacuna observada na literatura sobre a carência de estudos que unissem essas duas variáveis avaliadas através da ICG ao binômio ICFEr-TC6M, a tese foi elaborada.

2.2. Comprimento dos Telômeros, Insuficiência Cardíaca e Treinamento Físico

O envelhecimento pode ser caracterizado por uma diminuição orgânica e funcional, que não tem relação com doença, ou seja, um processo que acontece naturalmente com o passar do tempo. O envelhecimento biológico se expressa pela alteração estrutural e funcional, o qual é regulado por mecanismos celulares intrínsecos e modulado por diversos fatores ambientais. Essas alterações são responsáveis por desregular a homeostase corporal, a qual quando associada à idade cronológica avançada, podem determinar maior suscetibilidade ao aparecimento de doenças, incapacidades físicas e mentais, e consequentemente, aumentar a morbimortalidade.[26]

Um dos marcadores do processo de envelhecimento são os telômeros, que consistem em um complexo de sequências de DNA localizados nas extremidades do cromossomo.[27] Os telômeros são compostos por proteínas protetivas que têm a função de

proteger as informações do DNA (genoma).[28] A principal função dos telômeros é a de prevenir que o final do DNA cromossômico seja reconhecido como um final quebrado. Uma vez que os telômeros são responsáveis por reconstruir as informações perdidas durante a divisão celular devido ao desgaste das extremidades cromossômicas, telômeros "mais compridos" (saudáveis) são um sinal de que o processo de envelhecimento será mais lento, ou seja, também mais saudável.[29]

Por sua vez, a enzima telomerase é a estrutura responsável pela reconstrução e aumento do comprimento dos telômeros.[30] O comprimento dos telômeros pode ser menor em algumas doenças, como a ICFEr quando comparado a indivíduos de mesmas faixas etárias que não possuem a doença.[29, 30] A ICFEr é uma síndrome clínica complexa de mau prognóstico e alta prevalência. Tem como característica o cansaço, dispneia e intolerância aos esforços físicos devido à redução no DC, concomitante a alterações respiratórias, fraqueza em músculos periféricos e maior incidência de depressão.[5, 7]

Em indivíduos sedentários que não possuem IC, ocorre uma redução natural do tamanho dos telômeros, a qual está associada com o desenvolvimento precoce do processo de envelhecimento.[31] Por outro lado, em indivíduos com IC, ocorre uma maior redução no comprimento dos telômeros, em função de um maior estado inflamatório e oxidativo devido à doença.[32] Portanto, ter o diagnóstico de IC impacta negativamente no envelhecimento celular, já que nesta síndrome ocorre redução do comprimento do telômero.

Uma das estratégias mais promissoras para intervir no envelhecimento é a prática de exercício físico. No entanto, não se sabe os efeitos da prática de exercício físico sobre o comprimento dos telômeros de indivíduos com IC. Contudo, sabemos que o exercício físico impacta de forma positiva em pacientes com IC apresenta resultados positivos em relação a capacidade funcional, medida pelo VO_2pico.[33] Além disso, a melhora da capacidade funcional, ou seja, aumento do VO_2pico, está diretamente relacionada com o aumento do comprimento dos telômeros.[34-37] Em outras palavras, a prática regular de exercício físico é recomendada em pacientes com IC, sendo componente central na reabilitação cardiovascular adjuvante ao tratamento medicamentoso e a outras medidas não farmacológicas.

O exercício como estratégia de tratamento da IC pode ser eficiente em além de melhorar a capacidade funcional, melhorar a qualidade de vida e o prognóstico da doença.[11, 38] No entanto, existe uma carência de resultados concisos na literatura em relação aos efeitos crônicos do exercício aeróbico sobre o tamanho dos telômeros em

indivíduos com IC. Alguns estudos descrevem que o exercício melhora a atividade da enzima telomerase, o que seria um resultado positivo para a melhora do tamanho dos telômeros.[36, 39] Entretanto, outras evidências sugerem que o exercício possa gerar uma redução no tamanho dos telômeros por meio do desequilíbrio entre o estresse oxidativo acentuado e uma redução da ação de antioxidantes, o qual afeta diretamente a atividade da enzima telomerase.[40] Além disso, outros experimentos evidenciaram que o exercício aeróbico de alta intensidade pode promover um processo de catabolismo celular, o qual pode danificar a estrutura dos telômeros.[31, 41] Porém e em contrapartida, alguns autores demonstram que o exercício aeróbico de moderada intensidade pode promover um estado de redução do estresse oxidativo intracelular, por meio de uma maior atividade de propriedades antioxidantes, o que pode gerar efeitos benéficos ao comprimento dos telômeros.[36, 37, 42]

2.3. Transplante Cardíaco, Biomarcadores Inflamatórios e Treinamento Físico

O TxC é considerado tratamento padrão ouro em pacientes com IC terminal, ou seja, onde não há mais respostas positivas sob perspectiva do tratamento clínico convencional.[14] Nesse cenário hostil, embora seja considerado tratamento de escolha por melhorar o prognóstico do paciente e consequentemente sua qualidade de vida, muitos indivíduos permanecem com sua CF reduzida após o procedimento, além de apresentar um estado inflamatório exacerbado pelo uso de imunossupressores.[43] Esse quadro inflamatório é complexo, sendo composto por inúmeras substâncias que auxiliam no balanço inflamatório, que em situações de normalidade mantém um equilíbrio homeostático. Esse equilíbrio é determinado pelo sistema imunológico. Dentre as substâncias podemos destacar as citocinas, que são proteínas as quais modulam funções orgânicos no organismo humano. Sua principal produção é realizada por linfócitos e macrófagos. Dentre as citocinas do sistema imune podemos destacar a interleucina-6 (IL-6) e o fator de necrose tumoral alfa (TNF-alfa).[44] O aumento dessas duas proteínas gera uma cascata inflamatória causando um estado inflamatório exacerbado. Além disso, em indivíduos que realizaram TxC, em função do uso de imunossupressores, a produção de alguns hormônios é reduzida, como por exemplo, a adiponectina, que é sintetizada pelo tecido adiposo.[45] Sua função envolve diferentes processos metabólicos, incluindo uma melhora no controle glicêmico por aumento na sensibilidade à insulina e catabolismo de ácidos graxos. Sob a perspectiva de melhora do estado inflamatório geral, o exercício

físico parece ser uma alternativa segura e eficaz em diferentes populações, mas ainda não está claro seu papel em pacientes pós TxC.

O treinamento aeróbico (TA) regular é capaz de reduzir a concentração plasmática de IL-6, TNF-alfa e aumentar as concentrações de adiponectina em indivíduos com IC.[46] Em relação àqueles pós-TxC, existem evidências contraditórias sobre os efeitos do TA em relação a biomarcadores inflamatórios, e tal contradição gera uma dúvida em relação aos efeitos anti-inflamatórios do exercício físico nessa população. Pierce et al., 2008 demonstra que 12 semanas de TA de moderada intensidade é capaz de reduzir os níveis da ~~de~~ IL-6, mas, no entanto, não de maneira significativa (5,2 ± 3,9 vs 3,8 ± 2,4 pg/ml; P>0,05). Por outro lado, o TNF-alfa apresentou melhora significativa após o período de treinamento (1,66± 1 vs. 3 ± 1,1 pg/ml; P < 0.05).[46] Entretanto, Herman et al., 2011 após um programa de 8 semana de TA evidenciou não haver melhora significativa tanto para IL-6 quanto para TNF-alfa (P>0,05), apesar de terem apresentado redução absolutas nos valores.[47] Portanto, torna-se importante a investigação sobre os efeitos do TA nos biomarcadores inflamatórios em indivíduos pós-TxC.

3. JUSTIFICATIVA E OBJETIVOS

A lógica de estruturação da tese segue os três principais artigos, sendo a revisão da literatura a seguir com três principais tópicos:

- **Cardioimpedância e Teste de Caminhada de 6 Minutos:** nesse capítulo será abordado principais estudos sobre ICG e TC6M, trazendo um panorama geral sobre o assunto e expondo a lacuna na literatura para desenvolvimento do artigo número 1;
- **Comprimento dos Telômeros, Insuficiência Cardíaca e Treinamento Físico:** nesse capítulo será desenvolvido o racional teórico sobre a relação de telômeros com IC, sendo o comprimento do telômero um potencial marcador prognóstico. Além de ser conhecido como biomarcador de envelhecimento biológico e possuir associação com VO_2pico, no entanto, o conhecimento sobre os efeitos do treinamento físico sobre esse marcador ainda são pouco conhecidos. Este tópico será utilizado para explicitar a lacuna na literatura sobre o artigo número 2;
- **Transplante Cardíaco, Biomarcadores Inflamatórios e Treinamento Físico:** nesse último capítulo, abordaremos um tema muito relevante para a IC, que é o TxC. No entanto, observamos ser escassa a relação entre o treinamento físico e

biomarcadores inflamatórios no indivíduo que realizou TxC. Aqui, então, desenvolveremos uma abordagem que servirá para a leitura do artigo número 3.

Por fim, é importante descrever que os três assuntos estão diretamente relacionados. Primeiro, indivíduos com IC apresentam prejuízo no DC e sua relação com o exercício não é bem esclarecida. Da mesma forma, pacientes com IC apresentam redução no comprimento dos telômeros, o qual tem associação com VO_2pico. Apesar de não termos evidência sobre o comportamento dos telômeros após um programa de treinamento físico, sabemos que ele é capaz de melhorar o VO_2pico, por exemplo. Já o TxC possui relação direta com IC, visto que é o último tratamento indicado para a melhora do prognóstico da IC. Além disso, o estado inflamatório tem relação direta com os telômeros. Finalmente, a perspectiva é que o treinamento físico melhore o estado inflamatório geral, melhorando a qualidade de vida desses pacientes.

Objetivo primário: avaliar através da ICG a resposta da aceleração e desaceleração do DC ao TC6M comparando controles saudáveis com pacientes diagnosticados com ICFEr. Em paralelo, avaliaremos o comportamento hemodinâmico da amostra antes, durante e após o TC6M (variáveis SV, FC e IC) – artigo 1.

Objetivo secundário: descrever um estudo de protocolo (artigo 2), o qual foi desenhado para determinar o efeito de 16 semanas de TA (32 sessões) no comprimento de telômeros em pacientes com ICFEr; conduzir uma revisão sistemática e metanálise a respeito dos efeitos do TA sobre biomarcadores inflamatórios em indivíduos pós-TxC.

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5. ARTIGO 1

Title: Cardiodynamic noninvasive measured during a 6-minute walk test in heart failure patients using impedance cardiography.

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Keywords: Six-minute walk test; Heart failure; Impedance cardiography; Physical exercise.

Abstract

The 6-minute walk test (6MWT) is commonly used in the evaluation of heart failure patients. However, several clinical factors can influence the distance walked that are independent from this heart condition. Signal Morphology-Impedance cardiography (SM-ICG) is a useful tool for noninvasive hemodynamic assessment. As primary outcome, the present study aims to compare cardiac output (CO), heart rate (HR) and stroke volume (SV) acceleration and deceleration responses to the 6MWT among healthy and heart failure patients with reduced ejection fraction (HFrEF). A cross-section observational study was conducted with 27 subjects (13 with HFrEF; 14 healthy controls), all assessed by SM-ICG (PhysioFlow®). CO, HR, SV and cardiac index (CI) were evaluated before, during, and after the 6MWT. Descriptive analysis was performed, and statistical significance of differences in continuous variables was tested by independent sample *t*-test ($\alpha<0.05$). Mean age was similar (HFrEF, 64±8 years; control, 65±5 years; P=0.66). CO and HR acceleration differed significantly between groups (P<0.01; P=0.039, respectively), in favor of controls. In contrast, CO and HR deceleration did not differ between groups (P=0.07; P=0.385, respectively). There were significant differences in SV, CO, and CI between groups, especially for peak values (P<0.01). Linear regression showed impairment in contribution of SV (22.9% instead of 57.4% of control) to CO change in HFrEF, as expected. This study examined in real time the cardiodynamic behavior of HFrEF patients during a 6MWT. They showed impaired CO and HR acceleration during a submaximal exercise test compared with healthy controls, which may represent an imbalance in the autonomic response to exercise. SM-ICG is a reliable, noninvasive method for measuring cardiodynamic data in HFrEF patients.

Introduction

Heart failure is a complex syndrome that may be the ultimate consequence of most cardiovascular diseases. Contractility reduction is one of the core features of heart failure with reduced ejection fraction (HFrEF), where cardiac output (CO) impairment results in systemic hypoperfusion, which, in association with pulmonary, peripheral and neurohumoral changes, contributes to low tolerance to physical activity.[1, 2] This reduced capacity to perform physical activity at moderate and higher intensities is one of the major limitations experienced by individuals with HFrEF.[3]

The 6-minute walk test (6MWT) is a method widely used to evaluate acute responses to self-limited exercise. Total distance walked in the 6MWT has been proven to have prognostic value.[4] Furthermore, its execution is simple, inexpensive, and requires no specialized training.[5] However, it has been demonstrated that certain comorbidities, like HFrEF, significantly reduce the distance walked dependently from the actual heart performance (severity of disease), making supplementary assessment necessary through other tools that add information, whether clinical or prognostic.[6]

Therefore, signal-morphology impedance cardiography (SM-ICG) is a noninvasive method that provides accurate measures of CO, stroke volume (SV), heart rate (HR), and cardiac index (CI), and can be performed both in healthy individuals and in those with conditions such as HFrEF during the 6MWT, adding different information about health conditions.[7-10] A commercially available SM-ICG device (PhysioFlow®) has been shown to assess peak CO with strong accuracy when compared to Fick's and thermodilution methods, both at rest and during exercise.[11-13] Furthermore, SM-ICG can add predictive information for VO_2peak with a strong correlation between measured and predicted VO_2peak ($r = 0.931$, $p<0.001$), using values acquired during 6MWT for CO, SV and HR.[14]

SM-ICG provides useful hemodynamic data during a 6MWT in different clinical conditions, but we are not aware of data obtained in patients with HFrEF.[15] Furthermore, the CO acceleration and deceleration response to a 6MWT has not yet been demonstrated in these individuals, two variables that can represent an autonomic imbalance to exertion and recovery.[7, 11] Therefore, the primary aim of this study was to evaluate the CO, HR and SV acceleration and deceleration response to a 6MWT in healthy controls and HFrEF patients. In turn, the secondary outcome was to assess the hemodynamic behavior before, during and after the 6MWT (SV, HR and CI), also not previously described for this test.

Methods

Experimental design

Cross-section observational study of two groups, one composed of healthy individuals (control group - CG), and the other of HFrEF patients. The disease was defined by clinical signs and symptoms of HF in the setting of an ejection fraction <40%, assessed by echocardiography, while under optimal standard pharmacologic treatment. Patients included were stable for at least 3 months (no hospitalization, emergency department visits due to decompensated HFrEF, or change in drug therapy) and followed regularly at a specialized heart failure clinic. Patients with lung disease and peripheral vasculopathy were excluded from the sample. Controls were required to have no heart disease of any type and be sedentary for at least 6 months; enrolled controls were matched for age with HFrEF participants. Patients were invited to participate and received an explanation of the procedures that would be performed.[15] Figure 1 shows the screening, eligibility and assessment flowchart (Figure 1 – Study flowchart).

Signal Morphology Impedance Cardiography

Impedance (Z) is a measure of resistance level to an electric current. SM-ICG measurement is a method that evaluates the thoracic fluid content. From determination of current voltage, changes in impedance occur as a result of changes in blood volume that passes through the thorax.[16] Impedance variation (ΔZ) is filtered through the SM-ICG device software so it will not be influenced by variations due to inspired and expired volume, chest fluids, or other factors like obesity or electrode position, which impair conventional ICG.[11] Previous studies have demonstrated strong correlations between SM-ICG measurement and invasive methods of hemodynamic assessment,[17] both at rest and during exercise. [11, 12] It is noteworthy that SM-ICG can be applied as a diagnostic tool,[18] and it has been used as a predictor of cardiovascular prognosis.[19]

The height and weight of each participant were measured and the skin was prepared (shaved with a disposable razor blade and abrasive gel, sanitized with alcohol, and dried) for electrode placement. Six fresh and non-expired electrodes for cardiac monitoring (FS-50 Skintact, Skintact®, Austria) were attached through wires to a portable SM-ICG device (PhysioFlow® PF07 Enduro™, Paris, France; 11.5 x 8.5 x 1.8 cm; weight 200g) and the device to a Bluetooth adapter. The electrodes were placed in the left lateral region of the neck, supraclavicular fossa, center of the sternum, standard ECG positions V1 and V6, and in parallel with the spine at the height of the xiphoid process (Figure 2 – Schematical PhysioFlow electrodes positions and their electrical meanings). The wires and device were stabilized with a nylon strap at the patient's waist to reduce noise. Self-calibration of the system was performed through 30 consecutive heart beats, taken at full rest, establishing patient baseline signal morphology and resting hemodynamic values.

6-minute walk test

The 6MWT was conducted in accordance with American Thoracic Society Standard Guidelines.[20] The test was conducted along a 30-meter corridor; each participant was asked to walk the longest possible distance over the course of 6 minutes. The distance walked were recorded and expressed in meters.

Hemodynamic measurements

SM-ICG data were recorded at continuous beat to beat. False values were filtered with a manual extraction. CO was measured in liters per minute ($L \cdot min^{-1}$), SV in milliliters (mL), HR in beats per minute (bpm), and CI in liters per minute per square meter of body surface area ($L \cdot min^{-1} \cdot m^{-2}$).

For analysis, we used baseline values (represented by the mean of the last 2 minutes preceding evaluation with the patient still in the standing position, for practical reasons), the maximum value obtained during 6MWT, the delta (change, represented by the difference between maximum and baseline values), and values within the first minute of recovery.

Acceleration and deceleration – CO, HR and SV

Acceleration is represented by the difference between the value at rest and the mean of all values obtained during the first minute of the 6MWT, whereas deceleration is represented by the difference between the value measured at the end of the test and the mean of all values obtained during the first minute of recovery. These variables were captured during the first minute of walking (acceleration) and the first minute during recovery (deceleration), since they represent the moments during the 6MWT in which the most pronounced hemodynamic changes occur. Acceleration represents the variability of

hemodynamic response to the first minute of exercise, i.e., an increase in cardiovascular demand; deceleration represents the variability of response as soon as exercise ends, during the onset of recovery. All participants were monitored for a total of 18 minutes (6 minutes standing, 6 minutes throughout the walk test and 6 minutes' recovery standing).

Statistical analysis

Data were presented in mean and standard deviation or median and interquartile range according to normality test. Data distribution were assessed by Shapiro-Wilk test. To compare data between the groups, T-tests for independent samples were adopted when data presented normal distribution, and Mann-Whitney U test when data did not present normal distribution.

Pearson correlation was applied to identify the strength of association between variables. A multivariate linear regression with CO change as dependent variable was used to identify the contribution from the variable HR change and SV change.

Alpha (α) was defined as <0,05 indicating statistical significance. The statistics analyses were performed with the SPSS software, version 20.0 (IBM; ARMONK, NY, USA).

Results

Sample characteristic

The baseline characteristics of participants in each group are shown in Table 1. The mean age of controls and participants with HFrEF was 65±5 and 64±8 years, respectively (P=0.66). All controls were male (100%), while the HFrEF group was predominantly so (n=11).

Table 1. Baseline characteristics for both groups. Data are presented in mean and standard deviation or frequency (absolute and relative).

Characteristics	CG = 14	HFrEF = 13	P
Age (years)	65 ± 5	64 ± 8	0.66
Height (cm)	167 ± 11	171 ± 12	0.28
Weight (kg)	72 ± 15	79.5 ± 9	0.45
Ejection fraction (%)	63.4 ± 6.23	32.7 ± 5.25	0.17
Female gender	0 (0)	2 (15.4)	0.78
Race, n (%)			
Black	3 (21.4)	3 (23)	1.00
White	11 (78.6)	10 (77)	0.87
NYHA class, n (%)			
I	-	1 (7.7)	-
II	-	9 (69.2)	-
III	-	3 (23)	-
HF etiology, n (%)			
Ischemic	-	4 (30.8)	-
Non-ischemic	-	9 (69.2)	-
Left ventricular ejection (%)	-	32.7 ± 5.2	-
Medications, n (%)			
Warfarin	-	1 (7.7)	-
ACE inhibitors	-	9 (69)	-
Beta-blockers	-	13 (100)	-
Antihypertensive	-	9 (69)	-
Losartan	-	4 (30.7)	-
Diuretic	-	7 (53.7)	-
Digoxin	-	7 (53.8)	-
Isosorbide	-	2 (15.4)	-
Antidiabetic	-	4 (30.7)	-
Aspirin	-	3 (23)	-
Simvastatin	-	4 (30.7)	-

CG = control group; HFrEF = heart failure with reduced ejection fraction group; NYHA = New York Heart Association.

6-minute walk test

Acceleration and deceleration – CO, HR and SV

First, CO acceleration show a significant difference between groups (HFrEF: 1.89 ± 1.39 $l.min^{-1}.second^{-1}$; CG: 4.59 ± 2.75 $l.min^{-1}.second^{-1}$, P<0.01). In contrast, CO deceleration did not differ between groups (HFrEF: 0.62 ± 1.39 $l.min^{-1}.second^{-1}$; CG: 1.94 ± 2.11 $l.min^{-1}.second^{-1}$, P=0.07). (Figure 3 - Cardiac Output acceleration and deceleration responses to 6MWT between healthy individuals and HFrEF patients; **Note:** CO – cardiac output; CG – control group; HFrEF – heart failure with reduced ejection fraction). Likewise, HR acceleration show a significant difference between groups and HR deceleration did not show (HFrEF: 12 ± 12 bpm; CG: 24 ± 15 bpm, P=0.039 and HFrEF: 9 ± 8 bpm; CG 11 ± 9 bpm, P=0.385, respectively). (Figure 4 – Heart Rate acceleration and deceleration responses to 6MWT between healthy individuals and HFrEF patients; **Note:** HR – heart rate; CG – control group; HFrEF – heart failure with reduced ejection fraction**).** In contrast, VS acceleration and deceleration did not shown difference between groups (HFrEF: 15.51 ± 14.38 ml; CG: 25.12 ± 15.65 ml, P=0,110 and HFrEF 3.29 ± 9.01 ml; CG: 8.85 ± 16.98 ml, P=0.304).

Conventional measures

Traditional outcomes measured during 6MWT are listed in Table 2. Compared to controls, patients with HFrEF walked a shorter distance, with a similar HR at rest and during the test, but had a lower HR during the first minute of recovery. Patients with HFrEF presented significantly lower peak SV and CI values (Table 3).

Table 2. Traditional parameters measured during the six-minute walk test.

	CG = 14	**HFrEF = 13**	**P**
	Mean ± SD or Median (IR)	Mean ± SD or Median (IR)	
Walked distance (m)	559.50 ± 61,36	395.50 ± 87,63	< 0.01
HR baseline (bpm)	72 ± 8	71 ±9	0.927
HR maximum (bpm)	112 (108;138)	116 (91;146)	0.607
HR change (bpm)	43 (31;67)	48 (23;65)	0.837
HR recovery at 1 minute (bpm)	15 ± 9	8 ± 11	0.097

bpm = beats per minute; HR = heart rate; CG = control group; HFrEF = heart failure with reduced ejection fraction group; IQR = interquartile range.

Table 3. Hemodynamic parameters measured during the 6MWT.

	CG = 14	**HFrEF = 13**	**P**
	Mean ± SD or Median (IQR)	Mean ± SD or Median (IQR)	
SV baseline (ml)	83.77 ± 16.48	51.28 ± 13.78	< 0.01
SV maximum (ml)	147.03 ± 28.16	100.13 ± 27.96	< 0.01
SV change (ml)	60.36 (47.41; 69.82)	41.76 (30.76; 67.90)	0.13
SV recovery at 1 min (ml)	18.49 ± 13.38	5.34 ± 7.55	< 0.01
CO baseline ($l.min^{-1}$)	5.94 ±1.05	3.64 ± 1.03	< 0.01
CO maximum ($l.min^{-1}$)	16.35 (15.20; 17.70)	10.30 (7.31; 14.83)	< 0.01
CO change ($l.min^{-1}$)	10.15 (8.82; 12.20)	7.10 (3.64; 10.90)	0.05
CI baseline ($l.min^{-1}.m^{-2}$)	2.97 (2.70; 3.41)	1.99 (1.68; 2.19)	< 0.01
CI maximum ($l.min^{-1}.m^{-2}$)	8.67 ± 2.46	6.12 ± 1.90	< 0.01

CI change (l.min^{-1}.m^{-2})	5.61 ± 2.54	4.09 ± 2.03	0.09
CI recovery at 1min (l.min^{-1}.m^{-2})	1.77 ± 0.92	0.58 ± 0.70	< 0.01

CI = cardiac index; CO = cardiac output; SV = stroke volume; CG = control group; HFrEF = heart failure with reduced ejection fraction group; IQR = interquartile range.

6MWT distance walked, hemodynamic variables and functional class

The faster the CO acceleration, the greater the distance walked on 6MWT (r=0.49, P=0.01). Baseline CI (r=0.60, P<0.01), peak CI (r=0.67, P<0.01), ΔCI (r=0.63, P<0.01), and CI during the first minute of recovery (r=0.68, P<0.01) correlated significantly with distance covered on the 6MWT, as did SV during the first minute of recovery (r=0.50, P<0.01).

The distance walked during the 6MWT correlated with HR recovery in the first minute (r=0.68, P<0.01) and with NYHA class (r=0.62, P<0.01). In addition, baseline and peak SV correlated significantly with distance walked on 6MWT (r=0.51, P=0.01, r=0.60, P<0.01, respectively). Baseline CO, peak CO, and ΔCO also showed significant correlations with distance walked on 6MWT (r=0.52, P<0.01, r=0.67, P<0.01, r=0.61, P<0.01, respectively).

Contribution of variables in Cardiac Output change

In controls, change in HR explained 64.3% of the ΔCO, while in patients with HFrEF, it explained 70.3% of ΔCO. The contribution to ΔSV was 57.4% in controls and only 22.9% in HFrEF individuals. According to the β coefficients of linear regression, for each unit of change in HR, the ΔCO was modified by 1.121 units in controls and 0.92 unit in the HFrEF group. Regarding ΔSV, each unit of change in HR corresponded to a ΔCO change of 1.162 units in controls and 0.91 unit in the HFrEF group.

Discussion

In this experiment, we compared the cardiodynamic responses to the 6MWT between healthy individuals and HFrEF patients. The main finding was that these individuals showed blunted hemodynamic responses to the 6MWT compared to controls, especially in CO and HR acceleration. Additionally, significant differences were observed in distance walked during the 6MWT between groups, which demonstrates a reduction in functional capacity in individuals with HFrEF, as expected. Furthermore, during recovery, the HR response was lower in the HFrEF group.

To our knowledge, several studies have evaluated the hemodynamic profile in different diseases during 6MWT.[15, 21-23] However, only one evaluated the CO acceleration and deceleration, but in the pulmonary hypertension scenario, and do not evaluated HR and SV acceleration and deceleration.[22] These variables are very important in HFrEF, as they represent an imbalance in the autonomic response to exertion and recovery.[24, 25] We found a significant difference in CO acceleration between groups ($P<0.01$), but not in deceleration ($P=0.07$). The same occur for HR acceleration ($P=0.039$) and deceleration ($P=0.385$). In this context, however, both the acceleration and deceleration of CO and HR were lower in the HFrEF group, since these patients have a chronotropic deficit due to the disease "per se", as well as a consequence of the pharmacological effect of the beta-blocker (all patients were in beta-blocker therapy).

Given that acceleration and deceleration may represent sympathetic and parasympathetic activation, respectively, in HFrEF there is a greater sympathetic activation and a sympathovagal imbalance, which corroborates with findings of our study demonstrating lower CO and HR acceleration for the HFrEF group.[26, 27] Animal models demonstrate that sympathoexcitation and abnormal cardiovascular reflex function contribute to sympathetic nervous system activation in HFrEF.[28] In contrast, little is

known about the role of parasympathetic nerve activity in HFrEF. Hu et al.,[26] demonstrated that HR deceleration is an independent predictor of AMI and sudden cardiac death in HFrEF, being a stronger predictor than left ventricular ejection fraction and conventional HR variability measures. Despite these findings, Hu et al.,[26] only evaluated HR acceleration and deceleration response, therefore, our study is the first to evaluate the DC, HR and SV acceleration and deceleration in HFrEF. Although the CO deceleration did not show differences between groups, there is a tendency (P=0.07) that the HFrEF group has a loss in its response.

Ventricular impairment in HFrEF can trigger distinct compensatory mechanisms, which initially promote increased neurohormonal activation of the sympathetic nervous and renin-angiotensin-aldosterone system.[29] However, the chronic effect of these different compensatory mechanisms can be harmful, as it can result in pathological ventricular remodeling and, consequently, worsening the disease.[30] This prolonged exposure to sympathetic activation can result in downregulation of β-adrenergic receptors in cardiomyocytes, which contributes to a decrease in myocardial sensitivity to catecholamines and an associated reduction in the inotropic response, which can impair HR recovery after exercise. The findings of this study corroborate the aforementioned.[2]

Lower values of baseline and peak SV were found in HFrEF patients when compared to controls. Both, resting and exercising behavior in the HFrEF group represents a loss in ventricular contraction, that is, a reduction in SV in each systole performed by a sick myocardium.[31] In healthy individuals, the Frank–Starling principle works as a physiological mechanism that, in response to an initial reduction of ventricular contraction, promotes a compensatory increase in SV.[32] Soon after an initial reduction in ventricular contraction, SV reduces, generating an accumulation of blood in the ventricle at end-diastole, i.e., an increase in the end-diastolic volume.[32] Therefore, the

heart increases ventricle stretch, thus increasing the strength of subsequent ventricular contraction and increasing SV, which allows better physiological emptying of the left ventricle.[32] Individuals with HFrEF display a failure of this mechanism. The attendant reduction in cardiovascular reserve results in impairment and reduction of ventricular contractility, leading to a reduction in SV.[33] This phenomenon also corroborates the findings of the present study.

Significant differences were found for baseline and peak CI and CO values between groups as well. In these regards, the HR and SV compensatory mechanisms [21] corroborate and explain our results. It is evident that the decrease in both CI and CO in HFrEF individuals was due to ventricular impairment, which leads to compensatory mechanisms that are initially adjusted to promote an increase in these variables and maintain end-organ perfusion.[34] However, after prolonged exposure to these mechanisms, the myocardium undergoes remodeling, which reduces the ventricular inotropic capacity in addition to impairing the SV and, consequently, CI and CO.[2]

When subjected to submaximal exercise, individuals with HFrEF exhibit lower responses to both increase and decrease in oxygen consumption (VO_2) compared to healthy individuals.[35] These patients can also present a certain level of pulmonary hypertension, what can explain the behavior of CO and SV.[22, 36] This CO behavior may thus be due to the imbalance in autonomic response that affects SV.[37]

We tested whether hemodynamic parameters assessed through SM-IGC correlated with distance walked on the 6MWT and found that the faster the CO acceleration, the greater the distance walked during the test ($r=0.49$, $P=0.01$). In addition, individuals whose HR recovered quicker after the test were those who walked the longest. Even though this was not the main aim of the study, we found that hemodynamic assessment using SM-ICG during 6MWT can provide interesting results directly

indicative of functional capacity. In addition to distance on 6MWT, we found that NYHA functional class had a good association with maximum CO, CO deceleration, and HR during the first minute of recovery after the walking test.[38] As expected, we found differences between groups ($P<0.01$) for distance covered in 6MWT, which corroborates previous research.[39]

Finally, linear regression showed impairment in contribution of SV (22.9%) to CO change in HFrEF patients, as expected. In contrast, healthy controls showed normal values (57.4% of SV contribution to CO change). O_2 pulse, a surrogate of SV, can corroborate our findings, since it was lower in HFrEF patients compared to healthy controls according previous studies. SV impairment, as represented by low O_2 pulse, may reflect an insufficient systemic O_2 delivery during exercise, and/or reflect impaired O_2 utilization due to the reduction of mitochondrial function.[40, 41]

Some limitations of this study must be pointed out. First, it may have been underpowered to detect a significant difference in some comparisons due to the small sample size; however, the main purpose of this physiological study was to evaluate the hemodynamic behavior of individuals with HFrEF during a 6MWT and compare it to that of healthy individuals, in addition to determining the relative contribution of variations in HR, SV, and CO during the different phases of the walking test. Second, the study was not designed to test correlations and associations of SM-ICG parameters with distance walked, NYHA functional class, or ejection fraction.

Although 6MWT is safe, inexpensive, and easily used to assess functional capacity in patients with HFrEF, it has limitations both in clinical and research grounds,[20, 39] (e.g., it does not provide direct data in regard to hemodynamic behavior). Therefore, new technologies that add information to 6MWT findings may be important for clinical practice in this ominous syndrome. In this sense, technological advancement

has made it possible to develop a portable device to conduct real-time, noninvasive measurement of a wide range of hemodynamic parameters, such as CO, CI and others.

Conclusion

This is the first study to demonstrate real-time SM-ICG can be performed in patients with HFrEF during a 6MWT. Robust hemodynamic responses have been identified, with SV duplication, CO triplication and CI increase during exertion, but the magnitude of this response was still reduced in comparison to that of healthy controls. HFrEF individuals shown an impairment in CO and HR acceleration during submaximal exercise compared to healthy controls, and thus can represent an imbalance in autonomic response to effort. Further studies are needed to test whether changes in CO, SV, HR, and CI during 6MWT can provide information on disease prognosis.

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Figure 1.

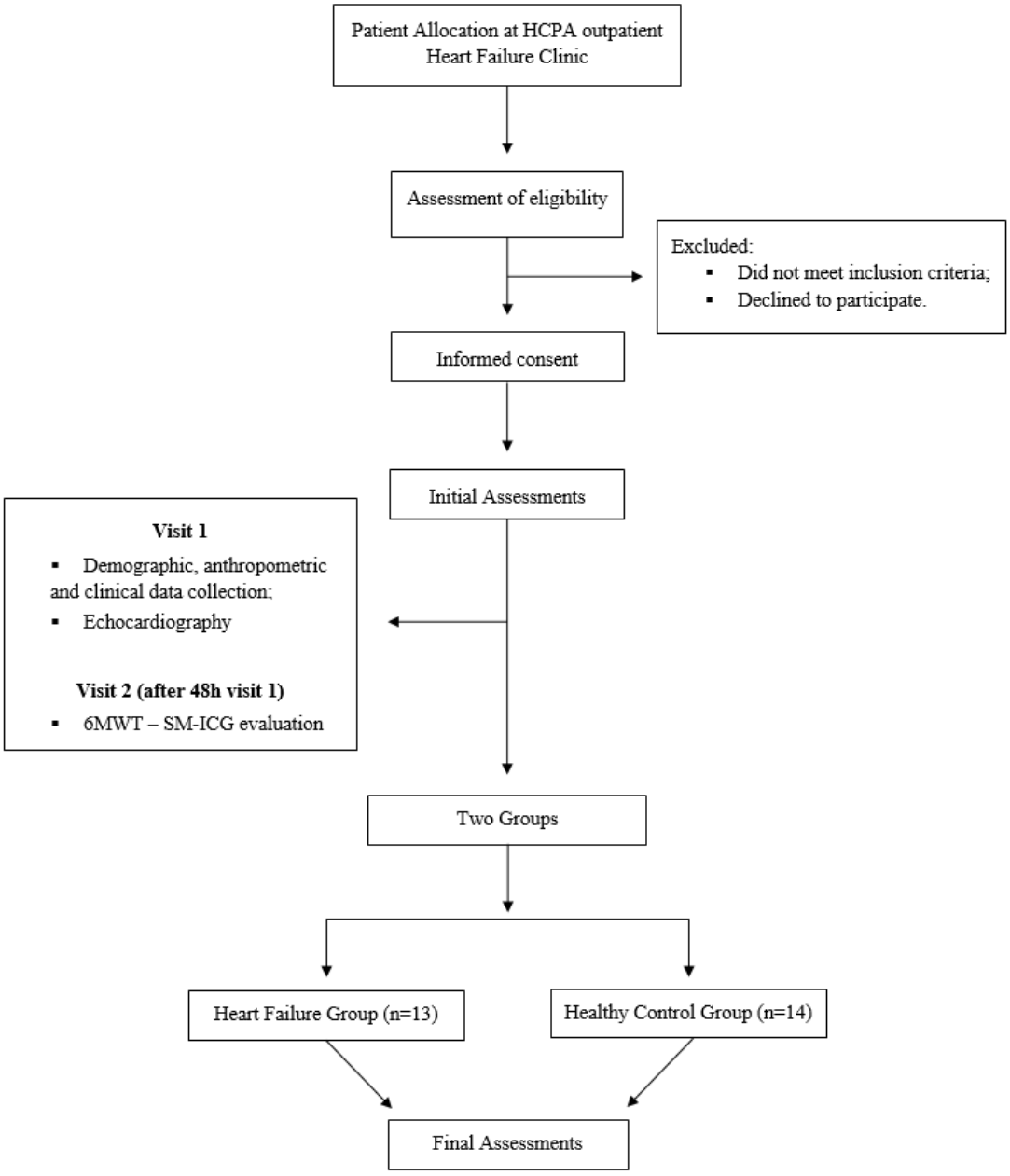

Patient Allocation at HCPA outpatient Heart Failure Clinic
Assessment of eligibility
Excluded:
Did not meet inclusion criteria;
Declined to participate.
Informed consent
Initial Assessments
Visit 1
Demographic, anthropometric and clinical data collection;
Echocardiography
Visit 2 (after 48h visit 1)
6MWT – SM-ICG evaluation
Two Groups
Heart Failure Group (n=13)
Healthy Control Group (n=14)
Final Assessments

Figure 2.

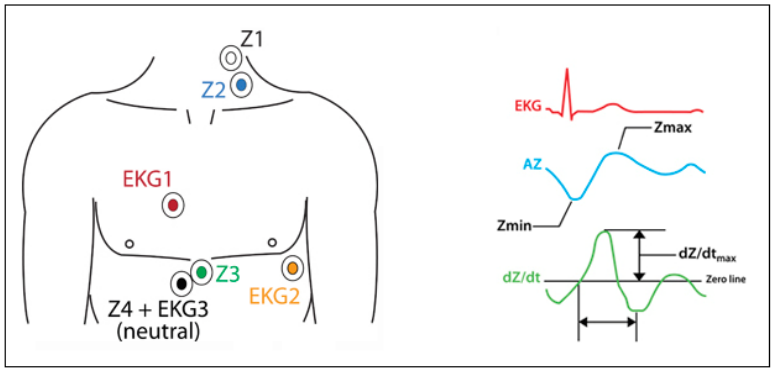


Figure 3.

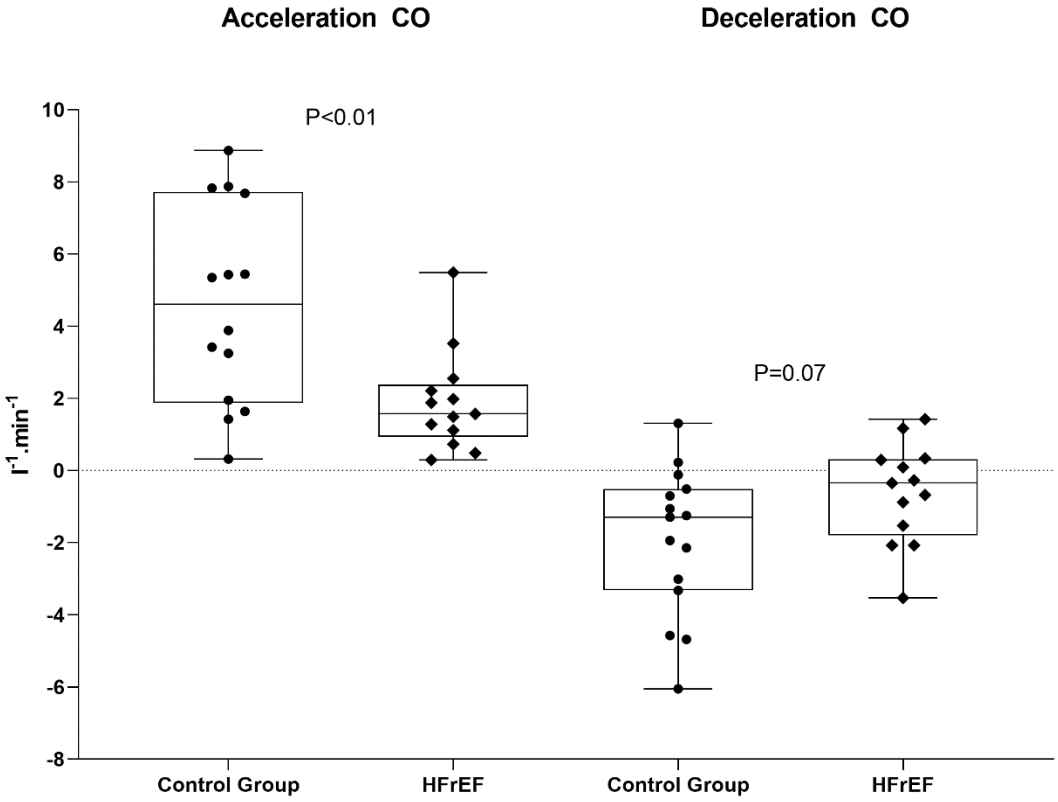
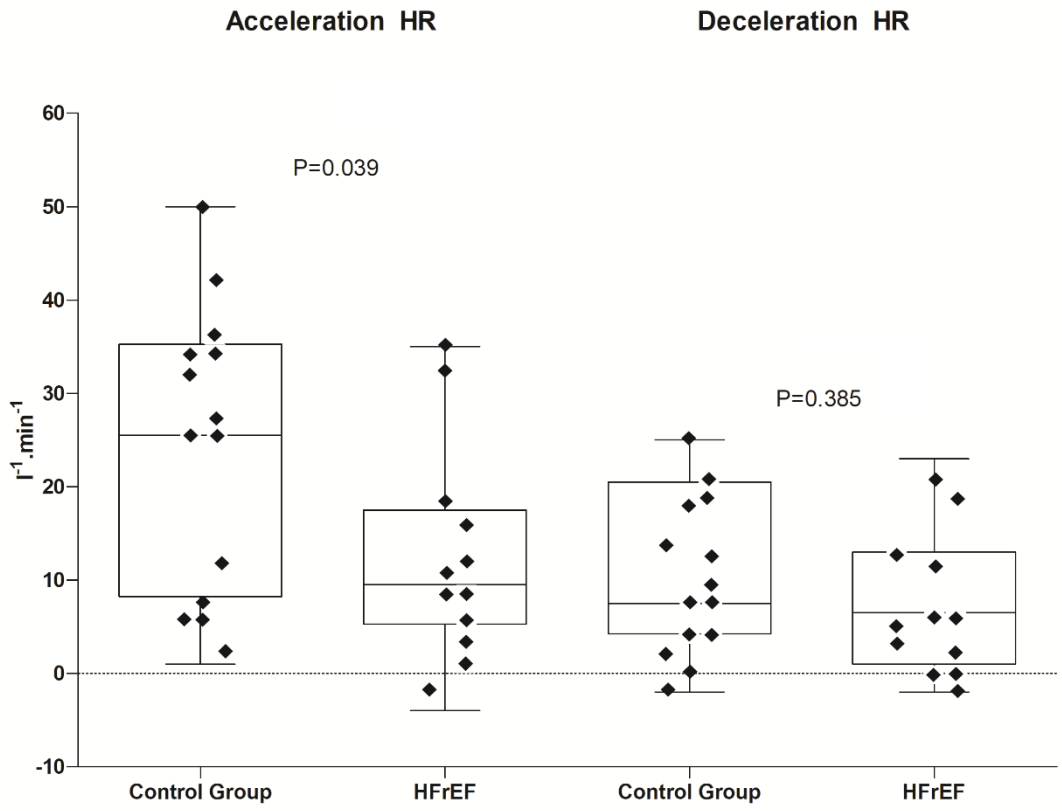


Figure 4.


Acceleration HR
Deceleration HR
P=0.039
P=0.385
60
50
40
30
20
10
0
-10
r-1.min-1
Control Group
HFrEF
Control Group
HFrEF

6. ARTIGO 2 (https://doi.org/10.1186/s13063-022-06257-1)

Title: Aerobic exercise and telomere length in patients with systolic heart failure: protocol study for a randomized controlled trial

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Abstract

Background: Heart failure (HF) with reduced ejection fraction (HFrEF) is a syndrome that leads to fatigue and reduced functional capacity due to disease-related pathophysiological mechanisms. Aerobic exercise (AERO) plays a key role in improving HF outcomes, such as increase in peak oxygen uptake (VO_2peak). In addition, HF promotes cell senescence, which involves reducing telomere length. Several studies have shown that patients with a worse prognosis (i.e., reduced VO_2peak) also have shorter telomeres. However, the effects of AERO on telomere length in patients with HFrEF are still unknown. In an attempt to fill this gap, we designed a study to determine the effects of 16 weeks of aerobic training (32 sessions) on telomere length in HFrEF patients.

Methods: In this single-center randomized controlled trial, men and women between 50-80 years old will be allocated into two different groups: a moderate-intensity aerobic training and a control group. Telomere length, functional capacity, echocardiographic variables, endothelial function and walking ability will be assessed before and after 16-week intervention period.

Discussion: Understanding the role of physical exercise in biological aging in HFrEF patients is relevant. Due to cell senescence, these individuals shown a shorter telomere length. AERO can delay biological aging according to a balance in oxidative stress through antioxidant action. Positive telomere length results are expected for aerobic training group.

Trial registration: ClinicalTrials.gov Identifier - NCT03856736. First registered: February 27, 2019 – Aerobic Exercise and Telomere Length in Patients With Heart Failure.

Keywords: Exercise training, Cardiac rehabilitation, Heart failure, Functional capacity, Biological ageing, Endothelium.

Background

HF affects more than 26 million people worldwide. It is considered a global public health problem and is expected to increase substantially with the ageing of the population. Globally, HFrEF is the most prevalent form of HF syndrome, affecting at least 60% of all patients.[1, 2] Despite different strategies for its management, most individuals with this syndrome will experience some limitation in exercise capacity during the natural course of the disease.[3, 4] In fact, exercise intolerance dominates the clinical presentation of moderate to severe HFrEF and is a major determinant of overall prognosis.[5-7] On the other hand, patients who exercise regularly have a better prognosis than sedentary ones,[8] since AERO improves VO_2peak[9-11] and TL.[12]

TL is a complex DNA sequence located at the ends of chromosomes.[13-15] It is important to point out that oxidative stress is the main factor that shortens TL in HFrEF[16-19] and accelerates the aging process.[20-22] Studies have shown that exercise can promote a reverse profile in oxidative stress, increasing TL or preventing telomere shortening.[23-26] However, changes in TL depend on exercise intensity. HIIT is described as short periods of exercise performed at a high intensity (>80%-85% heart rate reserve), with active recovery intervals at a moderate intensity (30-40% of HRR).[27] MIAT (40%-60% HRR), however, is the most commonly used AERO modality, and different HF guidelines recommended it.[28-30] Physiologically, very high intensity exercise can lead to decreased TL due to an imbalance between severe oxidative stress and reduced antioxidant mechanisms.[31, 32] In contrast, MIAT can lead to a reduction in oxidative stress through higher antioxidant activity, which can have beneficial effects on TL.[33-37]

In individuals who have not been diagnosed with HFrEF, conflicting results have been found regarding the effects of MIAT on TL. Some studies have shown that MIAT may increase TL,[12, 24, 33, 34] while others have not observed any modification in these

outcomes.[38-40] In patients with HFrEF, MIAT can improve functional capacity and has been demonstrated to be safe, effective and reproducible outside the hospital environment.[41-43] However, as far as we know, no studies have investigated MIAT and TL in the HFrEF setting and, since there is a gap in the literature, the main goal of this manuscript is to describe the study protocol of this unique randomized controlled trial.

Methods

We will compare TL in a MIAT group and a CG of HFrEF patients before and after 16 weeks of an exercise-based cardiac rehabilitation program. In addition, the secondary outcomes of this randomized controlled trial are to correlate TL with:

1. Different CPET parameters such as VE/VCO_2, oxygen pulse and oxygen uptake efficiency slope;
2. Changes in echocardiographic variables by Doppler echocardiogram;
3. Changes in endothelial function measured by FMD of the brachial artery;
4. Changes in walking ability measured by a SWSS.

Study design

This study will be a single-center randomized, controlled trial performed at a tertiary hospital in southern Brazil. Patients recruited to participate will assigned to the MIAT group, which will exercise twice a week for 16 weeks, or the CG, which will stretch and do low-intensity and low volume treadmill walking exercise (to mimic the intervention group) twice a week for 16 weeks. The public title for the work to patient recruitment is "Exercise to improve your heart and longevity". Procedures explaining the intervention and the benefits of exercise will be applied in recruiting the patient

Measurements will be taken before and at the end of the follow-up. For familiarization, all subjects will participate in a run-in period involving three treadmill exercise sessions before randomization. The allocation ratio will be 1:1 and the framework will be superiority.

An experienced researcher in cardiac rehabilitation who is not involved in the data collection will apply the protocol. The study named “Exercise for improve your health” will be conducted at the hospital cardiac rehabilitation center with support from the CardioEx. The trial protocol was registered in ClinicalTrials.gov (identifier: NCT03856736) and follows the recommendations of the SPIRIT 2013 statement (Standard Protocol Items: Recommendations for Interventional Trials). The schedule of enrollment, interventions, and assessments is presented in Table 1.

Participants

The volunteers will be recruited through the HF Outpatient Clinic of a tertiary public hospital in Porto Alegre, Brazil. Participants will be randomly allocated into two different groups: MIAT and the CG, which will engage in supervised low-intensity AERO with stretching.

Inclusion criteria:

- Primary diagnosis of HF with ejection fraction <40%;
- Clinically stable patients with at least three months on optimal HF treatment;
- Age between 50 and 80 years;
- NYHA functional class II to III;
- No contraindications to participate in an exercise program;
- Mentally able to understand instructions during the study.

Exclusion criteria:

- Severe valve disease;
- Peripheral artery disease with symptoms of intermittent claudication;
- Uncontrolled hypertension;
- Drug or alcohol abuse;
- Cognitive and/or osteomyoarticular conditions that prevent exercise;
- Logistical impossibility of attending the hospital intervention;
- Engaging in supervised physical exercise in the past three months;
- Do not complete the run-in period.

Study procedures

The protocol for both groups will be applied at a local tertiary hospital (HCPA). The study diagram can be seen in Figure 1. After confirming the eligibility criteria during first contact, the researchers will obtain the written informed consent.

Randomization

A researcher not involved in other phases of the study will perform the randomization and allocation protocols. The allocation list will be generated through the *randomization.com* website and the data will be managed through the REDCap software in order to provide the allocation concealment. The randomization will be stratified by sex and age (50 to 64yr; 65 to 80yr) and different size blocks will be employed in random order. Researchers involved in data analysis and assessments will be blinded to the participant's allocation group.

Demographic and clinical variables

Demographic, anthropometric and clinical data will be collected to characterize the sample. In addition, blood collection, CPET, echocardiography, endothelial function (assessed by brachial artery FMD measurement) and a SSWS test will be performed.

Age, gender, the presence of diabetes mellitus, systemic hypertension and dyslipidemia will be some of the data used for sample characterization, as well as medical history and current medications. We will also measure waist, abdomen and hip circumferences, as well as body mass and height, before participants begin the protocol.

Telomere length

A real-time qPCR will be utilized to quantify TL. This technique is based on extending the telomere sequence from a small amount of genomic DNA.

In the present study, relative TL will be specifically evaluated, which is obtained through two qPCR reactions for each sample. One reaction is used for amplifying the T, while the other is for a S, which is responsible for controlling the amplification and allowing the number of genome copies per sample to be calculated. Therefore, the T/S ratio will be calculated to obtain a value that correlates with the average length of the analyzed telomeres.[44, 45]

Blood collection

The participants will rest for 15 minutes prior to blood collection. After the rest period, 10mL of peripheral blood will be collected by trained personnel. The collected blood will be dispensed into 15mL tubes containing EDTA anticoagulant and will be homogenized by inversion. Subsequently, the blood will be transferred to 15mL tubes with a Histopaque® 1077 phase (density: 1.077 g/mL, Sigma Aldrich, St Louis, MO,

USA) at a 1:1 ratio and then centrifuged at 400 xg for 30 min. Thereafter, centrifugation-purified peripheral blood mononuclear cells will be collected, from which genomic DNA will be extracted for subsequent qPCR.

Cardiopulmonary exercise test

All evaluations will be performed during the morning shift at the HCPA noninvasive cardiology unit under controlled temperature (18 to 22ºC). The tests will always be performed by the same cardiologist, who is qualified by the Brazilian Society of Cardiology. The test will be performed on a treadmill (General Eletric T-2100, GE Healthcare, Waukesha, WI, USA) using a ramp protocol previously described in Nery et al. 2015.[46] VO_2, VCO_2, ventilatory anaerobic threshold, respiratory compensation point, peak respiratory exchange ratio, VE/VCO_2 slope, oxygen uptake efficiency slope, and O_2/HR will be measured and recorded breath by breath with a specific CPET system for measuring pulmonary gas exchange (Quark CPET, COSMED, Rome, Italy). Continuous 12-lead electrocardiographic monitoring (Nihon Kohden Corporation, Tokyo, Japan) will be performed following Mason & Likar 1966. Blood pressure measurement will be assessed with a sphygmomanometer (P.A. MED PA 2001, Brazil). Maximum tests will be considered when the peak respiratory exchange ratio is ≥ 1.05.

Transthoracic doppler echocardiogram

All evaluations will be performed by a trained cardiologist on the same equipment at the HCPA noninvasive medicine unit (Envisor C HD, Philips, USA) with a standard multifrequency sector transducer. Patients will be evaluated at rest in the left lateral supine position. Ultrasound equipment will be placed on the patient's chest and the signals will be transmitted and converted into a moving image on a monitor.

Subsequently, the diameters and volumes of the atrium and left ventricle will be measured. The ejection fraction will be calculated using the Teicholz formula from the parasternal long axis. However, for patients with regional wall motion abnormalities, Simpson's rule will be used. The assessment will proceed according to current guidelines of the American College of Cardiology and the American Heart Association.[47]

Endothelial function

The assessments will be performed according the recent expert consensus and evidence-based recommendations on flow-mediated dilatation in humans.[48] The volunteers will receive preparation instructions, such as the need to fast for six hours prior to evaluation; no smoking or tobacco consumption prior to measurement (>6 h); avoiding exercise (>24h), caffeine and alcohol (>12h) prior to the evaluation; recording medication used in the 24 hours prior to assessment; premenopausal women should record the day of the menstrual cycle, since the evaluation will be between the first and seventh cycle day.

The pre- and post-assessment will be performed at the same hour, in a room with controlled temperature (18 to 22ºC). The volunteers will have 10-15 minutes of supine rest prior to beginning their assessment. During the assessment, the volunteers will be asked to lie in the supine position with their left arm positioned comfortably. Endothelium-dependent and independent dilations will be measured by spectral Doppler ultrasound (Ultrasonix, Ultrasonix Medical Corporation, Richmond, Canada) with a modulated electrocardiogram and a high frequency vascular transducer (between 7.5 and 14 MHz). FMD will be expressed as the relative variation of the brachial diameter in the hyperemic phase and defined as [(post hyperemic diameter - baseline diameter)/baseline diameter] × 100.

Self-selected walking speed

This test will be performed during the first training session to determine the volunteers' SSWS. The test will be conducted in a 30-meter corridor, demarcated every 3 meters with cones, as previously described by Monteiro et al 2017.[49]

To balance any effects related to the participant's sensation of being evaluated and wanting to walk faster, timing will begin not with the first cone but the second. Since this test is a measure of self-selected speed, we must ensure that it is performed with no stimulus to walk faster, especially when the subject is approaching the final cone. However, the timer will be stopped prior to the final cone for the same reasons as the first cone. Therefore, the evaluation will consist of the time taken to walk 24 meters. To calculate the SSWS, the distance traveled will be divided by the time necessary to do so; three attempts will be performed and the mean time will be considered the SSWS.

Intervention protocols

The aerobic training model will follow a predefined schedule (Table 2). The first week will be a run-in period (Figure 2), consisting of three sessions of moderate intensity AERO with a progressive increase in session duration. Both groups will perform treadmill exercise and stretching. However, the intensity and duration of treadmill walking and stretching will change. Because this will be a blind randomized clinical trial, we must ensure that the volunteers do not know the group in which they will be participating. Both protocols will last 16 weeks and involve sessions twice a week, totaling 32 sessions for each group. The minimum frequency will be 85% of total sessions. In the event of three consecutive absences, the volunteer will be excluded from the study, as well as if the patient appears only one session per week for three consecutive weeks. To improve adherence, we will use daily motivation in each session, such as praise, reminders that

the treatment is good for him/her, or that the patient is doing well and this will make a lot of difference to their health, regardless of which group was randomized (exercise or control).

The training prescription will be individualized according to CPET. The target training zones will be defined through percentages of heart rate reserve and ventilatory thresholds, concomitant with the use of a modified Borg CR10 scale[50] at moderate intensity (40-60% of heart rate reserve; Borg 4-6). The protocol will begin with a warm up and will have a cool-down period. Variables such as speed and grade will be systematically adjusted each week according to each patient's chronotropic response, effort perception and condition. When necessary, it will be returned to the previous level until the individual adapts and can progress.

Exercise sessions will be prescribed and accompanied by an exercise physiologist who may be accompanied by undergraduate physical education, physical therapy or medicine students who will monitor and record HR, blood pressure and perceived exertion before, during and after exercise session. The training program will be performed on treadmills (Inbramed, Export, Porto Alegre, Brazil and TEB APEX 2000, São Paulo, Brazil).

Control group

The CG will undergo two sessions weekly, totaling 32 sessions. Stretching exercises, low intensity treadmill walking and body relaxation techniques will be performed. Given the literature gap about the effects of AERO on TL in HF patients, we decided to create a CG with the same intervention time and weekly frequency that performs low-intensity AERO on a treadmill to mimic the intervention group and

consequently investigate the real effects of MIAT advocated in different guidelines, with all participants blinded as far as possible to intervention type.

Statistical analysis

The calculated sample size is 10 patients for each group (20 total), considering a significance level of 5%, a power of 80%, a difference to be detected equal to a standard deviation of 0.0026 for TL, the primary outcome. Based on other studies, we estimate there will be a 20% loss, so it will be necessary to include 12 patients per group (24 patients in total). The difference to be detected is considered clinically relevant and the variability was based on Van der Harst et al. 2007.[16]

Descriptive statistics will be performed with mean and standard deviation or median and interquartile range when appropriate. The Shapiro-Wilk test will be performed to verify data normality. Baseline sample characteristics will be compared using Student's *t*-test or the Mann-Whitney U test for continuous variables and the chi-square and Fisher exact test for categorical variables. The outcomes for the MIAT and the CG during the pre- and post-training periods will be analyzed with generalized estimating equations. A Bonferroni post-hoc test will be used to identify differences between effects and interactions. Intention to treat will be applied

Discussion

Telomere and its length have been studied as a biological marker of aging and is considered a therapeutic target, not only in patients, but also in healthy individuals.[51,52] The larger the telomere, the greater the life expectancy of the individual.[53]

Acute AERO can promote the up-regulation of telomeres and the expression of white blood cell microRNAs, improving immune function and physical health.[54] In its

turn, chronic physical training plays an important role in maintaining or increasing the TL.[23] Some evidence suggests that only AERO (moderate or high intensity), can increase the TL after 6 months of intervention in healthy individuals[12] and there is already some evidence that MIAT can have a positive impact on TL in some pathological scenarios.[33-37]

HFrEF patients present a decrease in VO_2peak and an increase in oxidative stress, findings that goes towards a more reserved prognosis. It is important to point out that the worse the disease, the shorter the telomeres.[23] In contrast, MIAT can increase VO_2peak and reduce oxidative stress through greater antioxidant activity, acting positively on TL. However, specifically, the effect of MIAT on TL in the HFrEF scenario is unknown, but we will test if 6 months of this type of training can delay biological aging, promoting a positive impact on TL in these stable patients with this syndrome.

Abbreviations

Heart failure – HF; Heart failure with reduced ejection fraction – HFrEF; Aerobic exercise – AERO; Peak oxygen uptake – VO_2peak; Telomere length – TL; High-intensity interval training – HIIT; Heart rate reserve – HRR; Moderate-intensity aerobic training – MIAT; Control group – CG; Cardiopulmonary exercise testing – CPET; Ratio between ventilation and the carbon dioxide production – VE/VCO_2; Flow-mediated dilation – FMD; Self-selected walking speed test (SWSS); Exercise Cardiology Research Group – CardioEx; New York Heart Association – NYHA; Hospital de Clínicas de Porto Alegre – HCPA; Research Electronic Data Capture – REDCap; Polymerase chain reaction – qPCR; Telomeric sequence – T; Single-copy gene – S; Oxygen uptake – VO_2; Oxygen pulse – O_2/HR

Declarations

Ethics approval and consent to participate

This experiment will be conducted from March 2021 to December 2021. The study protocol was approved by the Institutional Review Board of Hospital de Clínicas de Porto Alegre (protocol 180651, version 1) in August 2018. Prior to the evaluation process and after the study's objectives, methods and the procedures for guaranteeing their anonymity is guaranteed are explained (including use of the data only for scientific purposes), the participants will provide written informed consent to participate, as recommended by Resolution 466/12 CNS/MS. Prior to the study, the participants will be informed about how testing and training will take place. In addition, the importance of the data obtained during the study will also be pointed out.

Both the research team and the institution will keep the identities of volunteers strictly confidential. The results of procedures evaluated in the research will be analyzed and allocated in tables, figures and/or graphs, and will be disseminated in lectures, conferences, scientific journals, or other media for transferring knowledge to society in accordance with the regulatory norms of national or international protection.

The expected benefits will have great importance to participants, the institution involved, the researchers, society and any other medium interested in advancing science, such as a better understanding how TL behaves in patients with HFrEF, given its importance in cell senescence and the potential biomarker of disease prognosis The expected results will demonstrate the effects of AERO on TL, which could contribute to improving rehabilitation programs and new research in the fields of exercise, cardiac rehabilitation and health promotion.

Consent for Publication

Not applicable.

Availability of data and material

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study. Relevant data from this study will be made available upon study completion and researchers request from the corresponding author.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

LTF conceptualized the study and wrote the first draft of the paper. ELG, AAP, SBM, MMA, OAB, MALS, ADL contributed to design and will be collecting and analyzing data during the study. AHP, LCD and RS have contributed to understanding pathophysiological mechanism of TL, heart failure and exercise, developing an intervention protocol and approaches to recruitment into the study. RS has contributed to theoretical planning, particularly on decision making and he is a head of the Exercise

Cardiology Group (CardioEx). All authors had input to revisions of the paper and approved the final version of manuscript.

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Figure 1. Study diagram.

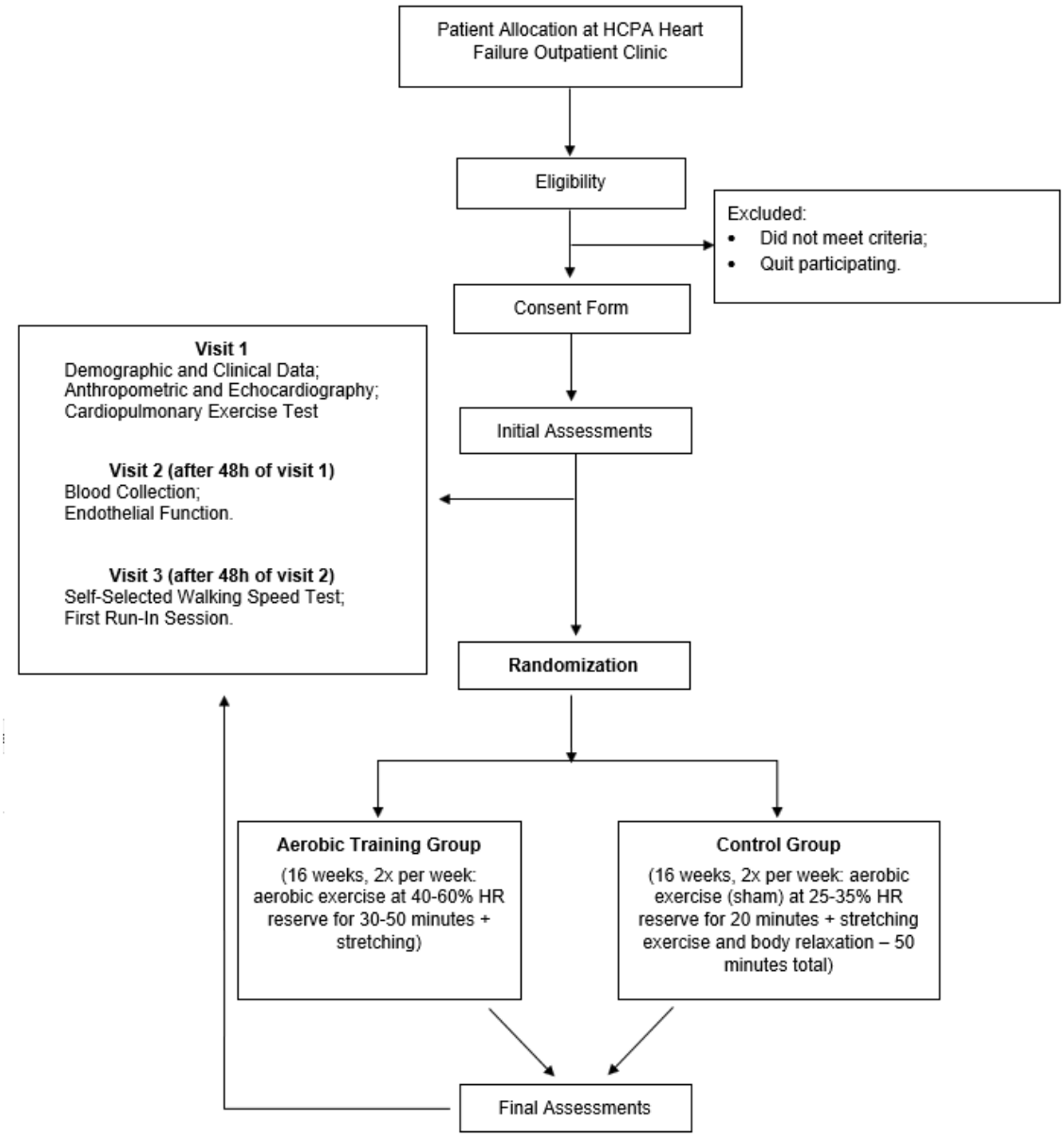

Figure 2. First week run-in period.

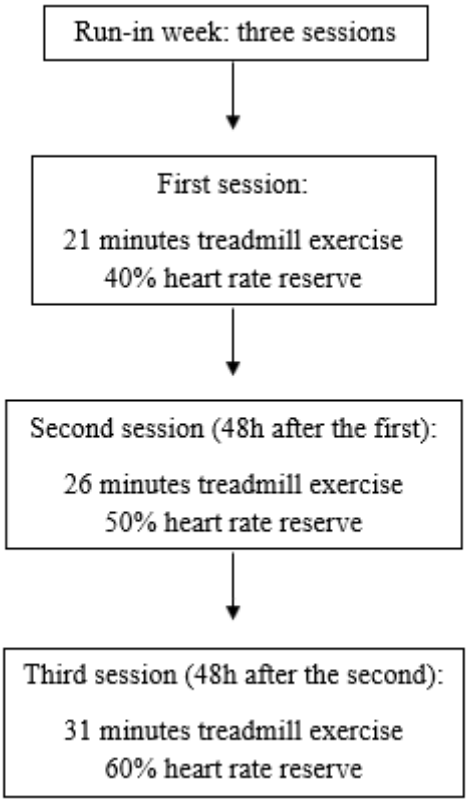

Table 1: Schedule of enrollment, interventions, and assessments.

	Enrollment	Pre-Allocation		Post Allocation		Close-out
Time point (months)*	$-t_1$	t_0	$t_{1/2}$	t_1	t_2	tx
Enrollment						
Eligibility screening	X					
Informed consent	X					
Allocation			X			
Interventions						
Moderate-Intensity Aerobic Training				⟷		
Control group				⟷		
Assessments:						
Telomere length		X				X
Cardiopulmonary exercise testing		X				X
Echocardiogram		X				X
Flow-mediated dilation		X				X
Self-Selected Walking Speed Test		X				X

Note: *–t1: enrollment; t0: baseline assessment before randomization; t1/2: allocation; t1: start of interventions; t2: final assessment after interventions; tx: analysis of variables; HF: heart failure.

Table 2. Predefined periodization for aerobic training group

Intervention Period	Exercise Prescription
Week 1-2	Time: 30 – 35 min – 40-45% HRR = equivalent % VO_2peak
Week 2-3	Time: 35 – 40 min – 45-50% HRR = equivalent % VO_2peak
Week 3-4	Time: 35 – 40 min – 50-55% HRR = equivalent % VO_2peak
Week 4-6	Time: 40 – 45 min – 55-60% HRR = equivalent % VO_2peak
Week 6-7	Time: 45 – 50 min – 50-60% HRR = equivalent % VO_2peak
Week 7-8	Time: 45 – 50 min – 50-60% HRR = equivalent % VO_2peak
Week 8-9	Time: 45 – 50 min – 50-60% HRR = equivalent % VO_2peak
Week 9-11	Time: 45 – 50 min – 50-60% HRR = equivalent % VO_2peak
Week 11-12	Time: 45 – 50 min – 50-60% HRR = equivalent % VO_2peak
Week 12-13	Time: 45 – 50 min – 50-60% HRR = equivalent % VO_2peak
Week 13-14	Time: 45 – 50 min – 50-60% HRR = equivalent % VO_2peak
Week 14-15	Time: 45 – 50 min – 50-60% HRR = equivalent % VO_2peak
Week 15-16	Time: 45 – 50 min – 50-60% HRR = equivalent % VO_2peak

HRR: Heart Rate Reserve.

8. CONSIDERAÇÕES FINAIS

Ao longo de meu andamento para me tornar Doutor em cardiologia e ciências cardiovasculares pelo PPG da cardiologia, desenvolvi três artigos com assuntos relacionados: o primeiro e central sobre ICG e TC6M em indivíduos com ICFEr; o segundo, um estudo de protocolo sobre comprimento de telômeros e treinamento físico em indivíduos com ICFEr; Por fim, o terceiro sobre TxC, biomarcadores inflamatórios e treinamento físico. Os três artigos têm relação direta com um tópico bastante emergente: cardiologia e exercício físico. Tanto no aspecto da IC, quanto no TxC, precisamos incentivar a prática do ~~o~~ exercício físico para população cardiopata, visto que os benefícios são inúmeros, baseados em evidências robustas os quais são corroborados com os resultados encontrados nos estudos discutidos na minha tese.