TESE DE DOUTORADO

2017

METABORREFLEXO MUSCULAR INSPIRATÓRIO EM INDIVÍDUOS HIPERTENSOS

Simone Regina Posser Barreiro

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE MEDICINA

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE: CARDIOLOGIA E CIÊNCIAS CARDIOVASCULARES

Metaborreflexo Muscular Inspiratório em Indivíduos Hipertensos

Aluna: Simone Regina Posser Barreiro

Orientadora: Profª. Drª. Leila Beltrami Moreira

Coorientadora: Profª. Drª. Carine Cristina Callegaro

Tese submetida como requisito para obtenção do grau de Doutorado ao Programa de Pós-Graduação em Cardiologia e Ciências Cardiovasculares, da Universidade Federal do Rio Grande do Sul.

Porto Alegre

2017

CIP - Catalogação na Publicação

Barreiro, Simone Regina Posser
Metaborreflexo muscular inspiratório em indivíduos hipertensos / Simone Regina Posser Barreiro. -- 2017.
94 f.
Orientadora: Leila Beltrami Moreira.

Coorientadora: Carine Cristina Callegaro.

Tese (Doutorado) -- Universidade Federal do Rio Grande do Sul, Faculdade de Medicina, Programa de Pós-Graduação em Ciências da Saúde: Cardiologia e Ciências Cardiovasculares, Porto Alegre, BR-RS, 2017.

1. Hipertensão. 2. Músculos respiratórios. 3. Fluxo sanguíneo. 4. Resistência vascular. I. Moreira, Leila Beltrami, orient. II. Callegaro, Carine Cristina, coorient. III. Título.

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

Dedico aos meus pais, José e Elisa, ao meu marido Jonas e meu filho Pedro.

Obrigada por acreditarem em mim e nunca me deixar desistir.

Sou muito grata por ter vocês na minha vida.

AGRADECIMENTOS

As orientadoras, Profª. Drª. Leila Beltrami Moreira e a Profª. Drª. Carine Cristina Callegaro, por desde o primeiro momento depositar confiança neste trabalho e se dedicarem para a concretização. Ficam os exemplos de profissionalismo, ética e humildade. E a amizade de regalo.

Ao Hospital de Clínicas de Porto Alegre, onde intensamente passei meus dias, e aprendi um pouco mais sobre o mundo da pesquisa.

A Sirlei Ferreira Reis, ex-secretária deste PPG, pela amizade e conselhos sempre muito bem vindos.

Aos colegas da sala 945, pela amizade que criamos e por toda disposição em compartilhar conhecimento. Cada um com seu jeito particular de ser, mas que deixavam a rotina diária mais divertida e menos complicada. Em especial Glaube Riegel, Fernanda D' Athayde, Maria Angélica Pires, Marcela Pedromo e Silvana Perini.

Aos bolsistas e voluntários Valéria Rampelotto, Mariana Pinto, Scarlet Sobragi, Kellen Sábio, Giulia Martins, Vitor Feuser da Rosa, Débora Hutten, Carlos Rangel de Castilhos, Alberto Pompeo e Paula Merello, por toda colaboração e dedicação na conclusão deste projeto. Sem o apoio de vocês nada seria possível.

Aos colegas do Laboratório de Fisiopatologia do Exercício (LaFiEx), Aline Macedo, Lucas Porto, Paula Figueiredo, Paula Zambeli, Vinicius Marques, Gaspar Guatimozin e Daniel Umpierre por terem compartilhado seus conhecimentos, habilidades, experiências e amizade.

A Andressa Schein, pessoa incrível que o doutorado me proporcionou conhecer. Grande amiga e companheira de todas as horas.

Aos meus pais que me incentivaram buscar o doutorado, mesmo sabendo que deixaria o convívio do lar, me apoiaram de todas as maneiras possíveis. Agradecer vocês é realmente muito difícil, pois sempre é pouco. Amo-os.

Finalmente a minha nova família que estou constituindo, meu marido Jonas e meu filho Pedro, que ainda se faz presente no meu ventre. Jonas, por todo tempo que passamos distantes e sofremos com isso, e mesmo assim sempre me incentivou e apoiou, não me deixando desistir. Obrigada também por me dar o presente mais espero das nossas vidas, nosso filho Pedro. Amo vocês eternamente.

Toda reforma interior e toda mudança para melhor dependem exclusivamente da aplicação do nosso próprio esforço.
Immanuel Kant.

SUMÁRIO

LISTA DE ABREVIATURAS E SIGLAS

ANSM - Atividade Nervosa Simpática Muscular

CO_2 - Gás Carbônico

DC - Débito Cardíaco

DM - Diabetes Mellitus

FC - Frequência Cardíaca

FS - Fluxo Sanguíneo

HAS - Hipertensão Arterial Sistêmica

HCPA - Hospital de Clínicas de Porto Alegre

IC - Insuficiência Cardíaca

IMC - Índice de Massa Corporal

IPAQ - International Physical Activity Questionnaire

MAPA - Monitorização Ambulatorial de Pressão Arterial

MRMI - Metaborreflexo muscular inspiratório

OMS - Organização Mundial de Saúde

O_2 - Oxigênio

PA- Pressão Arterial

PAD - Pressão Arterial Diastólica

PAM - Pressão Arterial Média

PAS - Pressão Arterial Sistólica

RV - Resistência Vascular

SNA - Sistema Nervoso Autônomo

SNS - Sistema Nervoso Simpático

SNP - Sistema Nervoso Parassimpático

TMI - Treinamento Muscular Inspiratório

RESUMO

Indivíduos com hipertensão arterial sistêmica podem apresentar o metaborreflexo muscular inspiratório exacerbado comparado a indivíduos normotensos. No primeiro estudo, comparamos as respostas hemodinâmicas e respiratórias da ativação deste reflexo entre hipertensos e normotensos. Em uma amostra de conveniência, 31 participantes realizaram o protocolo de indução do metaborreflexo muscular inspiratório com carga e o protocolo controle. No protocolo de indução com carga houve maior aumento da pressão arterial média e redução do fluxo sanguíneo da perna para os hipertensos em relação aos normotensos. Conclui-se que o metaborreflexo muscular inspiratório pode estar exacerbado nos indivíduos hipertensos**.** O segundo estudo, protocolo de ensaio clínico randomizado duplo cego, foi publicado e tem como objetivo testar a eficácia do treinamento muscular inspiratório sobre a redução dos níveis pressóricos de hipertensos.

ABSTRACT

Individuals with systemic arterial hypertension may present exacerbated inspiratory muscle metaboreflex compared to normotensive individuals. In the first study, we compared the hemodynamic and respiratory responses of the activation of this reflex between hypertensive and normotensive individuals. In a convenience sample, 31 participants performed the induction protocol of inspiratory muscle metaborreflex with load and the control protocol. In the protocol of induction with load there was a greater increase in mean arterial pressure and reduction of blood flow from the leg to hypertensive patients in relation to the normotensive ones. It is concluded that inspiratory muscle metaboreflex may be exacerbated in hypertensive subjects. The second study, a randomized double-blind clinical trial protocol, was published and aims to test the effectiveness of inspiratory muscle training on the reduction of blood pressure levels in hypertensive patients.

1. INTRODUÇÃO E JUSTIFICATIVA

O metaborreflexo muscular é um mecanismo ativado quando há um desequilíbrio entre o aporte de oxigênio (O_2) e o metabolismo muscular durante exercícios moderados a intensos, resultando em resposta pressórica aumentada, com restauração da perfusão sanguínea para a musculatura em isquemia.[1]

Esta resposta ocorre em consequência das contrações fatigantes dos músculos e consequente acúmulo de produtos metabólicos que estimulam os nervos frênicos aferentes tipo IV,[1-4] resultando em aumento pronunciado da atividade simpática vasoconstritora.[1,5-8] Este reflexo causa a redução do fluxo sanguíneo (FS) da musculatura periférica inativa e consequente aumento da resistência vascular (RV).[1,5,9]

Na hipertensão arterial sistêmica (HAS), condição crônica caracterizada por níveis elevados e sustentados de pressão arterial (PA)[10-12], o exercício de resistência de alta intensidade leva a aumento na atividade eferente simpática para o coração e os vasos sanguíneos, resultando em aumento moderado da pressão arterial média (PAM) e frequência cardíaca (FC).[13-16]

Estudos mostraram que sujeitos adultos hipertensos que nunca foram submetidos a tratamento farmacológico[17] e com tratamento farmacológico[18], em comparação com normotensos, apresentam redução do controle da atividade simpática periférica mediada pelo metaborreflexo muscular periférico e que a ativação do componente metabólico do metaborreflexo ou reflexo pressor no exercício está aumentada em humanos hipertensos mais velhos. Mais recentemente, Leal et al.[19]

mostrou que o metaborreflexo muscular periférico é hiperativado durante o exercício em ratos espontaneamente hipertensos.

O desencadeamento do metaborreflexo ativado pela contração dos músculos esqueléticos periféricos está bem documentado, mas apenas na última década foi descrito que ele também pode ser ativado pelo trabalho da musculatura respiratória.[5,7,8,20-22] Segundo Dempsey[23,24], o mecanismo chamado de "metaborreflexo muscular inspiratório" (MRMI), pode gerar vasoconstrição no músculo esquelético inativo, mas também no músculo ativo durante o exercício[7,8], limitando a capacidade funcional, desviando o FS a partir dos membros inativos para a musculatura respiratória[24], exacerbando a fadiga dos músculos periféricos[25]. Em homens jovens saudáveis, o exercício muscular inspiratório com cargas de 60% da pressão inspiratória máxima (PImáx) ativa o MRMI, ocasionando elevação tempo-dependente da atividade nervosa simpática muscular (ANSM), PAM e da RV, bem como diminuição do FS dos membros.[5,20,21]

O treinamento muscular inspiratório (TMI) é uma modalidade de exercício que tem sido utilizada em diversas situações clínicas, demonstrando vários benefícios similares aos exercícios convencionais, como melhora da capacidade cardiopulmonar e do controle autonômico cardiovascular, além de determinar melhora nos parâmetros da função pulmonar, na força e resistência dos músculos inspiratórios.[26,27] Também, dessa forma, é possível que reduza o acúmulo de metabólitos durante o trabalho muscular inspiratório, o que pode vir a atenuar a atividade do metaborreflexo.[28-33]

Foi demonstrado por Witt et al.[34] que o treinamento da musculatura inspiratória a 50% da PImáx atenua a resposta pressora, assim como reduz a fadiga dos

músculos flexores plantares durante a ativação do MRMI em indivíduos saudáveis.[35] Em pacientes com insuficiência cardíaca (IC), o TMI com carga de 30% da PImáx, resultou em melhora do FS dos membros em repouso.[36] Em outro estudo incluindo 13 participantes hipertensos, o TMI com carga de 30% da PImáx reduziu 7,9mmHg e 5,5mmHg do delta da pressão arterial sistólica (PAS) e pressão arterial diastólica (PAD) na média de 24hs, respectivamente, e também melhorou o controle autonômico cardiovascular.[37]

O conhecimento sobre o MRMI em indivíduos hipertensos ainda é escasso na literatura, havendo dados limitados que examinam este reflexo em humanos hipertensos. Além do que, o MRMI pode ser atenuado pelo TMI, como visto em pacientes com insuficiência cardíaca (IC)[36] e saudáveis,[34] podendo também reduzir os níveis pressóricos dessa população.[37] Neste contexto, cabe a realização de novos estudos para testar as hipóteses de que: 1) o MRMI pode estar exacerbado em indivíduos hipertensos; 2) o TMI atue na atenuação dos mecanismos envolvidos na ativação do MRMI nestes indivíduos, reduzindo os valores pressóricos.

2) REVISÃO DE LITERATURA

2.1) Metaborreflexo Muscular Inspiratório

O metaborreflexo muscular periférico já é bem conhecido por fisiologistas no mundo todo, porém nos últimos anos evidenciou-se que este reflexo também pode ser ativado pela musculatura respiratória.[5,7,8,20] A existência do MRMI é explicada pelo fato de que o diafragma compete com a musculatura periférica pelo FS durante exercícios com grande demanda metabólica, o que leva à fadiga diafragmática, aumentando a utilização dos músculos acessórios, provocando desequilíbrio entre o aporte de O_2 e o metabolismo muscular, agregando ainda algum nível de dispneia.[3,38] Como na musculatura dos membros, as contrações dos músculos respiratórios e acúmulo de metabólitos ativam fibras nervosas aferentes do tipo IV não mielinizadas[3], o que, por sua vez, aumenta a atividade vasoconstritora simpática via reflexo supra-espinhal.[1,5,7,17,20-22,39] Esses achados sugerem a existência de um metaborreflexo mediado simpaticamente que se origina de músculos inspiratórios fatigados.[34]

O metaborreflexo ocorre por meio de uma resposta pressórica aumentada desencadeada para restaurar a perfusão sanguínea e aporte de O_2 para a musculatura em isquemia. Durante exercício máximo, os músculos respiratórios requerem até 15% do consumo total de O_2 em indivíduos destreinados e 14 a 16% do débito cardíaco (DC),[25,40] levando a aumentos da RV periférica.[41] Segundo Rowell[9], este reflexo só é ativado a partir de um limiar de redução do FS e aporte de oxigênio para a musculatura, ocorrendo em intensidades moderadas e elevadas de isquemia.

A importância do metaborreflexo se dá no que concerne à fadiga e exaustão dos músculos periféricos durante exercícios de alta intensidade. O FS das pernas mostrou-se inversamente relacionado ao trabalho respiratório, e as consequentes alterações na RV das pernas demonstraram estar diretamente relacionadas ao grau de liberação de noradrenalina.[6] Segundo Dempsey et al.[1], a ativação do MRMI leva à ativação de sistema nervoso simpático que induz vasoconstrição periférica, diminuindo o FS aos membros e causando, consequentemente, fadiga e aumento da percepção de esforço.

Observou-se que, em homens jovens, o trabalho intenso do músculo inspiratório ativa o MRMI, levando a aumentos tempo-dependentes na atividade nervosa simpática muscular, PAM, resistência vascular periférica e FC, bem como diminuição do FS para o membro em repouso.[5,20,38,42] Dessa forma, a ativação do MRMI durante o exercício físico intenso que leva à fadiga muscular inspiratória[43] pode limitar o desempenho físico[1,24] dos músculos esqueléticos ativos[6], exacerbando a fadiga dos músculos periféricos.[25] Efeitos semelhantes também foram demonstrados em pacientes com insuficiência cardíaca.[44,32,36]

2.2) Hipertensão Arterial Sistêmica

Segundo organizações nacionais e internacionais a HAS se caracteriza por elevação sustentada da PA, atualmente definida como valores ≥ 140mmHg para pressão sistólica (PAS) e/ou 90mmHg para pressão diastólica (PAD)[10,45], embora para pacientes com 80 anos ou mais uma PAS de até 150mmHg possa ser considerada aceitável.[46,47] Entretanto, em valores maiores do que 115mmHg e 75mmHg, para PAS e

PAD, respectivamente, os riscos cardiovasculares absolutos começam a crescer constantemente.[48]

A HAS continua mantendo-se como fator de risco modificável mais importante para morte por todas as causas, aterosclerose e trombose e lesões em órgãos-alvo, como cardiopatia isquêmica, acidente vascular encefálico, doença renal crônica, além de ser causa também de cardiopatia hipertensiva, podendo levar a IC.[45-47] O risco propiciado pela HAS, pode também ser agravado pela presença concomitante de outros fatores de risco como dislipidemia, obesidade abdominal, intolerância à glicose e diabetes mellitus (DM).[45]

A prevalência de hipertensão está em torno de 30-45% na população geral, com um aumento acentuado com o envelhecimento, podendo haver diferenças notáveis nos níveis médios de PA entre países e regiões.[10] Meta-análise de estudos populacionais brasileiros[48], que incluiu mais de 120 mil pessoas, estimou que, nas últimas três décadas, houve redução em 6% na prevalência de hipertensão no Brasil, porém, ainda está próximo de 30%.

Os principais fatores de risco para HAS incluem história familiar de hipertensão[49], DM, aumento de peso (principalmente o perímetro da cintura)[50], sobrecarga de cloreto de sódio[51] e abuso de álcool[52,53]. Para prevenção primária de HAS, é indicado o controle dos fatores de risco e diversas evidências de ensaios clínicos randomizados sustentam os benefícios do uso de fármacos anti-hipertensivos na redução de morbimortalidade em indivíduos hipertensos[47] e também naqueles definidos como pré-hipertensos, reduzindo também a incidência de hipertensão[54]. O tratamento não medicamentoso envolve os fatores de risco, como perda ponderal,

medidas nutricionais, prática de atividades físicas, cessação do tabagismo e redução da ingestão de álcool.[45]

O mecanismo fisiopatológico da HAS pode ser explicado pelo aumento da RV, por mudanças na estrutura da relação parede/lúmen vascular, por remodelamento, que dificulta a contração da musculatura lisa vascular.[55]

A regulação da PA é determinada basicamente pela retenção de sódio, o balanço hídrico e o tônus vasomotor do indivíduo. Estes mecanismos, por sua vez, são regulados por diversos fatores genéticos e ambientais, além de serem controlados por vias hormonais, via sistema nervoso autônomo (SNA) e por mecanismos de retroalimentação parácrinos e intracelulares. As interações entre estes fatores podem variar, principalmente com a idade, levando a grande heterogeneidade das alterações hemodinâmicas que levam à elevação da PA e sua sustentação.[56]

Basicamente, o mecanismo fisiopatológico que leva à HAS é a necessidade de uma PA maior do que o normal para os rins excretarem o excesso de sódio. Para isso, o Sistema Renina-Angiotensina-Aldosterona é ativado, resultando em vasoconstrição arteriolar aferente, o que aumenta a pressão intraglomerular e a taxa de filtração glomerular, além de aumentar a reabsorção renal de cloreto de sódio .[57]

O papel do sistema nervoso autônomo (SNA) no controle da PA tem sido cada vez mais estudado, tendo grande importância tanto no mecanismo inicial como na manutenção da hipertensão. Já foi demonstrado que em indivíduos hipertensos, o fluxo simpático está aumentado, tanto nos rins, como na musculatura vascular esquelética.[58] A ativação dos receptores α_1- adrenérgicos nos rins causa aumento da reabsorção de sódio, cujo papel é fundamental na gênese da hipertensão arterial primária.[58,59] Além disso, os barorreceptores arteriais, localizados nas carótidas e no

arco aórtico, tem a sua sensibilidade diminuída. Sendo assim, estes receptores não sinalizam para que haja redução do fluxo simpático diante de determinadas elevações na PA.[60,61] Os quimiorreceptores centrais também estão alterados em indivíduos hipertensos, levando ao aumento excessivo do fluxo simpático quando ativados.[62] Outro mecanismo que pode contribuir para a hiperativação simpática na HAS trata-se do metaborreflexo muscular periférico mediado pelo exercício, que induz aumento exagerado da pressão arterial em animais e humanos.[18,19,63]

2.3) Metaborreflexo Muscular e Hipertensão Arterial Sistêmica

Em animais hipertensos, foi demonstrado que existe uma resposta da PAM e frequência cardíaca (FC) exagerada à contração muscular eletricamente induzida[63] e que o metaborreflexo muscular periférico, especificamente, é exacerbado durante o exercício intenso em ratos.[18] Porém, há dados limitados que examinam o metaborreflexo em humanos hipertensos.

Os mecanismos básicos do aumento da PA durante o exercício nas populações normotensas incluem o "comando central" e o "reflexo pressor do exercício" (composto pelo mecanorreflexo e o metaborreflexo). Esses mecanismos podem contribuir para a resposta aumentada da PA observada durante o exercício, em estudo com hipertensos.[64] O comando central é um sistema nervoso avançado originado no cérebro, que aumenta a PA pela retração da atividade do sistema nervoso parassimpático (SNP) e aumento da atividade nervosa simpática.[65-67] Já o reflexo pressor do exercício é um feedback do sistema nervoso periférico iniciado no músculo esquelético composto de mecanorreflexo e metaborreflexo, que provoca elevação da

PA através do grupo III (predominantemente mecânico sensível) e do grupo IV (predominantemente metabo sensível) dos nervos aferentes.[64]

No estudo de Rondom et al.[18] sujeitos hipertensos, que nunca foram submetidos a tratamento farmacológico, apresentaram redução no controle da ANSM pelo metaborreflexo muscular periférico quando comparados a sujeitos controles saudáveis (normotensos). Em outro experimento, um total de 15 indivíduos normotensos (64±1 anos) e 12 hipertensos (65±1 anos) tiveram o metaborreflexo isolado usando isquemia pós-exercício de *handgrip* dinâmico para examinar resposta da PA comparando os dois grupos. Os principais achados do estudo foram que a isquemia pós-exercício resultou em uma maior elevação sustentada na PAM nos indivíduos hipertensos em comparação com os indivíduos normotensos. Os autores sugeriram que os indivíduos hipertensos possuem maior sensibilidade ao metaborreflexo.[64]

2.4) Treinamento Muscular Inspiratório

O TMI recebeu considerável interesse nos últimos anos como um método destinado a melhorar o condicionamento e o desempenho dos músculos respiratórios no exercício em indivíduos saudáveis. Desde que foi demonstrado que os músculos respiratórios podem ser treinados e respondem satisfatoriamente com aumento de força e/ou resistência, a aplicação do TMI passou a ser uma opção terapêutica.[68]

O TMI pode ser realizado através de dispositivos que fazem resistência à inspiração por meio de um sistema de mola com uma válvula unidirecional. Em indivíduos saudáveis, o TMI resulta em aumento na proporção de fibras do tipo I,

aumento da capacidade oxidativa e da densidade capilar, assim como no número e tamanho das mitocôndrias[69], mudanças favoráveis nos níveis de lactato[70] e espessura do diafragma.[71]

Vários benefícios similares aos exercícios convencionais podem resultar do TMI, como melhora da capacidade cardiopulmonar, do controle autonômico, além de determinar melhorias nos parâmetros da função pulmonar.[26-32] Também, dessa forma, é possível que reduza o acúmulo de metabólitos durante o trabalho muscular inspiratório, o que pode vir a atenuar a atividade do metaborreflexo[33], já que a redução do trabalho dos músculos respiratórios previne a fadiga respiratória, reduzindo a ativação simpática reflexa e permitindo o aumento do FS para os músculos locomotores.[1]

2.4.1) Treinamento Muscular Inspiratório e Metaborreflexo Muscular Inspiratório

O TMI pode minimizar os efeitos da ativação do MRMI em indivíduos saudáveis[34] e pacientes com IC crônica,[36] assim como reduzir o consumo de O_2 dos músculos respiratórios em indivíduos saudáveis durante hipóxia.[72] Witt et al.[34] conduziram um estudo de cinco semanas de TMI com o objetivo de aumentar a força muscular respiratória e presumivelmente reduzir ou retardar a fadiga muscular respiratória. Neste estudo, TMI reduziu o aumento da FC e PAM que normalmente ocorre com o aumento do trabalho de respiração durante o exercício intenso em indivíduos saudáveis. Segundo os autores, essa diminuição da resposta cardiovascular se deu por redução da atividade de fibras aferentes quimicamente sensíveis que

inervam os músculos inspiratórios, possivelmente explicada por dessensibilização ou declínio na capacidade de resposta dos nervos aferentes de tipo III e IV aos estímulos químicos resultantes de uma resposta condicionada à exposição repetida ao acúmulo de metabólitos associados às TMI.

A ativação do MRMI pode limitar o desempenho físico quando o exercício ultrapassa 85% do consumo máximo de O_2 devido à fadiga da musculatura inspiratória, reduzindo o FS para os músculos ativos e exacerbando a fadiga dos músculos periféricos.[7,21,24,25,43] Esta hipótese é reforçada pelo estudo de Harms et al.[8] com ciclistas, no qual a redução do trabalho muscular inspiratório (via uma assistência ventilatória) aumentou o tempo de exercício em 14%, provavelmente por inibir o MRMI, atenuando a fadiga do quadríceps ao exercício.[25] Além disso, demonstraram que o tempo limite de tolerância durante o exercício de ciclismo intenso é inversamente relacionado ao nível predominante de trabalho muscular inspiratório. Gething et al.[25] ampliaram essas observações, informando que a magnitude da fadiga dos músculos dos membros induzidos pelo exercício também está inversamente relacionada ao nível predominante de trabalho muscular inspiratório. Esses estudos sugerem que o trabalho dos músculos inspiratórios é capaz de influenciar a tolerância ao exercício, e que isso provavelmente é mediado por sua influência na perfusão dos membros. McConnell and Romer[73] sugeriram também que um dos mecanismos que mediam essa resposta pode ser uma atenuação, abolição ou atraso do MRMI.

Em pacientes com IC, a redução do trabalho muscular inspiratório após TMI aumentou o FS na perna, permanecendo inalterado nos controles saudáveis.[36] Assim sendo, pacientes com IC estão mais susceptíveis à intolerância ao exercício devido aos efeitos da ativação do metaborreflexo pelo trabalho muscular inspiratório[75] e o TMI

atenua o MRMI nestes pacientes[36], que apresentam também fraqueza da musculatura inspiratória.[32] Moreno et al.[74] também em seu estudo em pacientes com IC demostrou que o TMI atenua a magnitude da mudança na saturação de oxigênio do músculo intercostal e do antebraço durante a fadiga respiratória, atenuando assim o MRMI.

Sendo assim, estudos evidenciaram que aumentando a força muscular respiratória, através de TMI, é possível atenuar o MRMI e diminuir a redução da perfusão muscular periférica.[8,20] Além disso, os exercícios respiratórios são uma intervenção não farmacológica que pode modular a atividade do SNA e reduzir a PA.[37,75] O TMI foi testado em um ensaio clínico randomizado que incluiu 11 participantes hipertensos, por oito semanas, atribuindo seis e sete indivíduos a grupos de intervenção e controle, respectivamente.[37] Os resultados mostraram redução da PAS em 7,9mmHg e PAD em 5,5mmHg, além de melhorar o controle autonômico cardiovascular. Embora esses achados sejam encorajadores, o estudo não avaliou a eficácia do TMI no controle da PA, já que realizaram somente análise intragrupo e os efeitos na redução da pressão arterial necessitam ser reproduzidos.

3) REFERÊNCIAS BIBLIOGRÁFICAS

1. Dempsey JA, Romer L, Rodman J, Miller J, Smith C. Consequences of exercise-induced respiratory muscle work. Respir Physiol Neurobiol. 2006;151(2-3):242-50.

2. Rodman JR, Henderson KS, Smith CA, Dempsey JA. Cardiovascular effects of the respiratory muscle metaboreflexes in dogs: rest and exercise. J Appl Physiol (1985). 2003;95(3):1159-69.

3. Hill JM. Discharge of group IV phrenic afferent fibers increases during diaphragmatic fatigue. Brain Res. 2000;856(1-2):240-4.

4. Hussain SN, Chatillon A, Comtois A, Roussos C, Magder S. Chemical activation of thin-fiber phrenic afferents. 2. Cardiovascular responses. J Appl Physiol (1985). 1991;70(1):77-86.

5. St Croix CM, Morgan BJ, Wetter TJ, Dempsey JA. Fatiguing inspiratory muscle work causes reflex sympathetic activation in humans. J Physiol. 2000;529 Pt 2:493-504.

6. Harms CA, Babcock MA, McClaran SR, Pegelow DF, Nickele GA, Nelson WB, et al. Respiratory muscle work compromises leg blood flow during maximal exercise. J Appl Physiol (1985). 1997;82(5):1573-83.

7. Harms CA, Wetter TJ, McClaran SR, Pegelow DF, Nickele GA, Nelson WB, et al. Effects of respiratory muscle work on cardiac output and its distribution during maximal exercise. J Appl Physiol (1985). 1998;85(2):609-18.

8. Harms CA, Wetter TJ, St Croix CM, Pegelow DF, Dempsey JA. Effects of respiratory muscle work on exercise performance. J Appl Physiol (1985). 2000;89(1):131-8.

9. Rowell LB. Neural control of muscle blood flow: importance during dynamic exercise. Clin and Experim Pharmac and Physiol. 1997; 24: 117-125.

10. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Bohm M, et al. 2013 ESH/ESC Guidelines for the management of arterial hypertension: the Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). J Hypertens. 2013;31(7):1281-357.

11. Nobre F. [Introduction: Brazilian guidelines on hypertension VI. Brazilian Society of Cardiology, Brazilian Society of Hypertension, Brazilian Society of Nephrology]. J Bras Nefrol. 2010;32 Suppl 1:Iii.

12. Williams B, Poulter NR, Brown MJ, Davis M, McInnes GT, Potter JF, et al. Guidelines for management of hypertension: report of the fourth working party of the British Hypertension Society, 2004-BHS IV. J Hum Hypertens. 2004;18(3):139-85.

13. Mitchell JH, Kaufman MP, Iwamoto GA. The exercise pressor reflex: its cardiovascular effects, afferent mechanisms, and central pathways. Annu Rev Physiol. 1983;45:229-42.

14. Mitchell JH, Haskell W, Snell P, Van Camp SP. Task Force 8: classification of sports. J Am Coll Cardiol. 2005;45(8):1364-7.

15. Mitchell JH, and Raven PB. Cardiovascular adaptation to physical activity. Champaign Human Kinetics Publishers, 1994.

16. Mitchell JH. Abnormal cardiovascular response to exercise in hypertension: contribution of neural factors. Am J Physiol Regul Integr Comp Physiol. 2017;312(6):R851-r63.

17. Delaney EP, Greaney JL, Edwards DG, Rose WC, Fadel PJ, Farquhar WB. Exaggerated sympathetic and pressor responses to handgrip exercise in older hypertensive humans: role of the muscle metaboreflex. Am J Physiol Heart Circ Physiol. 2010; 299(5):H1318-27.

18. Rondon MU, Laterza MC, de Matos LD, Trombetta IC, Braga AM, Roveda F, et al. Abnormal muscle metaboreflex control of sympathetic activity in never-treated hypertensive subjects. Am J Hypertens. 2006;19(9):951-7.

19. Leal AK, Williams MA, Garry MG, Mitchell JH, Smith SA. Evidence for functional alterations in the skeletal muscle mechanoreflex and metaboreflex in hypertensive rats. Am J Physiol Heart Circ Physiol. 2008;295(4):H1429-38.

20. Sheel AW, Derchak PA, Morgan BJ, Pegelow DF, Jacques AJ, Dempsey JA. Fatiguing inspiratory muscle work causes reflex reduction in resting leg blood flow in humans. J Physiol. 2001;537(Pt 1):277-89.

21. Sheel AW. Respiratory muscle training in healthy individuals: physiological rationale and implications for exercise performance. Sports Med. 2002;32(9):567-81.

22. Sheel AW, Derchak PA, Pegelow DF, Dempsey JA. Threshold effects of respiratory muscle work on limb vascular resistance. Am J Physiol Heart Circ Physiol. 2002;282(5):H1732-8.

23. Dempsey JA, Sheel AW, St Croix CM, Morgan BJ. Respiratory influences on sympathetic vasomotor outflow in humans. Respir Physiol Neurobl. 2002;130(1):3-20.

24. Dempsey JA, Miller JD, Romer L, Amann M, Smith CA. Exercise-induced respiratory muscle work: effects on blood flow, fatigue and performance. Adv Exp Med Biol. 2008;605:209-12.

25. Romer LM, Lovering AT, Haverkamp HC, Pegelow DF, Dempsey JA. Effect of inspiratory muscle work on peripheral fatigue of locomotor muscles in healthy humans. J Physiol. 2006;571(Pt 2):425-39.

26. Geddes EL, O'Brien K, Reid WD, Brooks D, Crowe J. Inspiratory muscle training in adults with chronic obstructive pulmonary disease: an update of a systematic review. Respir Med. 2008;102(12):1715-29.

27. Smart NA, Giallauria F, Dieberg G. Efficacy of inspiratory muscle training in chronic heart failure patients: a systematic review and meta-analysis. Int J Cardiol. 2013;167(4):1502-7.

28. Rodrigues F, Araujo AA, Mostarda CT, Ferreira J, de Barros Silva MC, Nascimento AM, et al. Autonomic changes in young smokers: acute effects of inspiratory exercise. Clin Auton Res. 2013;23(4):201-7.

29. Williams JS, Wongsathikun J, Boon SM, Acevedo O. Inspiratory muscle training fails to improve endurance capacity in athletes. Med Sci Sports Exerc. 2002;34(7):1194-8.

30. Gething AD, Passfield L, Davies B. The effects of different inspiratory muscle training intensities on exercising heart rate and perceived exertion. Eur J Appl Physiol. 2004;92(1-2):50-5.

31. Enright SJ, Unnithan VB, Heward C, Withnall L, Davies DH. Effect of high-intensity inspiratory muscle training on lung volumes, diaphragm thickness, and exercise capacity in subjects who are healthy. Phys Ther. 2006;86(3):345-54.

32. Dall'Ago P, Chiappa GR, Guths H, Stein R, Ribeiro JP. Inspiratory muscle training in patients with heart failure and inspiratory muscle weakness: a randomized trial. J Am Coll Cardiol. 2006;47(4):757-63.

33. Sinoway LI, Hill JM, Pickar JG, Kaufman MP. Effects of contraction and lactic acid on the discharge of group III muscle afferents in cats. J Neuroph. 1993;69(4):1053-9.

34. Witt JD, Guenette JA, Rupert JL, McKenzie DC, Sheel AW. Inspiratory muscle training attenuates the human respiratory muscle metaboreflex. J Physiol. 2007;584(Pt 3):1019-28.

35. McConnell AK, Lomax M. The influence of inspiratory muscle work history and specific inspiratory muscle training upon human limb muscle fatigue. J Physiol. 2006;577(Pt 1):445-57.

36. Chiappa GR, Roseguini BT, Vieira PJ, Alves CN, Tavares A, Winkelmann ER, et al. Inspiratory muscle training improves blood flow to resting and exercising limbs in patients with chronic heart failure. J Am Coll Cardiol. 2008;51(17):1663-71.

37. Ferreira JB, Plentz RDM, Stein C, Casali KR, Arena R, Lago PD. Inspiratory muscle training reduces blood pressure and sympathetic activity in hypertensive patients: a randomized controlled trial. International Journal of Cardiology. 2013;166(1):61-7.

38. Ward DS, Nitti GJ. The effects of sufentanil on the hemodynamic and respiratory response to exercise. Med Sci Sports Exerc. 1988; 20(6):579-86.

39. Derchak PA, Sheel AW, Morgan BJ, Dempsey JA. Effects of expiratory muscle work on muscle sympathetic nerve activity. J Appl Physiol (1985). 2002;92(4):1539-52.

40. Ward DS, Nitti GJ. The effects of sufentanil on the hemodynamic and respiratory response to exercise. Med Sci Sports Exerc. 1988; 20(6):579-86.

41. O'Leary DS, Sheriff DD. Is the muscle metaboreflex important in control of blood flow to ischemic active skeletal muscle in dogs? Am J Physiol. 1995;268(3 Pt 2):H980-6.

42. Augustyniak RA, Collins HL, Ansorge EJ, Rossi NF, O'Leary DS. Severe exercise alters the strength and mechanisms of the muscle metaboreflex. Am J Physiol Heart Circ Physiol. 2001;280(4):H1645-52.

43. Guenette JA, Martens AM, Lee AL, Tyler GD, Richards JC, Foster GE, et al. Variable effects of respiratory muscle training on cycle exercise performance in men and women. Appl Physiol Nutr Metab. 2006;31(2):159-66.

44. Johnson BD, Babcock MA, Suman OE, Dempsey JA. Exercise-induced diaphragmatic fatigue in healthy humans. J Physiol. 1993;460:385-405.

45. Borghi-Silva A, Carrascosa C, Oliveira CC, Barroco AC, Berton DC, et al. Effects of respiratory muscle unloading on leg muscle oxygenation and blood volume during high-intensity exercise in chronic heart failure. Am J Physiol Heart Circ Physiol. 2008; 294(6):H2465-72.

46. Malachias M, Plavnik FL, Machado CA, Malta D, Scala LCN, Fuchs S. 7th Brazilian Guideline of Arterial Hypertension: Chapter 1 - Concept, Epidemiology and Primary Prevention. Arq Bras Cardiol. 2016;107(3 Suppl 3):1-6.

47. Weber MA, Schiffrin EL, White WB, Mann S, Lindholm LH, Kenerson JG, et al. Clinical practice guidelines for the management of hypertension in the community:

a statement by the American Society of Hypertension and the International Society of Hypertension. J Clin Hypertens (Greenwich). 2014;16(1):14-26.

48. James PA, Oparil S, Carter BL, Cushman WC, Dennison-Himmelfarb C, Handler J, et al. 2014 evidence-based guideline for the management of high blood pressure in adults: report from the panel members appointed to the Eighth Joint National Committee (JNC 8). JAMA. 2014;311(5):507-20.

49. Picon RV, Fuchs FD, Moreira LB, Riegel G, Fuchs SC. Trends in prevalence of hypertension in Brazil: a systematic review with meta-analysis. PLoS One. 2012;7(10):e48255.

50. Moraes RS, Fuchs FD, Dalla Costa F, Moreira LB. Familial predisposition to hypertension and the association between urinary sodium excretion and blood pressure in a population-based sample of young adults. Braz J Med Biol Res. 2000;33(7):799-803.

51. Fuchs FD, Gus M, Moreira LB, Moraes RS, Wiehe M, Pereira GM, et al. Anthropometric indices and the incidence of hypertension: a comparative analysis. Obes Res. 2005;13(9):1515-7.

52. Zhao D, Qi Y, Zheng Z, Wang Y, Zhang XY, Li HJ, et al. Dietary factors associated with hypertension. Nat Rev Cardiol. 2011;8(8):456-65.

53. Steffens AA, Moreira LB, Fuchs SC, Wiehe M, Gus M, Fuchs FD. Incidence of hypertension by alcohol consumption: is it modified by race? J Hypertens. 2006;24(8):1489-92.

54. Briasoulis A, Agarwal V, Messerli FH. Alcohol consumption and the risk of hypertension in men and women: a systematic review and meta-analysis. J Clin Hypertens (Greenwich). 2012;14(11):792-8.

55. Fuchs SC, Poli-de-Figueiredo CE, Figueiredo Neto JA, Scala LC, Whelton PK, Mosele F, et al. Effectiveness of Chlorthalidone Plus Amiloride for the Prevention of Hypertension: The PREVER-Prevention Randomized Clinical Trial. J Am Heart Assoc. 2016;5(12).

56. Staessen JA, Wang J, Bianchi G, Birkenhager WH. Essential hypertension. Lancet. 2003;361(9369):1629-41.

57. Beevers G, Lip GY, O'Brien E. ABC of hypertension: The pathophysiology of hypertension. Bmj. 2001;322(7291):912-6.

58. Jan Danser AH. Renin and prorenin as biomarkers in hypertension. Curr Opin Nephrol Hypertens 2012; 21:508.

59. Grassi G. Role of the sympathetic nervous system in human hypertension. J Hypertens. 1998;16(12 Pt 2):1979-87.

60. Parati G, Esler M. The human sympathetic nervous system: its relevance in hypertension and heart failure. Eur Heart J. 2012;33(9):1058-66.

61. Gribbin B, Pickering TG, Sleight P, Peto R. Effect of age and high blood pressure on baroreflex sensitivity in man. Circ Res. 1971;29(4):424-31.

62. Hajduczok G, Chapleau MW, Johnson SL, Abboud FM. Increase in sympathetic activity with age. I. Role of impairment of arterial baroreflexes. Am J Physiol. 1991;260(4 Pt 2):H1113-20.

63. Somers VK, Mark AL, Abboud FM. Potentiation of sympathetic nerve responses to hypoxia in borderline hypertensive subjects. Hypertension. 1988;11(6 Pt 2):608-12.

64. Smith SA, Williams MA, Leal AK, Mitchell JH, Garry MG. Exercise pressor reflex function is altered in spontaneously hypertensive rats. J Physiol. 2006;577(Pt 3):1009-20.

65. Sausen MT, Delaney EP, Stillabower ME, Farquhar WB. Enhanced metaboreflex sensitivity in hypertensive humans. Eur J Appl Physiol. 2009;105(3):351-6.

66. Krogh A, Lindhard J. The regulation of respiration and circulation during the initial stages of muscular work. J Physiol. 1913;47(1-2):112-36.

67. Ciriello J, Caverson MM, Polosa C. Function of the ventrolateral medulla in the control of the circulation. Brain Res. 1986;396(4):359-91.

68. Goodwin GM, McCloskey DI, Mitchell JH. Cardiovascular and respiratory responses to changes in central command during isometric exercise at constant muscle tension. J Physiol. 1972;226(1):173-90.

69. Padula CA, Yeaw E. Inspiratory muscle training: integrative review of use in conditions other than COPD. Res Theory Nurs Pract. 2007;21(2):98-118.

70. Powers SK, Grinton S, Lawler J, Criswell D, Dodd S. High intensity exercise training-induced metabolic alterations in respiratory muscles. Respir Physiol. 1992;89(2):169-77.

71. Spengler CM, Roos M, Laube SM, Boutellier U. Decreased exercise blood lactate concentrations after respiratory endurance training in humans. Eur J Appl Physiol Occup Physiol. 1999;79(4):299-305.

72. Downey AE, Chenoweth LM, Townsend DK, Ranum JD, Ferguson CS, Harms CA. Effects of inspiratory muscle training on exercise responses in normoxia and hypoxia. Respir Physiol Neurobiol. 2007;156(2):137-46.

73. Turner LA, Tecklenburg-Lund SL, Chapman R, et al. The Effect of Inspiratory Muscle Training on Respiratory and Limb Locomotor Muscle Deoxygenation During Exercise with Resistive Inspiratory Loading. Int J Sports Med 2016; 37:598-606.

74. McConnell AK, Romer LM. Respiratory muscle training in healthy humans: resolving the controversy. Int J Sports Med 2004;25:284–293.

75. Moreno AM, Castro RR, Silva BM, Villacorta H, Sant'Anna Junior M, Nobrega AC. Intercostal and forearm muscle deoxygenation during respiratory fatigue in patients with heart failure: potential role of a respiratory muscle metaboreflex. Braz J Med Biol Res. 2014;47(11):972-6.

76. Hunt BE, Fahy L, Farquhar WB, Taylor JA. Quantification of mechanical and neural components of vagal baroreflex in humans. Hypertension. 2001;37(6):1362-8.

4) HIPÓTESES CONCENTUAIS

Com base nos conhecimentos científicos apresentados acima e na necessidade de melhor compreender o metaborreflexo muscular, investigamos mais profundamente os efeitos deste mecanismo, onde nossas hipóteses norteadoras foram:

I) Em comparação com indivíduos normotensos, os indivíduos hipertensos apresentam metaborreflexo muscular inspiratório exacerbado.

II) O treinamento muscular inspiratório reduz os valores pressóricos de indivíduos hipertensos.

III) O treinamento muscular inspiratório atenua o metaborreflexo muscular inspiratório em indivíduos hipertensos.

5) OBJETIVOS GERAIS

Estudo 1:

Comparar a ativação do metaborreflexo muscular inspiratório entre indivíduos com hipertensão arterial sistêmica e indivíduos normotensos.

Estudo 2:

Avaliar o efeito do treinamento muscular inspiratório com carga em relação ao treinamento placebo sobre a pressão arterial em indivíduos com hipertensão arterial sistêmica: protocolo de estudo de um ensaio clínico randomizado duplo-cego.

6) RESULTADOS

6.1) Estudo 1:

Altered Inspiratory muscle metaboreflex in hypertensive individuals

Barreiro SRP[1]; Callegaro CC[1,2]; Rosa VF[3]; Huten DO[3]; Merello PN[3]; Sobragi SM[4]; Souza KS[4]; Alves VM[5]; Moreira LB[1]

[1] Postgraduate Program in Cardiology, School of Medicine. Universidade Federal do Rio Grande do Sul (UFRGS), R. Ramiro Barcelos 2600, CEP 90035-003 Porto Alegre, RS, Brazil

[2] Department of Physical Therapy and Rehabilitation. Physiology and Rehabilitation Laboratory. Universidade Federal de Santa Maria (UFSM), Santa Maria, Rio Grande do Sul, Brazil. E-mail: ccallegaro84@gmail.com

[3] Cientific Initiation Program, School of Medicine.Universidade Federal do Rio Grande do Sul (UFRGS)

[4] Academic Physiotherapy

[5] Physical Educator

Correspondig author

Professor Leila Beltrami Moreira

Hospital de Clínicas de Porto Alegre

Rua Ramiro Barcelos 2350, Porto Alegre, RS, Brazil

e-mail: lbmoreira@hcpa.edu.br

Abstract

Previous studies suggest that hypertensive individuals have altered peripheral metaboreflex. Fatiguing inspiratory muscle work also induces metaboreflex activation resulting in pressor response and peripheral vasoconstriction, but the effects of inducing inspiratory metaboreflex (IMMR) in hypertensive individuals remain uninvestigated.

Objective: To evaluate if hypertensive individuals present altered IMMR compared to normotensive individuals.

Methods: The participants should be ≥35 and ≤65 years old, body mass index < 30 kg/m^2, practice less than 150 minutes of moderate or intense physical activity per week and have ≥70% of predicted maximal inspiratory pressure. Hypertension was defined as systolic blood pressure (SBP) ≥140mmHg and/or diastolic blood pressure (DBP) ≥90mmHg and the controls SBP should be <125mmHg and DBP <75mmHg in the 24 hours mean of ambulatory blood pressure monitoring.

The IMMR induction consisted in inspiring against a threshold loading of 60% of maximal inspiratory pressure (MIP) and after 40 min rest, participants performed the sham protocol, inspiring against 2cmH_2O. The values of BP, heart rate (HR) and leg blood flow (LBF) were monitored.

Results: Sixteen hypertensive subjects (53 ± 8 years old, 60% male, 24 h BP: 135 ±12 / 86 ±10 mmHg) and 15 healthy controls (48 ± 6 years, 44% male; 114 ±8/73 ±8mmHg) were recruited. During metaboreflex induction protocol, there was a higher increase in mean arterial pressure (MAP) in hypertensive than in normotensive individuals (from

111 ±16 mmHg to 128 ±17 mmHg and 95 ±15mmHg to 101 ±18mmHg, respectively; p <0.05). There was a more pronounced reduction of LBF in the hypertensive than in the normotensive ones (from 4 ±3ml /min /100ml to 3 ±3ml /min /100ml and 3 ±2ml/ min /100ml to 3 ±1ml/min /100ml, respectively, p <0.05). Peripheral vascular resistance (PVR) increased in both groups. In the sham protocol, there was no difference in HR, LBF and MBP variation, while PVR had a greater increase in normotensive subjects (time * group: 0.04).

Conclusion: Metaboreflex activation induced a greater MAP response and reduction of LBF in hypertensive individuals, suggesting IMMR exacerbation compared to normotensive individuals.

Keywords: systemic arterial hypertension; hypertension; inspiratory muscles; metaboreflex; blood flow; vascular resistance.

1. Introduction

The imbalance between oxygen (O_2) supply and muscular metabolism activates inspiratory muscle metaboreflex (IMMR) during moderate to intense exercise (1-3). The fatiguing contractions of the inspiratory muscles causes accumulation of metabolic products that stimulate the afferent phrenic nerves type IV, resulting in increased sympathetic vasoconstrictive activity (1,4,7-10). The activated IMMR causes blood flow (BF) reduction in the inactive peripheral musculature, and consequent increase of the peripheral vascular resistance (PVR) (3,4,11).

Several pathological conditions may alter the IMMR. Patients with heart failure and inspiratory muscle weakness exhibit exacerbation of IMMR (12). Similar response can be found in patients with diabetes mellitus, regardless of the presence of neuropathy (12). In addition, interventions that improve inspiratory muscle conditioning such as aerobic and inspiratory muscle training, can attenuate IMMR (12, 13,14). Those same interventions reduce blood pressure (BP) in hypertensive individuals (15) and in healthy normotensives (16).

In hypertension (HT), high intensity resistance exercise is associated with increasing sympathetic efferent activity in the heart and blood vessels, resulting in a moderate increasing in mean arterial pressure (MAP) and heart rate (HR) (17-20). Interestingly, hypertensive subjects who never underwent pharmacological treatment showed increased muscle sympathetic nerve activity (MSNA) at rest compared to normotensive subjects. However, during handgrip exercise at 30% of maximal voluntary contraction there was a lower increase in MSNA in hypertensive individuals,

suggesting a reduction in metaboreflex (21). In contrast, in another experiment, hypertensive individuals had a higher sustained BP elevation compared to normotensive individuals during ischemia after dynamic exercise with handgrip, suggesting a greater sensitivity to metaboreflex (22). Likewise, in the Delaney et al. (23) study, hypertensive individuals exhibited exaggerated sympathetic and pressor responses to wrist exercise, maintained during the post-ischemic period mediated by the metaboreflex.

Based on the knowledge about activation of peripheral muscle metaboreflex in hypertensive individuals, we hypothesized that inspiratory muscle metaboreflex (IMMR) may be altered in hypertensive individuals in relation to normotensive individuals. The present study aimed to evaluate whether hypertensive individuals present altered IMMR compared to normotensive individuals.

2) Methods

2.1) Study Design and Sample Size Calculation

An experimental study was conducted, including hypertensive and normotensive individuals of both gender, recruited from the hypertension outpatient clinic or the primary care unit of the Hospital de Clínicas de Porto Alegre (HCPA), and from the general population. The Institutional Review Board approved the study, in agreement to Helsinki Declaration and all participants signed a consent form.

To calculate the sample size, it was considered a difference between hypertensive and normotensive individuals of 0.038 (SD 0.01)cm/s in BF measurement (13); significance

level of 0.05 and 90% power. Adding 20% of losses, 16 participants per group should be included.

2.2) Inclusion and Exclusion Criteria

Males and females, aged ≥35 and ≤65 years were eligible. Hypertensive individuals who did not use pharmacological therapy or who took only thiazide diuretics monotherapy were eligible for the study. HT was defined as systolic blood pressure (SBP) ≥140mmHg and / or diastolic blood pressure (DBP) ≥90mmHg. To be eligible for the control group, SBP should be <125mmHg and DBP <75mmHg in the 24 hours mean of ambulatory blood pressure monitoring (ABPM). Participants should have a body mass index (BMI) <30 kg/m^2 , practice less than 150 minutes of moderate or intense physical activity per week (24) and have a maximal inspiratory pressure (MIP)≥70% of predicted according the gender and sex (25).

Exclusion criteria were: dyspnea; diabetes mellitus (DM); orthopedic, musculoskeletal or neurological limitations; cognitive impairment; current or past history of deep venous thrombosis; history of myocardial infarction or stroke in the last 6 months; heart failure; angina pectoris; pulmonary disease of any etiology (including asthma and chronic obstructive pulmonary disease) or active smoker; pregnant or lactating women. Individuals with any other condition that impaired participation in the study were excluded.

Participants were also instructed not to change pharmacological or non-pharmacological treatment for hypertension (including changes in prescription or changes in physical activity level) during study participation.

2.3) Baseline assessment prior IMMR induction

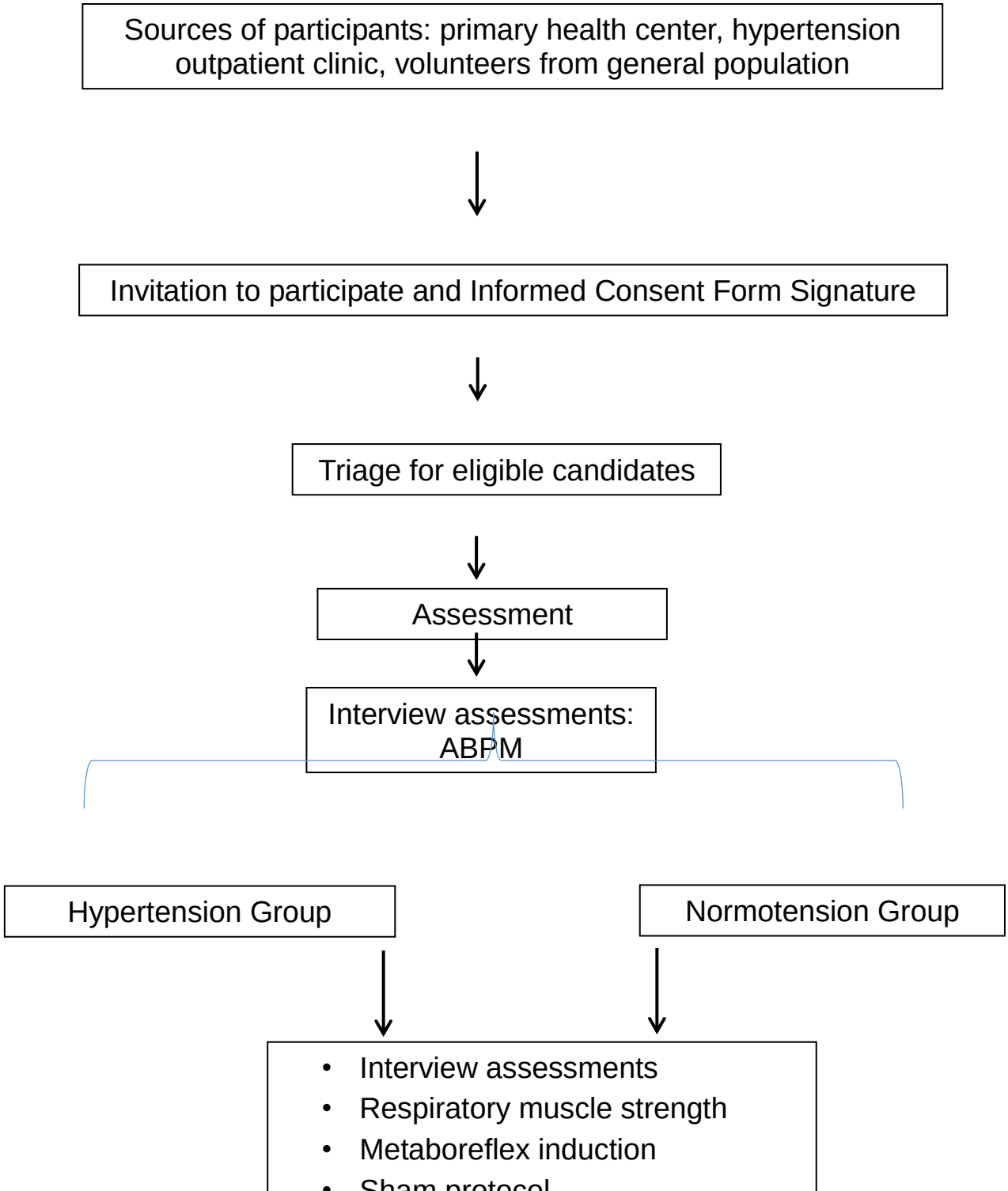
Both hypertensive and normotensive individuals came to the laboratory on two separate days for completion of the study protocols. At the first laboratory visit, baseline assessment and collection of socio-demographic data were performed. Body mass index (BMI) was calculated by dividing weight (kg) by the square of height (m) and classified as recommended by the World Health Organization (WHO). After that, respiratory muscle strength was measured. MIP and maximal expiratory pressure (MEP) were evaluated with a pressure transducer ranging from -300 to + 300cmH_2O (MVD-300 Microhard System Digital Manovacuometer, Globalmed, Porto Alegre-RS, Brazil). The MIP and MEP were expressed in cmH_2O, as well as percentage of predicted values for age and sex (25,26). During the evaluation, the subjects remained seated with a 90° angle between the trunk and the legs, with a nasal clip to avoid exhaust air. The lips remained sealed and connected to a buccal with a 2mm hole to avoid increasing the pressure of the oral cavity generated by unwanted contraction of the oropharyngeal muscles (26-28). MIP was measured during forced inspiration, initiated from residual volume and MEP, was initiated from total lung capacity. The participants were able to perform up to 12 maneuvers to provide 6 measures with variation <10% in order to avoid the effects of the learning curve. The highest peak pressure sustained for at least one second among the 6 measurements was used in the analysis (22).

Confirmation of HT was obtained by ABPM of 24 hours. The ABPM was performed with Spacelabs device 90207 (Redmond, WA, USA), with cuff size (regular or large) according to the brachial circumference. SBP, DBP and MAP were recorded every 15

minutes during the day (6am to 10am) and every 20 minutes during the night (10pm to 6am). ABPM was considered satisfactory when were obtained at least 16 valid readings during the day and 8 during the night.

IMMR induction protocol was performed in the second visit. The flowchart of the study is depicted in Figure 1.

Figure 1) Study flowchart.



2.4) IMMR and Sham induction protocols

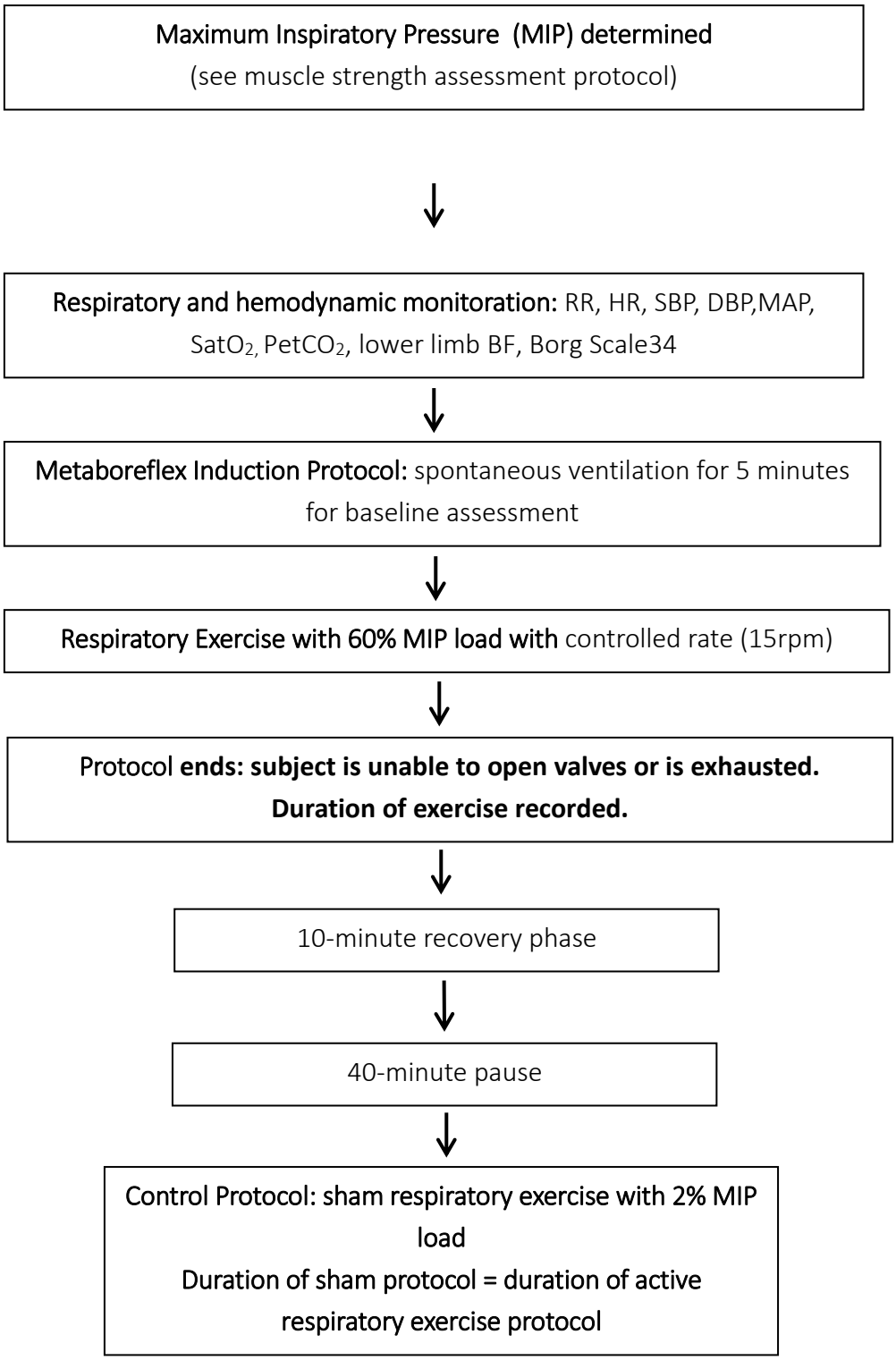
Participants were instructed not perform physical activities for 48 hours prior the test, not drink alcohol or caffeine for at least 12 hours before, and to remain on rest for 2 hours prior protocol initiation. Peripheral oxygen saturation ($SatO_2$) was monitored by pulse oximetry. SBP, DBP and MAP were measured with automated sphygmomanometer (Dinamap, DASH 2000, General Electric, CT) on the contralateral leg to the limb where LBF was measured. The end-expired carbon dioxide ($PetCO_2$) and respiratory rate (RR) were monitored by infrared capnography (Takaoka, USA). LBF was determined by venous occlusion plethysmography (Hokanson, WA) on the calf of the non-dominant lower limb every 10 seconds, expressed in ml/min/100ml of tissue (29). VR was calculated by dividing MAP by LBF (30).

First, the participant was placed in a semi-supine position with a nasal clip and instructed to breathe through a two-way respiratory system (Hans Rudolph, KS) for 5 minutes in spontaneous breathing, while baseline physiological parameters were recorded. An inspiratory resistance generator (PowerBreath PLus, UK) was then connected to the respiratory system to generate inspiratory load equal to 60% of the MIP (2,3). Participants should maintain the respiratory rate at 15 bpm and the ratio inspiratory time/total time (Ti/Ttot) of the cycle at 0.75. A pacemaker metrometer designed by the HCPA Biomedical Engineering Service helped keep pace. The device emits sounds and lights that signal the moment of inspiration and expiration. During each cycle, participants were instructed to 1) perform diaphragmatic breathing to avoid the use of accessory muscles and 2) maintain constant inspiratory pressure with

the aid of visual feedback. The inspiratory pressure was recorded and displayed continuously on a screen. The inspiratory effort was assess every 1 minute during exercise and at the end of the protocol using the modified Borg Scale34. The IMMR induction with 60% MIP load ended when: 1) the participant was unable to open the valve of the resistance device; or 2) a pressure of less than 90% of predicted MIP was observed in three consecutive breathing. When one of the criteria was met, the inspiratory resistance device was removed and the participant instructed to maintain the same breathing pattern guided by the metrometer. The subject was then monitored for 10 minutes in the recovery phase.

After a 40-minute pause, participants were submitted to sham protocol, with load of two cmH_2O (13). The same procedures adopted for the IMMR induction protocol were repeated. The duration of the sham protocol was determined by the duration of the IMMR induction protocol for each participant. Data from both protocols were described as baseline means at the first and second minutes (pre-exercise) and 1 minute (last minute) after the individuals were no longer able to perform the inspiratory exercise with the device. The protocol flowchart can be seen in Figure 2.

Figure 2. IMMR induction protocol and Sham protocol flowchart.



2.5) Statistical analysis

Numerical continuous variables were described as mean ± standard deviation (SD). Student's t-test was used to compare the characteristics between normotensive and hypertensive groups. The hemodynamic and respiratory responses during IMMR induction protocol and in the sham protocol were evaluated by two-way analysis of variance (ANOVA) for repeated measures (basal versus final). Value of $p < 0.05$ was considered statistically significant. All analyzes were performed with SPSS 18® (International Business Machines Corp).

3) Results

The sample consisted of 31 participants, 16 hypertensive and 15 normotensive subjects, with a mean age of 50 ±7 years, tending hypertensive individuals to be older than normotensive subjects. Of the hypertensive individuals, eight were using antihypertensive medication (thiazide diuretic). Respiratory variables did not differ between groups (Table 1).

Table 1. Characterization of the sample.

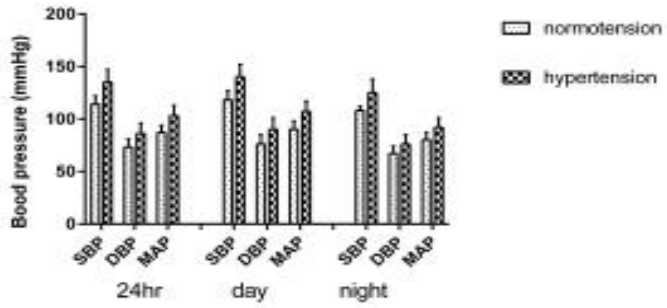
	Normotensive (n = 15)	Hypertensive (n = 16)	P
Gender (Male/Female)	9/6 (60%/40%)	7/9 (44% / 56%)	0.38
Age (years)	48 ± 6	53 ± 8	0.06
BMI (kg/m^2)	26 ± 3	27 ± 3	0.24
MIP (cmH_2O)	108 ± 31	98 ± 30	0.33
MIP % predicted	104 ± 2	101 ± 2	0.19
MEP (cmH_2O)	161 ± 44	154 ± 40	0.63

MEP % predicted	146 ± 2	100 ± 2	0.20

Data are presented as mean ± SD. BMI: body mass index; MIP: Maximum inspiratory pressure; MEP: Maximum expiratory pressure; SBP: Systolic blood pressure; PAD: Diastolic blood pressure; PAM: Mean blood pressure.

During the induction of IMMR, SBP did not show statistically significant variation in hypertensive (from 167 ± 29 mmHg to 188 ± 33 mmHg) and normotensive participants (from 145 ± 30 mmHg to 144 ± 43 mmHg, Time: 0.09, Group: 0.005, Time * Group: 0,05). DBP increased in the IMMR induction protocol in both groups (hypertensive: from 84 ± 10mmHg to 98 ± 13mmHg, normotensive: from 71 ± 7mmHg to 79 ± 17mmHg, Time: 0.000, Group: 0.000, Time * Group: 0, 27) (Figure 3).

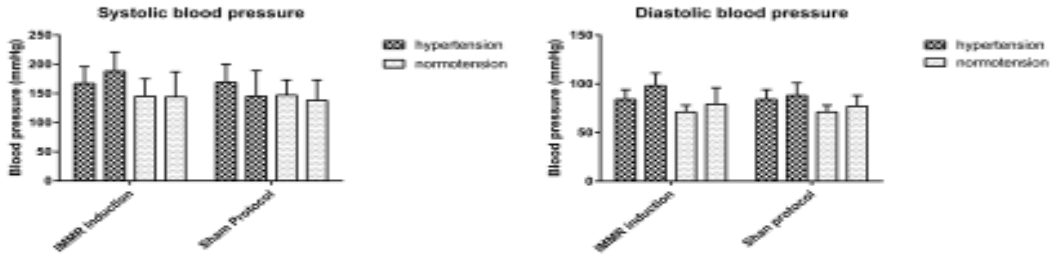
Figure 3. Values of 24-hour, daytime, and nighttime BP measurements of hypertensive and normotensive subjects in the sample.



In the sham protocol (Figure 4), SBP decreased in both groups (from 169 ± 31 mmHg to 145 ± 44 mmHg in hypertensive and from 147 ± 26 mmHg to 138 ± 35 mmHg in

normotensive participants (Tempo: 0,02; Grupo: 0,19; Tempo*Grupo: 0,29). DBP increased in hypertensive group from 84 ± 10 mmHg to 88 ± 13mmHg and in normotensive group from 71 ± 7mmHg to 77 ± 11mmHg (Time: 0.000; Group: 0.000; Time * Group: 0.38).

Figure 4. Response of systolic and diastolic blood pressure during the inspiratory muscle metaborreflex induction test with load (60% of MIP) and sham protocol (2cmH_2O) in hypertensive and normotensive individuals.



In the IMMR induction protocol (Figure 5), there was a higher increase in MAP in hypertensive patients compared to normotensive participants (time * group: 0.006); HR and peripheral VR increased in both groups and LBF had a greater reduction in hypertensives participants (from 4 ± 3ml/min/100ml to 3 ± 3ml/min/100ml, group* time: 0.01). In the sham protocol, performed after 40 minutes of rest, there was no difference in MAP, HR and BF variations, while peripheral VR had a higher increase in normotensive subjects (time * group: 0.04).

Figure 5. Hemodynamic responses during the inspiratory muscle metaborreflex induction test with load (60% of MIP) and sham protocol (2cmH_2O) in hypertensive and normotensive individuals.

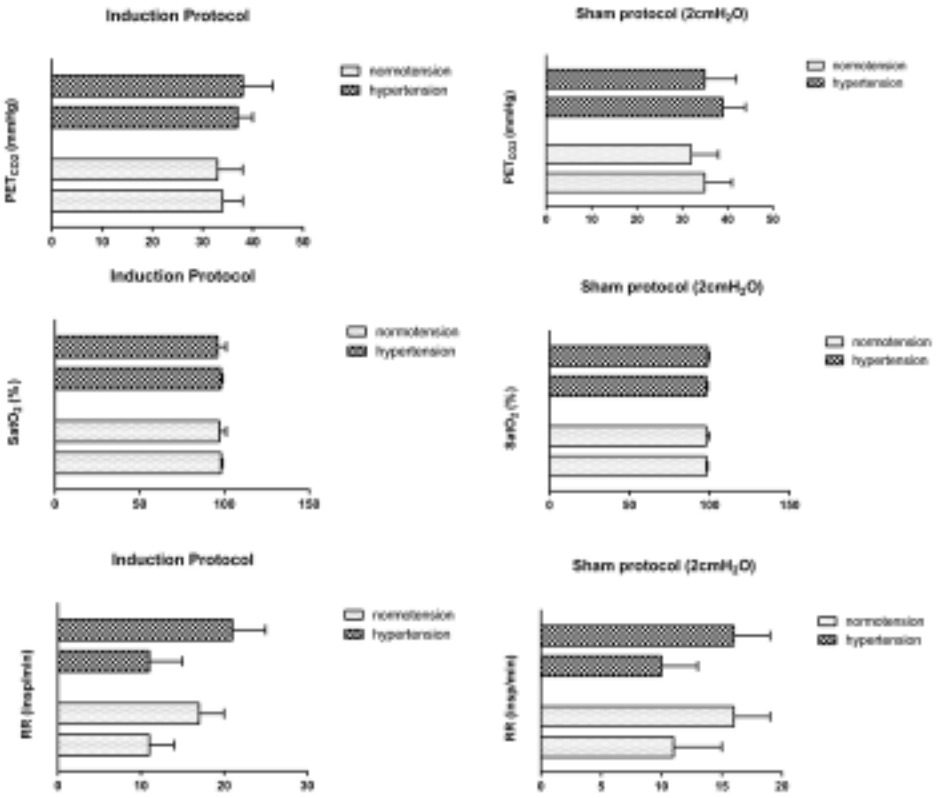
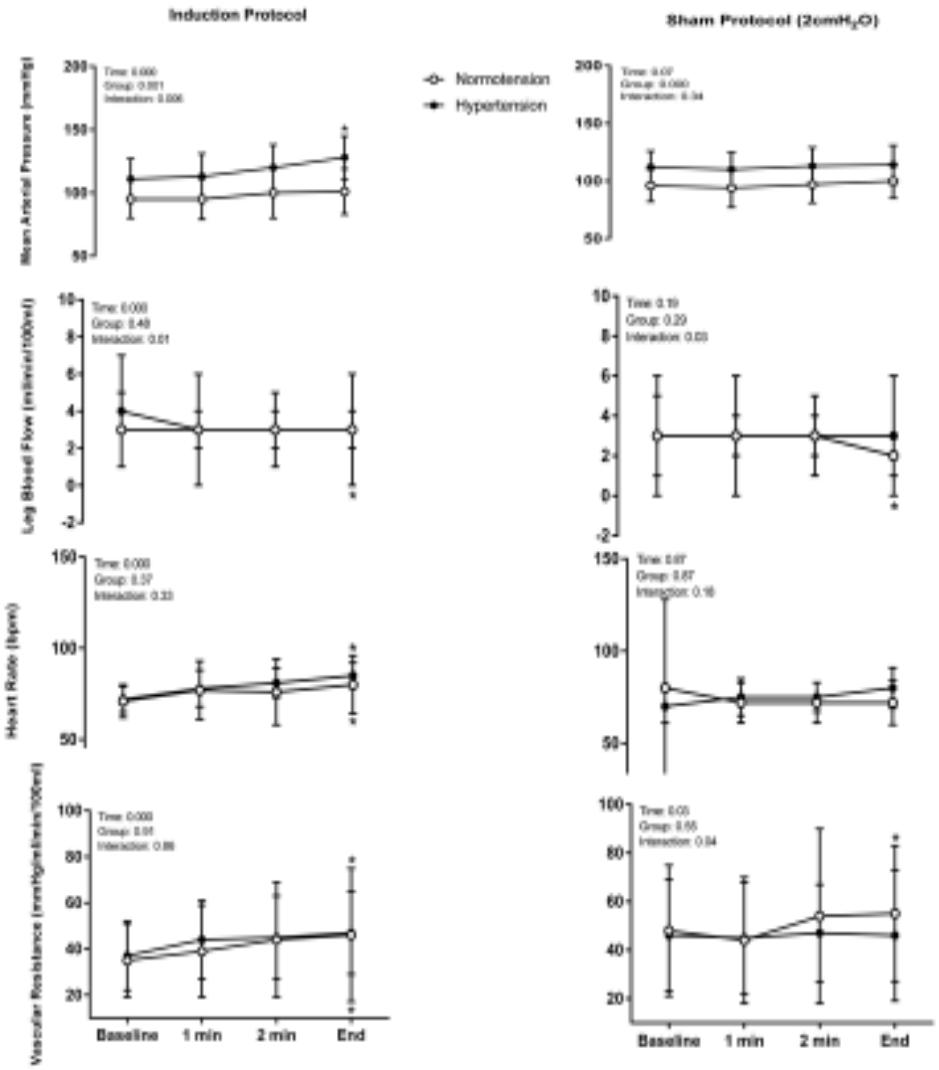


Figure 6 shows respiratory variables. $PetCO_2$ was maintained stable during IMMR induction (Time: 0.94; Group: 0.01; Time * Group: 0.24), but there was a reduction during the sham protocol (Time: <0.001; Group: 0.13; Time * Group: 0.47). The $SatO_2$ was reduced in both groups during IMMR induction (Time: 0.04; Group: 0.48; Time*Group: 0.49) and remained unchanged in the sham protocol (Time: 0.62; Group: 0.41; Time * Group: 0.21). There was an increase in respiratory frequency in the IMMR

induction (Time: 0.00; Group: 0.05; Time * Group: 0.06) as well as in the sham protocol (Time: 0.00; Group: 0.87; Time * Group: 0.57).

Figure 6. Respiratory response during the inspiratory muscle metaborreflex induction with load (60% of MIP) and sham protocol (2cmH_2O) in hypertensive and normotensive individuals.



4) Discussion

Compared with normotensive individuals, hypertensive individuals, without pharmacological treatment or using thiazide diuretics as monotherapy, presented a

more marked increase in MAP and a tendency of increase in SBP in response to fatiguing inspiratory muscle exercise with load. HR and peripheral VR increased in both groups, while the LBF had a greater reduction in hypertensive individuals.

Studies (31-33) have shown that in hypertensive individuals there are greater BP, HR and efferent sympathetic nervous activity than in normotensive individuals. The elevation of MAP observed in our study is in accordance with the study by Delaney et al. (23), which evaluated the behavior of MAP and MSNA in normotensive and hypertensive individuals during static maneuver at 30 and 40% of maximal voluntary contraction of the peripheral musculature. Both MAP and MSNA increased significantly during static handgrip exercise in older hypertensive individuals. Thus, the authors suggested that hypertensive individuals exhibit enhanced sympathetic and pressor responses to handgrip exercise maintained during the post-exercise ischemia post-ischemic period mediated by muscle metaboreflex. Similar results were observed in a dynamic handgrip exercise study (21), with MAP deltas higher in hypertensive than in normotensive individuals (12 ± 3 mmHg and 6 ± 1 mmHg, respectively, $p < 0.05$). It has been suggested that hypertensive individuals present greater sensitivity to metaboreflex, which supports the hypothesis that they present enhanced muscular metaboreflex in relation to normotensive individuals. Kaufman et al. (34) demonstrated that the accumulation of metabolites due to intense muscular work increases type III fibers activity (mechanosensitive activity). Thus, it is possible that this activity had contributed to the MAP response in our study during the protocol with 60% MIP load (3).

Consistent with previous results (2,3,29), VR increased in the legs in parallel with the work intensity of inspiratory muscles, without difference between groups. Comparing healthy adult men and women, Smith et al. (35) observed that intense inspiratory muscle work leads to time-dependent neural and cardiovascular consequences, specifically on MSNA (2), leading to an increase in MAP and VR (2,3,7). Thus, metabolites accumulation and decreased muscle oxygenation may have led to the combined effects of slight increases in cardiac debit and small decreases in systemic vascular conductance (13). This would explain the fact that VR does not differ between hypertensive and normotensive individuals, despite the higher increase in MAP and decrease of LBF in hypertensive participants.

In contrast, in the sham protocol, we observed significant increase in VR in the leg in normotensive individuals. One hypothesis is that a reduction in tissue perfusion secondary to the HR reduction observed in the first minute of the sham protocol caused the VR elevation. The HR reduction could be due to the initial response to the stimulation of the carotid sinuses and aortic receptors activated by the sympathetic nervous system (36-38). Another factor leading to increased VR and blood pressure in human and animals is tissue hyperoxia, which acts as a local vasoconstrictor (39, 40). In our study, however, $SatO_2$ did not present variation during the sham protocol.

MMR is the adaptive redistribution of BF from the peripheral circulation to the vascular bed of metabolically active muscles (4). Considering the IMMR, Callegaro et al (13) demonstrated reduction of LBF in the calf in sedentary individuals when submitted to fatiguing inspiratory muscle work. In another study, the increase in inspiratory muscle work, by means of inspiratory load, reduced LBF during maximal

bicycle exercise (8). Previous studies have shown that resistive inspiratory exercise leads to the redistribution of blood flow from peripheral muscles to inspiratory muscles (2,3,19). These findings are consistent with our results that can be explained by the increased demand for O_2 by inspiratory muscles and accumulation of metabolites, such as lactic acid (19), and reduced O_2 transport (3,4) which causes peripheral blood flow reduction (42-44) and blood redistribution to inspiratory muscles.

The increase in respiratory rate and the decrease of $PetCO_2$ are associated with a greater loss of tissue carbon dioxide in respiratory muscles and consequent influence on sympathetic activation (3). Therefore, it was offered a gas mixture containing O_2 at 21% and CO_2 at 13%, and there were no significant changes in $PetCO_2$ during the protocol conduction, as was also observed by other authors (41).

The main limitation of the study is the sample size that may have been insufficient to detect differences in the increase in SBP and peripheral VR between hypertensive and normotensive individuals after the activation of inspiratory metaboreflex. The LBF difference between groups was smaller and with greater variability than the expected effect size considered in the sample size calculation, which reduced the study power.

In conclusion, the higher MAP elevation and the reduction in LBF in response to fatiguing inspiratory muscular exercise with load suggest IMMR exacerbation in hypertensive compared to normotensive individuals.

Acknowledgment: Exercise Pathophysiology Research Laboratory, Hospital de Clínicas de Porto Alegre, Porto Alegre, Rio Grande do Sul, Brasil

Conflict of interests

The authors declare that there is no conflict of interest.

Financing

This work was funded by the Research Incentive Fund of Hospital de Clínicas de Porto Alegre (HCPA-FIPE). The funding agency had no participation in the design and implementation of the study, as well as in the analysis and interpretation of data or in the decision to publish the results. SRP received grant from the Coordination Foundation for the Improvement of Higher Education Personnel (CAPES-Brazil).

References

1. Hill JM. Discharge of group IV phrenic afferent fibers increases during diaphragmatic fatigue. Brain Res. 2000;856(1-2):240-4.

2. Sheel AW, Derchak PA, Pegelow DF, Dempsey JA. Threshold effects of respiratory muscle work on limb vascular resistance. Am J Physiol Heart Circ Physiol. 2002;282(5):H1732-8.

3. Sheel AW, Derchak PA, Morgan BJ, Pegelow DF, Jacques AJ, Dempsey JA. Fatiguing inspiratory muscle work causes reflex reduction in resting leg blood flow in humans. J Physiol. 2001;537(Pt 1):277-89.

4. Dempsey JA, Romer L, Rodman J, Miller J, Smith C. Consequences of exercise-induced respiratory muscle work. Respir Physiol Neurobiol. 2006;151(2-3):242-50.

5. Rodman JR, Henderson KS, Smith CA, Dempsey JA. Cardiovascular effects of the respiratory muscle metaboreflexes in dogs: rest and exercise. J Appl Physiol (1985). 2003;95(3):1159-69.

6. Hussain SN, Chatillon A, Comtois A, Roussos C, Magder S. Chemical activation of thin-fiber phrenic afferents. 2. Cardiovascular responses. J Appl Physiol (1985). 1991;70(1):77-86.

7. St Croix CM, Morgan BJ, Wetter TJ, Dempsey JA. Fatiguing inspiratory muscle work causes reflex sympathetic activation in humans. J Physiol. 2000;529 Pt 2:493-504.

8. Harms CA, Babcock MA, McClaran SR, Pegelow DF, Nickele GA, Nelson WB, et al. Respiratory muscle work compromises leg blood flow during maximal exercise. J Appl Physiol (1985). 1997;82(5):1573-83.

9. Harms CA, Wetter TJ, McClaran SR, Pegelow DF, Nickele GA, Nelson WB, et al. Effects of respiratory muscle work on cardiac output and its distribution during maximal exercise. J Appl Physiol (1985). 1998;85(2):609-18.

10. Harms CA, Wetter TJ, St Croix CM, Pegelow DF, Dempsey JA. Effects of respiratory muscle work on exercise performance. J Appl Physiol (1985). 2000;89(1):131-8.

11. Rowell LB. Neural control of muscle blood flow: importance during dynamic exercise. Clin and Experim Pharmac and Physiol. 1997; 24: 117-125.

12. Chiappa GR, Roseguini BT, Vieira PJ, Alves CN, Tavares A, Winkelmann ER, et al. Inspiratory muscle training improves blood flow to resting and exercising limbs in patients with chronic heart failure. J Am Coll Cardiol. 2008;51(17):1663-71.

13. Callegaro CC, Ribeiro JP, Tan CO, Taylor JA. Attenuated inspiratory muscle metaboreflex in endurance-trained individuals. Respir Physiol Neurobiol. 2011;177(1):24-9.

14. Galdino G, et al. Association between respiratory muscle strength and reduction of arterial blood pressure levels after aerobic training in hypertensive subjects. J Phys Ther Sci. 2016; 28(12): 3421–3426.

15. Jones CU, et al. Slow Breathing Training Reduces Resting Blood Pressure and the Pressure Responses to Exercise. Physiol. Res. 2015; 64: 673-682.

16. DeLucia CM, De Assis RM, Bailey EF. Daily inspiratory muscle training lowers blood pressure and vascular resistance in healthy men and women. Exp Physiol 2018; 103(2):201-211.

17. Mitchell JH, Kaufman MP, Iwamoto GA. The exercise pressor reflex: its cardiovascular effects, afferent mechanisms, and central pathways. Annu Rev Physiol. 1983;45:229-42.

18. Mitchell JH, Haskell W, Snell P, Van Camp SP. Task Force 8: classification of sports. J Am Coll Cardiol. 2005;45(8):1364-7.

19. Mitchell JH, and Raven PB. Cardiovascular adaptation to physical activity. Champaign Human Kinetics Publishers, 1994.

20. Mitchell JH. Abnormal cardiovascular response to exercise in hypertension: contribution of neural factors. Am J Physiol Regul Integr Comp Physiol. 2017;312(6):R851-r63.

21. Rondon MU, Laterza MC, de Matos LD, Trombetta IC, Braga AM, Roveda F, et al. Abnormal muscle metaboreflex control of sympathetic activity in never-treated hypertensive subjects. Am J Hypertens. 2006;19(9):951-7.

22. Sausen MT, Delaney EP, Stillabower ME, Farquhar WB. Enhanced metaboreflex sensitivity in hypertensive humans. Eur J Appl Physiol. 2009;105(3):351-6.

23. Delaney EP, Greaney JL, Edwards DG, Rose WC, Fadel PJ, Farquhar WB. Exaggerated sympathetic and pressor responses to handgrip exercise in older hypertensive humans: role of the muscle metaboreflex. Am J Physiol Heart Circ Physiol. 2010;299(5):H1318-27.

24. Van Holle V, De Bourdeaudhuij I, Deforche B, Van Cauwenberg J, Van Dyck D. Assessment of physical activity in older Belgian adults: validity and reliability of an adapted interview version of the long International Physical Activity Questionnaire (IPAQ-L). BMC Public Health. 2015;15:433.

25. Neder JA, Andreoni S, Lerario MC, Nery LE. Reference values for lung function tests. II. Maximal respiratory pressures and voluntary ventilation. Braz J Med Biol Res. 1999;32(6):719-27.

26. Steier J, Kaul S, Seymour J, Jolley C, Rafferty G, et al. The value of multiple tests of respiratory muscle strength. Thorax. 2007;62(11):975-80.

27. American Thoracic Society/European Respiratory Society. ATS/ERS Statement on respiratory Muscle Testing. Am J Respir Crit Care Med 2002;166:518-624.

28. Sobush DC, Dunning M, 3rd. Assessing maximal static ventilatory muscle pressures using the "bugle" dynamometer. Suggestion from the field. Phys Ther. 1984;64(11):1689-90.

29. Correa AP, Antunes CF, Figueira FR, de Castro MA, Ribeiro JP, Schaan BD. Effect of acute inspiratory muscle exercise on blood flow of resting and exercising limbs and glucose levels in type 2 diabetes. PLoS One. 2015;10(3):e0121384.

30. Roseguini BT, Alves CN, Chiappa GR, Stein R, Knorst MM, Ribeiro JP. Attenuation of muscle metaboreflex in chronic obstructive pulmonary disease. Med Sci Sports Exerc. 2008;40:9–14.

31. Anderson EA, Sinkey CA, Lawton WJ, Mark AL. Elevated sympathetic nerve activity in borderline hypertensive humans. Evidence from direct intraneural recordings. Hypertension. 1989;14(2):177-83.

32. Aoki K, Sato K, Kondo S, Pyon CB, Yamamoto M. Increased response of blood pressure to rest and handgrip in subjects with essential hypertension. Jpn Circ J. 1983;47(7):802-9.

33. Floras JS, Hara K. Sympathoneural and haemodynamic characteristics of young subjects with mild essential hypertension. J Hypertens. 1993;11(6):647-55.

34. Kaufman MP, Waldrop TG, Rybicki KJ, Ordway GA, Mitchell JH. Effects of static and rhythmic twitch contractions on the discharge of group III and IV muscle afferents. Cardiovasc Res. 1984;18(11):663-8.

35. Smith JR, Alexander AM, Hammer SM, Didier KD, Kurti SP, Broxterman RM, et al. Cardiovascular Consequences of the Inspiratory Muscle Metaboreflex: Effects of Age and Sex. Am J Physiol Heart Circ Physiol. 2017.

36. Narkiewicz K, Pesek CA, van de Borne PJ, Kato M, Somers VK. Enhanced sympathetic and ventilatory responses to central chemoreflex activation in heart failure. Circulation. 1999;100(3):262-7.

37. Somers VK, Mark AL, Abboud FM. Potentiation of sympathetic nerve responses to hypoxia in borderline hypertensive subjects. Hypertension. 1988;11(6 Pt 2):608-12.

38. Dalymde B, Hazzledine JL, Howe A. Reflex respiratory and peripheral vascular responses to stimulation of the isolated perfused aortic arch chemoreceptors of the dog. J Physiol. 1965;177:300-22.

39. Cassuto Y, Farhi LE. Circulatory response to arterial hyperoxia. J Appl Physiol Respir Environ Exerc Physiol. 1979;46(5):973-7.

40. Lombard JH, Hess ME, Stekiel WJ. Enhanced response of arterioles to oxygen during development of hypertension in SHR. Am J Physiol. 1986;250(5 Pt 2):H761-4.

41. Miller JD, Smith CA, Hemauer SJ, Dempsey JA. The effects of inspiratory intrathoracic pressure production on the cardiovascular response to submaximal exercise in health and chronic heart failure. Am J Physiol Heart Circ Physiol. 2007;292(1):H580-92.

42. Moreno AM, Castro RR, Silva BM, Villacorta H, Sant'Anna Junior M, Nobrega AC. Intercostal and forearm muscle deoxygenation during respiratory fatigue in patients with heart failure: potential role of a respiratory muscle metaboreflex. Braz J Med Biol Res. 2014;47(11):972-6.

43. Guenette JA, Martens AM, Lee AL, Tyler GD, Richards JC, Foster GE, et al. Variable effects of respiratory muscle training on cycle exercise performance in men and women. Appl Physiol Nutr Metab. 2006;31(2):159-66.

44. Derchak PA, Sheel AW, Morgan BJ, Dempsey JA. Effects of expiratory muscle work on muscle sympathetic nerve activity. J Appl Physiol (1985). 2002;92(4):1539-52.

6.2) ESTUDO 2 – publicado na revista TRIALS Journal

Effect of inspiratory muscle training with load compared to sham training on blood pressure in individuals with hypertension: study protocol of a double-blind randomized clinical trial

Simone Regina Posser[1]; Carine Cristina Callegaro[2]; Marina Beltrami-Moreira[3], Leila Beltrami Moreira[4]*.

1. PT, MSc; Graduate Studies Program in Health Sciences: Cardiology and Cardiovascular Sciences, Universidade Federal do Rio Grande do Sul (UFRGS)
   Av. Jerônimo de Ornelas, 721, Porto Alegre, RS, Brazil 90040-341
   E-mail: sposser@hcpa.edu.br
2. PT, PhD; Graduate Program in Integral Attention to Health (PPGAIS- UNICRUZ/UNIJUI). Universidade de Cruz Alta.
   Rodovia Municipal Jacob Della Méa, Km 5.6 Cruz Alta, RS, Brazil, 98020-290
   E-mail: ccallegaro@unicruz.edu.br
3. MD, PhD; Graduate Studies Program in Health Sciences: Cardiology and Cardiovascular Sciences, Universidade Federal do Rio Grande do Sul (UFRGS)
   Av. Jerônimo de Ornelas, 721, Porto Alegre, RS, Brazil 90040-341
   E-mail: mbmoreira@hcpa.edu.br

4. MD, PhD; Graduate Studies Program in Health Sciences: Cardiology and Cardiovascular Sciences, Universidade Federal do Rio Grande do Sul (UFRGS), RS, Brazil; Hospital de Clínicas de Porto Alegre, RS, Brazil.
Rua Ramiro Barcelos, 2350, room 943. ZIP: 90035-903 Porto Alegre-RS/Brazil.
Email: lbmoreira@hcpa.edu.br * Corresponding author

Abstract

Background: Hypertension is a complex chronic condition characterized by elevated arterial blood pressure. Management of hypertension comprises non-pharmacologic strategies, which may include techniques that effectively reduce autonomic sympathetic activity. Respiratory exercises improve autonomic control over cardiovascular system and attenuate muscle metaboreflex. Because of these effects, respiratory exercises may be useful to lower blood pressure in subjects with hypertension.

Methods: This randomized, double-blind clinical trial will test the efficacy of inspiratory muscle training in reducing blood pressure in adults with essential hypertension. Subjects are randomly allocated to intervention or control group. Intervention consists of inspiratory muscle training loaded with 40% of maximum inspiratory pressure readjusted weekly. Control sham intervention consists of unloaded exercises. Systolic and diastolic blood pressures are co-primary endpoints assessed with 24h ambulatory blood pressure monitoring. Secondary outcomes include cardiovascular autonomic control, inspiratory muscle metaboreflex, cardiopulmonary capacity, and inspiratory muscle strength and endurance.

Discussion: Previously published work suggests that inspiratory muscle training lowers blood pressure in persons with hypertension, but the effectiveness of this intervention is yet to be established. We propose an adequately sized randomized clinical trial to test rigorously this hypothesis. If an effect is found, this study will allow for investigating putative mechanisms mediating this effect, including autonomic cardiovascular control and metaboreflex.

Trial registration: ClinicalTrials.gov identifier NCT02275377, registered September 30, 2014, last updated May 18, 2015.

Keywords: respiratory exercises; blood pressure; physical therapy; fitness; sympathetic nervous system; hypertension; clinical trial.

Background

Essential hypertension is a multifactorial disease characterized by chronically elevated systolic blood pressure (SBP, ≥140 mmHg) and/or diastolic blood pressure (DBP, ≥90 mmHg) (1-4). Hypertension is a major risk factor for target-organ dysfunction, cardiovascular disease, and premature mortality (3, 4). The prevalence of hypertension in Brazil has been decreasing over the past three decades, but 30% of persons over 18 years of age still bear this condition (5).

The autonomic nervous system (ANS) is a major determinant of systemic blood pressure and it may be a therapeutic target in hypertension (6, 7). Hyperactivity of sympathetic nervous system plays a role in abnormal elevations of blood pressure (BP) (6-8). BP variability is increased in hypertensive persons. This phenomenon has been linked to desensitization of baroreceptors leading to anomalous autonomic response

(9, 10). Beta-adrenergic receptor blockers are an example of effective pharmacologic approach to target the ANS in hypertension (11).

Respiratory exercises are a non-pharmacologic intervention that may modulate the ANS activity and reduce blood pressure levels (12, 13). An exercise composed of controlled respiratory patterns with slow respiratory rate has improved autonomic control and reduced BP in hypertensive subjects (12). Inspiratory muscle training (IMT) – a different modality of respiratory exercise – was tested in one randomized clinical trial (RCT) including 13 participants (13). IMT has reduced 7.9 and 5.5 mmHg of 24h-SBP and 24h-DBP, respectively, and has also improved autonomic cardiovascular control. Respiratory muscle training may have reduced sympathetic activity through attenuation of muscle metaboreflex (14, 15).

Methods / Design

Study Aim

This study aims to test the efficacy of inspiratory muscle training (IMT) in reducing BP in subjects with essential hypertension. It will further investigate the effects of this intervention on inspiratory metaboreflex – a putative mechanism for blood pressure reduction. The conceptual hypothesis is that IMT reduces mean 24h blood pressure through modulation of sympathetic nervous system activity and muscle metaboreflex.

Study design

This is a double-blind, parallel-group, sham-controlled randomized clinical trial. Participants are randomly assigned to receive inspiratory muscle training (IMT) with a

load equivalent to 40% of maximum inspiratory pressure (MIP) or IMT sham (no load) for 8 weeks. The study flowchart is depicted on Figure 1. Participants are followed on weekly visits. Study outcomes are assessed at the end of the 8th week. Study timeline is summarized on Figure 1S in Additional File 1, where detailed information on methods of assessment and protocols can be found.

Study context

Participants are recruited among patients on follow up at the hypertension clinic or the primary health care center at Hospital de Clínicas de Porto Alegre, a tertiary, university-affiliated teaching hospital. Public announcements (radio, newspapers, and website) also call for volunteers among residents of the city of Porto Alegre.

Eligibility criteria

The study includes both male and female subjects, with age ≥ 35 and < 65 years, previously diagnosed with essential hypertension, and a SBP ≥140 mmHg and/or DBP ≥ 90mmHg. Subjects receiving no pharmacologic therapy or taking only a thiazide diuretic are eligible to enter the study. Participants must have BMI<30 kg/m^2 (16), practice less than 150 minutes of moderate or intense physical activity per week according to International Physical Activity Questionnaire (IPAQ) (17, 18), and have inspiratory muscle strength ≥70% of predicted (19).

Participants are excluded from the study if: office BP ≥ 160/100 mmHg; participant has severe dyspnea, diabetes mellitus, orthopedic, musculoskeletal, or neurologic limitations, significant cognitive impairment, current or past history of deep venous thrombosis, history of myocardial infarction or stroke in the past 6 months, congestive

heart failure, unstable angina, pulmonary disease of any etiology (including asthma and COPD), or is an active smoker. Women who are pregnant or nursing are excluded from this study. Subjects are not eligible if they participated in another clinical trial in the past 30 days, or if there is any other condition that may impair the subject's ability to participate in study or follow protocol, at the discretion of the recruiter.

Participants are also oriented not to change pharmacologic or non-pharmacologic treatment for hypertension (including changes in prescription or changes in the level physical activity) during their participation in this study.

Study outcomes

Co-primary outcomes: Changes from baseline to end of study (delta) in mean 24h SBP and mean 24h DPB evaluated by ambulatory blood pressure monitoring (ABPM).

Secondary outcomes: Delta mean SBP and DBP mean, SBP and DBP night mean delta, heart rate variability, blood pressure variability, aerobic capacity and inspiratory metaboreflex.

Randomization, allocation, blinding, and confidentiality

Randomization sequence has been generated at Randomization.org in blocks of 4 to either group 1 – IMT with 40% of maximal inspiratory pressure (MIP) – or group 2 – sham IMT. An independent person not involved in this study possesses the computer-generated randomization sequence. A research assistant not involved in participant assessment obtains the code and sets the load in each device according to randomization. A black tape hides the loading spring from participant and from investigators assessing outcomes. There are no foreseen circumstances when un-blinding is permissible. When data collection is completed, the person responsible for

the randomization list will provide assignment information to the investigators. Patient's forms and other data sources are kept in a locked cabinet. Data is anonymized when entered into database to protect confidentiality. Only investigators formally associated to the project will have access final study database.

Studied variables and methods of assessment

Participants are assessed as described in Additional file 1. Briefly, baseline physical activity is quantified with IPAQ long form (20). Body mass index (BMI) is calculated as weight (kg) divided by the square of the height (m) and categorized as recommended by the World Health Organization (21). ABPM is performed with Spacelabs 90207 devices (Redmond, WA, USA). The protocol includes BP measures every 15 minutes during daytime (6AM to 10PM) and every 20 minutes during nighttime (10PM to 6AM) (22). Functional capacity is assessed during exercise through O_2 and CO_2 expiration fraction in a metabolic system. Maximum oxygen consumption (VO_2max) in each breath is recorded in ml/kg/min (23, 24).

Maximum inspiratory pressure (MIP) and maximum expiratory pressure (MEP) serve as indicators of inspiratory and expiratory muscle strength, respectively. MIP and MEP are assessed with a digital pressure manometer (MVD-300, Microhard System, Globalmed, Porto Alegre, Brazil) (25, 26).

Inspiratory muscle endurance is determined by the incremental test proposed by Martyn and col (27) using the Powerbreathe Plus® system (London, UK). Test is stopped when the subject cannot open the inspiratory valve or desires to stop the test due to respiratory exhaustion (quantified with the modified Borg scale).

Blood pressure (BP) variability and heart rate (HR) variability are assessed to evaluate autonomic cardiovascular control. Non-invasive continuous BP curves, HR, and electrocardiogram tracing are obtained simultaneously at a frequency of 1000Hz with Biopac MP150 (Biopac, California, EUA). Data are registered in a computer with biologic signals conversion capabilities.

The muscle metaboreflex is the adaptive blood flow redistribution from peripheral circulation into the vascular bed of metabolically active (exercised) muscles (28). The metaboreflex intensity is inversely related to one's fitness. Inspiratory muscle metaboreflex is assessed by causing diaphragmatic fatigue and detecting peripheral blood flow reduction (28, 29). Monitored parameters include: oxygen saturation ($SatO_2$), carbon dioxide partial expiratory tension ($PETCO_2$), mean arterial pressure, and venous occlusion plethysmography. Detailed protocol can be found in Additional File 1.

Intervention

Participants are extensively instructed on how to perform home-based IMT assisted by a Power Breathe Plus® (London, UK) device. For participant's safety, research staff is responsible for device hygiene before and after intervention period. To perform the exercise training, subjects should be seated, obstruct nose with a nasal clip, and breathe in and out through the mouthpiece. The goal is to keep diaphragmatic breathing at 10-15 respirations per minute for a total of 30 minutes per day, 7 days a week.

Participants receiving active IMT (intervention group) perform the exercises with a load equivalent to 40% of maximal inspiratory pressure. Those receiving sham

intervention (control group) follow the same instructions but have no load applied to the respiratory exercises. Sham intervention was chosen as comparator for it is the most similar intervention available. It allows for blinding of investigators and participants, and it is not expected to reproduce the effects of loaded exercises.

The complete study protocol consists of 56 daily training sessions completed over 8 weeks. During the scheduled weekly visit to the study center, participants are questioned about possible discomforts associated to the intervention, which may include: headaches, nausea, intense muscle fatigue, muscle cramps, or chest pain. Respiratory muscle strength is then reassessed; inspiratory threshold loading is adjusted accordingly, keeping a 40% MIP load for the intervention group throughout the study. The staff member who receives information about treatment allocation and adjusts the load does not participate in any assessments nor communicates with participants.

Participants in both groups receive a journal to register date, time, duration of training session, and possible symptoms or discomforts associated to the intervention. This serves as a mean to stimulate and estimate adherence to intervention. Adherence to the protocol is also reinforced on weekly follow-up phone calls.

Sample size calculation

Sample size was estimated based on a small RCT on the effect of inspiratory muscle training on BP of hypertensive subjects in Brazil (13). That study found an 8-mmHg difference in SBP between the active treatment and the control group (SD 10mmHg). To detect this difference with power of 80%, and assuming alpha error of 5%, the study should include 26 participants per group. We opted for a 20% inflation of this number

to account for loss of follow-up and consent withdrawal – therefore, a total of 60 participants will be included and assigned to either intervention or sham treatment.

Data management and statistical analysis plan

Data recorded in printed forms are kept in locked cabinets. Information is entered on Microsoft Excel spreadsheets by one person and reviewed by another. Numeric continuous variables, including BP measures in the primary outcome, will be described as mean ± standard deviation or as median (interquartile range) and comparison between groups will be performed with Student's t test or Mann-Whitney U test, depending on variable distribution. As a superiority trial, differences in autonomic cardiovascular control and metaboreflex between groups will be tested with two-sided repeated-measures ANOVA. Results will be analyzed as per intention-to-treat. Participants who drop out and do not complete outcome assessment will be compared to those who completed the protocol, but their data will be excluded from analyses. P values <0.05 will be considered statistically significant. All analyses will be performed with PASW Statistics 18® (International Business Machines Corp., New York, NY).

Results communication

A summary of the main findings in lay words will be sent to participants after study completion. Scientific papers written according to the CONSORT recommendations will be submitted for peer-reviewed publication. Study results will also be communicated as abstracts and presentations in local, national, and international scientific meetings.

Discussion

The effect of inspiratory muscle training (IMT) on blood pressure (BP) in hypertensive individuals is not yet established. A significant reduction in BP was observed in a small RCT with 6 and 5 subject allocated to intervention and control group, respectively (13). Although these findings do not provide enough evidence to support the efficacy of IMT training in BP control, they are encouraging. We propose an adequately-sized RCT to test the hypothesis that IMT reduces BP in persons with hypertension. If the hypothesis is confirmed, this study will also allow for investigation of putative mechanisms mediating such effect. We will assess the effects of IMT on autonomic cardiovascular control and metaboreflex in subjects with hypertension – data that to the best of our knowledge is lacking in the medical literature.

Evaluation of inspiratory muscle metaboreflex is one of the strengths of this study. IMT attenuated the metaboreflex in healthy subjects (14) and in persons with chronic heart failure (31). In healthy subjects, decrease inspiratory work through assisted ventilation increases exercise time on a cycle ergometer by 14% (32); a similar intervention has been shown to attenuate quadriceps fatigue during exercise (9). This effect is likely due to inhibition of inspiratory muscle metaboreflex (10).

The effects of inspiratory muscle training deserve to be better understood given the potential to help control blood pressure in hypertensive patients. The results of this study will provide evidence on the effects of IMT on blood pressure, in addition to inspiratory muscle metaboreflex, autonomic cardiovascular control, and other physiologic and metabolic aspects. These data will provide mechanistic insights as well as useful clinical information for the management of hypertension.

Status of the study

Trial is enrolling participants.

Ethics approval and consent to participate

Participants receive thorough oral and written description of study goals, intervention, potential risks and benefits involved, and efforts in place to protect personal and health information. Participants have the opportunity to ask questions, which are answered by the investigator. After information, consent form is offered for participant to sign (in Portuguese only, available upon request). It is clarified that they can withdraw the consent and quit the study at any time. If any medical attention is needed while participant is performing assessments in the research center, immediate medical care will be provided at Hospital de Clínicas de Porto Alegre. The study protocol and its informed consent form has been approved by the Institutional Review Board of Hospital de Clinical de Porto Alegre (protocol number 140405) and is registered at ClinicalTrials.gov (NCT2275377). Any protocol amendments are subjected to IRB approval and later published at ClinicalTrials.gov.

Competing interests

The authors have no conflict of interests to disclose.

Funding

This project is funded by the Research and Graduate Education Group (GPPG) at Hospital de Clínicas de Porto Alegre. The funding agency had no role in study design and implementation, and will have no role in data analysis and interpretation, or the decision to publish trial results.

Author´s contributions

SRP projected the trial protocol, elaborated this manuscript, performs data collection, and will perform data analysis and interpretation. MBM offered suggestions on trial design and data analysis plan, collaborated on the writing and critical review of this manuscript, and will collaborate on data interpretation. LBM and CCC contributed to protocol development and writing of this manuscript; they will perform data analysis and interpretation, critical revision and final approval of future research communications. All authors read and approved the final version of this manuscript.

Authors' information

SRP, PT, MSc, is a PhD candidate in the Graduate Studies Program in Health Sciences: Cardiology and Cardiovascular Sciences, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS.

CCC, PT, PhD is an Professor of physiotherapy at University of Cruz Alta (UNICRUZ), Cruz Alta, RS.

MBM is MD, PhD, and collaborates as a volunteer visiting scholar in this project.

LBM, MD, PhD is a cardiologist and Professor of Clinical Pharmacology at UFRGS, Porto Alegre, RS, Brazil.

Additional Files

Additional File 1: Supplementary Material - Methods for assessment of study outcomes and other variables (page 66)

Additional File 2: SPIRIT checklist (page 72)

Figure 1. Study flow diagram (page 79)

Figure 1S: Schedule of enrollment, intervention, and assessments (page 80)

Figure 2S: Inspiratory muscle metaboreflex induction protocol flow-chart (page 81)

List of abbreviations

ANOVA: analysis of variance; ANS: autonomic nervous system; BMI: body mass index; BP: blood pressure; CO_2: carbon dioxide; CONSORT: Consolidated Standards of Reporting Trials; DBP: diastolic blood pressure; HF: high frequency; HR: heart rate; IPAQ: International Physical Activity Questionnaire; LF: low frequency; MEP: maximum expiratory mouth pressure; MIP: maximum inspiratory mouth pressure; O_2: oxygen; $PetCO_2$: expired carbon dioxide pressure; RR: respiratory rate; RCT: randomized clinical trial; SBP: Systolic blood pressure; SatO2: oxygen saturation; IMT: inspiratory muscle training; VO2max: maximum oxygen consumption; WHO: world health organization.

References

1. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Bohm M, et al. 2013 ESH/ESC guidelines for the management of arterial hypertension: the Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Eur Heart J. 2013;34(28):2159-219.

2. Nobre F. [Introduction: Brazilian guidelines on hypertension VI. Brazilian Society of Cardiology, Brazilian Society of Hypertension, Brazilian Society of Nephrology]. J Bras Nefrol. 2010;32 Suppl 1:Iii.

3. Williams B, Poulter NR, Brown MJ, Davis M, McInnes GT, Potter JF, et al. Guidelines for management of hypertension: report of the fourth working party of the British Hypertension Society, 2004-BHS IV. J Hum Hypertens. 2004;18(3):139-85.

4. 2013 Practice guidelines for the management of arterial hypertension of the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC): ESH/ESC Task Force for the Management of Arterial Hypertension. J Hypertens. 2013;31(10):1925-38.

5. Picon RV, Fuchs FD, Moreira LB, Riegel G, Fuchs SC. Trends in prevalence of hypertension in Brazil: a systematic review with meta-analysis. PLoS One. 2012;7(10):e48255.

6. Callegaro CC, Moraes RS, Negrao CE, Trombetta IC, Rondon MU, Teixeira MS, et al. Acute water ingestion increases arterial blood pressure in hypertensive and normotensive subjects. J Hum Hypertens. 2007;21(7):564-70.

7. Rondon MU, Laterza MC, de Matos LD, Trombetta IC, Braga AM, Roveda F, et al. Abnormal muscle metaboreflex control of sympathetic activity in never-treated hypertensive subjects. Am J Hypertens. 2006;19(9):951-7.

8. Guzzetti S, Piccaluga E, Casati R, Cerutti S, Lombardi F, Pagani M et al. Sympathetic predominance in essential hypertension: a study employing spectral analysis of heart rate variability. J Hypertens 1988; 6:711–717.

9. Romer LM, Lovering AT, Haverkamp HC, Pegelow DF, Dempsey JA. Effect of inspiratory muscle work on peripheral fatigue of locomotor muscles in healthy humans. J Physiol. 2006;571(Pt 2):425-39.

10. Ribeiro JP, Chiappa GR, Callegaro CC. The contribution of inspiratory muscles function to exercise limitation in heart failure: pathophysiological mechanisms. Rev Bras Fisioter. 2012;16(4):261-7.

11. Wiysonge CS, Bradley HA, Volmink J, Mayosi BM, Mbewu A, Opie LH. Beta-blockers for hypertension. Cochrane Database of Systematic Reviews 2012, Issue 11. Art. No.: CD002003. DOI: 10.1002/14651858.CD002003.pub4.

12. Hunt BE, Fahy L, Farquhar WB, Taylor JA. Quantification of mechanical and neural components of vagal baroreflex in humans. Hypertension. 2001;37(6):1362-8.

13. Ferreira JB, Plentz RDM, Stein C, Casali KR, Arena R, Lago PD. Inspiratory muscle training reduces blood pressure and sympathetic activity in hypertensive patients: a randomized controlled trial. International Journal of Cardiology. 2013;166(1):61-7.

14. Witt JD, Guenette JA, Rupert JL, McKenzie DC, Sheel AW. Inspiratory muscle training attenuates the human respiratory muscle metaboreflex. J Physiol. 2007;584(Pt 3):1019-28.

15. McConnell AK, Lomax M. The influence of inspiratory muscle work history and specific inspiratory muscle training upon human limb muscle fatigue. J Physiol. 2006;577(Pt 1):445-57.

16. Jensen MD, Ryan DH, Donato KA, Apovian CM, Ard JD, Comuzzie AG, et al. Executive summary: Guidelines (2013) for the management of overweight and obesity in adults: A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The Obesity Society Published by The Obesity Society and American College of Cardiology/ American Heart Association Task Force on Practice Guidelines. Based on a systematic review from the Obesity Expert Panel, 2013. Obesity (Silver Spring). 2014;22 Suppl 2:S5-s39.

17. Hallal PC, Victora CG, Wells JC, Lima RC. Physical inactivity: prevalence and associated variables in Brazilian adults. Med Sci Sports Exerc. 2003;35(11):1894-900.

18. Hallal PC, Simoes E, Reichert FF, Azevedo MR, Ramos LR, Pratt M, et al. Validity and reliability of the telephone-administered international physical activity questionnaire in Brazil. J Phys Act Health. 2010;7(3):402-9.

19. Neder JA, Andreoni S, Lerario MC, Nery LE. Reference values for lung function tests. II. Maximal respiratory pressures and voluntary ventilation. Braz J Med Biol Res. 1999;32(6):719-27.

20. Adams MA, Ding D, Sallis JF, Bowles HR, Ainsworth BE, Bergman P, et al. Patterns of neighborhood environment attributes related to physical activity across 11 countries: a latent class analysis. Int J Behav Nutr Phys Act. 2013;10:34.

21. World Health Organization. Body Mass Index – BMI. http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi. Last accessed May 19 2016.

22. [V Brazilian guidelines for ambulatory monitoring of arterial pressure and III Brazilian guidelines for home monitoring of blood pressure]. J Bras Nefrol. 2011;33(3):365-88.

23. ATS/ACCP Statement on cardiopulmonary exercise testing. Am J Respir Crit Care Med. 2003;167(2):211-77.

24. Winkelmann ER, Chiappa GR, Lima CO, Viecili PR, Stein R, Ribeiro JP. Addition of inspiratory muscle training to aerobic training improves cardiorespiratory responses to exercise in patients with heart failure and inspiratory muscle weakness. Am Heart J. 2009;158(5):768.e1-7.

25. Sobush DC, Dunning M, 3rd. Assessing maximal static ventilatory muscle pressures using the "bugle" dynamometer. Suggestion from the field. Phys Ther. 1984;64(11):1689-90.

26. Callegaro CC, Taylor JA. Age-related effects of vagotonic atropine on cardiovagal baroreflex gain. Neurobiol Aging. 2012;33(2):368-74.

27. Martyn JB, Moreno RH, Pare PD, Pardy RL. Measurement of inspiratory muscle performance with incremental threshold loading. Am Rev Respir Dis. 1987;135(4):919-23.

28. Dempsey JA, Romer L, Rodman J, Miller J, Smith C. Consequences of exercise-induced respiratory muscle work. Respir Physiol Neurobiol. 2006;151(2-3):242-50.

29. St Croix CM, Morgan BJ, Wetter TJ, Dempsey JA. Fatiguing inspiratory muscle work causes reflex sympathetic activation in humans. J Physiol. 2000;529 Pt 2:493-504.

30. Callegaro CC, Ribeiro JP, Tan CO, Taylor JA. Attenuated inspiratory muscle metaboreflex in endurance-trained individuals. Resp Physiol Neuro. 2011;177: 24–29.

31. Chiappa, G.R., Roseguini, B.T., Vieira, P.J., Alves, C.N., Tavares, A., Winkelmann, E.R.,Ferlin, E.L., Stein, R., Ribeiro,J.P. Inspiratory muscle training improves blood flow to resting and exercising limbs in patients with chronic heart failure.Journal of the American College of Cardiology. 2008; (51), 1663–1671.

32. Harms CA, Wetter TJ, St Croix CM, Pegelow DF, Dempsey JA. Effects of respiratory muscle work on exercise performance. J Appl Physiol (1985). 2000;89(1):131-8.

7) CONCLUSÕES E CONSIDERAÇÕES FINAIS

A prevalência da hipertensão é em torno de 30-45% na população adulta. Além de prevalente, é o mais importante fator de risco modificável para doenças cardiovasculares, o que ressalta a importância de medidas efetivas para seu controle. Sabendo-se que o sistema nervoso autônomo simpático tem grande importância tanto no mecanismo inicial como na manutenção da hipertensão, intervenções não farmacológicas que atuem sobre esse sistema e reduzam os níveis pressóricos podem constituir-se em mais uma alternativa para melhorar o seu controle.

No Artigo 1 desta tese, foram investigados mecanismos pelos quais o treinamento muscular inspiratório poderia reduzir a pressão arterial. O primeiro

mecanismo investigado foi o comportamento de variáveis hemodinâmicas e respiratórias no metaborreflexo muscular inspiratório em indivíduos hipertensos.

Podemos verificar que, tanto o fluxo sanguíneo da perna quanto a pressão arterial média dos hipertensos, apresentaram-se alterados no protocolo de indução do metaborreflexo, diferindo do grupo de normotensos o que sugere que o metaborreflexo possa estar exacerbado em indivíduos hipertensos comparado aos normotensos. Entretanto, sugere-se a realização de estudos futuros com maior tamanho amostral para confirmar nossos achados, pois a variabilidade nas variáveis estudadas pode ter reduzido o poder do estudo para detectar diferenças estatisticamente significativas nas variáveis pressão aretrail sistólica, pressão arterial diastólica e resistência vascular periférica. Mesmo assim, os achados de nosso estudo não descartam a hipótese de que o treinamento possa induzir efeito hipotensor, por atenuar o metaborreflexo.

No Artigo 2, um ensaio clínico randomizado foi desenhado para testar a eficácia do treinamento muscular inspiratório sobre a redução dos níveis pressóricos em pacientes hipertensos através do mecanismo de atenuação do metaborreflexo e melhora do controle autonômico cardiovascular. O protocolo do estudo foi publicado na revista *Trials Journal* (2016). A aplicação do protocolo está em andamento, tendo sido suspenso temporariamente por questões logísticas, com 12 participantes incluídos, os quais realizaram todas as etapas do estudo (avaliação pré intervenção, intervenção e avaliação final), sendo que a meta é que em 2018 seja finalizado.

Os efeitos do treinamento muscular inspiratório merecem ser melhor compreendidos, dado o potencial já conhecido para melhora da capacidade funcional,

redução da fadiga e também atuação sobre o sistema nervoso simpático. Os resultados deste estudo fornecerão informações clínicas importantes para melhor compreender o efeito do exercício respiratório com carga no gerenciamento da hipertensão.

8) ANEXOS E APÊNDICES

8.1) ANEXO 1 – CAPA DO ESTUDO 2 PUBLICADO

Posser et al. Trials (2016) 17:382
DOI 10.1186/s13063-016-1514-y

Trials

STUDY PROTOCOL Open Access

Effect of inspiratory muscle training with load compared with sham training on blood pressure in individuals with hypertension: study protocol of a double-blind randomized clinical trial

Simone Regina Posser[1], Carine Cristina Callegaro[2], Marina Beltrami-Moreira[1] and Leila Beltrami Moreira[1,3*]

Abstract

Background: Hypertension is a complex chronic condition characterized by elevated arterial blood pressure. Management of hypertension includes non-pharmacologic strategies, which may include techniques that effectively reduce autonomic sympathetic activity. Respiratory exercises improve autonomic control over cardiovascular system and attenuate muscle metaboreflex. Because of these effects, respiratory exercises may be useful to lower blood pressure in subjects with hypertension.

Methods/design: This randomized, double-blind clinical trial will test the efficacy of inspiratory muscle training in reducing blood pressure in adults with essential hypertension. Subjects are randomly allocated to intervention or control groups. Intervention consists of inspiratory muscle training loaded with 40 % of maximum inspiratory pressure, readjusted weekly. Control sham intervention consists of unloaded exercises. Systolic and diastolic blood pressures are co-primary endpoint measures assessed with 24 h ambulatory blood pressure monitoring. Secondary outcome measures include cardiovascular autonomic control, inspiratory muscle metaboreflex, cardiopulmonary capacity, and inspiratory muscle strength and endurance.

Discussion: Previously published work suggests that inspiratory muscle training reduces blood pressure in persons with hypertension, but the effectiveness of this intervention is yet to be established. We propose an adequately sized randomized clinical trial to test this hypothesis rigorously. If an effect is found, this study will allow for the investigation of putative mechanisms to mediate this effect, including autonomic cardiovascular control and metaboreflex.

Trial registration: ClinicalTrials.gov NCT02275377. Registered on 30 September 2014.

Keywords: Blood pressure, Clinical trial, Fitness, Hypertension, Physical therapy, Respiratory exercises, Sympathetic nervous system

* Correspondence: lbmoreira@hcpa.edu.br
[1]Graduate Studies Program in Health Sciences: Cardiology and Cardiovascular Sciences, Universidade Federal do Rio Grande do Sul (UFRGS), Av. Jerônimo de Ornelas, 721, Porto Alegre, RS 90040-341, Brazil
[3]Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos, 2350, room 943, 90035-903 Porto Alegre, RS, Brazil
Full list of author information is available at the end of the article

Fonte: www.trialsjournal.com, acessado em 05/05/2017.

8.2) APÊNDICE A - Additional File 1: Supplementary Material - Methods for assessment of study outcomes and other variables (ESTUDO 2)

Additional File 1 - Supplementary material

Methods for assessment of study outcomes and other variables

- Ambulatory blood pressure monitoring (ABPM)

ABPM is performed with Spacelabs 90207 devices (Redmond, WA, USA). Cuff size (regular or large) is chosen based on brachial circumference. The protocol includes blood pressure (BP) assessments every 15 minutes during daytime (6AM to 10PM) and every 20 minutes during nighttime (10PM to 6AM). ABPM is considered satisfactory if at least 16 valid readings during daytime and 8 valid readings during nighttime are obtained (1). The timeline of outcomes assessment and intervention are depicted in Figure 1S.

- Functional capacity

A cardiopulmonary exercise protocol tests cardiopulmonary conditioning. It is performed on a treadmill (T-2100 Treadmill, GE Healthcare, Idaho, USA), with a breath-by-breath gas analyzer system (Metalyzer 3B, CPX System, Cortex, Leipzig, Germany), and an aneroid sphygmomanometer. Equipment is calibrated before each test, performed in a room with controlled temperature (19 to 21^{o}C) and humidity (relative humidity 60-70%). Participants maintain their usual medical treatment, if any, and receive thorough information about test procedures before the session begins.

Test lasts for 8 to 12 minutes in an incremental ramp protocol; this protocol is indicated for individuals with comorbidities or with functional or age-related

limitations. Speed is increased by 0.3-0.7 km/min while inclination is increased by 0.5 to 1%/min, depending on the individual's estimated physical conditioning. Test will be stopped per participant's request or per criteria described in the American Thoracic Society/American College of Chest Physicians consensus for cardiopulmonary exercise testing (2). Gas fraction analysis in open circuit evaluates peak oxygen consumption (VO_2, in ml/kg/min) in each breath. Ventilatory threshold is defined as established in the literature (2, 3). Functional capacity is reassessed with the same protocol after the 8 weeks of intervention. In case of intercurrences or emergencies, the test center is equipped with emergency kit including ACLS defibrillator and medications.

- Maximum inspiratory pressure (MIP) and maximum expiratory pressure (MEP): respiratory muscle strength

Subjects are seated with an angle of 90° between trunk and legs and with a nasal clip in place throughout the assessment. The manometer is connected to a mouthpiece, and participants are instructed to seal the lips around it. The mouthpiece is designed to avoid oral cavity pressure increase generated by undesired contraction of oropharyngeal muscles (4). Participants are instructed to perform diaphragmatic respiration. MIP is then measured during forced inspiration starting from the residual volume; MEP is measured during forced expiration from the total lung capacity. Participants will perform up to 12 maneuvers to provide 6 measurements with variation <10% and avoid effects of learning curve. The highest 1-second peak pressure among the 6 measurements will be used in the analysis (5). MIP and MEP are considered surrogates of inspiratory muscle strength; they will be measured as described above before and after the intervention.

- Inspiratory muscle endurance

Participants ventilate through a circuit made up by two unidirectional valves with linear pressure resistance (Powerbreathe Plus®, London, UK). An initial load of 50% of MIP is applied. Every 3 minutes the load is increased by 10% of MIP. Test is stopped when subject cannot open the inspiratory valve or desires to stop the test due to respiratory exhaustion, quantified with the modified Borg scale (ranging between 0 – no respiratory fatigue – to 10 – maximal respiratory exhaustion) (6).

- Autonomic control

BP variability and HR variability are assessed to evaluate autonomic cardiovascular control. Non-invasive continuous blood pressure monitoring is used to register pressure waves are detected by a cuff placed on the intermediate phalange of the third finger. After 20 of rest, continuous BP curves, heart rate, and electrocardiogram tracing are obtained with Biopac MP150 (Biopac, California, EUA) at a frequency of 1000Hz and registered simultaneously in a computer with biologic signals conversion capabilities. These signals are subjected to analytic protocols to provide BP and HR variability with the use of fast Fourier transform during exercise phase and in post-exercise rest phase.

- Inspiratory muscle metaboreflex

The metaboreflex induction protocol consists of causing respiratory muscle fatigue through exercises and measuring leg arterial blood flow reduction simultaneously. Participants are instructed not to perform physical activities for 48h before the test;

they should not have caffeinated or alcoholic beverages for at least 12 hours before the test, and they are instructed to fast for 2h before the protocol begins.

First, maximum inspiratory pressure is determined for each individual as described above. Subject is then positioned on a bed with elevated head (semi-supine), and monitoring of physiologic parameters begins. Respiratory rate and peripheral O_2 saturation is monitored with pulse oximetry. Heart rete is monitored with electrocardiography tracing. An automated sphygmomanometer cuff (Dinamap, DASH 2000, General Electric, CT) is placed on the non-dominant arm over the brachial artery to measure systolic, diastolic, and mean arterial blood pressures. Blood pressure and heart rate variability are assessed as described above ('autonomic control' section). End-tidal CO_2 partial pressure is monitored with infrared capnography (Takaoka, USA).

Blood flow is determined by venous occlusion plethysmography (Hokanson, WA) in the non-dominant lower limb every 10 seconds, expressed in ml/min/100ml. Simultaneously, mean arterial blood pressure will be monitored with automated sphygmomanometer (Dinamap, DASH 2000, General Electric, Bloomfield, CT, USA) on the popliteal artery contralateral to the limb where blood flow is measured. Vascular resistance is calculated dividing mean arterial blood pressure by blood flow.

After 15 minutes of rest, baseline autonomic control assessment begins. A nasal clip is placed and participant is instructed to breathe through a two-way respiratory system (Hans Rudolph, KS). Phase 1 of this assessment consists of 10 minutes of spontaneous ventilation; phase 2 consists of 10 minutes of controlled respiratory rate (15rpm). After this step, subjects have a 30-minute break.

After the break, metaboreflex induction protocol begins. Subjects are instructed to keep spontaneous breathing for 5 minutes, while baseline measurements are

recorded. A breathing trainer (PowerBreath, UK) is then connected to the respiratory system to create an inspiratory load equal to 60% of the maximum inspiratory pressured assessed at the beginning of the session. After that, a period of controlled ventilation starts.

Participants should keep respiratory rate at 15rpm and the ratio inspiratory time/total cycle time at 0.75. A metrometer designed by the Biomedical Engineering Service at Hospital de Clínicas de Porto Alegre aids subjects to maintain the correct rhythm through sounds and lights that signal for inspiration and expiration. During each cycle, subjects are instructed to 1) perform diaphragmatic breathing to avoid use of accessory muscles, and 2) maintain the inspiratory pressure constant, with the aid of visual feedback as the inspiratory pressure is continuously recorded and displayed on a screen. Modified Borg scale assesses respiratory effort every 1 minute during the exercise. Borg's scale quantifies perceived exertion at the end of the protocol.

Metaboreflex induction exercise with 60% MIP load ends when: 1) subject is unable to open the resistance device's valve; or 2) a pressure less than 90% of the predicted MIP is observed in 3 consecutive breaths. When either of the criteria is met, respiratory device is unloaded and participant is instructed to keep the same respiratory pattern guided by the metrometer. Subject is then monitored for 10 minutes in this recovery phase.

After a 40-minute break, the sham protocol starts with 2% MIP load. The same procedures adopted for the induction protocol are repeated. The duration of the sham protocol is determined by the duration of the induction protocol for each participant.

References:

1. [V Brazilian guidelines for ambulatory monitoring of arterial pressure and III Brazilian guidelines for home monitoring of blood pressure]. J Bras Nefrol. 2011;33(3):365-88.
2. ATS/ACCP Statement on cardiopulmonary exercise testing. Am J Respir Crit Care Med. 2003;167(2):211-77.
3. Winkelmann ER, Chiappa GR, Lima CO, Viecili PR, Stein R, Ribeiro JP. Addition of inspiratory muscle training to aerobic training improves cardiorespiratory responses to exercise in patients with heart failure and inspiratory muscle weakness. Am Heart J. 2009;158(5):768.e1-7.
4. Sobush DC, Dunning M, 3rd. Assessing maximal static ventilatory muscle pressures using the "bugle" dynamometer. Suggestion from the field. Phys Ther. 1984;64(11):1689-90.
5. Callegaro CC, Taylor JA. Age-related effects of vagotonic atropine on cardiovagal baroreflex gain. Neurobiol Aging. 2012;33(2):368-74.
6. Martyn JB, Moreno RH, Pare PD, Pardy RL. Measurement of inspiratory muscle performance with incremental threshold loading. Am Rev Respir Dis. 1987;135(4):919-7.
7. Callegaro CC, Moraes RS, Negrao CE, Trombetta IC, Rondon MU, Teixeira MS, et al. Acute water ingestion increases arterial blood pressure in hypertensive and normotensive subjects. J Hum Hypertens. 2007;21(7):564-70.

8.4)

APÊNDICE B - 

Additional File 2: SPIRIT checklist (ESTUDO 2)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	-
Protocol version	3	Date and version identifier	3
Funding	4	Sources and types of financial, material, and other support	13
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 2, 13
	5b	Name and contact information for the trial sponsor	1, 2

	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	10, 11
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	NA
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3, 4
	6b	Explanation for choice of comparators	8
Objectives	7	Specific objectives or hypotheses	4
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	4, 6, 9
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5

Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	5, 6
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8 and additional file 1
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	-
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	9
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	6
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	6
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	5 and additional file 1 (Figure 1S)
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	7

Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	5

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	6
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	6
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	6
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	6

Methods: Data collection, management, and analysis

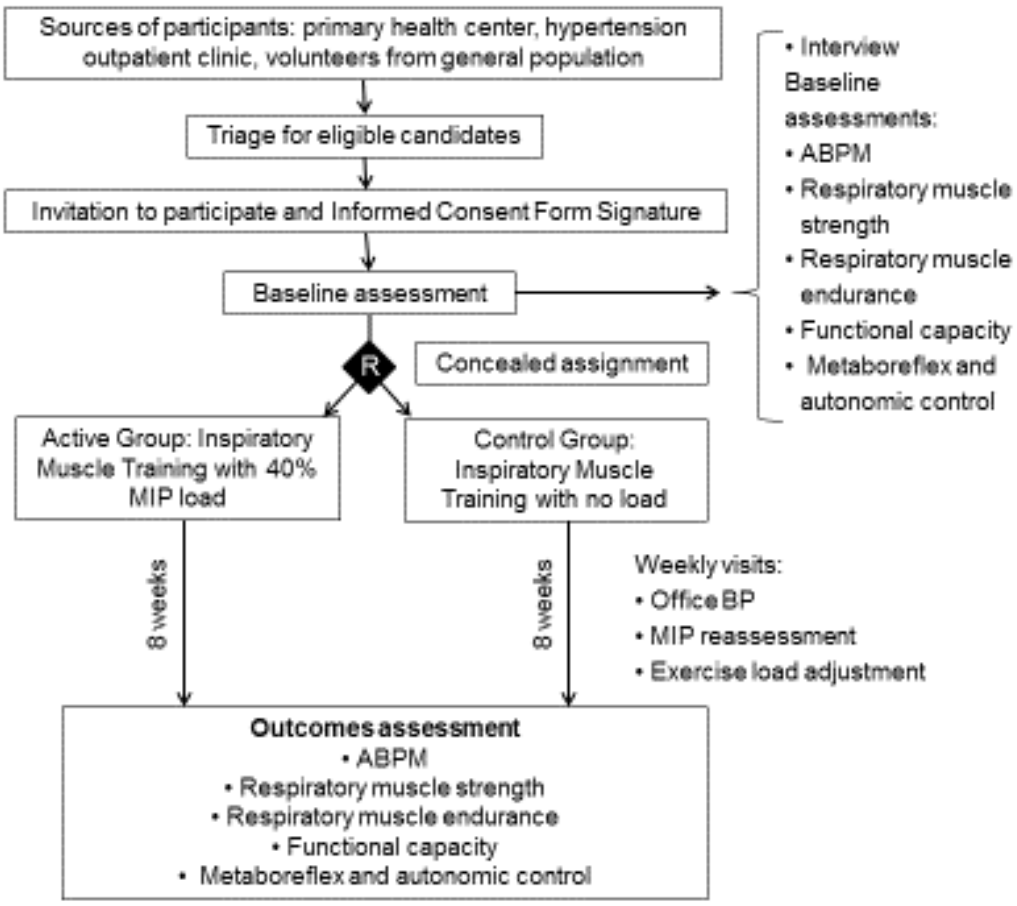
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	6, 7, 8 and additional file 1
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	-
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	9
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	10
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	-
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	10
Methods: Monitoring			

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	12
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	NA
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	8, 9
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	NA
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	12
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	12
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	12 and Figure 1
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA

Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	6, 7
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	13
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	7
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	12
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	11
	31b	Authorship eligibility guidelines and any intended use of professional writers	-
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	-
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	12
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the

8.5) APÊNDICE C - Figure 1. Study flow diagram (ESTUDO 2)



8.6) APÊNDICE D - Figure 1S: Schedule of enrollment, intervention, and assessments (ESTUDO 2)

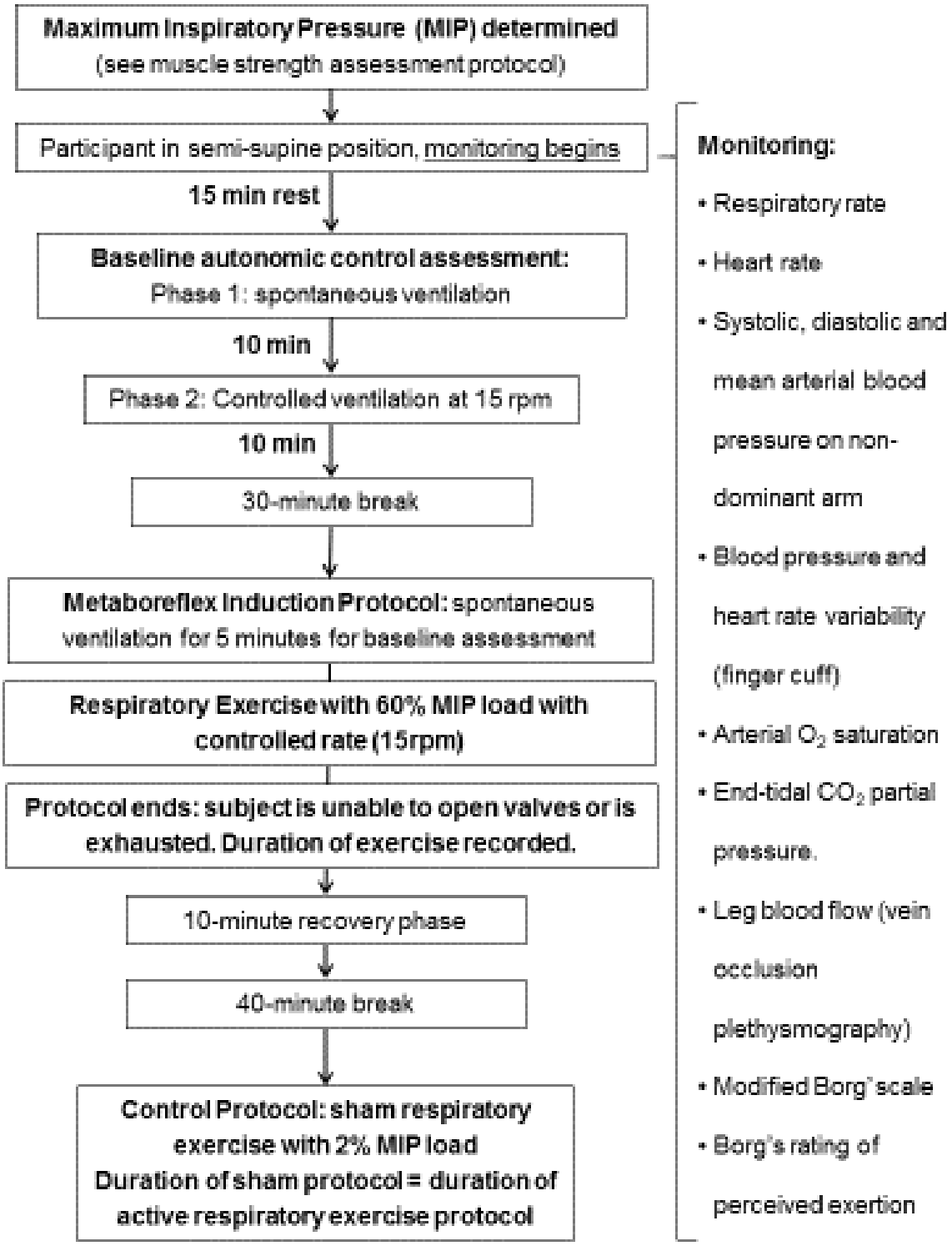
Figure 1S: Schedule of enrollment, intervention, and assessments

	STUDY PERIOD					
	Enrolment	Allocation	Baseline assessment	Intervention period	Primary endpoint	Close-out
TIME POINT	Week -2-0		Week 0	Week 1-8	Week 8	Week 9-12
ENROLMENT:						
Eligibility screen	**X**					
Informed consent	**X**					
Allocation		**X**				
INTERVENTION:						
Inspiratory muscle training			→	→	→	
Sham respiratory training (control group)			→	→	→	
ASSESSMENTS:						
IPAQ, RMS, height, weight			**X**			
ABPM			**X**		**X**	

Metaboreflex inspiratory			**X**			**X**
Functional capacity			**X**			**X**
Respiratory muscle strength tests			**X**	**X**	**X**	**X**
Respiratory muscle endurance			**X**			**X**

IPAQ: International Physical Activity Questionnaire; RMS: respiratory muscle strength; ABPM: ambulatory blood pressure monitoring.

8.6) APÊNDICE E - Figure 2S: Inspiratory muscle metaboreflex induction protocol flow-chart (ESTUDO 2)


Maximum Inspiratory Pressure (MIP) determined
(see muscle strength assessment protocol)
Participant in semi-supine position, monitoring begins
15 min rest
Baseline autonomic control assessment:
Phase 1: spontaneous ventilation
10 min
Phase 2: Controlled ventilation at 15 rpm
10 min
30-minute break
Metaboreflex Induction Protocol: spontaneous ventilation for 5 minutes for baseline assessment
Respiratory Exercise with 60% MIP load with controlled rate (15rpm)
Protocol ends: subject is unable to open valves or is exhausted. Duration of exercise recorded.
10-minute recovery phase
40-minute break
Control Protocol: sham respiratory exercise with 2% MIP load
Duration of sham protocol = duration of active respiratory exercise protocol
Monitoring:
• Respiratory rate
• Heart rate
• Systolic, diastolic and mean arterial blood pressure on non-dominant arm
• Blood pressure and heart rate variability (finger cuff)
• Arterial O_2 saturation
• End-tidal CO_2 partial pressure.
• Leg blood flow (vein occlusion plethysmography)
• Modified Borg' scale
• Borg's rating of perceived exertion