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AJUSTES CARDIOVASCULARES A DIFERENTES MODALIDADES DE EXERCÍCIO EM POPULAÇÕES DE ALTO RISCO

Aluna: Ana Paula dos Santos Corrêa

Orientadora: Beatriz D'Agord Schaan (UFRGS)

Co-orientadora: Maria A. Fiatarone Singh (UNISYD)

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Ana Paula dos Santos Corrêa

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Orientadora:
Profa. Dra. Beatriz D´Agord Schaan

Banca Examinadora:
Prof. Dr. Bruno Tesini Roseguini
Prof. Dr. Daniel Umpierre de Moraes
Profa. Dra. Ticiana da Costa Rodrigues

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LISTA DE ABREVIATURAS

DM2: Diabetes mellitus do tipo 2

EDGF: Fator de crescimento derivado do endotélio

FGF: Fator de crescimento de fibroblastos

IC: Insuficiência cardíaca

NO: Oxido nítrico

PDGF: Fator de crescimento derivado de plaquetas

TI/Ttot: Tempo inspiratório e duração total do ciclo respiratório

TMI: Treinamento muscular inspiratório

VO_2: Consumo de oxigênio

VO_2máx: Consumo máximo de oxigênio

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RESUMO

Os objetivos desta tese foram avaliar as respostas hemodinâmicas e cardiovasculares ao exercício de diferentes modalidades em populações de alto risco para o desenvolvimento de doença cardiovascular. **Métodos**. Foram desenvolvidos um artigo de revisão e dois estudos de intervenção. O artigo de revisão teve por objetivo investigar a interação dos diversos fatores envolvidos na intolerância ao exercício físico em pacientes com DM2 e também, analisar a influência do exercício de força sobre a rigidez arterial em indivíduos idosos. No primeiro estudo, foram avaliados os músculos inspiratórios e o controle reflexo da circulação em indivíduos com DM2 com e sem neuropatia autonômica. Os indivíduos foram submetidos a protocolos para testar o metaborreflexo muscular inspiratório através de carga inspiratória de 60% da PImáx. A glicemia foi avaliada durante este protocolo em um subgrupo de pacientes. No segundo experimento foram analisados os efeitos agudos de 1 RM sobre a rigidez arterial em indivíduos idosos. Os dados coletados em 5, 30 e 60 minutos após o teste de 1 RM foram comparados com os valores basais. **Resultados**. Concluímos que indivíduos com DM2 apresentam metaborreflexo muscular inspiratório exacerbado, independente da presença de neuropatia autonômica e evidenciamos que a carga inspiratória de 60% da PImáx reduziu abruptamente a glicemia. Também mostramos que o teste de 1 RM não altera a rigidez arterial em indivíduos idosos agudamente, sendo este seguro para os mesmos, que buscam realizar exercícios de força para tratar ou prevenir doenças cardiovasculares.

Palavras-Chaves: Resposta cardiovascular, Músculos inspiratórios, Força dinâmica máxima, Metaborreflexo, Rigidez arterial.

ABSTRACT

The objectives of this thesis were to evaluate the hemodynamic and cardiovascular responses to exercise in different modalities in populations at high risk for developing cardiovascular disease. **Methods**. We developed a review article and two intervention studies. The review article aimed to investigate the interaction of the various factors involved in exercise tolerance in patients with DM2 and also to analyze the influence of resistance exercise on arterial stiffness in elderly individuals. In the first study, we evaluated the inspiratory muscles and the reflex control of the circulation in individuals with DM2 with and without autonomic neuropathy. The subjects underwent a protocol to test the metaboreflex inspiratory muscle through inspiratory load of 60% of PImáx. Blood glucose was assessed during this protocol in a subset of patients. The second experiment examined the acute effects of 1 RM on arterial stiffness in elderly individuals. Data collected at 5, 30 and 60 minutes after the 1 RM test were compared with baseline. **Results**. We conclude that individuals with DM2 have an exacerbated inspiratory metaboreflex, regardless of the presence of autonomic neuropathy and evidenced that the inspiratory load of 60% of PImáx abruptly reduced the blood glucose. We also showed that 1 RM test does not alter arterial stiffness in elderly subjects, which is safe for them, who seek to accomplish strength exercises to treat or prevent cardiovascular diseases.

CAPÍTULO I

CAPÍTULO 1

1. INTRODUÇÃO

A fadiga muscular respiratória é conhecida por comprometer o desempenho no exercício físico em indivíduos saudáveis (1; 2). Evidências de que contrações fatigantes da musculatura inspiratória podem afetar o desempenho no exercício pelo acúmulo de metabólitos, como o ácido lático nos músculos respiratórios, (3) já são claras em pacientes com insuficiência cardíaca (IC) (4) e em indivíduos saudáveis (5). Esse aumento do trabalho muscular inspiratório provoca aumento da atividade simpática, com consequente vasoconstrição nos membros durante o exercício, acarretando fadiga muscular e redução da capacidade funcional (6; 7). No entanto, para o nosso conhecimento não há estudos avaliando essa condição em indivíduos com diabetes mellitus do tipo 2 (DM2) e neuropatia autonômica.

Além da vasoconstrição que ocorre nos membros durante o exercício, uma função vascular normal é essencial para adequada resposta durante o exercício. Evidências suportam a prática do exercício aeróbico para prevenir e tratar complicações cardiovasculares (8). Diversos estudos mostraram que o exercício aeróbico contribui de maneira benéfica para uma boa complacência arterial (9), função endotelial (10) e rigidez arterial (11) em pacientes com DM2 (12) e hipertensão (13). No entanto, o exercício resistido também é uma modalidade recomendada por diretriz para prevenir e tratar hipertensão, obesidade, osteoporose e sarcopenia (14). Sabe-se ainda que doenças cardiovasculares são relacionadas com o avanço da idade (15) e a indicação da prática dessa modalidade de exercício é fortemente recomendada com o objetivo de prevenir e tratar problemas crônicos como o DM2 (14). Uma vez que as diretrizes (14, 16) recomendam a realização do teste de força dinâmica máxima (1RM) antes de se iniciar um

programa de musculação, ao nosso conhecimento não há estudos avaliando os efeitos agudos de um teste de 1 RM sobre a rigidez arterial em indivíduos idosos.

Uma vez que a intolerância ao exercício também pode ser influenciada pelo baixo desempenho dos músculos inspiratórios, ela pode ocorrer em algumas condições clínicas em que a fraqueza da musculatura inspiratória está presente (17; 18; 19). Indivíduos com DM2 e neuropatia autonômica apresentam alta taxa de mortalidade e morbidade por doenças cardiovasculares, bem como diminuição da capacidade funcional e fraqueza da musculatura inspiratória (20; 21). Além disso, a fraqueza da musculatura inspiratória está associada com a disfunção autonômica nesta população (21). Justifica-se a busca de entendimento dos mecanismos pelos quais a musculatura inspiratória pode influenciar a tolerância ao exercício em indivíduos com DM2, o que será abordado nesta tese.

2. OBJETIVOS

Objetivo Geral

Avaliar as respostas cardiovasculares ao exercício de diferentes modalidades em populações de alto risco para o desenvolvimento de doença cardiovascular.

Objetivos Específicos

- Avaliar as respostas hemodinâmicas e cardiovasculares durante o exercício agudo da muscular inspiratória em pacientes com DM2 com e sem neuropatia autonômica;

- Investigar as respostas hemodinâmicas e cardiovasculares ao teste de exercício de força dinâmica máxima em idosos.

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CAPÍTULO II

CAPÍTULO 2

ARTIGO DE REVISÃO

Contribuição da musculatura inspiratória na limitação ao exercício físico em indivíduos com diabetes tipo 2

Ana Paula S. Corrêa[1,2], Beatriz D. Schaan[3,4]

[1]Laboratório de Fisiopatologia do exercício, [2]Cardiovascular, e [3]Divisão de Endocrinologia, Hospital de Clínicas de Porto Alegre, Porto Alegre, BRASIL; e [4]Departmento de Medicina, Faculdade de Medicina, Universidade Federal do Rio Grande do Sul, Porto Alegre, BRASIL;

RESUMO

A neuropatia autonômica é frequente complicação do diabetes mellitus do tipo 2 (DM2) e está associada à limitação ao exercício físico e fraqueza da musculatura inspiratória, que ocorre em 1/3 dos pacientes com DM2. **Objetivos:** O objetivo desta revisão foi descrever a complexa interação de diversos fatores envolvidos na intolerância ao exercício físico em pacientes com DM2. Essas alterações incluem anormalidades nas respostas autonômicas central, vascular e ventilatória durante o exercício. **Métodos:** Foi realizada busca nas bases de dados eletrônicos MEDLINE, EMBASE e PREMEDLINE incluindo registro de estudos mais antigos até 2013. **Resultados:** A fraqueza da musculatura inspiratória está associada com baixa capacidade funcional e com disfunção autonômica em indivíduos com DM2. Estes pacientes podem apresentar redução do fluxo sanguíneo da perna durante o exercício inspiratório. As alterações na função vascular que ocorrem no DM2 estão relacionadas com as doenças cardiovasculares e apresentam associação com o envelhecimento. **Conclusão:** Pacientes com DM2 podem apresentar função muscular inspiratória prejudicada; a presença da disfunção endotelial pode agravar a intolerância ao exercício nesta população.

Palavras Chaves: Fluxo sanguíneo, musculatura inspiratória, endotélio, função vascular, metaborreflexo.

REVIEW ARTICLE

The contribution of inspiratory muscle function to exercise limitation in type 2 diabetes mellitus

Ana Paula S. Corrêa[1,2], Beatriz D. Schaan[3,4]

[1]Exercise Pathophysiology Research Laboratory, [2]Cardiovascular, and [3]Endocrinology Divisions, Hospital de Clínicas de Porto Alegre, Porto Alegre, BRAZIL; and [4]Department of Medicine, Faculty of Medicine, Universidade Federal do Rio Grande do Sul, Porto Alegre, BRAZIL;

ABSTRACT

Background: Autonomic neuropathy is a common complication of diabetes mellitus type 2 (DM2) and is associated with impaired exercise capacity and inspiratory muscles weakness, which occurs in one third of patients with DM2. **Objectives:** The aim of this review was to describe the complex interaction of several factors limiting exercise tolerance in patients with DM2. These changes include abnormalities in the central autonomic responses, and vascular capacity during exercise. **Methods:** We performed searches in the electronic databases MEDLINE, EMBASE and PREMEDLINE including older studies until 2013. **Results:** Inspiratory muscle weakness is associated with poor functional capacity and autonomic dysfunction in individuals with DM2. These patients may have reduced blood flow to the leg during exercise inspiratory. Changes in vascular function that occur in DM2 are related with cardiovascular diseases, and are associated with aging. **Conclusion:** Patients with type 2 diabetes may have impaired inspiratory muscle function, the presence of endothelial dysfunction may worsen exercise intolerance in this population.

Key Words: Blood flow; Inspiratory muscles; Endothelium; Vascular function; Metaboreflex.

INTRODUÇÃO

Por muitos anos no ensino da fisiologia do exercício se afirmava que o sistema respiratório em indivíduos saudáveis era construído para não limitar o desempenho físico. Hoje se sabe que a disfunção dos músculos ventilatórios pode limitar o exercício nesses indivíduos (1; 2) e contribuir para o desenvolvimento da insuficiência respiratória em pacientes com diferentes desordens neuromusculares (3-5). As manifestações clínicas dessas desordens caracterizam-se por fadiga, dispneia e intolerância ao exercício.

As mudanças fisiológicas e fisiopatológicas na função dos músculos ventilatórios vêm sendo bastante estudadas. Nas últimas décadas, muitas linhas de pesquisa foram desenvolvidas com o intuito de investigar os mecanismos pelos quais o sistema respiratório influencia o desempenho ao exercício físico. Talvez a principal delas tenha sido a constituída pelo grupo do Professor Jerome Dempsey, que vem mostrando que o sistema respiratório pode limitar o desempenho ao exercício em indivíduos saudáveis, altamente treinados. Mais recentemente, o grupo de pesquisa liderado pelo Professor Jorge Pinto Ribeiro vem preenchendo a lacuna de que essa limitação ao exercício possa estar exacerbada em indivíduos com condições patológicas, tais como insuficiência cardíaca (6; 7), doença pulmonar obstrutiva crônica (DPOC) (8) e diabetes mellitus do tipo 2 (DM2) (9).

A intolerância ao exercício resulta de uma complexa interação de diversos fatores que incluem anormalidades na hemodinâmica central em respostas autonômicas, vasculares e ventilatórias durante o exercício. Diversos estudos envolvendo populações saudáveis (10), pacientes com doença arterial coronariana (11), com insuficiência cardíaca (IC) (12) e indivíduos com diabetes (13) têm mostrado forte associação entre diminuição da capacidade ao exercício com evento cardiovascular futuro.

Especial atenção vem sendo dada ao diabetes, principalmente por causa do aumento da prevalência e incidência de diabetes mellitus do tipo 2 (14). O DM2 é importante causa de mortalidade prematura e morbidade relacionada a doenças cardiovasculares, renais e do sistema nervoso (15). O exercício físico tem sido reconhecido como um marco para o manejo e tratamento desses pacientes (16). Embora a atividade física regular e o exercício estruturado possam impedir ou retardar o aparecimento do DM2 (17) e ser peça fundamental no controle glicêmico (18), a maioria das pessoas com DM2 não são ativas fisicamente (19). Talvez esse seja um dos maiores desafios nas práticas de saúde pública, pois a não aderência ao exercício físico por parte destes pacientes possa estar relacionada às anormalidades da função pulmonar (9; 20-22) capazes de limitar as respostas durante o exercício físico (4).

As alterações da função pulmonar que podem acometer indivíduos com diabetes incluem a redução dos volumes pulmonares (21; 23; 24) e da difusão do monóxido de carbono (25), bem como a redução da complacência e do recolhimento elástico do pulmão (20), e ainda da força muscular inspiratória (9; 22; 26; 27), em especial na presença da neuropatia autonômica cardiovascular (28; 29). O desempenho dos músculos inspiratórios tornou-se de interesse particular porque pode influenciar a tolerância ao exercício em algumas condições clínicas em que a fraqueza da musculatura inspiratória está presente (30-32).

A diminuição da resposta das capacidades pulmonares e da força muscular inspiratória que podem estar presentes nos pacientes com DM2 durante o exercício tem sido pouco estudada, assim como os mecanismos pelos quais há redução no desempenho dos músculos ventilatórios. A fraqueza da musculatura inspiratória em indivíduos com DM2 pode estar associada com neuropatia periférica (27; 33; 34) e autonômica (9; 22). Estudos sugerem que a diminuição no desempenho desses músculos

seja resultado de alterações nas fibras simpáticas e parassimpáticas, por diferentes graus de denervação autonômica (29), pela disfunção endotelial (35) e mitocondrial que ocorre nas células musculares demonstrados pela presença de mitocôndrias menores, redução da atividade das enzimas oxidativas mitocondriais e aumento do conteúdo intramolecular de triglicerídeos (36).

Outra possível explicação para a tolerância diminuída ao exercício em pacientes com DM2 poderia ser a ativação do metaborreflexo muscular inspiratório, que ocorre quando contrações fatigantes dos músculos inspiratórios durante o exercício causam acúmulo de produtos metabólicos locais. No entanto, para o nosso conhecimento, o papel da fadiga da musculatura inspiratória e do controle do fluxo sanguíneo durante o exercício, em pacientes com DM2 que apresentam neuropatia autonômica, ainda não foram estudados.

Resposta Autonômica ao Exercício

Redistribuição do fluxo sanguíneo

As alterações hemodinâmicas e musculares durante o exercício físico podem refletir nas respostas do sistema nervoso autonômico. Sabe-se que a participação autonômica está presente desde a condição de repouso, a qual se intensifica durante todas as fases do exercício físico e continua atuante na fase de recuperação. Por outro lado, a atividade física no sistema cardiovascular pode melhorar o controle autonômico, promovendo benefícios hemodinâmicos no repouso e durante o exercício físico (37-39).

Durante o exercício, a hemodinâmica cardiovascular é alterada pela interação símpato-vagal sobre o coração e vasos sanguíneos (40). No início do exercício físico, há uma diminuição da atividade vagal e aumento da atividade simpática, provocando um

aumento do ritmo cardíaco. O débito cardíaco aumenta linearmente com o aumento do consumo de oxigênio (VO_2). Com o aumento da intensidade do exercício há uma predominância da atividade simpática, que pode chegar ao quase completo bloqueio da atividade vagal (38). Os fatores metabólicos locais sobre os vasos sanguíneos também são responsáveis pelo aumento dos disparos simpáticos para o leito vascular sistêmico, causando vasoconstrição dos músculos esqueléticos inativos. A ativação dos receptores do tipo III e IV, que são constituídos por fibras mielínicas e amielínicas, respectivamente, modulam as atividades dos mecanorreceptores e do metaborreceptores. A deformação mecânica muscular esquelética ativa os mecanorreceptores da unidade músculotendinosa, e o acúmulo de metabólitos nos músculos ativos, por sua vez, ativam os metaborreceptores que estimularão o sistema nervoso para o aumento da atividade simpática, um efeito resultante do "metaborreflexo muscular" (41). Este aumento da atividade simpática, tanto central, quanto local, é essencial para a redistribuição do fluxo sanguíneo das áreas inativas, das regiões renal, esplênica e mesentérica para os músculos esqueléticos em atividade e também para o miocárdio, favorecendo a adaptação durante o exercício. No período de recuperação, há uma diminuição da atividade simpática e aumento da atividade vagal, causando uma diminuição da frequência cardíaca até próximo a níveis de repouso (40; 42).

No DM2, a relação entre a atividade autonômica e o controle do fluxo sanguíneo muscular é um pouco diferente. Muitos estudos em diabetes experimental mostraram uma disfunção do sistema nervoso autonômico, tanto em coelhos (43), como em ratos (44-48). Estes estudos mostraram que ratos com diabetes induzido por estreptozotocina, precocemente apresentam alteração do reflexo autonômico cardiovascular, alteração da frequência cardíaca e diminuição da variabilidade da pressão arterial, sugerindo que essas anormalidades possam resultar da redução da atividade vagal gerada

principalmente pela disfunção do sistema nervoso central. Essa disfunção parassimpática observada neste modelo experimental pode estar relacionada a alterações nos receptores cardíacos (44) e à inibição do barorreflexo arterial. Essa redução do barorreflexo arterial pode causar um aumento da resistência vascular periférica, diminuindo assim o fluxo sanguíneo vascular.

Por outro lado, em humanos, a neuropatia autonômica apresenta um progresso gradual com o aumento da duração do diabetes, o qual está relacionado com o mau controle glicêmico (49). Sendo um fator agravante, o tempo de exposição a essa complicação da doença, a taxa de mortalidade aproxima-se de 44% dentro de 2, 5 anos de diagnóstico da neuropatia autonômica (50). Em adição, as manifestações iniciais da neuropatia autonômica podem envolver ambas as alças eferentes, simpática e parassimpática (51).

A diminuição do tônus vascular com consequente aumento do fluxo sanguíneo muscular esquelético foi sugerida pelo aumento da liberação de oxido nítrico (NO) a partir do endotélio vascular ou a partir de uma diminuição da atividade nervosa simpática. Por outro lado, Martin e colaboradores. (52) avaliaram o fluxo sanguíneo da perna em pacientes com DM2 e não encontraram diferença entre os indivíduos com DM2 e os sujeitos controle. No entanto, alguns estudos mostraram uma redução da resposta do fluxo sanguíneo da perna durante o exercício em pacientes com DM2 (53) e neuropatia autonômica cardiovascular (51) como resultado da deficiência das respostas vagal/simpática que normalmente diminuem o débito cardíaco e o fluxo sanguíneo periférico direto para os músculos da perna.

Outros estudos mostraram que a hiperatividade simpática está presente em indivíduos com DM2 (54; 55) e a descarga neural simpática periférica está aumentada na presença de hipertensão arterial (56) e neuropatia autonômica (51), o que poderia

reduzir o fluxo sanguíneo muscular da perna e limitar o desempenho ao exercício físico. Sendo uma das maiores manifestações clínicas da neuropatia autonômica diabética a tolerância diminuída ao exercício (51), Kahn e colaboradores (57) estudaram indivíduos com e sem neuropatia autonômica cardiovascular e mostraram uma resposta reduzida da frequência cardíaca e da pressão arterial durante o exercício em indivíduos com neuropatia. Roy e colegas, (58) também mostraram uma redução do débito cardíaco em resposta aos exercícios em indivíduos com neuropatia diabética.

A gravidade da neuropatia autonômica cardiovascular também tem sido inversamente correlacionada com o aumento da frequência cardíaca, a qualquer momento durante o exercício e com o aumento máximo na frequência cardíaca (58). Observou-se também uma redução da fração de ejeção, disfunção sistólica e diastólica nesses indivíduos com neuropatia autonômica, o que contribuiria ainda mais para a redução da tolerância ao exercício (59). Dado o potencial para a tolerância ao exercício diminuída em pacientes com diabetes e neuropatia autonômica cardiovascular, pesquisas adicionais são necessárias para avaliar a relação entre a atividade do sistema nervoso autonômico e o controle do fluxo sanguíneo muscular durante o exercício. Entender os mecanismos fisiológicos pelos quais a regulação do tônus vascular está alterada no DM2 poderá explicar, em parte, a baixa capacidade funcional em pacientes com DM2, em especial na presença da neuropatia autonômica cardiovascular.

Resposta Ventilatória ao Exercício

Metaborreflexo muscular inspiratório

Durante o exercício, a ventilação pulmonar precisa ser aumentada para manter adequada a oxigenação e a remoção de gás carbônico dos tecidos (60). Entender o papel

dos músculos ventilatórios durante o exercício físico e as consequências das suas disfunções foi os fatores determinantes para o estudo da tolerância diminuída ao exercício em pacientes com DM2. Dados do nosso grupo, Corrêa e colaboradores (9) mostraram que a fraqueza da musculatura inspiratória é frequente em pacientes com DM2, ocorrendo em 29% dos indivíduos rastreados. Este estudo também mostrou que um programa de treinamento muscular inspiratório (TMI) por 8 semanas foi capaz de reverter a perda de força muscular inspiratória desses pacientes. Entretanto, o treinamento que mostrou aumento da força muscular inspiratória não foi acompanhado por mudanças na capacidade funcional ou da modulação autonômica.

Em indivíduos saudáveis, o TMI melhora a força e resistência muscular inspiratórias, resultando em aumento na proporção de fibras musculares do tipo I, aumento da capacidade oxidativa e da densidade capilar das células musculares, assim como no número e tamanho das mitocôndrias (61). No entanto, apesar destas adaptações, o TMI não melhorou a capacidade funcional nesses indivíduos saudáveis (62). Em uma meta-análise foi mostrado que o TMI melhora a resistência muscular inspiratória em indivíduos saudáveis com maiores melhorias em indivíduos menos aptos e em esportes de longa duração (63). À semelhança do que aconteceu com os nossos pacientes com DM2, uma revisão sistemática mostrou que em pacientes com doença pulmonar obstrutiva crônica, o TMI foi capaz de aumentar a força e a resistência dos músculos inspiratórios, mas não alterou a capacidade funcional (64).

Em contrapartida, temos mostrado consistentemente que o programa TMI utilizado no nosso estudo (9) melhora a fraqueza da musculatura inspiratória, bem como a capacidade funcional em pacientes com insuficiência cardíaca crônica (7; 65). Em recente revisão sistemática o TMI não mostrou benefício adicional na qualidade de vida em pacientes com insuficiência cardíaca sem fraqueza da musculatura inspiratória (66).

No entanto, as razões para as diferentes respostas do TMI sobre a capacidade funcional entre as condições médicas não são aparentes, mas pode ser devido à fadiga muscular inspiratória e ao controle circulatório.

Dempsey e colegas (41; 67-72) na sua série contínua de investigações elegantes sobre os músculos respiratórios, sistema nervoso autônomo e regulação cardiovascular em humanos descreveram a existência de um "metaborreflexo muscular inspiratório". Em seus estudos mostraram que contrações fatigantes da musculatura inspiratória elevam os níveis de noradrenalina, reduzindo o fluxo sanguíneo da perna durante exercício máximo (67) e diminuição do consumo de oxigênio para o membro ativo (67; 68). Este aumento do trabalho muscular ventilatório resulta em acúmulo de produtos metabólicos que ativam as fibras frênicas do tipo IV, resultando em pronunciado aumento na atividade vasoconstritora simpática.

Em trabalhos subsequentes deste mesmo grupo mostrou que o aumento do trabalho muscular inspiratório causa uma redistribuição do fluxo sanguíneo dos músculos periféricos ativos para o diafragma, correspondendo de 14-16% do débito cardíaco (67; 68). Além disso, contrações fatigantes da musculatura inspiratória com esforço inspiratório intenso de 60% da Pimáx associado com o sustentado tempo inspiratório e duração total do ciclo respiratório [TI / Ttot] de 0,70 aumentam a atividade nervosa simpática muscular (72) e reduzem o fluxo sanguíneo da perna inativa (70; 71). Esses achados evidenciam a existência de um metaborreflexo inspiratório (71) que é especialmente importante durante exercícios de alta intensidade sustentada em que ocorre modulação da concorrência para o fluxo de sangue entre os músculos respiratórios e locomotores (Figura 1).

Posteriormente, o mesmo grupo demonstrou que a redução do trabalho muscular inspiratório, via assistência ventilatória, aumenta o desempenho dos músculos

respiratórios em aproximadamente 14% (69) e atenua a fadiga do quadríceps durante o exercício em ciclistas (1), provavelmente por inibir o metaborreflexo inspiratório. Segundo esses achados, o metaborreflexo inspiratório pode limitar o desempenho físico (73) quando o exercício ultrapassa 85% do consumo máximo de oxigênio (VO_2máx), pois induz a fadiga muscular diafragmática (74), até mesmo em atletas de elite (75). Recentemente, Callegaro e colaboradores (76) mostraram que atletas que realizaram treinamento aeróbico apresentam um atenuado metaborreflexo inspiratório, sugerindo que este tipo de exercício pode atenuar o metaborreflexo muscular inspiratório.

O efeito do metaborreflexo inspiratório sobre o desempenho físico pode ser relevante para indivíduos portadores de IC, de DPOC e de DM2. Nos pacientes com IC, observou-se que o metaborreflexo muscular inspiratório está exacerbado e que o TMI foi capaz de melhorar o fluxo sanguíneo no exercício pela atenuação do metaborreflexo muscular inspiratório (7). A redução do trabalho muscular inspiratório aumenta a tolerância ao exercício e melhora a oxigenação muscular periférica (77). Em indivíduos com DPOC a ventilação não-invasiva melhora a saturação periférica de O_2 e reduz a fatigabilidade do músculo quadríceps durante exercício isocinético (78). Atualmente, ao nosso conhecimento, não há nenhum estudo que avalie a atividade metaborreflexa inspiratória em pacientes com DM2 que apresentam neuropatia autonômica.

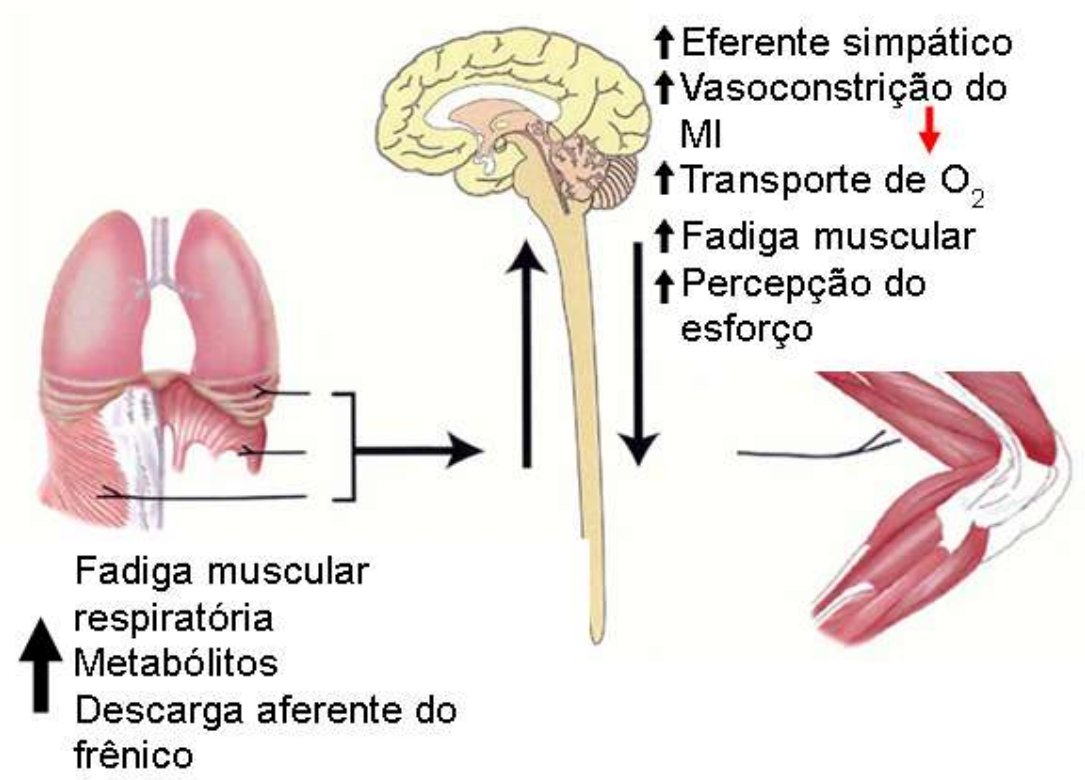

Figura 01: Ilustração do metaborreflexo inspiratório. Adaptado de Dempsey J.A. et al. Respir Physiol & Neurobiol. 2006;151:242-50.

Resposta Vascular ao Exercício

Endotélio vascular e complacência arterial

Os danos aos nervos do sistema nervoso autônomo no indivíduo com DM2 podem causar distúrbios da função motora, sensorial e reflexa nos sistemas digestivo, urogenital, sudoral e de modo especial no sistema cardiovascular. A neuropatia diabética no sistema cardiovascular pode provocar dano nas fibras nervosas autonômicas, que inervam o coração e os vasos sanguíneos, podendo resultar em anormalidades no controle da frequência cardíaca e da dinâmica vascular (51).

O controle do tônus vascular é determinado principalmente, pelo balanço entre os mecanismos vasoconstritores e vasodilatadores mediados pelo endotélio vascular, tanto no repouso quanto no exercício físico (79). Sabe-se que diversas substâncias são produzidas pelo endotélio vascular em resposta aos diferentes estímulos. Dentre estas substâncias destacam-se a bradicinina, prostaciclina, fator hiperpolarizante derivado do endotélio e NO (79), que possuem propriedades vasodilatadoras. Além do NO ser um importante vasodilatador endógeno, ele é considerado um forte mediador do aumento do fluxo sanguíneo (80). Por outro lado, o endotélio vascular também libera substâncias vasoconstritoras como a endotelina (81) e a angiotensina II (79; 82). Além disso, o endotélio libera fatores que promovem o crescimento celular, dentre os quais se destacam o fator de crescimento derivado de plaquetas (PDGF), fator de crescimento de fibroblastos (FGF) e o fator de crescimento derivado do endotélio (EDGF) (83). Estudos que avaliaram a dinâmica vascular durante o exercício em indivíduos com DM2 mostraram um aumento plasmático da concentração de endotelina-1 circulante (84) e redução das concentrações das substâncias vasodilatadoras mediada pelo endotélio (53) como o NO [85; 86]. Estes resultados podem resultar em baixo fluxo de sangue muscular em repouso (87) e durante o exercício muscular (53; 88; 89) em pacientes com diabetes.

O exercício físico promove importantes alterações cardiovasculares e tem impacto direto sobre a função vascular (90). Dentre essas alterações destacam-se o aumento do fluxo sanguíneo para a musculatura em atividade, redução da resistência vascular periférica proporcional ao aumento do débito cardíaco e, consequentemente, elevação da pressão arterial sistólica. Fatores regulatórios que geram redução da resistência vascular periférica e, consequentemente, da pressão arterial são primariamente dependentes do endotélio (91). A habilidade das células endoteliais de reconhecer e responder às mudanças no fluxo sanguíneo se faz essencial na regulação

do tono vascular, propiciando o remodelamento da parede arterial e manutenção da integridade do endotélio [92]. O aumento do fluxo sanguíneo pulsátil e a pressão que o sangue exerce sobre a parede vascular produzem a chamada força de cisalhamento (*shear stress*) que atua sobre a camada íntima dos vasos onde residem as células endoteliais. A força de cisalhamento é um poderoso estímulo para a geração do agente vasodilatador NO no sistema vascular (93). Associado a esse fenômeno, o exercício físico é um importante estímulo para o aumento do fluxo sanguíneo e, consequentemente, promove aumento na produção de NO. Este desencadeia efeitos benéficos, como relaxamento vascular e inibição da agregação plaquetária, prevenindo doenças como a hipertensão arterial e a aterosclerose (92). O avanço da doença vascular no diabetes mellitus está relacionado a esses mecanismos descritos e à influência de alterações hormonais como a resistência à insulina e a hiperglicemia (94). Além disso, fatores genéticos e ambientais podem influenciar na gênese das complicações micro e macrovasculares no diabetes.

A importância do endotélio na manutenção da função vascular normal tem sido cada vez mais reconhecida. O efeito do exercício físico na função vascular tem sido amplamente estudado, principalmente no que diz respeito à complacência arterial e à função endotelial. Outro importante parâmetro a ser estudado é a rigidez arterial, que é caracterizada pelo espessamento das paredes dos vasos sanguíneos com subsequente diminuição da distensibilidade arterial. Sendo a rigidez arterial considerada um dos principais determinantes da pressão arterial sistólica e da pressão de pulso, que representa o componente pulsátil da pressão arterial, ela sofre influência direta do exercício físico (95-97). Em adição, a diminuição da distensibilidade arterial é reconhecida como um potencial marcador de doença cardiovascular subclínica (98) e é independentemente associada à morbidade e mortalidade cardiovascular (99-101). As

alterações na função arterial podem ocorrer em várias condições de doença, em particular em pacientes com hipertensão arterial, DM2 e aterosclerose (102-104). Além disso, a rigidez arterial aumenta com o avanço da idade (105).

Estudos têm mostrado benefícios de exercícios aeróbicos regulares sobre a rigidez arterial, principalmente na melhora da capacidade funcional e na (96) atenuação do enrijecimento arterial em adultos saudáveis (95-97). Do mesmo modo, o exercício físico regular em indivíduos mais velhos mostrou ser capaz de reduzir a rigidez arterial e diminuir a degeneração da parede vascular, a qual está relacionada com o envelhecimento (106; 107). Importante lembrar que a redução da rigidez arterial após exercício aeróbico regular é independente da redução nos fatores de risco cardiovasculares tradicionais (96; 108).

O treinamento de força é outra modalidade de exercício recomendada pelas diretrizes do Colégio Americano de Medicina do Esporte (109; 110), que ajuda a prevenir e tratar a osteoporose, sarcopenia, obesidade e muitos outros problemas crônicos como o diabetes mellitus. No DM há melhora do controle glicêmico tanto quanto em exercícios aeróbicos (18). No entanto, há poucos estudos avaliando os efeitos do treinamento de força sobre a função arterial, e os que existem são contraditórios. Alguns estudos mostraram que o treinamento de força de alta intensidade e alto volume aumentou a rigidez arterial em homens de meia-idade (111; 112), assim como programas de exercício de força agudo mostraram resultados de aumento da rigidez arterial em indivíduos jovens (113). No entanto, outros estudos mostraram que o treinamento de força composto de alta intensidade, sem aumentos simultâneos no volume, não determinou aumento da rigidez arterial (114). Recente meta-análise (115) concluiu que o treinamento de força foi associado com o aumento da rigidez arterial em

jovens com valores basais baixos de rigidez arterial, enquanto que intensidade moderada de treinamento de força em população de meia-idade essa associação não ocorreu.

Assim, os mecanismos pelos quais o exercício de força afeta a rigidez arterial não são completamente compreendidos. Estudos adicionais são necessários para explicar os efeitos agudos de um teste dinâmico de força máxima (1RM) sobre as mudanças hemodinâmicas centrais e sobre a rigidez arterial em indivíduos de meia idade, já que o teste de 1RM é recomendado antes do ingresso a qualquer tipo de intervenção de exercício de força, principalmente em indivíduos de meia idade que buscam esta modalidade de exercício como forma de prevenção e/ou controle do DM2. Além disso, para o nosso conhecimento não há estudos relacionando a disfunção endotelial com a atividade dos músculos inspiratórios nesses indivíduos.

Capacidade Funcional no Diabetes Mellitus tipo 2

Evidências demonstram que a inatividade física está envolvida na progressão do metabolismo normal da glicose para diabetes tipo 2 (116, 117). Além disso, vários estudos demonstraram a relação entre o nível de atividade física e a predisposição ao diabetes tipo 2 (118, 119). Diversos programa de atividade física para pessoas com diabetes tipo 2 sem complicações significativas ou restrições mostraram que a atividade física contribui para a prevenção de doenças cardiovasculares e previne inicial descontrole glicêmico (120, 121, 122).

Tais programas devem incluir apropriados exercícios aeróbicos e de força muscular para desenvolvimento e manutenção da aptidão cardiorrespiratória, composição corporal, força e resistência muscular. No entanto, se preconiza que todo e qualquer tipo de treinamento deve-se atender tempo, intensidade e freqüência adequada

para cada modalidade (tabela 1) (123). O treinamento de resistência deve ser realizado pelo menos 2 dias por semana, com um mínimo de 8 a 10 exercícios envolvendo grandes grupos musculares em 10-15 repetições para alcançar a fadiga. Para a maioria das pessoas com DM tipo 2, intensidade baixa a moderada correspondente a 40-70% do Vo2máx é recomendada para melhorar condicionamento cardiorespiratório e obter favoráveis efeitos metabólicos como redução da glicose sanguínea e aumento da sensibilidade à insulina (124), porém, implementando-se a atividade com intensidade de baixa a moderada para pessoas com DM tipo 2 minimiza-se os riscos e maximiza os benefícios aumentando a aderência e diminuindo injúria músculo-esquelético e possíveis traumas nos pés, particularmente quando há excesso de peso. Evidências de que baixos níveis de treinamento de resistência (40-50% de máxima repetição) em combinação com exercício aeróbio moderado melhoram HbA1c similarmente ao treinamento de alta resistência (125), além de também reduzir vários fatores de risco cardiovasculares. Quanto ao exercício aeróbio à quantidade e intensidade recomendada para melhora do controle glicêmico com manutenção do peso e redução do risco de doença vascular coronária é de pelo menos 150min/sem de exercício aeróbio de intensidade moderada (40-60% do Vo2máx ou 50-70% da frequência cardíaca máxima) e/ou pelo menos 90 min/sem de exercício aeróbio intenso (> 60% do Vo2máx ou > 70% da frequência cardíaca máxima). Atividade aeróbia com > 4h/sem de intensidade moderada a intensa e/ou exercício de resistência associado conduzem a uma grande redução do risco de doença vascular coronariana (18).

Tabela 1: Classificação da intensidade do exercício de acordo com a duração da Atividade com duração acima de 60 min.

	Intensidade relativa		
Intensidade	**Vo2máx (%)**	**Frequência cardíaca máxima (%)**	**Percepção subjetiva do esforço**
Muito leve	< 20	< 35	< 10
Leve	20-39	35-54	10-11
Moderada	40-59	55-69	12-13
Intensa	60-84	70-89	14-16
Muito intensa	> 85	> 90	17-19
Máxima	100	100	20

Fonte: ZINMAN et al., 2003.
Frequência cardíaca máxima = 220 – idade; Percepção subjetiva do esforço escala de 6-20.

Implicações Clínicas

Os diversos fatores envolvidos na tolerância diminuída ao exercício em indivíduos com DM2 são decorrentes das anormalidades das respostas autonômicas, vasculares e ventilatórias. Conhecer e entender esses mecanismos são a chave para um bom manejo e intervenção nessa doença durante o exercício físico. A indicação de intervenções não farmacológicas como os exercícios orientados e estruturados é quase que rotina para profissionais médicos aos indivíduos com DM2. Com o intuito de buscar não só um melhor desempenho da musculatura esquelética mas também da musculatura ventilatória estudos estão sendo desenvolvidos para avaliar os benefícios dos exercícios musculares sobre a glicemia nos indivíduos com DM2. No entanto, mais estudos nesta área são esperados para investigar também as alterações hemodinâmicas durante o exercício em especial sobre a musculatura inspiratória, pois como foi mostrada ela pode influenciar na baixa capacidade funcional nesta população.

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CAPÍTULO III

CAPÍTULO 3

ORIGINAL ARTICLE

Exacerbated inspiratory muscle metaboreflex and endothelial dysfunction: potential determinants of exercise intolerance in type 2 diabetes regardless of the presence of autonomic neuropathy

Running Title: Inspiratory metaboreflex in type 2 diabetes

Article type: Original Investigation

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ANA PAULA S. CORRÊA, MSc[1,2] · JORGE P. RIBEIRO, ScD[1,2,3*] · CRISTIANO F. ANTUNES, Ac[1,2]· FRANCIELE R. FIGUEIRA, MSc[1,4] · MARINA A. CASTRO, Ac[1], and BEATRIZ D. SCHAAN, ScD[3,4]

[1]Exercise Pathophysiology Research Laboratory, Hospital de Clínicas de Porto Alegre, Porto Alegre, Rio Grande do Sul, Brasil; [2]Postgraduate Program in Cardiology, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brasil; [3]Department Internal Medicine, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brasil; [4]Postgraduate Program in Endocrinology, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brasil.

*Dr. Jorge Pinto Ribeiro passed away on 23 August 2012. Although he had been fully involved in the conception and design of the study and in data analysis and interpretation, the final version of this manuscript is responsibility of the other authors.

Address for correspondence:

Beatriz D'Agord Schaan, MD, ScD
Serviço de Endocrinologia- Hospital de Clínicas de Porto Alegre
Rua Ramiro Barcelos 2350, prédio 12, 4º andar,
Porto Alegre, RS 90035-003
Brazil
Phone/Fax: 5133598127 beatrizschaan@gmail.com

Abstract

OBJECTIVE. To evaluate the effects of inspiratory loading on blood flow of resting and exercising limbs in patients with diabetic autonomic neuropathy.

RESEARCH DESIGN AND METHODS. Ten patients without autonomic neuropathy (DM), 10 patients with diabetic autonomic neuropathy (DM-CAN) and 10 healthy controls (C) were selected. An inspiratory muscle load to 60% of maximal inspiratory pressure (PImax) was applied while blood flow to the resting calf (CBF) and exercising forearm (FBF) were measured. Flow-mediated dilatation (FMD) was also evaluated. Six diabetic patients also performed two sessions of 2% (placebo) or 60% (inspiratory muscle metaboreflex) of PImax for assessment of glucose levels (continuous glucose monitoring system).

RESULTS. Mean age was 58 ± 8 years, and mean HbA1c, 7.8% (62 mmol/mol) (DM and DM-CAN). A PImax of 60% caused reduction of CBF in DM-CAN and DM ($P<0.001$), but not in C ($P<0.001$), whereas calf vascular resistance (CVR) increased in DM-CAN and DM ($P<0.001$), but not in C ($P<0.001$). The increase in FBF during forearm exercise was blunted during 60% of PImax in DM-CAN and DM, and augmented in C ($P<0.001$). Glucose levels decreased by 40 ± 18.8% ($P<0.001$) at 60%, but not at 2%, of PImax. A negative correlation was observed between FMD and changes in CVR (Beta coefficient = -0.44, $P = 0.034$).

CONCLUSIONS. Inspiratory muscle loading caused an exacerbation of the inspiratory muscle metaboreflex in patients with diabetes, regardless of the presence of neuropathy, but influenced by endothelial dysfunction. High-intensity exercise that recruits the diaphragm can abruptly reduce glucose levels.

KEYWORDS: Breathing exercise, Type 2 diabetes mellitus, Diabetic neuropathies, Respiratory muscles, Glucose.

Abbreviations:

C = Healthy controls
CBF = Calf blood flow
CGMS = Continuous subcutaneous glucose monitoring system
CVR = Calf vascular resistance
CO2 = Carbon dioxide
DM = Type 2 diabetes without autonomic neuropathy
DM-CAN = Type 2 diabetes with autonomic neuropathy
$ETCO_2$ = End-tidal carbon dioxide
***f*b** = Breathing frequency
FBF = Forearm blood flow
FEV1 = Forced expiratory volume in 1 s
FMD = Flow-mediated dilatation
FVC = Forced vital capacity
FVR = Forearm vascular resistance
GEE = Generalized estimating equations
HR = Heart rate
MAP = Mean arterial pressure
MVV = Maximal voluntary ventilation
PImax = Maximal inspiratory mouth pressure
Pthmax = Inspiratory muscle endurance
Pthmax/PImax = Percentage of PImax
R = Respiratory exchange ratio
SPO_2 = Arterial oxygen saturation by pulse oximetry
T_I/T_{Tot} = Duty cycle
$\dot{V}O_{2peak}$ = Peak oxygen uptake

INTRODUCTION

Cardiovascular autonomic neuropathy causes increased morbidity and mortality to diabetic patients (1). Exercise intolerance is one of its major clinical manifestations, which translates into low levels of physical activity and poor cardiorespiratory fitness; this, in turn, is an independent predictor of all-cause mortality (2). The limited exercise response experienced by patients with diabetic autonomic neuropathy may result from reduced responses in heart rate, blood pressure, and cardiac output (3), as well as decreased strength and endurance of inspiratory muscles (4; 5) and impaired endothelium-dependent vasodilatory responses (6).

Respiratory muscle weakness in diabetic patients may be associated with peripheral (7) and autonomic neuropathy (4; 5). Another possible explanation may be the activation of the inspiratory muscle metaboreflex, which occurs when fatiguing contractions of the inspiratory muscles during exercise cause accumulation of metabolic products. These products stimulate skeletal muscle neural afferents, resulting in increased sympathetic vasoconstrictor activity (8), leaving preferential blood flow for the skeletal muscles to the detriment of the inspiratory muscles (9). Although physiologic, the inspiratory muscle metaboreflex may affect patients' symptoms and the ventilatory response to exercise (10). Blunting of the inspiratory muscle metaboreflex with improvement in limb blood flow and endurance capacity can be obtained by endurance training in healthy individuals (11) and in patients with heart failure (12), who can also attain this benefit with inspiratory muscle training (13). Accordingly, improved strength of the inspiratory muscles can also be obtained by inspiratory muscle training in diabetic patients (5)

As muscle metaboreflex activity and brachial artery flow-mediated dilatation are both impaired in hypertensive and diabetic patients (15), endothelial dysfunction could partially explain the impaired leg blood flow regulation response to exercise, further limiting exercise

capacity in diabetic subjects (16). It is possible that activation of the inspiratory muscle metaboreflex would cause excessive reduction in blood flow control during exercise in the presence of diabetic autonomic neuropathy, as these patients would experience greater impairment of endothelium-dependent vasodilation (15), but this has yet to be investigated. The present study was conducted to evaluate the effects of inspiratory loading on blood flow of resting and exercising limbs in patients with diabetic autonomic neuropathy, comparing their responses to those elicited by the same protocol in controls and diabetic patients without autonomic neuropathy.

RESEARCH DESIGN AND METHODS

Patients and design. Ten patients with type 2 diabetes without autonomic neuropathy (DM) were matched to 10 patients with type 2 diabetes with moderate and severe cardiovascular autonomic neuropathy (DM-CAN). They were recruited from the Outpatient Endocrinology Clinic of the Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil. Exclusion criteria were body mass index (BMI) $\geq$ 30 kg.m^2, exercise-induced asthma, infectious, osteoarticular, cardiac or pulmonary diseases, as well as regular alcohol or tobacco consumption in the preceding 6 months. A group of 10 healthy controls (C) were selected to match the DM and DM-CAN groups in age, gender, and BMI. The protocol was approved by the Hospital de Clínicas de Porto Alegre Research Ethics Committee, and all subjects provided written informed consent for participation.

Protocol. Subjects came to the laboratory on three separate days for completion of the study protocols (Supplemental Figure 1). On the first day, pulmonary function, inspiratory muscle function and cardiovascular autonomic function were evaluated. On the second day, individuals underwent endothelial function assessment and cardiopulmonary exercise testing. On the third day, at least two days after the above-described evaluations, subjects underwent

a protocol designed to elicit the inspiratory muscle metaboreflex, so as to evaluate its impact on resting hemodynamic parameters, maximal inspiratory muscle pressure, and calf blood flow (CBF). One subgroup of patients with diabetes underwent this evaluation while wearing a continuous subcutaneous glucose monitoring system (CGMS), so as to determine whether glucose changes occurred concomitantly with cardiovascular changes. All experiments were performed in a temperature-controlled room and all subjects were in the fasting state (at least for 3 hours), had refrained from consuming caffeinated beverages and alcohol for at least 12 hours, and had not exercised for at least 48 hours prior to the evaluation.

Pulmonary function. Measurements of forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1), and maximal voluntary ventilation (MVV) were obtained with a computerized spirometer (Eric Jaeger, GmbH, Würzburg, Germany), as recommended by the American Thoracic Society (17), and results were expressed as percentage of predicted value (18).

Inspiratory muscle function. Inspiratory muscle function testing was performed using a pressure transducer (MVD-500 V.1.1 Microhard System; Globalmed, Porto Alegre, Brazil), connected to a system with two unidirectional valves (DHD Inspiratory Muscle Trainer, Chicago, IL) as previously described (13). In short, the maximal inspiratory mouth pressure (PImax) was determined during deep inspiration from residual volume against an occluded airway with a small air leak. The test was repeated several times to obtain 6 measurements with less than 10% variation (19). Predicted values were corrected for age and gender (20). Inspiratory muscle endurance (Pthmax) was determined by an incremental test and expressed as a percentage of PImax (Pthmax/PImax).

Cardiovascular autonomic function testing. The presence of cardiovascular autonomic neuropathy was verified using the five noninvasive cardiovascular reflex tests proposed by Ewing, as previously described (21) and standardized in our institution (22). Three tests were

used to evaluate the heart rate response to *1*) deep breathing, *2*) lying-to-standing heart rate ratio, and *3*) the Valsalva maneuver. Two tests of blood pressure control were also calculated during *1*) orthostatic hypotension and *2*) sustained handgrip. All tests were performed three times, and the mean value was used for analysis. Two or more abnormal tests were deemed diagnostic of cardiovascular autonomic neuropathy. In short, heart rate (HR) variation was assessed while patients were asked to breathe deeply at a rate of 6 breaths per minute while being monitored on 3-lead electrocardiography. The maximum and minimum HR during each breathing cycle were measured, and the mean difference of 6 cycles was calculated. The lying-to-standing heart rate ratio test was determined after 10 min of rest in the supine position, and HR variability was determined by calculating the maximal to minimal HR ratio: the longest R-R interval, measured around the 30^{th} beat after standing up, to the shortest R-R interval, measured around the 15^{th} beat after standing up. The Valsalva test consisted of forced exhalation into a mouthpiece with a pressure of 40 mmHg for 15 s, and the ratio of the maximum R-R after the maneuver to the minimum R-R during the maneuver was calculated. Orthostatic hypotension was defined as a decrease in systolic blood pressure of 30 mmHg when changing from the supine to the standing position. Measurements were obtained every minute for at least 3 min. Sustained muscle contraction as measured by handgrip dynamometer causes a rise in systolic and diastolic blood pressure and heart rate. The dynamometer was first squeezed to isometric maximum; then, patients were asked to hold at 30% maximum for 5 min. A rise in diastolic blood pressure of < 10mmHg is considered an abnormal response.

Endothelial function. Reactive hyperemia was measured using cuffs placed at the upper and lower arm. In the lower arm, a rapid cuff inflator was used to occlude venous outflow (50–60 mmHg), and three blood flow recordings were obtained each minute for 3 min. Thereafter, the upper-arm cuff, which induces hyperemia, was occluded at 250 mmHg for 5 min, with

pressure released in 10 s intervals over 1 min. Flow-mediated dilatation (FMD) was calculated using the percentage of dilatation after reactive hyperemia in relation to baseline (23).

Cardiopulmonary exercise testing. The maximal incremental exercise test was performed on an electrically braked cycle ergometer (ER-900, Ergoline, Jaeger, Würzburg, Germany) with minute increments of 10 W for DM-CAN, 15 W for DM, and 20 W for C. During testing, gas exchange variables were measured by a previously validated system (Oxycon Delta, VIASYS, Healthcare GmbH, Jaeger, Germany). The HR was determined from a 12-lead electrocardiogram and blood pressure was measured every 2 min with a standard arm cuff.

Resting calf experiment during respiratory exercise at 2% and 60% of PImax. Induction of the inspiratory muscle metaboreflex was performed according to apreviously described protocol (12). In short, subjects breathed continuously through a two-way valve (Hans Rudolph, 2600 series, Shawnee, KS, USA) with low resistance (2% of PImax) connected to an inspiratory resistance obtained by a threshold inspiratory muscle trainer (DHD inspiratory muscle trainer, Chicago, IL) or to a POWERbreathe® inspiratory muscle trainer (Southam, UK) for higher inspiratory pressures (60% of PImax). Blood flow responses in the resting calf were assessed as subjects breathed under high (60% of PImax) and low (2% of PImax) inspiratory pressures. The sequence of performing the experiments, induction of the inspiratory muscle metaboreflex (60 % of PImax) or allocation to the placebo experiment (2 % of PImax) was randomly assigned, and the experiments were separated by a 40 min interval. Baseline data were collected during 5 minutes of spontaneous breathing in both protocols. After this period, controlled breathing was initiated, and individuals maintained a breathing frequency (*f*b) of 15 breaths per min and a duty cycle (T_I/T_{Tot}) of 0.7 by listening to an audio signal with distinct inspiratory and expiratory tones in both protocols. Inspiratory

pressure was continuously recorded and displayed on a computer screen and a 10-point Borg scale (24) was used to access inspiratory effort at task failure. Inability to maintain breathing was defined as a reduction of PImax to less than 80% of the prescription during 3 consecutive breaths. For the placebo experiments (2% of PImax), measures were interrupted at 5 min.

Forearm exercise experiment after respiratory exercise at 2% and 60% of PImax. Immediately after induction of the inspiratory muscle metaboreflex and the placebo experiment, subjects began the forearm exercise, which consisted of repetitive maximal voluntary contractions on a hand dynamometer (Kratos, DLC, Cotia, Brazil) that was measured at baseline, maintained for 10 s and released for 30 s until task failure.

Ventilatory and hemodynamic measures. During induction of the inspiratory muscle metaboreflex and the placebo experiment, ventilatory and hemodynamic variables were measured as previously described (12). In short, *f*b, arterial oxygen saturation by pulse oximetry (Sp_{O_2}), and end-tidal carbon dioxide ($ETCO_2$) were measured by oxycapnography (Takaoka Oxicap, São Paulo, Brazil). Mean arterial blood pressure (MAP) was measured on the non-dominant arm with an automated sphygmomanometer at each minute of the test (Dinamap 1846 SX/P, Critikon, Tampa, USA). The CBF was measured by venous occlusion plethysmography (Hokanson, TL-400, Bellevue, USA) and calf vascular resistance (CVR) was calculated as MAP/CBF (25). End-tidal carbon dioxide was maintained at eupneic levels during both fatiguing and control protocols via addition of CO_2 into the inspiratory circuit.

Metabolic evaluation by the continuous subcutaneous glucose monitoring system (CGMS). One subgroup of 6 patients with diabetes was randomly assigned to two sessions of inspiratory muscle training either with 2% of PImax (placebo condition) or 60% of PImax (inspiratory muscle metaboreflex), on two different days. Subjects were admitted to the laboratory in the morning, when the CGMS device was placed (Medtronic Mini-Med,

Northridge, CA). The sensor was inserted through a needle into the subcutaneous tissue of the abdominal wall using a spring-loaded device (Senserted Medtronic, Northridge, USA) and kept for 24h. Glucose measurements were obtained every 10 s and averaged every 5 min, for a total of 288 readings per day. Sensor readings were calibrated with a glucose monitor (Accu-Check Performa, Roche Diagnostics, Mannheim, Germany) using 4 finger stick blood samples obtained over the 24-h period. Patients were previously instructed about the operation of the monitor, which included event registration for meals, inserting capillary glucose values as calibration, general care and completing a detailed food recall during the monitoring period. Subjects were asked to closely match their daily nutritional intake.

Statistical Analysis. A sample size of 10 individuals per group was estimated to detect a 10% difference in CBF response, with for a statistical power of 0.8 and an alpha of 0.05. Values are reported as mean ± SD. All analyses were performed using the Statistical Package for the Social Sciences 18.0 for Windows software (SPSS Inc., Chicago, IL). Generalized estimating equations (GEE) were used to compare differences in patients' characteristics and baseline values among groups. The GEE model for repeated measures was used to compare respiratory and hemodynamic measurements during the inspiratory muscle metaboreflex protocol, followed by Bonferroni's *post-hoc* test for multiple comparisons. Pearson's correlation coefficient and multiple linear regression analyses were performed to determine the relationship between VO2 peak, delta of CVR and baseline HbA1c and PImax variables after the protocols. Categorical variables were compared by means of Fisher's exact test. Findings were considered significant at $p < 0.05$.

RESULTS

Baseline characteristics. The baseline characteristics, resting hemodynamics, pulmonary/ inspiratory muscle function and cardiopulmonary exercise testing results of the subjects are

shown in Table 1. Groups were similar in age, weight, and BMI. As expected, plasma glucose and glycated hemoglobin (HbA1c) levels were higher in the DM and DM-CAN groups as compared to C. Resting HR was higher in DM-CAN as compared to the other groups. Lower MVV, PImax, endurance time, R_{peak} and $\dot{V}O_{2peak}$ were observed in DM and DM-CAN as compared to C. Lower Pthmax/PImax, and HR_{peak} were observed in DM-CAN as compared to C. The FEV_1 was lower in DM-CAN as compared with the C and DM groups. The FVC was lower in DM and DM-CAN as compared to C, and even lower in the DM-CAN as compared with the DM group.

Resting calf blood flow is reduced during inspiratory loading in diabetes. These data are shown in Supplemental Table S1 and Figure 1. Patients from the DM-CAN group did not maintain breathing during inspiratory effort (128 ± 29 s) as long as the others, quitting earlier than both DM (207 ± 30 s) and C (326 ± 44 s) ($P < 0.01$). All subjects were able to maintain a breathing frequency of ~15 breaths/min and a prolonged T_I/T_{Tot} of ~0.7 for fatiguing and control protocols. The HR was lower at the end point of the 60% protocol in DM-CAN when compared with C and DM. The $ETCO_2$ remained at eupneic levels during both protocols. Figure 1 shows the reduction in CBF and increment in CVR and MAP in the DM and DM-CAN groups as compared with C as the inspiratory muscle metaboreflex was induced.

Forearm blood flow increment is blunted after inspiratory loading in diabetes. These data are shown in Supplemental Table S2 and Figure 2. Time to fatigue during forearm exercise was reduced in the 60% protocol in the DM (209 ± 31 s) and DM-CAN (159 ± 34 s) groups as compared with C (326 ± 50 s), $P< 0.001$. The intermittent static handgrip exercise with inspiratory loading at 60% of PImax resulted in similar SpO_2 and HR responses among the groups studied. Figure 2 shows the blunted increment in FBF during forearm exercise for the induction period of the inspiratory muscle metaboreflex in DM and DM-CAN as

compared with C. Conversely, forearm vascular resistance (FVR) and MAP during exercise were higher in DM and DM-CAN than in C.

Decreased endothelial function in diabetes. Figure 3 depicts flow-mediated dilatation in the study groups. It was decreased in both DM (6.3 ± 2%) and DM-CAN (4.9 ± 3%) subjects as compared with controls (12.5 ± 2%); P<0.001.

Metabolic evaluation (CGMS) showing lowering of glucose levels during the metaboreflex induction in diabetes. Supplemental Figure S2 shows glucose levels obtained during the metaboreflex induction in a random subgroup of six diabetic patients, two without cardiovascular autonomic neuropathy and four with cardiovascular autonomic neuropathy. At the induction of the inspiratory muscle metaboreflex (60% PImax), glucose levels decreased significantly and remained low until the end of the test (15 min, P<0.001). This was not seen during the placebo experiment, when a minimal PImax (2% PImax) was applied. Glucose levels remained low during the recovery period (30 min, P<0.001).

Calf vascular resistance was negatively associated with basal Pimax (r = -0.492; P=0.006), FMD (r = - 0.746, P<0.001) and VO2peak (r = -0.680; P<0.001) on pooled analysis of all groups. On multiple linear regression, only FMD remained significantly correlated with changes in delta CVR (Beta coefficient = -0.44, P < 0.034).

CONCLUSIONS

This study shows that voluntary efforts against an inspiratory resistive load cause exaggerated vasoconstriction of the resting calf in patients with type 2 diabetes, suggesting an exacerbation of the inspiratory muscle metaboreflex. These fatiguing levels of inspiratory muscle force translated into reduced circulatory responses to forearm exercise. These derangements were determined by the diabetic state *per se*, not by autonomic neuropathy, as no differences were observed between patients with or without this complication. In addition,

changes in the peripheral vasoconstrictor response to inspiratory metaboreflex activation were inversely related to flow-mediated vasodilation. This study also shows that glucose levels decreased significantly during the inspiratory muscle metaboreflex activity, and remained low for at least 30 minutes after cessation of exertion.

Several studies have shown that the inspiratory metaboreflex generated by fatiguing inspiratory muscle work elicits a sympathetically mediated vasoconstrictor response in the resting limbs of healthy subjects (8; 26). The prevailing level of inspiratory muscle work is inversely related to the time to the limit of tolerance during very heavy cycling exercise (8; 10) and to the magnitude of exercise-induced limb muscle fatigue (10; 27). It has also been suggested that inspiratory muscle work can alter exercise tolerance, a phenomenon probably mediated by its influence on limb perfusion. In healthy subjects, inspiratory metaboreflex activation may be particularly important during sustained heavy-intensity exercise, as it modulates the competition for blood flow between the respiratory and locomotor muscles (9). In agreement with these findings, inspiratory muscle fatigue increased forearm vascular resistance immediately at the onset of exercise and the time to fatigue during handgrip exercise was reduced in diabetic patients after inspiratory loading when compared with non diabetic individuals, . These findings suggest that inspiratory muscle metaboreflex activation modified the forearm hyperemic response to handgrip exercise, possibly by boosting sympathetic vasoconstrictor activity (28). Interestingly, these findings in diabetic patients are similar to those reported previously in chronic heart failure, where excessive inspiratory muscle work contributes to augmented respiratory muscle metaboreflex stimulation, derangements that are associated with exercise limitation (12).

It is known that autonomic neuropathy can impair hemodynamic responses to cardiovascular stress, and also cause decreased heart rate variability (1) and damage to the autonomic nerve fibers that innervate the heart and blood vessels. These abnormalities in

heart rate control and vascular dynamics may be involved in changes in leg blood flow caused by limb vasoconstriction, leading to an impaired inspiratory muscle metaboreflex (29). However, although a clear rationale implicates autonomic neuropathy as a possible determinant of exacerbated metaboreflex in diabetic patients, this was not demonstrated in the present study. Although our patients with autonomic dysfunction exhibited greater derangements in pulmonary function as compared to those without neuropathy, exercise tolerance was low in both diabetes groups, regardless of the presence of neuropathy. These results suggest diabetic-specific causes of exercise intolerance rather than neuropathy. These could be a combination of pulmonary derangements (4; 5) and impaired endothelium-dependent vasodilatory responses (6).

Leg blood flow is reduced in type 2 diabetic subjects during exercise, a response that is correlated with endothelial function (16). This impaired leg blood flow response to exercise may be involved in the limited exercise capacity in diabetes, as this was shown to be associated with longer duration of diabetes and the microvascular (30) and macrovascular complications of the disease (16). Another potential mechanism involved in the leg blood flow response to exercise would be metabolic control, as acute hyperglycemia attenuates endothelium-dependent vasodilation in humans (31). The significant correlation between calf vascular resistance changes and flow-mediated dilatation in diabetes may represent a mechanical disadvantage in the control of blood flow. These data suggest that exacerbation of the inspiratory muscle metaboreflex is a manifestation of diabetes itself, and that impairment in endothelial function may at least partly account for this phenomenon, contributing to exercise limitation in patients with diabetes.

The abrupt decrease in glucose levels observed during inspiratory loading (~40% reduction at 60% of PImax) was of similar magnitude to the acute reduction in glucose levels observed after acute aerobic exercise in a similar population (32). It is well known that

conventional exercise can acutely reduce glucose levels in diabetic subjects (32; 33), as it results in acute increases in insulin sensitivity and high muscle glucose uptake (33; 34). However, very intense limb exercise can actually prevent glucose levels from declining if it is intense enough to elicit a rise in counter-regulatory hormones, sometimes even resulting in elevated glucose levels (35). This is consistent with subsequent findings of increased GLUT4 protein content in the sheep diaphragm induced by chronic inspiratory resistive flow (36).

Some limitations of our study bear stressing. We did not study specific protocols for inspiratory muscle loading and sustained handgrip separately; thus, we could not compare the impact of each of these interventions on the rapid, abrupt decline in glucose levels observed in patients with type 2 diabetes. However, as far as clinical implications are concerned, our results provide evidence that inspiratory loading at 60% of PImax markedly decreases glucose levels and warrants formal testing in controlled experiments comparing its effects to those of other types of exercise in limb muscles. We did not evaluate muscle sympathetic nerve activity in our patients, but other investigators have demonstrated that exercise-induced diaphragmatic fatigue caused important peripheral vasoconstriction secondary to sympathetic activation (26).

In conclusion, inspiratory muscle loading caused an exacerbation of the inspiratory muscle metaboreflex in subjects with diabetes. This phenomenon occurred regardless of the presence of neuropathy, but was influenced by endothelial dysfunction. High-intensity exercise that recruits the diaphragm can reduce glucose levels abruptly, and this is an avenue that should be explored for its potential relevance to future clinical practice. Our findings support the hypothesis that specific exercises targeting the inspiratory muscles could be used as an alternative tool for glucose control in patients with type 2 diabetes.

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Duality of interest. The authors declare that there is no duality of interest associated with this manuscript.

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Author contribution paragraph. APSC, FRF, CFA, and MAC contributed to data acquisition and revised the manuscript. APSC and BDS contributed to the conception and design of the study and to data analysis and interpretation, and revised the manuscript. APSC and JPR contributed to the conception and design of the study. APSC, FRF, CFA, MAC and BDS approved the final version.

Figure Legends

Figure 1: Calf blood flow (CBF), calf vascular resistance (CVR), and mean arterial pressure (MAP) at baseline, minute 1, minute 2 and at the end of the placebo experiment (2% PImax, left panels) and during induction of the inspiratory muscle metaboreflex (60% PImax., right panels). Groups: Controls (C, closed squares), patients with type 2 diabetes without autonomic neuropathy (DM, open circles), and patients with type 2 diabetes and cardiovascular autonomic neuropathy (DM-CAN, closed circles). Data expressed as mean ± SD. Analysis: Generalized estimating equations (GEE) for repeated measures, followed by Bonferroni's post hoc test. Panel A: group ($p<0.001$), time ($p=0.72$) and interaction ($p<0.001$); Panel B: group ($p=0.03$), time ($p=0.34$) and interaction ($p=0.001$); Panel C: group ($p=0.84$), time ($p=0.22$) and interaction ($p=0.15$); Panel D: group ($p<0.001$), time ($p=0.002$) and interaction ($p<0.001$); Panel E: group ($p<0.001$), time ($p<0.001$) and interaction ($p<0.001$); Panel F: group ($p<0.001$), time ($p<0.001$) and interaction ($p<0.001$); $^{\dagger}p<0.05$ *vs.* C; $^{€}p<0.05$ *vs.* DM.

Figure 2: Forearm blood flow (FBF), forearm vascular resistance (FVR), and mean arterial pressure (MAP) immediately after the end of inspiratory loading (onset of forearm exercise), at minute 1, minute 2 and at the end of forearm exercise. Groups: Controls (C, closed squares), patients with type 2 diabetes without autonomic neuropathy (DM, open circles), and patients with type 2 diabetes and cardiovascular autonomic neuropathy (DM-CAN, closed circles). Data expressed as mean ± SD. Analysis: Generalized estimating equations (GEE) for repeated measures, followed by Bonferroni's post hoc test. Panel A: group ($p<0.05$), time ($p<0.05$), interaction ($p<0.05$); Panel B: group ($p=0.29$), time ($p=0.49$), interaction ($p=0.34$); Panel C: group ($p=0.34$), time ($p=0.74$), interaction ($p=0.16$); Panel D: group ($p<0.001$), time ($p<0.001$), interaction ($p<0.001$); Panel E: group ($p<0.001$), time ($p<0.001$), interaction ($p<0.001$); Panel F: group ($p<0.001$), time ($p<0.001$), interaction ($p<0.001$); $^{\dagger}p<0.05$ *vs.* C.

Figure 3: Percentage of flow-mediated dilatation (FMD) after reactive hyperemia in relation to baseline. Groups: Controls (C, white bar), patients with type 2 diabetes without autonomic neuropathy (DM, grey bar), and patients with type 2 diabetes and cardiovascular autonomic neuropathy (DM-CAN, black bar). Data expressed as mean ± SD. Analysis: Generalized estimating equations (GEE) for repeated measures, followed by Bonferroni's post hoc test. * $p<0.05$ *vs.* C.

Supplements Figure Legends

Supplemental Figure S1: Timeline showing the steps of the protocol.

Supplemental Figure S2: Glucose levels as measured by the continuous subcutaneous glucose monitoring system (CGMS) at rest, breath control (grey bar), load (dark bar),

handgrip exercise (black bar) and recovery period during placebo experiment (2% PImax) and during induction of the inspiratory muscle metaboreflex (60% PImax) in a random subgroup of 6 diabetic patients, both without cardiovascular autonomic neuropathy and with cardiovascular autonomic neuropathy. Data expressed as mean ± SD. Analysis: Generalized estimating equations (GEE) for repeated measures, followed by Bonferroni's post hoc test. Group (Pimax load 2% *vs.* Pimax load 60%, $p<0.05$), time ($p<0.001$) and interaction ($p<0.05$). §$p<0.05$ *vs.* baseline; † $p<0.05$ *vs.* PImax load 2%.

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Table 1. Clinical characteristics, resting hemodynamics, pulmonary/inspiratory muscle function and cardiopulmonary exercise testing of the three study groups

	C (n=10)	DM (n=10)	DM-CAN (n=10)
General			
Male: female ratio	6/4	4/6	4/6
Age, y	56 ± 7	59 ± 8	59 ± 9
Body mass index, $kg.m^{-2}$	25 ± 3	26 ± 7	27 ± 1
Diabetes duration, y	-	8 (6 – 12)	11 (6 – 15)
HbA1c %	5 (5-6)	7 (7-9)*	8 (8-9)*†
Fasting plasma glucose,$mg.dL^{-1}$	89 ± 6	144 ± 21*	141 ± 26*
Total cholesterol, $mg.dL^{-1}$	165 ± 26	190 ± 23	186 ± 30
HDL cholesterol, $mg.dL^{-1}$	42 ± 9	45 ± 8	45 ± 8
GFR, $ml/min/1.73m^2$	92.8 ± 20.8	79.2 ± 20.3	72.8 ± 14.5
Resting hemodynamics			
MAP, mmHg	93 ± 8	91 ± 7	95 ± 7
HR, beats/min	68 ± 6	74 ± 11	80 ± 7*
CBF, ml/min.100 ml	3.6 ± 1	3.5 ± 0	2.7 ± 0
CVR, units	27 ± 7	28 ± 7	33 ± 4
Handgrip force, N	39 ± 13	32 ± 5	34 ± 12
Medications, n (%)			
Metformin	-	8 (80)	9 (90)
Sulfonylureas	-	2 (20)	1 (10)
ACE inhibitor	-	1 (10)	3 (30)
Diuretics	-	4 (40)	2 (20)
Statins	-	1 (10)	1 (10)
Beta blockers	-	1 (10)	1 (10)
Pulmonary function			
FEV1, % predicted	92 ± 2	88 ± 4	81 ± 5*†
FVC, % predicted	99 ± 1	99 ± 2	94 ± 4*†
MVV, % predicted	116 ± 13	80 ± 1*	79 ± 1*
Inspiratory muscle function			

PI_{max}, cmH_2O	111 ± 13	76 ± 17*	80 ± 16*
PI_{max}, % predicted	109 ± 10	86 ± 10*	84 ± 9*
Pthmax/PImax, %	73 ± 14	70 ± 20	50 ± 17*
Endurance time, s	538 ± 129	302 ± 58*	238 ± 84*
Cardiopulmonary exercise test			
HR_{peak}, beats/min	168 ± 13	159 ± 12	145 ± 17*
$\dot{V}O_{2peak}$, $mL.min^{-1}.kg^{-1}$	37 ± 6	25 ± 3*	20 ± 1*
R_{peak}	1.2 ± 0	1.1 ± 0*	1.1 ± 0*

Values expressed as mean ± SD or n (%). C, control, DM, diabetes mellitus; DM-CAN, diabetes mellitus and cardiovascular autonomic neuropathy; GFR, glomerular filtration rate; MAP, mean arterial pressure; CBF, calf blood flow; CVR, calf vascular resistance. HR, heart rate; ACE, angiotensin-converting enzyme; HbA1c, glycated hemoglobin; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; MVV, maximal voluntary ventilation; PImax, maximal inspiratory pressure; Pthmax, inspiratory endurance determined during incremental test; $\dot{V}O_2$peak, peak oxygen uptake; R, respiratory exchange ratio. Analysis: Generalized estimating equations (GEE) for repeated measures. * P<0.05 *vs.* C; † P<0.05 *vs.* DM.

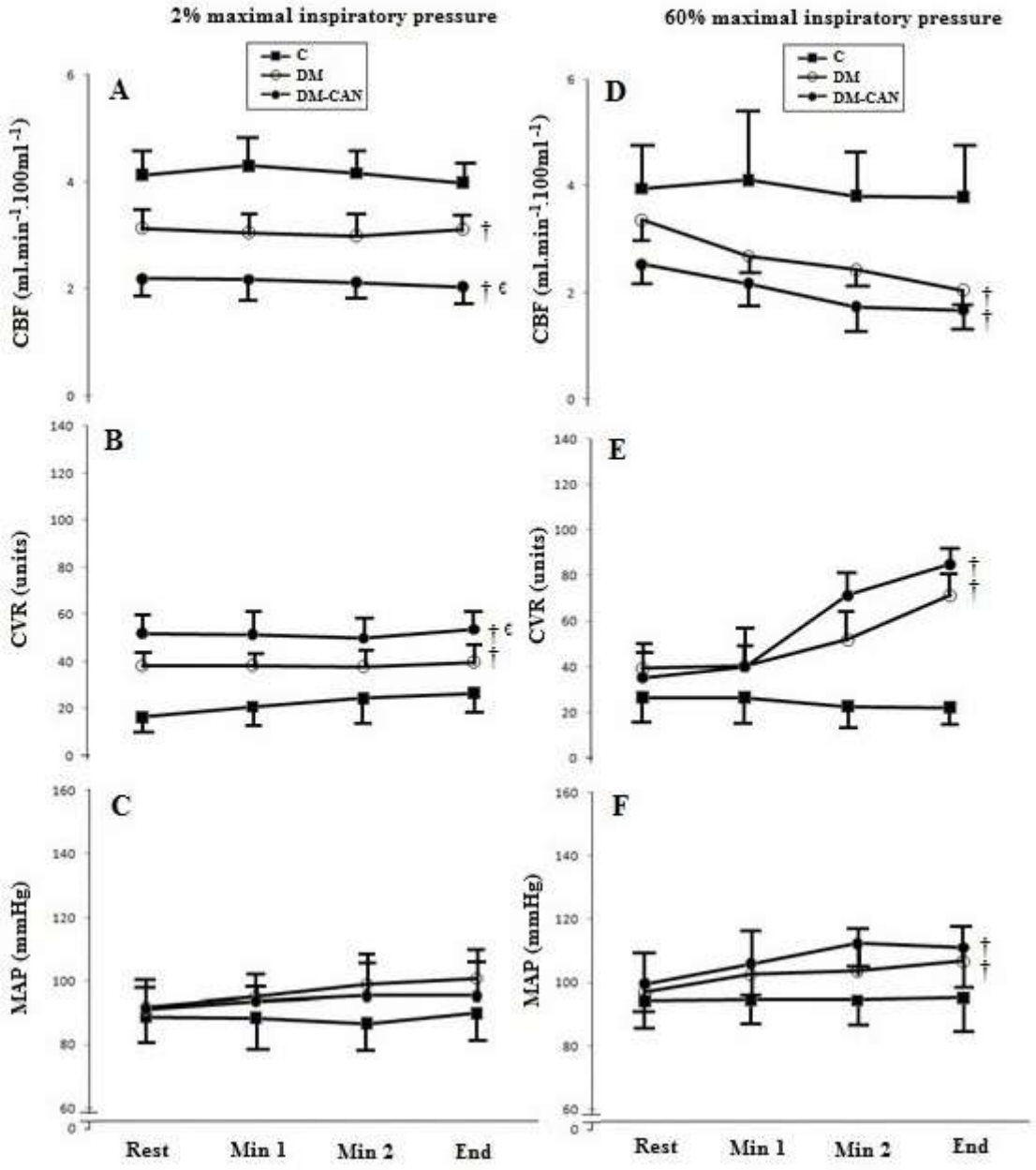

2% maximal inspiratory pressure
60% maximal inspiratory pressure
C
DM
DM-CAN
A
B
C
D
E
F
CBF (ml.min-1.100ml-1)
CVR (units)
MAP (mmHg)
Rest
Min 1
Min 2
End

Figure 1:

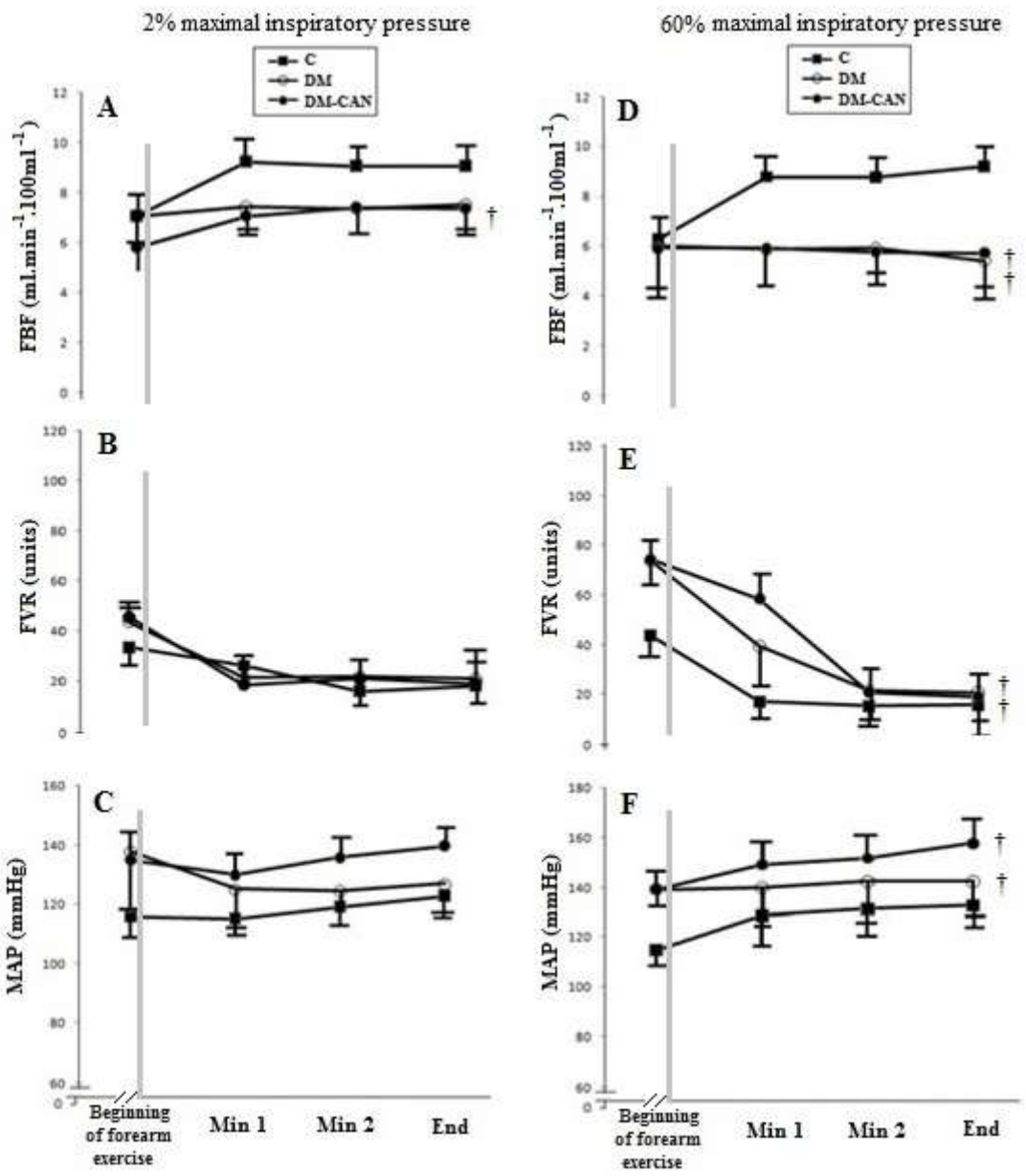

2% maximal inspiratory pressure
60% maximal inspiratory pressure
C
DM
DM-CAN
A
B
C
D
E
F
FBF (ml.min-1.100ml-1)
FVR (units)
MAP (mmHg)
†
Beginning of forearm exercise
Min 1
Min 2
End

Figure 2:

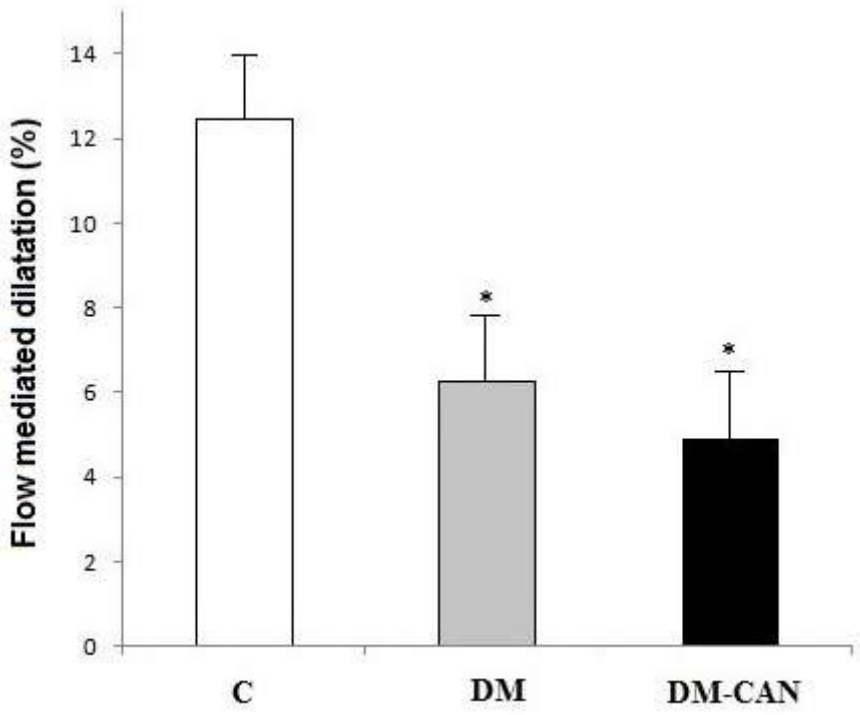


Figure 3:

Exacerbated inspiratory muscle metaboreflex and endothelial dysfunction: potential determinants of exercise intolerance in type 2 diabetes regardless of the presence of autonomic neuropathy

Supplemental Table S1. Resting calf experiment during respiratory exercise at 2% and 60% of PImax in the subjects studied

Supplemental Table S2. Forearm exercise experiment after respiratory exercise at 2% and 60% of PImax in the subjects studied

Supplemental Figure S1. Experimental design. CGMS: Continuous subcutaneous glucose monitoring system; CB: controlled breathing; CBF: calf blood flow; CVR: calf vascular resistance; FBF: forearm blood flow; FVR: forearm vascular resistance.

Supplemental Figure S2. Glucose levels as measured by the continuous subcutaneous glucose monitoring system (CGMS) at rest, breath control (grey bar), load (dark bar), handgrip exercise (black bar) and recovery period during placebo experiment (2% PImax) and during induction of the inspiratory muscle metaboreflex (60% PImax) in a random subgroup of 6 diabetic patients, both without cardiovascular autonomic neuropathy and with cardiovascular autonomic neuropathy. Data expressed as mean ± SD. Analysis: Generalized estimating equations (GEE) for repeated measures, followed by Bonferroni's post hoc test. Group (Pimax load 2% *vs*. Pimax load 60%, $p<0.05$), time ($p<0.001$) and interaction ($p<0.05$). §$p<0.05$ *vs*. baseline; † $p<0.05$ *vs*. PImax load 2%.

Supplemental Table S1. Resting calf experiment during respiratory exercise at 2% and 60% of PImax in the subjects studied

		Resting Calf			
		Baseline	**1 min**	**2 min**	**End**
2% PImax; TI/TTot, 0.7					
HR (beats.min^{-1})	C	83 ± 3	82 ± 8	83 ± 10	82 ± 9
	DM	72 ± 11	74 ± 10	75 ± 12	75 ± 12
	DM-CAN	75 ± 15	74 ± 15	75 ± 16	75 ± 12
f_b (breaths/min^{-1})	C	15 ± 0	16 ± 1	16 ± 1	15 ± 1
	DM	16 ± 1	15 ± 0	15 ± 1	15 ± 0
	DM-CAN	15 ± 0	16 ± 1	16 ± 1	15 ± 1
$ETCO_2$ (mmHg)	C	29 ± 3	31 ± 1	30 ± 0	30 ± 1
	DM	24 ± 3	23 ± 4	23 ± 4	23 ± 3
	DM- CAN	24 ± 3	22 ± 2	23 ± 2	22 ± 3
SpO_2 (%)	C	98 ± 1	97 ± 1	98 ± 1	98 ± 0
	DM	98 ± 0	97 ± 1	97 ± 1	97 ± 1
	DM-CAN	98 ± 0	98 ± 0	98 ± 0	97 ± 1
60% PImax; TI/TTot, 0.7					
HR (beats.min^{-1})	C	71 ± 9	74 ± 8	80 ± 9	81 ± 4
	DM	80 ± 13	86 ± 8	94 ± 4	81 ± 13
	DM-CAN	82 ± 2 ¥	78 ± 7	98 ± 1	76 ± 8*†€¥
f_b (breaths/min^{-1})	C	15 ± 0	15 ± 0	15 ± 1	15 ± 0
	DM	15 ± 1	16 ± 1	16 ± 1	15 ± 1
	DM-CAN	16 ± 0	18 ± 1	17 ± 0	16 ± 1

$ETCO_2$ (mmHg)	C	32 ± 2	33 ± 0	31 ± 0	33 ± 2
	DM	23 ± 4	22 ± 3	24 ± 3	22 ± 3
	DM-CAN	24 ± 2 ¥	26 ± 1	24 ± 0	25 ± 0
SpO_2 (%)	C	98 ± 0	98 ± 0	98 ± 0	97 ± 1
	DM	98 ± 0	98 ± 0	98 ± 1	97 ± 1
	DM-CAN	98 ± 0	98 ± 1	98 ± 1	97 ± 1

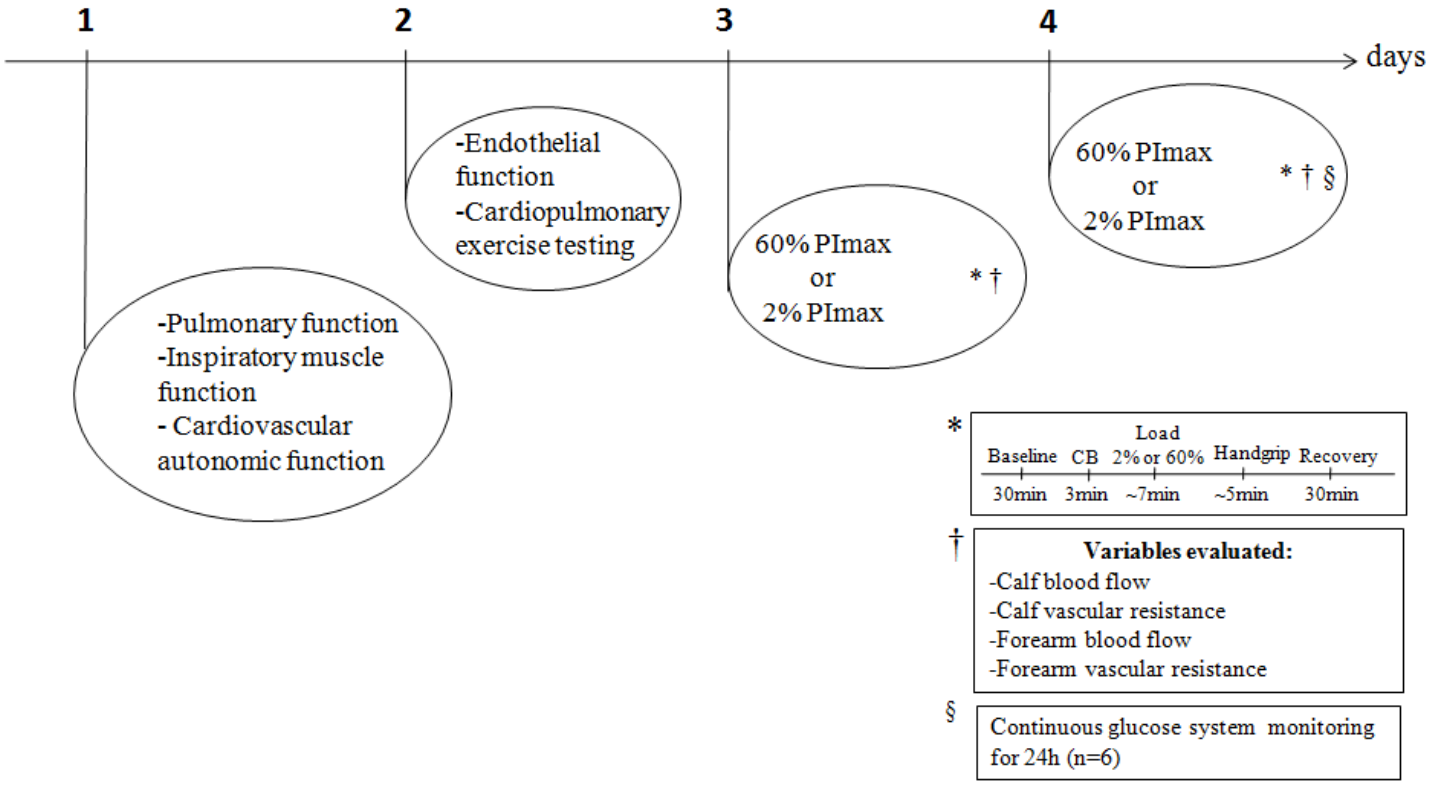
Values expressed as means ± SD. C, control, DM, diabetes mellitus; DM-CAN, diabetes mellitus and cardiovascular autonomic neuropathy; HR, heart rate; *f*b, breathing frequency; SpO_2, oxygen saturation; $ETCO_2$, end-tidal carbon dioxide; TI/TTot, duty cycle. Analysis: Generalized estimating equations (GEE) for repeated measures. * P<0.05 *vs.* C; † P<0.05 *vs.* DM; € P<0.05 different from baseline; ¥ different from protocol at 2% PImax.

Supplemental Table S2. Forearm exercise experiment after respiratory exercise at 2% and 60% of PImax in the subjects studied

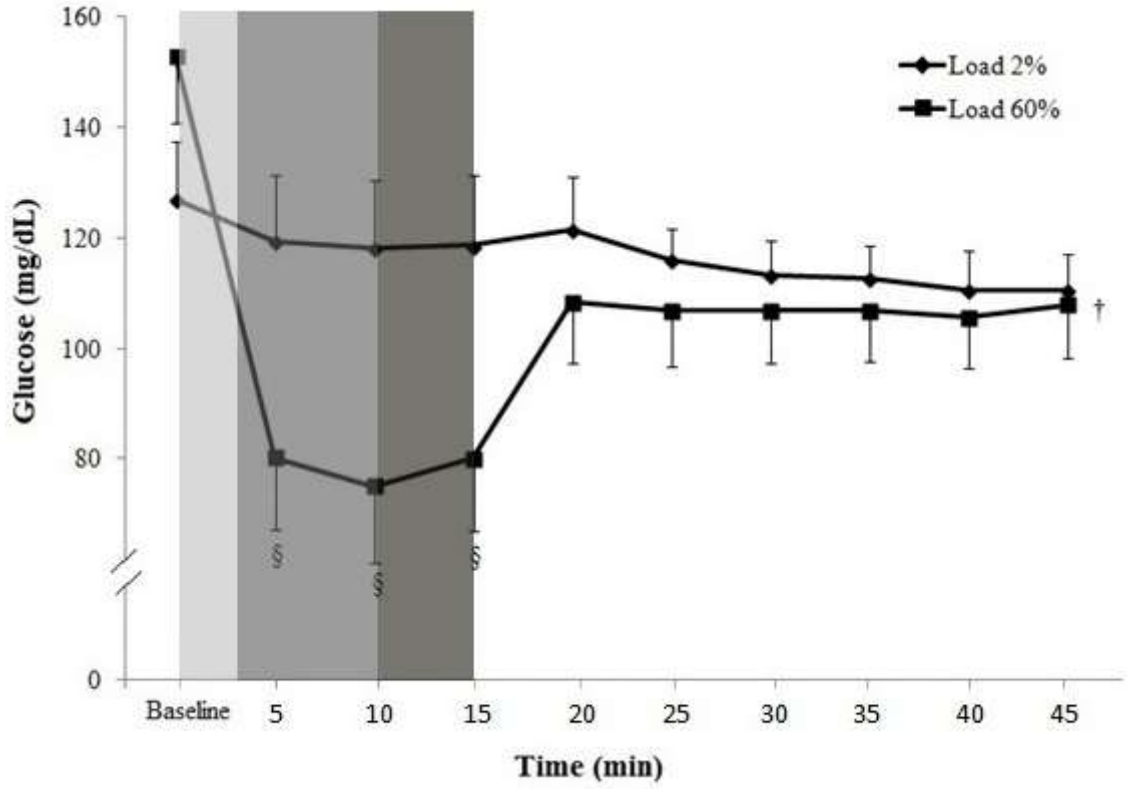
			Forearm exercise		
		End-Inspiratory Load	**1 min**	**2 min**	**End**
2% PImax; TI/TTot, 0.7; f_b, 15					
HR (beats.min^{-1})	C	71 ± 8	71 ± 8	73 ± 9	75 ± 7
	DM	72 ± 8	71 ± 10	68 ± 12	77 ± 12
	DM-CAN	77 ± 7	77 ± 8	75 ± 8	79 ± 8
SpO_2 (%)	C	98 ± 0	98 ± 0	98 ± 0	98 ± 0
	DM	98 ± 0	97 ± 1	97 ± 1	97 ± 2
	DM-CAN	98 ± 0	96 ± 2	95 ± 4	97 ± 1
60% PImax; TI/TTot, 0.7; f_b, 15					
HR (beats.min^{-1})	C	76 ± 14	76 ± 13	80 ± 17	81 ± 12
	DM	73 ± 10	70 ± 7	72 ± 15	76 ± 15
	DM-CAN	83 ± 3	77 ± 7	78 ± 6	76 ± 8
SpO_2 (%)	C	97 ± 1	97 ± 1	98 ± 0	98 ± 1
	DM	97 ± 1	97 ± 1	97 ± 1	96 ± 4
	DM-CAN	96 ± 3	95 ± 4	93 ± 8	95 ± 3

Values are means ± S.D. C, control, DM, diabetes mellitus; DM-CAN, diabetes mellitus and cardiovascular autonomic neuropathy; HR, heart rate; SpO_2, oxygen saturation; $ETCO_2$, end-tidal carbon dioxide; TI/TTot, duty cycle. Analysis: Generalized estimating equations (GEE) for repeated measures. There were no differences among groups.

Supplemental Figure S1.



Supplemental Figure S1:



Supplemental Figure S2:

CAPÍTULO IV

CAPÍTULO 4

ORIGINAL RESEARCH

Maximal dynamic strength testing increases heart rate variability with no alteration on arterial stiffness in older adults

Ana Paula S. Corrêa[1,2], Beatriz D. Schaan[3,4], Michael Baker[5,6], Jacinda Meiklejohn[5,7], Guy Wilson[5], Nidhi Jain[5], and Maria Fiatarone Singh[5,7,8]

[1]Exercise Pathophysiology Research Laboratory, [2]Cardiovascular and [3]Endocrine Divisions, Hospital de Clínicas de Porto Alegre, Porto Alegre, BRAZIL [4]Department of Medicine, Faculty of Medicine, Universidade Federal do Rio Grande do Sul, Porto Alegre, BRAZIL; [5]School of Exercise and Sport Sciences, Faculty of Health Sciences, University of Sydney, Lidcombe, NSW, Australia; [6]Australian Catholic University, NSW, Australia; [7]Balmain Hospital, Sydney, NSW, Australia; [8]Hebrew SeniorLife, Boston;

Running Title: Strength testing does not alter arterial stiffness

Article type: Original Investigation

Word count (entire text): 2.570
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Number of Figure: 3

Address for correspondence:

Maria Antoinette Fiatarone Singh, MD, FRACP
University of Sydney,
K221, Cumberland Campus
75 East Street, Lidcombe, 2141
NSW, Australia
Phone/Fax: (02) 9351-9204 m.singh@usyd.edu.au

Abbreviations:

1-RM = maximal dynamic strength testing
baPWV =brachial-ankle pulse wave velocity
AIx = aortic augmentation index
HRV = hear rate variability
LF = low frequency
HF = high frequency
CVD = cardiovascular disease
BP = blood pressure
PWA = pulse wave analysis
AIx@75 aortic AIx normalized to a heart rate of 75 beats/ min
PAI = peripheral augmentation index
BMI = body mass index

ABSTRACT

Background: Arterial stiffness is a marker of cardiovascular diseases and has a progressive increase with age. Process of physiological aging is lead for changes in autonomic modulation. **Objective:** To quantify the acute changes in arterial stiffness, central hemodynamic parameters and autonomic modulation after maximal dynamic strength testing (1-RM) in older adults. **Methods:** Twenty independent older adults (69 ± 7) and community dwelling were recruited. Brachial-ankle pulse wave velocity (baPWV) and aortic augmentation index as indices of aortic stiffness were measured using applanation tonometry. Measurements were made at baseline, 5, 30 and 60 minutes after 1 RM testing. Hear rate variability (HRV) measures including normalized low frequency (LF), high frequency (HF), LF/HF ratio, HRV triangular index and RMSSD were all evaluated via SphygmoCor. HRV was measured at rest, 15, 40 and 70 minutes after 1-RM testing. **Results:** Brachial systolic blood pressure, central systolic blood pressure and brachial pulse pressure increased after 5 minutes of dynamic strength testing ($P<0.05$). Triangular index was higher after 1 RM testing as compared with baseline time ($P=0.01$). There was no difference in baPWV and augmentation index after 1RM testing. The triangular index increased significantly from a resting value ($P=0.01$). The remainders of HRV variables were unchanged over time. **Conclusion:** Maximal dynamic strength testing in older adults with stable chronic disease is associated with modest and transient rises in brachial and central aortic pressures, but does not acutely alter arterial stiffness. The clinical significance and duration of the increases in the triangular index measure of HRV observed requires further study, as this measure has been directly associated with higher aerobic fitness levels and lower risk of cardiovascular events in many cohorts.

Keywords: Weight lifting; Exercise test; Aged; Vascular stiffness; Hemodynamics

INTRODUCTION

A decrease in arterial distensibility is recognized as a potential marker of subclinical cardiovascular disease (CVD) (Meaume, 2001) and is independently associated with cardiovascular morbidity and mortality (Sutton-Tyrrell 2005, Willum 2006). Alterations in arterial function can occur in several diseases including hypertension, diabetes mellitus and atherosclerosis (Laurent 2001), that are closely related to heart disease and stroke (Rosamond 2007). Importantly, arterial stiffness has a progressive increase with age (Longo 2006), as the risk for cardiovascular diseases (Mattace-Raso, 2006), and is associated with reduced heart rate variability (HRV) and altered cardiac sympathovagal balance in hypertensive patients (Kosch et al, 1999). As sympathetic overactivity may be the contributor of reduced arterial distensibility observed in hypertensive patients (Acampa M et al, 2009), and the process of physiological aging can lead to reduced HRV, these changes may also be shared by aged (Jensen-Urstad, 1997).

Exercise programs have traditionally emphasized aerobic exercises, because of its well-known cardiovascular, metabolic and endurance benefits (Nelson 2007). In addition, regular aerobic exercise training can minimize the loss in arterial compliance with advancing age (Tanaka 2000), and reduce structural degeneration of the vascular wall related to aging, thereby reducing arterial stiffness (Seals 2008), effects that are independent of the amelioration of traditional cardiovascular risk factors (Moreau KL, 2003, 2005).

Although aerobic exercise had been traditionally emphasized, there is data suggesting that complementary resistance training has favorable effects on muscular strength and endurance, cardiovascular function, metabolism, coronary risk factors, and psychosocial well-being (Pollock 2000). However, there are few studies addressing the

effect of resistance training on arterial function and HRV, and their results are contradictory (Rakobowchuk 2005; Yoon 2010; Bertovic 1999; Miyachi 2003). For example, after three months of resistance training in young men central arterial compliance was unaltered (Rakobowchuk 2005), but other studies suggest that acute (Yoon 2010) and chronic, high intensity, high-volume resistance training increase arterial stiffness in both young and middle-aged men (Bertovic 1999; Miyachi 2003). Moreover, only 6 days of intense endurance exercise can induce HRV changes in healthy young males (Currie et al, 2009), but no data was published in aged. As the prescription of resistance training depends on results obtained from a maximal dynamic strength testing (1-RM), this test is usually performed in those who begin this kind of training. However, the acute effect of maximal dynamic strength testing (1-RM) on arterial stiffness and HRV in older adults has never been investigated. Therefore, this study aims to quantify acute changes in arterial stiffness, central hemodynamic parameters and autonomic modulation after 1-RM testing in aged adults.

METHODS

Research design and participants. This is a cross-sectional analysis of arterial stiffness and augmentation index (AIx) responses to acute 1-RM testing. A sample of 20 participants aged 50 years and older, independent and community dwelling were recruited from the Study of Mental and Regular Training (SMART study). All participants were studied at their final 18 months follow up visit. The SMART study was a randomized clinical trial of 6 months of supervised training followed by a 12 months observational period without any continued training exposure. The study was approved by the Central Sydney Area Health Service Ethics Review Committee and the

University of Sydney Human Ethics Committee, and all subjects signed a written informed consent form.

Primary inclusion criteria were self-reported memory complaint, a clinical dementia rating $\leq$ 1.0 (not demented), no significant functional impairment due to memory; Mini-Mental Status Examination score of 23-29 and no unstable disease precluding planned exercise. The exclusion criteria included depression, rapidly progressive or terminal illness, recent stroke, myocardial infarction in the past 6 months, unstable cardiovascular or metabolic disease, neuromuscular or musculoskeletal disorders severely disrupting voluntary movement, currently symptomatic hernias or severe hemorrhoids, or non-ambulatory status.

Protocol. Subjects came to the laboratory to perform physical assessments, blood pressure (BP) assessment, arterial stiffness (assessed by brachial pulse wave velocity (baPWV), Aix (assessed by pulse wave analysis (PWA) and HRV assessed by SphygmoCor machine (AtCor Medical, Australia) (Van Bortel 2002). Twelve hours before the exercise session, participants were refraining from alcohol, caffeine and exercise. On the exercise day the participants ate a light breakfast 3h before the test. All baseline measurements were executed for at least 10 min in a quiet and stable room temperature (23-26^{o}), and the exams were initiated around the same time of day (~10am) to minimize possible diurnal changes in the dependent variables (Van Bortel 2002).

Central and peripheral hemodynamics. The applanation tonometry is a non-invasive technique that is currently used to assess the radial artery waveform (Mackenzie 2002; O'Rourke 2001) reflecting systemic arterial stiffness. The aortic pulse contour, derived from the radial pulse waveform, is used to evaluate central hemodynamics including aortic pulse pressure and AIx (Nichols 2005; O'Rourke 2001; Laurent, 2006). After the

supine resting period, the peripheral systolic and diastolic blood pressure measurements in the left arm were taken by means of standard mercury sphygmomanometer. The average of two BP measurements was used for resting values and the intervals between measurements were set at 2 minutes. After the 1RM test the BP was measured once, approximately 1 minute before each PWA measurements.

Arterial stiffness and augmentation index assessment. The baPWV and aortic AIx measurements were evaluated according to guidelines specified by the Clinical Application of Arterial stiffness, Task Force III (Van Bortel 2002). For measurement of brachial/ankle baPWV, the distance from the brachial location to the sternal notch was subtracted from the distance between the sternal notch and the posterior tibial (ankle) site of measurement using a stadiometer. The aortic AIx was obtained by analyzing the pulse wave that was recorded from the radial artery. Its magnitude was expressed as a percentile value. Because the aortic AIx is affected by changes in heart rate, aortic AIx normalized to a heart rate of 75 beats/ min (AIx@75) were concurrently calculated (Wilkinson 2000). To enhance the accuracy of measurements, only values with quality index exceeds 80% were used. Prior to strength testing, the measurement of PWA was performed in the resting condition and then repeated at 5min., 30min. and 60 minutes after 1RM. The baPWV was measured before 1RM, at 10min., 35min. and 65 minutes after 1RM. The HRV was calculated at baseline before 1RM, at 15min., 40min. and 70 minutes after 1RM, and the duration of HRV was at least 5 minutes for each measurement. The peripheral augmentation index (PAI) was calculated using the following formula, [(P2-P1/peripheral pulse pressure)*100] (Munir 2008). All variables were measured by the same researcher to minimize variability due of technique.

Heart rate variability. The participants lay comfortably supine and the electrocardiogram (ECG) with a 3-lead ECG connected to an electronics

module was used to record heart rate. The ECG was recorded for at least 5 minute and the signal was digitized and stored using a commercially available PC-based system SphygmoCor (AtCor Medical, Australia). Heart rate variability was evaluated in the frequency, time domains and geometric index. Normalized low frequency and high frequency bandwidths, in addition to LF/HF balance ratio were recorded to determine sympathovagal balance and autonomic activity. The time domain measures of HRV were assessed and included RMSSD (estimate of short-term components of HRV), and HRV triangular index (estimate of overall HRV) (Task Force, 1996). Electrodes remained on the participants before and after the 1-RM test to reduce alterations in heart rate measurements.

Acute exercise bout with 1-RM testing. An acute bout of dynamic muscle strength test was comprised of dynamic contractions for approximately 40 min. of duration for each session. Large upper and lower body muscle groups (bilateral leg press, unilateral knee extension, unilateral hip abduction, seated row and chest press) were assessed using pneumatic-resistance equipment (Keiser Sports Health Equipment Inc., Fresno, CA) at the University of Sydney, Lidcombe. The 1RM is defined as the maximum load that can be lifted once throughout the full range of motion while maintaining a good technique. The 1-RM was determined in approximately 8 to 10 repetitions. Participants were instructed to not hold their breath at any time during the lifts. A rest period of 30 to 60 seconds was given between repetitions. For each repetition the cadence was of approximately 3 s concentric / 3 s eccentric.

Statistical analysis. All statistical analysis was performed using Statistical Package for the Social Sciences (SPSS, version 16.0 for Windows). Descriptive data were inspected visually and statistically for normality. Values are presented as mean ± standard

deviation (SD). Linear regression was used to reveal pertinent relationships between descriptive characteristics, central hemodynamic and arterial stiffness variables response at rest. Repeated measures analysis of variance (ANOVA) was used to compare hemodynamic response during 1-RM testing. The Bonferroni correction was used for all post hoc comparisons. Statistical significance was accepted at $p \leq 0.05$.

RESULTS

Baseline characteristics of the subjects are presented in Table 1. Men and women were of similar age (P = 0.72), with men having higher body mass index (BMI) (25.9 ± 2.3 *vs*. 29.3 ± 2.1 $kg.m^{-2}$; P = 0.04). Baseline central and peripheral BP were similar in both men and women, as well as medications in use.

Changes in hemodynamic variables 5, 30 and 60 min after the acute resistance exercise are shown in Table 2. Brachial pulse pressure was higher at 5 min after maximal dynamic strength testing (P < 0.05), but recovery was observed at 30 min after the exercise. The aortic Aix% and Aix@75 did not change after 1-RM testing. Brachial systolic BP had increased by 9% immediately (5 min) after 1-RM exercise, but recovery was observed 25 min later. Central (aortic) BP increased by 7% at the same time (5min) after the 1-RM testing and recovery occurred also 25 min later (Figure 1).

Heart rate variability indices are shown in table 3. Indices of HRV and spectral analysis did not changed within time. However, the triangular index of HRV was higher at 10, 35 and 65 min as compared with baseline (P=0.01) (Figure 2).

Figure 3 depicts regional baPWV at baseline and after each time of maximal dynamic strength testing, showing no change in any of the periods evaluated.

DISCUSSION

The primary finding of this study is that peripheral arterial stiffness did not acutely change after maximal dynamic strength testing in aged adults, but brachial BP increased along with changes in central systolic BP immediately after the test. In addition, the heart rate variability increased at the end of the test.

Previous studies have shown different results for arterial stiffness response after a bout of resistance exercise (Yoon 2010; Collier, 2010; Haffernan 2006; Haffernan 2007; DeVan et al. 2005): some of them showed increments (Yoon 2010; Collier, 2010; Haffernan 2007), and others showed decreases in central and peripheral arterial stiffness in young individuals. However, no study showed before the acute arterial stiffness evaluation after the 1-RM test in older adults, as we did. Our results are probably different from others cited above because we evaluated a total percent of 1-RM, whereas those assessed a certain percent of 1-RM. We also suggest that even with high intensity resistance exercises, moderate volumes of 1-RM are required to induce acute increments in peripheral arterial stiffness. Another possible difference between our results and previous ones is that the evaluations were performed at the end of the 12 month observational period after subjects were submitted to supervised resistance training for 6 months. We cannot exclude that individuals have been adapted to hemodynamic and vascular changes of weight lifting that modules arterial stiffness, thus previous training would be determinant of different responses to the 1-RM test.

We also showed that one bout of resistance exercise increased the brachial BP along with changes in central systolic BP at 5 min after 1-RM testing. In agreement, Heffernant et al. (2006) showed that the lower limb unilateral resistance at 85% of 1-RM exercises increased systolic BP from baseline to 5 minutes after exercise. However,

Yoon et al. (2010) showed that an acute bout of resistance exercise at 60% of 1RM could not change BP in young men. These results also showed that there are different effects of 1-RM intensity and volumes on BP.

The present study showed that 1-RM testing increases the overall variability of heart rate, as evaluated by the triangular index (Task Force 1996). To our knowledge, this is the first study to demonstrate that the geometric index of HRV increases after acute 1-RM testing in older adults. Improved vagal modulation of heart rate at submaximal exercise intensities and increses in HRV were shown after training in young (Hu 2009) and older subjects (Schuit 1999). These changes are based on shift in very low and low frequency components, which represent the vasomotor tone and peripheral vascular resistance modulated by sympatho-vagal activity (Task Force, 1996). It is reasonable to think that changes in HRV as a result of endurance training may be at least partly the result of the accumulation of the acute effects of exercise bouts, rather than or in addition to, adaptation to the stress of chronic training.

The changes in cardiac autonomic function observed in the present study are similar to those seen in investigations of long-term resistance training (Hu 2009, Schuit 1999). These changes might suggest a turn in autonomic function toward increased parasympathetic nervous system activity and decreased sympathetic nervous system activity. These results may provide further evidence of the cardioprotective effects of a maximal dynamic strength testing. However, the clinical significance and duration of the increases in the triangular index measure of HRV observed requires further study, as this measure has been directly associated with higher aerobic fitness levels and lower risk of cardiovascular events in many cohorts.

CONCLUSION

The results of this study showed that maximal dynamic strength is a safe test for older adults who are engaged in regular resistance exercise as a means of improving muscular strength.

Duality of interest. The authors declare that there is no duality of interest associated with this manuscript.

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Author contribution paragraph. APSC, BDS, MB, JM, GW, and NJ contributed to data acquisition and revised the manuscript. APSC and MFS contributed to the conception and design of the study and to data analysis and interpretation, and revised the manuscript. APSC, BDS, MB, JM, GW, NJ and MFS approved the final version.

Figure Legends

Figure 1: Brachial systolic blood pressure (Brachial SBP, black bars) and aortic systolic blood pressure (Aortic SBP, gray bars) at baseline, minute 5, minute 30 and minute 60 post maximal dynamic strength testing (1-RM). Data expressed as mean ± SD. Analysis: Generalized estimating equations (GEE) for repeated measures, followed by Bonferroni's post hoc test. * ($p<0.05$).

Figure 2: Triangular index of heart rate variability (HRV) at baseline (light grey bar), minute 15 (mid grey bar), minute 40 (dark grey bar) and minute 70 (black bar) post maximal dynamic strength testing (1-RM). Data expressed as mean ± SD. Analysis: Generalized estimating equations (GEE) for repeated measures, followed by Bonferroni's post hoc test. * ($p<0.05$).

Figure 3: Brachial-ankle pulse wave velocity (baPWV) at baseline (light grey bar), minute 10 (mid grey bar), minute 35 (dark grey bar) and minute 65 (black bar) post maximal dynamic strength testing (1-RM). Data expressed as mean ± SD. Analysis: Generalized estimating equations (GEE) for repeated measures, followed by Bonferroni's post hoc test.

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Table 1: Clinical characteristics and resting hemodynamics

Characteristic	Mean ± SD
General	
Male/Female	6/14
Age, yr	69 ± 7 (54 – 83)
Body mass index, $kg.m^{-2}$	27.5 ± 4.4
Isometric strength dominant handgrip, Kg	29.7 ± 7.7
Isometric strength non-dominant handgrip, Kg	28.1 ± 7.1
Chest press 1-RM, Kg	9.4 ± 4.2
Seated Row 1-RM, Kg	15.4 ± 5.7
Leg press 1-RM, Kg	162.1 ± 54.0
Left hip abduction 1-RM, Kg	9.4 ± 3.4
Right hip abduction 1-RM, Kg	8.9 ± 4.2
Left knee extension 1-RM, Kg	4.0 ± 2.0
Right knee extension 1-RM, Kg	4.7 ± 1.7
Medications, n (%)	
Anti-hypertensive	10 (50)
Multivitamins	5 (25)
Vitamin E	2 (10)
Vitamin D	13 (65)
Fish Oil	8 (40)
Resting hemodynamics	
Brachial SBP, mmHg	123.5 ± 12.8
Brachial DBP, mmHg	72.9 ± 8.8
Brachial MAP, mmHg	91.0 ± 9.8
Brachial PP, mmHg	50.5 ± 11.8
Heart Rate, beats/min	59.2 ± 10.6

Central SBP, mmHg	116.8 ± 13.0
Central DBP, mmHg	73.7 ± 9.1
Central MAP, mmHg	91.0 ± 9.8
Central PP, mmHg	43.0 ± 11.2

Values presented as mean ± SD or n (%). SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; PP: pulse pressure; 1-RM: single-repetition maximum lift.

Table 2: Changes in hemodynamic parameters at baseline, 5, 30 and 60 minutes after maximal dynamic strength testing (1-RM)

Variables	Baseline	5 min after	30 min after	60 min after
Heart rate, beats/min	59.2 ± 10.6	61.6 ± 12.0	61.4 ± 10.3	61.2 ± 10.0
Brachial DBP, mmHg	74.5 ± 8.4	74.8 ± 12.2	72.4 ± 12.5	71.5 ± 10.6
Brachial MAP, mmHg	92.7 ± 9.4	97.2 ± 14.0	91.1 ± 12.8	90.1 ± 12.8
Brachial PP, mmHg	51.1 ± 12.6	62.2 ± 13.7*	51.4 ± 7.1	51.5 ± 7.2
PAI, %	-6.2 ± 20.2	-10.7 ± 17.4	-11.1 ± 14.5	-7.1 ± 18.7
Aortic DBP, mmHg	75.2 ± 8.8	76.1 ± 12.7	73.1 ± 12.8	72.5 ± 11.0
Aortic MAP, mmHg	92.7 ± 9.4	97.2 ± 14.0	91.1 ± 12.8	90.2 ± 12.4
Aortic PP, mmHg	43.9 ± 11.9	51.5 ± 11.9	43.5 ± 7.3	43.8 ± 7.4
AIx@75, %	27.4 ± 9.2	27.6 ± 5.8	28.6 ± 7.9	28.7 ± 9.5
AIx, %	33.5 ± 10.8	34.4 ± 7.7	36.2 ± 10.5	36.2 ± 11.4

Values presented as mean ± SD or n (%). SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; PP: pulse pressure; pAI: Peripheral Augmentation Index; AIx@75: aortic augmentation index at a heart rate of 75 beats per minute; AIx: aortic augmentation index. * $P < 0.05$ vs. Baseline

Table 3: Heart rate variability at baseline, 15, 40 and 70 minutes after maximal dynamic strength testing (1-RM)

Variables	Baseline	15 min after	40 min after	70 min after
RMSSD, ms	36.0 ± 25.3	47.5 ± 56.0	43.2 ± 36.4	46.2 ± 41.1
LF, nu	53.3 ± 22.1	40.7 ± 17.9	54.3 ± 19.3	46.6 ± 16.7
HF, nu	45.7 ± 22.0	58.2 ± 17.9	44.6 ± 19.2	52.3 ± 16.7
LF/HF, ratio	1.37 ± 1.86	0.53 ± 1.21	1.47 ± 2.31	0.68 ± 0.94

Values presented as mean ± SD. RMSSD: estimate of short-term components of HRV; LF: low frequency; HF: high frequency; nu:normalized units . * $P < 0.05$ vs. Baseline

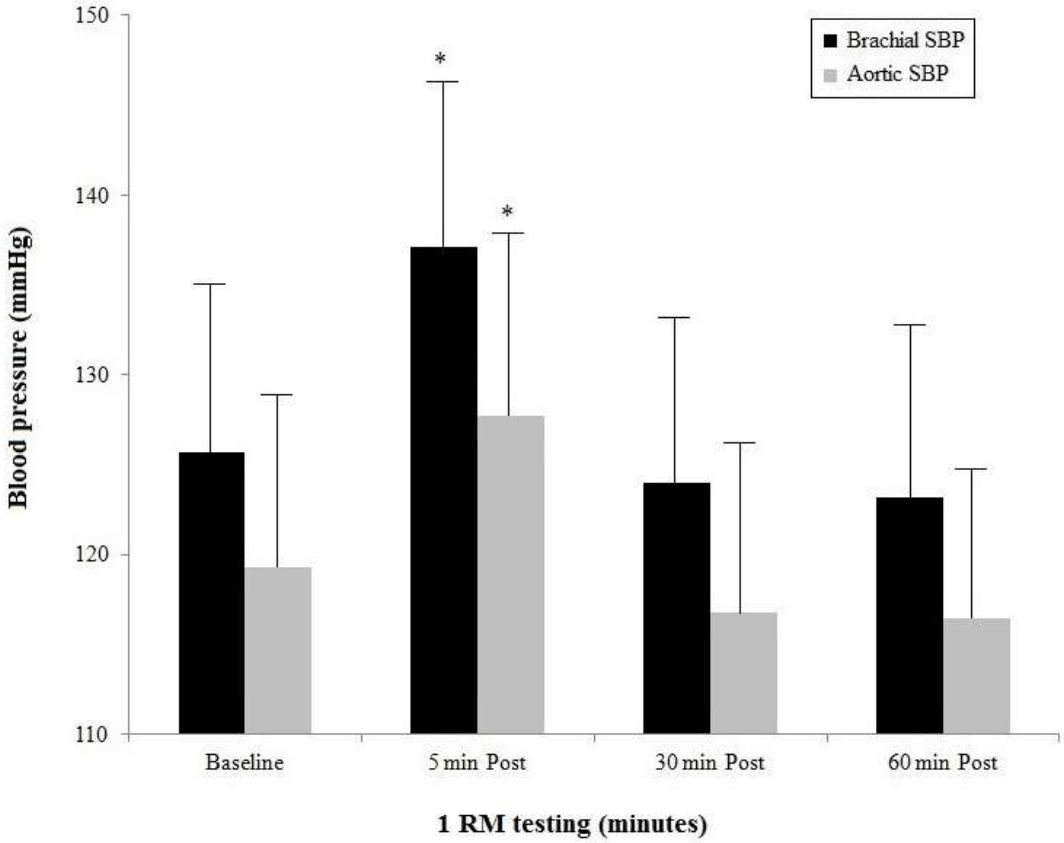
150
140
130
120
110
Blood pressure (mmHg)
Brachial SBP
Aortic SBP
*
*
Baseline
5 min Post
30 min Post
60 min Post
1 RM testing (minutes)

Figure 1:

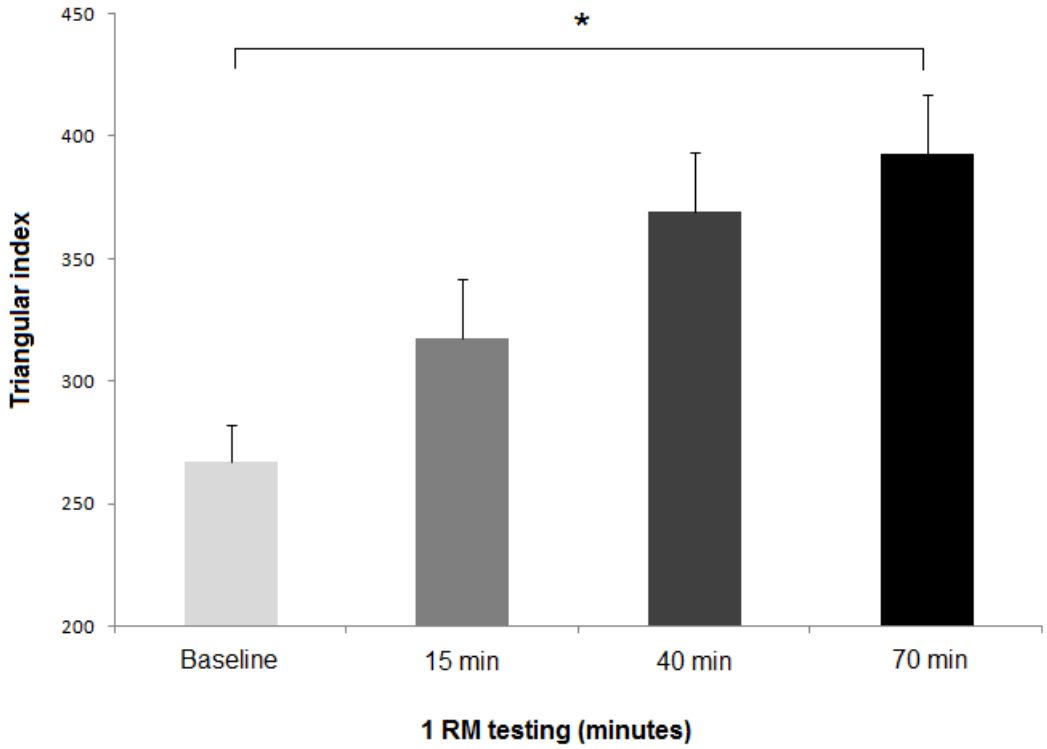
*
450
400
350
300
250
200
Triangular index
Baseline
15 min
40 min
70 min
1 RM testing (minutes)

Figure 2:

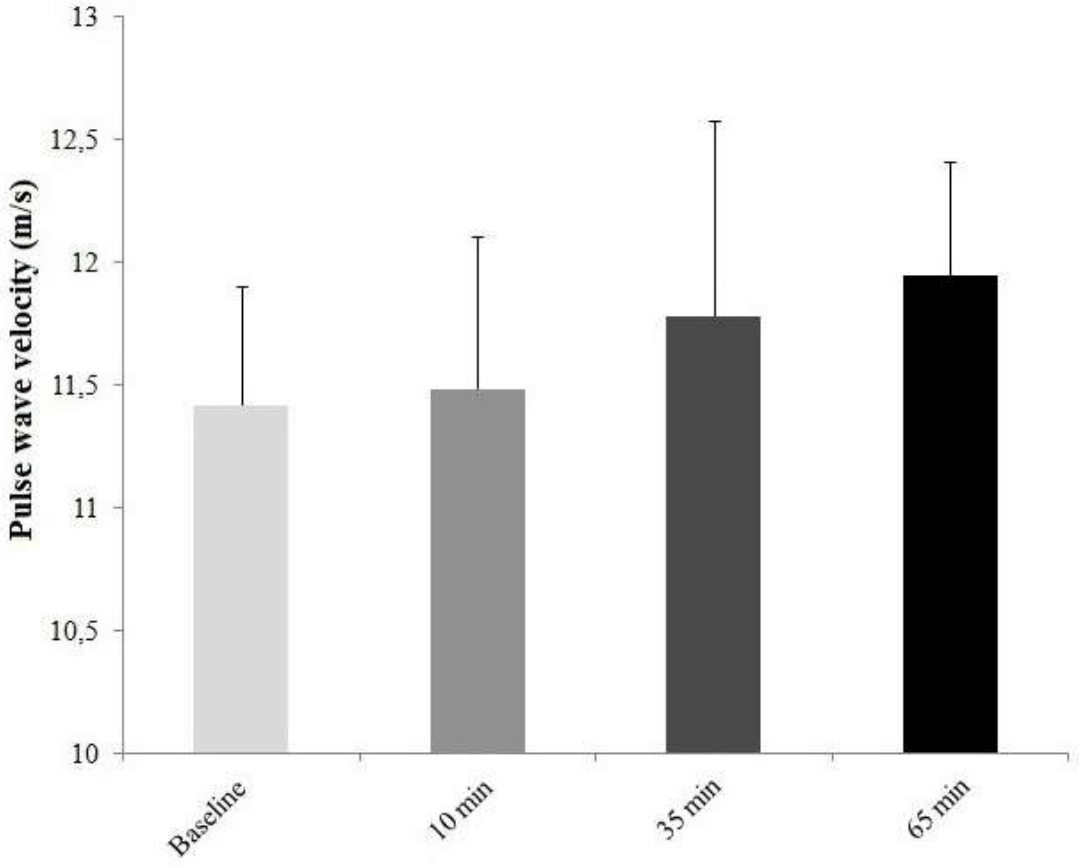
13
12,5
12
11,5
11
10,5
10
Pulse wave velocity (m/s)
Baseline
10 min
35 min
65 min

Figure 3:

CAPÍTULO V

CONCLUSÕES

1. Exercício agudo da musculatura inspiratória de alta intensidade determina exacerbação do metaborreflexo muscular inspiratório em pacientes com diabetes mellitus tipo 2. Isso ocorreu independentemente da presença da neuropatia autonômica cardiovascular, mas provavelmente influenciada pela disfunção endotelial. Além disso, níveis fatigantes da musculatura inspiratória refletiram-se em resposta circulatória reduzida ao exercício do antebraço. Em adição, o exercício muscular inspiratório utilizando uma carga de 60% da PImáx reduziu abruptamente os níveis de glicose nesses indivíduos.

2. O teste de exercício de força máxima não alterou a rigidez arterial em idosos, o que o torna seguro para esta população que deseja realizar exercícios resistidos regulares e para tal necessita programação deste tipo de treinamento.

ANEXO 1

Produção científica durante o doutorado no Brasil

1. **Corrêa APS**, Schaan BD, Antunes CF, Figueira FR, Castro MA, Ribeiro JP. Exacerbated inspiratory muscle metaboreflex and endothelial dysfunction: potential determinants of exercise intolerance in type 2 diabetes regardless of the presence of autonomic neuropathy. Under review to *Diabetes Care*.
2. **Corrêa AP**, Schaan BD, Antunes CF, Figueira FR, Castro MA, Ribeiro JP. Exacerbated inspiratory muscle metaboreflex in diabetic patients with autonomic neuropathy. Abstract presented at 1st American Diabetes Association Middle East Congress "Diabetes Prevention and Treatment", December 4-6 2002, Dubai, UAE. The Journal of Clinical and Applied Research and Education. *Diabetes Care.* 2012;9(Suppl 4):S14.
3. Chiappa GR, Vieira PJ, Umpierre D, **Corrêa APS**, Berton DC, Ribeiro JP, Neder A. Inspiratory resistance decreases limb blood flow in COPD patients with heart failure. European Respiratory *Journal. Epub* 2013.
4. **Ana P. S. Corrêa**, Franciele R. Figueira, Daniel Umpierre, Karina R. Casali and Beatriz D. Schaan. Inspiratory Loading or Aerobic Exercise, but not Aerobic plus Resistance Exercise Reduces Glucose Variability in Type 2 Diabetes. Abstract accepted to be presented at the World Diabetes Congress 2013. Melbourne. December 2 – 6, 2013.
5. Franciele R. Figueira, **Ana P. S. Corrêa**, Daniel Umpierre, Karina R. Casali, Beatriz D. Schaan. Effects of inspiratory loading, aerobic and aerobic plus resistance exercise on glucose variability in type 2 diabetes. Abstract presented at 1st International Symposium on Cardiovascular Epidemiology. Hospital de Clínicas de Porto Alegre, Porto Alegre, Brasil. May 21 – 23, 2013. Abstract Awarded with Honorable Mention.
6. **Corrêa APS**, Viecili PRN, and Callegaro CC. Heart rate recovery is impaired in soccer athletes during a competitive season. Submitted to *International Journal of Sports Physiology and Performance Journal*.
7. **Corrêa APS**, Viecili PRN, and Callegaro CC. Heart rate recovery is impaired in soccer athletes during a competitive season. Abstract submitted to ACSM's 61st Annual Meeting, 5th World Congress on Exercise is Medicine and World

Congress on the Role of Inflammation in Exercise, Health and Disease to be held May 27-31 in Orlanda, Florida.

Produção científica durante o Estágio Sanduíche – *The University of Sydney*

1. **Corrêa APS**, Schaan BD, Meiklejohn J, Wilson GC, Jain N, Baker MK, and Fiatarone Singh MA. Maximal dynamic strength testing does not alter arterial stiffness in older adults. To be submitted to *Vascular Health and Risk Management Journal*.
2. **Correa APS**, Schaan BD, Baker MK, Meiklejohn J, Wilson GC, Jain N, Costa-Hong V, Bortolotto L, and Fiatarone Singh MA. Maximal dynamic strength testing does not alter arterial stiffness in older adults. Abstract submitted to ACSM's 61st Annual Meeting, 5th World Congress on Exercise is Medicine and World Congress on the Role of Inflammation in Exercise, Health and Disease to be held May 27-31 in Orlanda, Florida.
3. **Correa APS**, Schaan BD, Baker MK, Meiklejohn J, Wilson GC, Jain N, Costa-Hong V, Bortolotto L, and Fiatarone Singh MA. Maximal dynamic strength testing increases HRV in older adults. Abstract submitted to ACSM's 61st Annual Meeting, 5th World Congress on Exercise is Medicine and World Congress on the Role of Inflammation in Exercise, Health and Disease to be held May 27-31 in Orlanda, Florida.
4. Parmenter BJ, Baker MK, Mavros Y, Gates NJ, Anderberg KA, Meiklejohn J, Jain N, Wilson GC, Kay S, Wang Y, **Correa APS**, and Fiatarone Singh MA. Muscle strength is impaired in older adults with peripheral arterial disease and predicts walking ability. Abstract submitted to ACSM's 61st Annual Meeting, 5th World Congress on Exercise is Medicine and World Congress on the Role of Inflammation in Exercise, Health and Disease to be held May 27-31 in Orlanda, Florida.
5. Baker MK. Gates N, Jain N, Wilson GC, Meiklejohn J, Sachdev P Brodaty H, Wen W, Singh N, Baune BT, Suo C, Foroughi N, Wang Y, **Correa APS**, Valenzuela M, and Fiatarone Singh MA. Cognitive impairment is associated with arterial stiffness in older adults with mild cognitive impairment. To be submitted to GSA Annual Meeting. Vancouver, British Columbia, Canada. 19-22 October, 2014.