Tese de doutorado

EFEITO DO EXERCÍCIO MUSCULAR INSPIRATÓRIO AGUDO SOBRE O PERFIL GLICÊMICO EM PACIENTES COM DIABETES TIPO 2

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SUMÁRIO

LISTA DE ABREVEATURAS E SIGLAS

DM: Diabetes mellitus

DLCO: Difusão do monóxido de carbono

CV: Capacidade vital

CVF: Capacidade vital forçada

VEF1: Volume expiratório forçado no primeiro segundo

HbA1c: Hemoglobina glicada

GLUT 4: Transporte de glicose tipo 4

CGMS: Sistema de monitorização contínua da glicose

SMBG: Auto-monitorização da glicemia capilar

MAGE: Média de amplitude de excursão glicêmica

CV: Coeficiente de variação da glicose

DP: Desvio padrão

M-value: Valor M

J-index: Índice de J

CONGA: Ação glicêmica contínua

MODD: Média absoluta das diferenças diárias

TMI: Treinamento muscular inspiratório

RESUMO

Objetivo: Avaliar o efeito do exercício muscular inspiratório (EMI) nos níveis de glicose, variabilidade glicêmica e controle autonômico cardiovascular em pacientes com diabetes tipo 2. Métodos: Quatorze indivíduos foram randomizados para realizar EMI com carga de 2% ou 60% da pressão inspiratória máxima (PImax). Durante o EMI, os níveis de glicose e a variabilidade glicêmica foram avaliados por monitorização contínua da glicose. O controle autonômico foi avaliado no domínio do tempo e da freqüência. Resultados: Os níveis de glicose e variabilidade glicêmica reduziram após o EMI com ambas as cargas. O EMI com carga de 60% PImax determinou redução no componente de alta frequência da variabilidade da frequência cardíaca e maior variabilidade da pressão arterial. Conclusões: O EMI com carga de 60% da PImax não demonstrou melhoras nos níveis de glicose e variabilidade glicêmica, quando comparado com à carga 2% da PImax. Entretanto, o EMI com carga de 60% da PImax levou a alterações na modulação vagal cardíaca e maior variabilidade da pressão arterial.

Objective: To evaluate the effect of inspiratory muscle exercise (IME) on glucose levels, glycemic variability and cardiovascular autonomic control in patients with type 2 diabetes. Methods: Fourteen subjects were randomized to perform IME with a of maximum inspiratory pressure (PImax) load of 2% or 60%. During IME, glucose levels and glycemic variability were assessed through continuous glucose monitoring. Autonomic control was evaluated in the domain of time and frequency. Results: Glucose levels and glycemic variability decreased after IME with both loads. IME with a load of 60% PImax resulted in a reduction in the high frequency component of heart rate variability and greater variability of blood pressure. Conclusions: IME with loads of 60% PImax fails to demonstrate improvement in glucose levels and glycemic variability, when compared to loads of 2% PImax. However, IME with 60% loading of PImax led to changes in vagal cardiac modulation and greater variability of blood pressure.

INTRODUÇÃO

A prevalência do diabetes mellitus (DM) vem crescendo ao longo dos anos ao redor do mundo, e fatores que podem contribuir para essa estimativa são o envelhecimento, aumento da prevalência de obesidade e a expansão populacional. O aumento de pessoas vivendo com DM tem ocorrido principalmente em países de baixa e média renda [1]. Em 1995 foi estimado que 135 milhões de pessoas apresentavam DM [2]; já em 2000 essa doença atingiu 171 milhões de indivíduos [3], apresentando um aumento de 79% em apenas 5 anos. Dados recentes da Federação Internacional de Diabetes (IDF) mostraram que atualmente a estimativa global de DM é de 425 milhões de pessoas, e, se este aumento continuar, haverá, em 2045, 693 milhões de pessoas com DM na população mundial [4]. No Brasil também foi observado o crescimento dessa doença. Em 2000, a prevalência de DM foi estimada em 4,6 milhões de pessoas e o país estava entre os dez países com maiores números de casos de DM no mundo ocupando a posição de oitavo lugar [3]. Após 15 anos houve um aumento de 32% (14,3 milhões de pessoas) de brasileiros com a doença e o Brasil atingiu a posição de quarto lugar com maior número absoluto de indivíduos com DM [5].

O impacto econômico global com a saúde de pessoas com DM está cada vez mais elevado, em 2014 foram gastos pelo menos US$ 612 bilhões de dólares e as regiões com maiores despesas com a saúde no DM foram o norte da América e região Caribenha [6]. Já em 2015 foram gastos US$ 673 bilhões de dólares e as projeções futuras visam um aumento de 25,4% no orçamento [7]. No Brasil, o Sistema único de Saúde (SUS) gasta US$ 2,108,287 a cada mil pacientes por ano com DM tipo 2 e os gastos individualmente são aumentados a medida que os pacientes desenvolvem complicações crônicas relacionadas à doença (microvasculares: US$ 2062, macrovasculares: US$ 2517 e ambas: US$3199) [8]. Essas estimativas de custos demonstram o impacto relevante economicamente, para a sociedade, sistemas de saúde e para os próprios pacientes [9].

As perspectivas são de aumentar ainda mais as despesas com a saúde, juntamente com a prevalência de DM, gerando um grave problema de saúde pública. Altos índices de mortalidade devido à presença de diabetes têm sido observados e os fatores de risco para a mortalidade nesses pacientes envolvem complicações microvasculares [10, 11] e macrovasculares [12] que são decorrentes da hiperglicemia sustentada [13]. Grandes estudos como o *Diabetes Control and Complications Trial* (DCCT) [14] e o *UK Prospective Diabetes Study* (UKPDS) [15] contribuíram de forma

significativa para o conhecimento científico, quando demonstraram que a hiperglicemia é o principal determinante do desenvolvimento e progressão das complicações do DM.

Recentente, foi realizado um estudo com 1280 indivíduos com DM tipo 2 e foi observado que os pacientes que apresentaram microalbuminúria tinham maior descontrole glicêmico e duração do DM quando comparados com os indivíduos sem microalbuminúria [16]. No estudo de Zoungas e colaboradores foi avaliado se a idade, idade do diagnóstico de DM e duração de DM se associava com risco de complicações micro e macrovasculares em 11.140 pacientes com DM tipo 2 que foram acompanhados durante 5 anos. Todos esses fatores foram associados independentemente com o risco de complicações macrovasculares e morte, enquanto apenas a duração do DM foi associada com complicações microvasculares [17].

Além destas complicações classicamente conhecidas, alterações da função pulmonar foram descritas no DM tipo 2 [18-20], de forma que alguns autores sugerem o pulmão também como órgão alvo nessa população [21]. Dentre essas alterações podem ser destacadas a diminuição da difusão do monóxido de carbono (DLCO) [22-24] e limitações nos volumes e capacidades pulmonares [18, 25], bem como na capacidade vital (CV), na capacidade vital forçada (CVF) e no volume expiratório forçado no primeiro segundo (VEF1). A redução desses fatores está associada com tempo de duração da doença [25, 26], exposição glicêmica [21, 27] e severidade do DM [25]. Além dessas alterações na função pulmonar, foi observada redução da força muscular inspiratória em pessoas com DM tipo 2 que apresentavam neuropatia autonômica diabética quando comparados com pacientes sem neuropatia autonômica [28].

Uma revisão sistemática de Van den Borts e colaboradores avaliou a função pulmonar em 3.182 indivíduos com DM e demonstrou que tanto o DM tipo 1 quanto o DM tipo 2 estão associados com diminuição da função pulmonar [29]. Resultados similares foram observados no estudo de Yu e colaboradores, que avaliaram a capacidade pulmonar em chineses e australianos com síndrome metabólica (n=1401), DM tipo 2 (n=472) e indivíduos saudáveis (n=3951). Todos os participantes foram submetidos ao teste de espirometria para avaliar o VEF1 e a CVF e ambos os parâmetros apresentaram-se menores quando comparado com o grupo controle ($p>0,001$) [30].

O controle glicêmico é fundamental para evitar o início e progressão das complicações do DM. Uma revisão sistemática de grandes ensaios clínicos randomizados (ACCORD, ADVANCE, UKPDS, VADT) demonstrou que o controle

glicêmico intensivo é capaz de reduzir complicações microvasculares em indivíduos com DM tipo 2 [31]. Benefícios cardiovasculares, como a diminuição do risco de infarto agudo do miocárdio também foram observados nesses pacientes [32].

O tratamento preconizado para indivíduos com DM tipo 2, o mais prevalente dos tipos de DM, consiste em modificação no estilo de vida através da realização de dieta e prática de exercício físico [33, 34] e tratamento farmacológico (insulina e/ou antidiabéticos orais). Além disso, o cuidado multidisciplinar através de programas de educação e autogestão tem sido descrito como forma de tratamento [35] no manejo desses pacientes.

Diversas medidas são realizadas para alcançar o bom controle glicêmico, dentre elas se destacam medidas de glicose capilar que permitem que os pacientes e médicos identifiquem se os alvos foram atingidos, determinando mudanças terapêuticas. O objetivo é atingir e manter alvo de 80 a 130mg/dL para glicemia pré prandial e <180 mg/dL para a glicemia pós prandial. Também glicemia de jejum e mensuração de hemoglobina glicada (HbA1c), com alvo de <7% [36] são importantes com este propósito. A HbA1c é o padrão ouro para determinar o controle glicêmico refletindo esse controle em longo prazo (três meses).

O exercício físico é recomendado por diretrizes pela sua importância como intervenção não farmacológica em indivíduos com DM, promovendo muitos benefícios, o principal deles, a melhora do controle glicêmico [37, 38] o que foi confirmado em revisão sistemática recente [39]. Qualquer tipo de exercício estruturado (aeróbico, resistido ou combinado) é capaz de melhorar o controle glicêmico em pacientes com DM tipo 2 de forma similar demonstrando reduções significativas nos níveis de HbA1c [40]. Além disso, o exercício aumenta a sensibilidade à insulina [41, 42], eleva o conteúdo e translocação de GLUT 4 nas células musculares [43] e aumenta a força muscular esquelética [44]. Outros benefícios como a redução da gordura abdominal [41, 45], diminuição dos níveis de pressão arterial [46, 47], e melhora da função autonômica [48, 49] foram observados nesses pacientes. Neste contexto, a literatura é consistente quanto aos benefícios dos diferentes tipos de exercício em indivíduos com DM.

O tratamento farmacológico do DM tipo 2 envolve múltiplas opções para uso oral e insulina, fármacos estes que são usados em geral em sequência na busca de atingimento da meta de controle glicêmico individualizada [36]. Apesar disso, existem alguns indivíduos que desenvolvem complicações crônicas da doença, mesmo tendo atingido níveis ideais de HbA1c. Por este motivo novas formas de avaliação têm sido buscadas. Além do mais, a HbA1c não demonstra, sozinha, informações sobre as

oscilações agudas dos níveis de glicose, bem como a relação dessas oscilações aguda de glicose com as complicações do DM.

VARIABILIDADE GLICÊMICA

Novas métricas do controle glicêmico têm despertado o interesse de pesquisadores por meio da análise completa do papel da glicemia pós-prandial, glicemia de jejum e hipoglicemia no controle do DM, surgindo o conceito de variabilidade glicêmica.

A variação glicêmica pode ser mensurada por meio da monitorização contínua da glicose (CGMS - *Continuous glucose monitoring system*) [50] através da colocação de um sensor no tecido subcutâneo do paciente. Na maioria dos sistemas de monitoramento, os valores de glicose intersticial são registrados a cada cinco minutos, totalizando 288 medidas em 24h. O CGMS pode ser profissional (retrospectivo ou cegado), ou seja, os pacientes não conseguem visualizar os valores de glicose e os dados são baixados posteriormente ao exame [51, 52], ou pessoal (em tempo real) em que os pacientes conseguem acompanhar as medidas de glicose em tempo real e saber quando ocorre uma hipo ou hiperglicemia [52, 53]. Outro método de avaliar as oscilações glicêmicas é pela auto-monitorização da glicemia capilar (SMBG – *Self monitoring blood glucose*) [54] que é realizada pelo próprio paciente com a utilização de um glicosímetro, com o qual são feitas diversas medidas de glicemia capilar por dia [55, 56].

Diversos métodos matemáticos são utilizados para quantificar a variabilidade glicêmica [57, 58]. Dentre estes métodos podemos destacar a média de amplitude de excursão glicêmica (MAGE), o coeficiente de variação da glicose (CV), o desvio padrão da glicose (DP), o valor M (M-value), o índice J (J-index), a ação glicêmica global contínua (CONGA) e a média absoluta das diferenças diárias (MODD), bem como outros índices que são demonstrados na figura 1 [57]. As informações das oscilações da glicose podem ser reportadas como: 1. variabilidade intra dia, avaliadas por SMBG ou CGMS sendo calculada através do DP, CV, MAGE, M-value, J-index e CONGA; 2.variabilidade entre os dias, pode ser analisada por DP da glicose em jejum e MODD, e 3. variabilidade ao longo do tempo, avaliada pelo DP e CV da glicose em jejum ou da HbA1c. Apesar da diversidade de métodos usados para determinar a variabilidade glicêmica não existe um método considerado padrão ouro e tampouco um consenso de qual índice é melhor para ser utilizado [59].

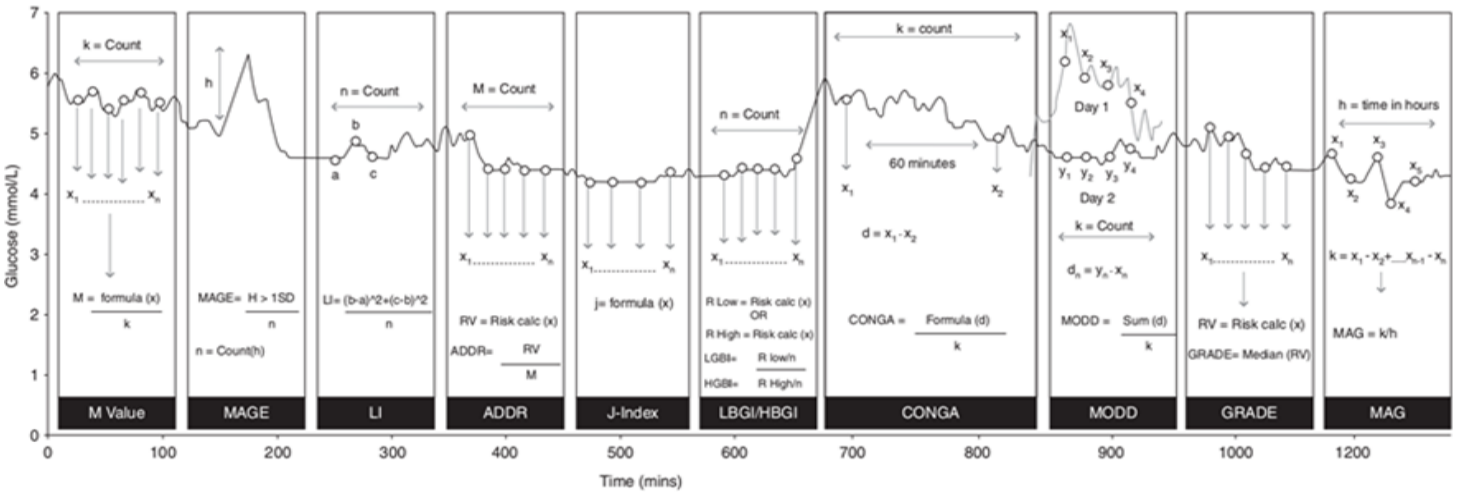

Figura 1: Índices de variabilidade glicêmica representados graficamente [57].

Existem resultados controversos na literatura se a variabilidade glicêmica em curto prazo é um fator de risco, independentemente da HbA1c para complicações relacionadas ao DM. Estudos demonstram que a variabilidade glicêmica pode contribuir para o desenvolvimento e progressão de complicações macrovasculares. Ela foi associada com a presença e gravidade de doença arterial coronariana em indivíduos com DM [60, 61]. Em relação às complicações microvasculares, a variabilidade glicêmica foi associada com neuropatia autonômica cardíaca em indivíduos mal controlados ou recentemente diagnosticados com DM tipo 2 [62, 63]. Uma revisão sistemática demonstrou que a variação glicêmica aumentada pode ser um fator de risco no desenvolvimento das complicações microvasculares [64].

Em contraste, em publicação recente, não foi observado associação entre a variabilidade glicêmica e doença arterial coronariana, infarto agudo do miocárdio, acidente vascular cerebral e doença arterial periférica [65]. Esses resultados foram confirmados por estudos publicados anteriormente [54]. De forma similar, as complicações microvasculares não foram associadas à variabilidade glicêmica em curto prazo em indivíduos com DM [65, 66]. No estudo de Jin e colaboradores nenhuma associação foi encontrada entre as oscilações glicêmicas e a extensão de albuminúria [67].

Baseado nos achados da literatura a relação entre complicações micro e macrovasculares com a variabilidade glicêmica permanece incerta. Diversos fatores podem ser considerados para esses resultados controversos; 1. delineamento dos estudos, já que todos os estudos citados acima são observacionais, sujeitos portanto a vieses de confusão; 2. diferentes condições clínicas do DM (recém diagnosticados,

internados, mal controlados, com e sem tratamento com antidiabéticos ou insulina); 3. diversidade de índices de variabilidade glicêmica utilizada; 4. método de mensuração da variabilidade glicêmica (CGMS ou SBMG). É importante que haja estudos prospectivos em longo prazo, que utilizem o CGMS, atualmente o método mais detalhado para avaliação de variabilidade glicêmica, para determinar a real contribuição das flutuações glicêmicas no desenvolvimento das complicações relacionadas ao DM.

Esse componente de disglicemia tem sido utilizado como avaliação de impacto de diferentes formas de tratamento, algumas delas mostrando de fato impactar sobre melhora da variabilidade glicêmica em indivíduos com DM. Pimazoni-Netto e colaboradores randomizaram indivíduos com DM tipo 2 para receber tratamento intensivo (10h de programa de educação multidisciplinar, visitas semanalmente até a sexta semana para aferição do controle da glicemia e atendimento clínico) ou tratamento controle (2h de programa de educação multidisciplinar). Ambos os grupos tiveram visitas na semana 0, 6 e 12. Redução da variabilidade glicêmica foi mostrada após 12 semanas de terapia intensiva multidisciplinar quando comparado com o tratamento controle. Algumas limitações podem ser destacadas como, por exemplo, a mensuração da variabilidade glicêmica que foi realizada através do SMBG e com pouco tempo de duração (12 semanas), uma vez que o controle glicêmico deve ser feito por anos nesses indivíduos [68].

Diminuição da variabilidade glicêmica também foi observada após a realização de uma sessão de exercício combinado em pacientes com DM tipo 2 [69] e exercício aeróbico em indivíduos com hiperglicemia [70], o que pode determinar efeito benéfico e adicional à redução da glicemia avaliada em níveis absolutos [69, 71]. Ambos os estudos são ensaios clínicos randomizados cruzados. O estudo de Nygaard e colaboradores apresentaram grupo controle (sessão sem exercício), enquanto o estudo de Figueira utilizou duas modalidades diferentes de exercício. Porém, ambos os estudos realizaram exercício agudo, o que limita muito o entendimento de benefício em longo prazo, o qual é o que de fato impactaria na prevenção de complicações do DM.

No estudo de Karstoft e colaboradores, um ensaio clínico randomizado cruzado, os pacientes com DM tipo 2 foram submetidos a três intervenções 1) nenhuma intervenção; 2) treinamento de caminhada contínuo e 3) treinamento de caminhada intervalado (três min com baixa intensidade e três min com alta intensidade). Ao todo foram realizadas 10 sessões de exercícios, durante 60min cinco vezes por semana. O estudo demonstrou que o treino intervalado foi capaz de reduzir o MAGE (pré: 7.1 ±

0.6mg/dL e pós: 5.4 ± 0.4mg/dL). Entretanto foram incluídos poucos pacientes no estudo, o que pode ter influenciado no tamanho do efeito de resposta ao exercício [72].

Outro estudo foi realizado em indivíduos com DM tipo 2 tratados com e sem insulina que foram randomizados para sessão de exercício agudo na bicicleta com intensidade moderada ou para uma sessão sem exercício em que os pacientes ficaram sentados pelo mesmo período de tempo da sessão de exercício (controle). A ordem de realização das intervenções foi randomizada e todos os indivíduos fizeram ambas as intervenções. O exercício foi capaz de reduzir a variabilidade glicêmica (avaliada pelo CONGA) e os episódios de hiperglicemia nesses indivíduos [73]. No entanto, o exercício foi realizado de maneira aguda e foram incluídos indivíduos tratados com insulina, que demonstraram maior variabilidade glicêmica quando comparado aos indivíduos não tratados com insulina. O efeito do exercício na variabilidade glicêmica não foi observado quando separado por tipo de tratamento antidiabético, apenas quando comparados os indivíduos como um todo que realizaram e não realizaram o exercício.

Apesar dos benefícios que o exercício físico proporciona para os indivíduos com DM, nem todos os pacientes toleram os exercícios convencionais, ou mesmo, têm dificuldades em aderir a programas de exercício físico, alternativas devem ser pensadas com o objetivo de prover este tipo de intervenção a eles, desde que estas alternativas sejam tão eficazes quanto o exercício convencional.

EXERCÍCIO MUSCULAR INSPIRATÓRIO

O exercício muscular inspiratório é uma modalidade de exercício que tem sido utilizada, ao longo dos anos, em diversas situações clínicas [74-76], tanto de forma aguda (sessão de exercício muscular inspiratório), como de forma crônica [treinamento muscular inspiratório (TMI)], demonstrando vários benefícios similares aos exercícios convencionais, como melhora da capacidade cardiopulmonar em indivíduos com insuficiência cardíaca [77], do controle autonômico em hipertensos [78] e idosos com resistência a insulina [79], aumento da força muscular inspiratória em indivíduos com lesão medular [80], aumento da resistência dos músculos inspiratórios em pacientes com DM tipo 2 [81], melhora da sensibilidade à insulina em indivíduos com resistência insulínica [82] e do controle glicêmico em pequena amostra de indivíduos com DM [83].

Recentemente Kauwachi e colaboradores demonstraram melhora na distância de caminhada e aumento da força muscular inspiratória e periférica após o exercício com baixa e moderada intensidade [84]. Em pacientes hipertensos foi observado aumento na

força muscular inspiratória em 47%, redução dos níveis de pressão arterial e melhora no controle autonômico após o treinamento muscular inspiratório quando comparados com grupo placebo [78]. Em indivíduos com DM tipo 2, neuropatia autonômica cardiovascular e fraqueza da musculatura inspiratória, oito semanas de TMI com carga de 30% da PImáx determinou aumento de resistência e força dos músculos inspiratórios, o que não ocorreu no grupo controle, que fez a mesma intervenção, porém com carga placebo (2% da PImáx) [81].

Alguns autores sugerem que os benefícios do exercício inspiratório tanto agudo como crônico sobre o controle autonômico podem ser explicados pelo padrão respiratório que desencadeia mudanças no controle autonômico, sensibilidade barorreflexa e quimiorreflexa [85], quando ocorre um aumento do volume corrente a modulação vagal pode ser aumentada, enquanto a modulação simpática se mantém reduzida. A elevação da expansão torácica, através do aumento do volume corrente, melhora a oxigenação e consequentemente diminui a atividade quimiorreflexa, na qual está associada a redução da atividade nervosa simpática [86]. Autores sugerem que esse mecanismo poderia justificar os ajustes autonômicos cardiovasculares em resposta ao exercício muscular inspiratório [87-89].

Outra hipótese é que o aumento da força muscular inspiratória, em resposta ao exercício muscular inspiratório pode minimizar o efeito da ativação do metaborreflexo, através da melhora da capacidade oxidativa da musculatura inspiratória que gera diminuição do acúmulo de metabólitos resultando em menor atividade das fibras aferentes quimiosensíveis melhorando o desempenho físico [90]. Callegaro e colaboradores avaliaram a resposta do metaborreflexo em indivíduos treinados e sedentários. O estudo demonstrou que o metaborreflexo é atenuado em indivíduos treinados quando comparado com sedentários [91].

O exercício muscular inspiratório realizado de forma aguda tem demonstrado benefícios no controle autonômico. O estudo de Rodrigues e colaboradores demonstrou que uma sessão de exercício muscular inspiratório de baixa intensidade foi capaz de reduzir os valores pressóricos e a modulação simpática, enquanto um aumento na modulação vagal foi observado em jovens fumantes [87]. Esses resultados estão de acordo com o estudo de Archiza e colaboradores que avaliaram o controle autonômico com diferentes tipos de intensidades de carga inspiratória (30%, 60% e 80% da Pimax) em idosos saudáveis e demonstraram que cargas de intensidade baixa melhoram a modulação vagal cardíaca [92]. Quando o exercício inspiratório foi realizado durante sete

dias com a mesma carga dos estudos citados anteriormente, demonstrou aumento da modulação do sistema nervoso vagal e redução da modulação simpática em mulheres com síndrome metabólica [89]. Entretanto poucos estudos avaliaram o exercício inspiratório agudo de alta intensidade (80% da Pimax) sobre o controle autonômico e seus resultados são contraditórios [88, 92].

Entretanto, o benefício do TMI, em longo prazo, no controle autonômico em indivíduos com DM tipo 2 é controverso. No estudo de Kamininski e colaboradores foi observada redução da modulação simpática vascular nos indivíduos que fizeram o exercício inspiratório com carga de 30% da PImax, todos os dias por 30min durante oito semanas [93]. Por outro lado, não foi observada alteração na modulação autonômica cardíaca quando o mesmo protocolo de exercício (carga: 30% da Pimax, sessão: todos os dias, duração da sessão: 30min e tempo de intervenção: oito semanas) foi implementado em indivíduos com DM tipo 2 com neuropatia autonômica e fraqueza muscular inspiratória [81].

Recente revisão sistemática de ensaios clínicos randomizados e não randomizados avaliou o efeito do TMI no sistema nervoso autonômico cardiovascular, incluindo seis estudos. Observou-se, na maioria deles, que o TMI com carga de baixa intensidade se associa a diminuição da modulação simpática e aumento da modulação parassimpática cardíaca em pacientes hipertensos, diabéticos e com insuficiência cardíaca. Dois dos seis estudos não encontraram diferenças no controle autonômico após o TMI. Porém, como a revisão incluiu poucos estudos e o exercício da musculatura inspiratória foi realizado em diversas populações com diferentes protocolos, ainda não se pode concluir com certeza que haja efeitos benéficos do TMI sobre o controle autonômico cardiovascular [94].

A melhora da força muscular inspiratória, resultante do TMI, pode gerar adaptações metabólicas através da melhora da capacidade respiratória do diafragma e aumentar a translocação de GLUT 4 que consequentemente diminui a resistência à insulina e eleva a captação de glicose pelas células insulino-sensíveis [82, 95]. Estes efeitos podem ocorrer após exercícios agudos e se manter ao longo do treinamento [95, 96].

No entanto, a literatura é inconsistente quanto ao efeito do exercício muscular inspiratório nos níveis de glicose (Tabela 1). Um ensaio clínico não randomizado foi realizado por Dos Santos Silva e colaboradores em 14 idosos com resistência à insulina que foram submetidos ao TMI de 12 semanas com carga de 40% da Pimáx ou com uma carga mínima do treinador muscular inspiratório (carga placebo). A resistência à

insulina foi determinada através do HOMA-IR e foram coletadas glicemia e insulinemia. Após o treinamento, ocorreu redução da insulinemia, do HOMA-IR e da glicemia [82]. Posteriormente, o mesmo grupo publicou uma carta mostrando que em 38 idosos com hiperglicemia de jejum e sem DM, os níveis de glicose foram reduzidos após o treinamento no grupo experimental. O TMI foi realizado com a mesma carga e tempo de sessão do estudo descrito anteriormente, porém a duração do treinamento foi menor (oito semanas) [97].

Já o estudo de Ahamad e colaboradores submeteu mulheres com DM tipo 2 ao TMI com carga moderada de 30% da Pimáx. O exercício foi realizado cinco vezes por semana durante oito semanas, com tempo de sessão progressiva. Nenhuma alteração foi observada nos níveis de glicose após o final do período de treinamento [98]. Os primeiros dois estudos realizaram o TMI com mais tempo de sessão, número de sessões por semana e com carga mais alta do que no estudo de Ahmad e colaboradores, podendo explicar os resultados controversos. Por outro lado faltam estudos bem estruturados com delineamento adequado (como ensaios clínicos randomizados) para determinar se o TMI é eficaz para reduzir os níveis de glicose.

Em relação ao exercício muscular inspiratório agudo, foi realizado um estudo experimental em mulheres saudáveis e com síndrome metabólica, que foram submetidas ao exercício inspiratório com carga de 30% da Pimáx, e se observou que após sete sessões de exercício os níveis de glicose não se alteraram [89]. Neste estudo o curto período de exercício e a carga de baixa intensidade podem ter influenciado nos resultados não significativos dos níveis de glicose. Além disso, o estudo foi limitado a apenas mulheres não incluindo homens com e sem síndrome metabólica. Em um estudo piloto do nosso grupo, com seis indivíduos com DM tipo 2 que utilizaram o CGMS durante uma sessão de exercício muscular inspiratório, demonstramos redução abrupta de 40% nos níveis de glicose após exercício muscular inspiratório com carga alta resistência (60% da PImáx) [83]. Além disso, no estudo de Correa, indivíduos com DM tipo 2 foram submetidos a duas modalidades de exercício agudo, inspiratório e os convencionais (aeróbico e combinado), e demonstrou-se reduções na glicemia de 24% após o exercício muscular inspiratório, 25% depois do exercício aeróbico e 11% após o exercício combinado. Além disso, redução da variabilidade glicêmica ocorreu também após as sessões agudas de exercício aeróbico e inspiratório [99]. Apesar desses benefícios observados sobre o controle glicêmico em DM tipo 2 ambos os estudos foram realizados com pequeno número de pacientes e em protocolo em que outras

intervenções foram aplicadas no mesmo dia o qual podem ter influenciado nesses resultados.

Tabela 1: Estudos de exercício crônico e agudo da musculatura inspiratória

Autor/ data	**Amostra**	**Tipo de publicação**	**Intensidade (carga)**	**Tempo da sessão de exercício**	**Tempo de intervenção**	**Resultados**
Estudos de exercício muscular inspiratório crônico sobre os níveis de glicose						
Ahamad et al, 2017	28 Mulheres com DM2	Artigo	14 TMI (30% da Pimax) 14 Controle	5x/semanas Primeiras 2 semanas (5 séries de 20 respirações) Próximas 3 semanas (5 séries de 30 respirações- 15-20min) Últimas 3 semanas (8 séries de 30 respirações- 25-30min)	8 semanas	Não houve alterações nos níveis de glicose, HOMA-IR.
Dos santos silva et al, 2015	38 idosos com hiperglicemia	*Short-comunication*	18 TMI (40% da Pimax) 20 Controle (menor carga do Threshold)	7x/semanas por 30min	8 semanas	Reduziu nível de glicose e HOMA-β no grupo experimento após as 8 semanas.
Dos santos silva, 2012	14 idosos com resistência a insulina	Artigo	TMI (40% da Pimax) Controle (sem carga)	7x/semanas por 30min	12 semanas	Reduziu glicose, insulina e HOMA-IR no grupo TMI com carga.
Estudos de exercício muscular inspiratório agudo sobre os níveis de glicose						
Feriani et al, 2017	16 indivíduos com SM 12 sem SM	Artigo	30% da Pimax	7 dias (3X 15 min)	7 dias	Não houve alterações nos níveis de glicose após o EMI.
Correa et al, 2015	6 DM2 (4 com NAC e 2 sem NAC) 14 DM2	Carta	EMI (60% da Pimax) Controle (2% da Pimax) Aeróbico (70% do pico de FC)	1 sessão - EMI: [5min basal, 3min VC, EMI (2% por 5 minutos ou 60% até exaustão diafragmática), hangrip)]. Teve uma semana de washout e o protocolo se repetiu. - Aeróbico [bicicleta 40 min]	1 sessão	Reduziu níveis de glicose 24%, 11% e 25% após uma sessão de aeróbico, combinado e EMI com 60% da Pimax, respectivamente.

			Combinado (70% do pico de FC e 65% de 1RM).	- Combinado [20 min de bicicleta e 4 exercícios de força 3 séries de 12 repetições]		Reduziu VG após aeróbico e EMI 60% da Pimax.
Correa et al, 2015	6 DM2 (4 com NAC e 2 sem NAC)	Estudo piloto	EMI (60% da Pimax) Controle (2% da Pimax)	1 sessão [5min basal, 3min VC, EMI (2% por 5 minutos ou 60% até exaustão diafragmática), hangrip)]. Teve uma semana de washout e o protocolo se repetiu.	1 sessão	Reduziu os níveis de glicose após a sessão com 60% da Pimax.

DM2, diabetes mellitus tipo 2; TMI, treinamento muscular inspiratório; EMI, exercício muscular inspiratório; HOMA-IR, *homeostatic model assessment- insulin resistance* ; SM, síndrome metabólica; Pimax, pressão inspiratória máxima; HOMA-β, *homeostatic model assessment- beta* ; NAC, neuropatia autonômica cardiovascular; VC, ventilação controlada; FC, frequência cardíaca; VG, variabilidade glicêmica; RM, repetição máxima.

Justificativa e Objetivos

O efeito do exercício muscular inspiratório sobre a glicose ainda não está definido. A literatura reporta poucos estudos, com diferentes tipos de protocolo de exercícios inspiratórios (intensidade da carga, frequência de exercício e duração), com situações clínicas diversas. É necessária a realização de estudo especificamente desenhado para testar a hipótese de que o exercício agudo da musculatura inspiratória reduz a glicose em pacientes com DM tipo 2. É possível que este efeito se relacione a alterações do controle autonômico cardiovascular induzido pelo exercício. Assim, este estudo pretende avaliar o efeito de uma sessão de exercício muscular inspiratório de alta intensidade sobre os níveis de glicose, variabilidade glicêmica e controle autonômico cardiovascular em indivíduos com DM tipo 2.

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ARTIGO 1

Publicado no Trial

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STUDY PROTOCOL

Are glucose levels, glucose variability and autonomic control influenced by inspiratory muscle exercise in patients with type 2 diabetes? Study protocol for a randomized controlled trial

ClinicalTrials.gov Identifier: NCT02292810.

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Abstract

Background: Physical exercise reduces glucose levels and glucose variability in patients with type 2 diabetes. Acute inspiratory muscle exercise has been shown to reduce these parameters in a small group of patients with type 2 diabetes, but these results have yet to be confirmed in a well-designed study. The aim of this study is to investigate the effect of acute inspiratory muscle exercise on glucose levels, glucose variability, and cardiovascular autonomic function in patients with type 2 diabetes. Methods/design: This study will use a randomized clinical trial crossover design. A total of 14 subjects will be recruited and randomly allocated to two groups to perform acute inspiratory muscle loading at 2 % of maximal inspiratory pressure (PImax, placebo load) or 60 % of PImax (experimental load). Discussion: Inspiratory muscle training could be a novel exercise modality to be used to decrease glucose levels and glucose variability.

Keywords: breathing exercises, exercise, glucose, type 2 diabetes.

Background

Hyperglycemia is the main cause of the clinical manifestations and acute and chronic complications of diabetes mellitus. Reducing glucose levels through nonpharmacological [1, 2] and pharmacological therapy is the cornerstone of type 2 diabetes management [3, 4]. Goals for optimal diabetes management include reducing glycated hemoglobin (HbA1c) levels to 7% and implementing a number of cardiovascular risk reduction strategies [5, 6].

However, chronic complications of diabetes can develop even when these targets are met. This can be attributed to genetic and epigenetic factors [7], disruption of repair mechanisms [8], and, possibly, high glucose variability even when optimal HbA1c levels are achieved. Glucose variability is the fluctuation of glucose levels during the day, including episodes of hypoglycemia and hyperglycemia [9]. Measurement of glucose levels in intersticium throughout the day by continuous glucose monitoring systems [10, 11] or periodic capillary blood-glucose measurements [12, 13] have been used to evaluate glucose variability. Several conventional measures are available to quantify this parameter and have been widely reported in the literature over the past years [14, 15], including the mean amplitude of glycemic excursions [16], the standard deviation of glucose, and the mean of daily differences [17]. Non-conventional methods, which use mathematical and statistical analysis of the dynamic characteristics of glucose levels, as measured using continuous glucose monitoring systems, through spectral analysis and symbolic analyses, were recently reported [18].

Exercise training, consisting of aerobic exercise, resistance exercise, or a combination of both, is a useful tool for reducing HbA1c levels in type 2 diabetes [2, 19, 20]. It is also associated with several other benefits, including improved blood

pressure control [21], reduction of skeletal muscle frailty [22], reduction of inflammation [23], and improved cardiovascular autonomic function [24]. Additionally, a recent study showed that an acute bout of aerobic or resistance exercise is associated with lower glucose variability [18], opening a new venue of benefits to explore.

Considering that many diabetic patients, especially those with autonomic neuropathy, have poor exercise tolerance or are completely unable to perform conventional exercises [25], alternatives should be sought. Inspiratory muscle training is a modality that has proven benefits in several clinical situations [26, 27], such as improving cardiopulmonary capacity and normalizing derangements in autonomic function [27, 28]. In patients with type 2 diabetes, this exercise modality can improve the strength and resistance of inspiratory muscles [29] and reduce glucose levels and glucose variability [30]. We speculate that, in type 2 diabetes, beyond reflecting the well-known improvement in insulin sensitivity induced by muscle contractions, inspiratory exercise would also yield improvements in cardiovascular autonomic function [27, 28]. Thus, the aim of this study is to investigate the effect of acute inspiratory muscle exercise on glucose levels, glucose variability, and autonomic control in patients with type 2 diabetes.

Methods/design

Study design

This study is a randomized clinical trial with a crossover design.

Outcome measures

The difference in glucose levels before and after exercise (evaluated by continuous glucose monitoring systems; glucose level after exercise minus glucose level

before exercise) is the primary outcome measure. The secondary outcome measures are: (1) changes in glucose variability, which will also be evaluated by continuous glucose monitoring systems through conventional (mean amplitude of glucose excursion, variation of glucose, coefficient of variation of glucose, and standard deviation of glucose) and non-conventional indices (spectral analysis and symbolic patterns: no variation); (2) changes in cardiovascular autonomic control, which will be evaluated by blood pressure variability and heart rate variability (measures of power spectral density of low frequency, high frequency and very low frequency bands). Table 1 shows the measures that will be evaluated to achieve the desired results.

Sample size and power calculation

According to preliminary results obtained in our laboratory [31], the sample size was estimated in 14 individuals with type 2 diabetes, allowing for a dropout rate of 10 %. This sample size would detect a 34 mg/dl difference in glucose levels between the intervention inspiratory load of 60 % and the placebo inspiratory load of 2 %, taking into account a standard deviation of 30 mg/dl, a statistical power of 80 %, and a type I error rate of 5 %. WinPepi software was used to calculate the sample size.

Inclusion and exclusion criteria

The study will be conducted in patients with type 2 diabetes, diagnosed according to the National Diabetes Data Group criteria [5]. The inclusion criteria will be age 30 years and older, HbA1c between 7.5 and 10 %, and stable clinical condition, to ensure safe performance of the trial protocols. Patients will be recruited from the Outpatient Endocrinology Clinic of Hospital de Clínicas de Porto Alegre, Brazil. All study methods described are in accordance with the CONSORT Statement [32].

The exclusion criteria will consist of: insulin treatment, pregnancy, pulmonary disease (history of exercise induced asthma), cardiac arrhythmias, chronic kidney disease (glomerular filtration rate <30 ml/min), exclusive use of beta blockers as antihypertensive therapy, current smoking, varicose veins, and musculoskeletal conditions that would hinder safe completion of the proposed exercise protocols.

Randomization

The randomization sequence will be generated by computer. Randomization to the low-resistance inspiratory muscle exercise group (2 % of maximal inspiratory pressure (PImax), placebo load) or the high-resistance inspiratory muscle exercise group (60 % of PImax, experimental load) will be performed by an investigator assigned exclusively to this task, who will not participate in the recruitment, assessment, or intervention phases of the study.

Data collection

Patients will be instructed to attend on three different days at the study laboratory for baseline evaluations, as follows:

Day 1: Urine collection and fasting blood draw for assessment of glucose, HbA1c, creatinine, and microalbuminuria; physical examination; Ewing tests for autonomic assessment; and completion of the International Physical Activity Questionnaire.

Day 2: Resting 12-lead electrocardiogram, inspiratory muscle and pulmonary function tests.

Day 3: Cardiopulmonary exercise testing

Autonomic neuropathy evaluation

The presence of cardiovascular autonomic neuropathy will be evaluated using digital electrocardiography (VNS-Rhythm Neurosoft, Ivanovo, Russia) as previously described [33] and the five noninvasive cardiovascular tests proposed by Ewing [34], as standardized in our institution [35]. The heart rate, electrocardiographically monitored, will be evaluated after 5 min of resting and before and after deep breathing, the Valsalva maneuver, and standing. Three tests will be used to evaluate the heart rate response to (1) deep breathing, (2) lying-tostanding heart rate ratio, and (3) a Valsalva maneuver. Two tests of blood pressure control will be performed during (1) orthostatic hypotension and (2) sustained handgrip. Two or more abnormal test results will be deemed diagnostic of cardiovascular autonomic neuropathy. Heart rate variation will be assessed while patients are asked to breathe deeply at a rate of six breaths per minute while being monitored using three-lead electrocardiography. The maximum and minimum heart rate during each breathing cycle will be measured, and the mean difference over six cycles will be calculated. The heart rate ratio test will be performed after a period of rest in the supine position, and heart rate variability will be determined by calculating the maximal to minimal heart rate ratio: the ratio of the longest RR interval, measured around the 30th beat after standing up, to the shortest RR interval, measured around the 15th beat after standing up. The Valsalva maneuver will consist of forced exhalation into a mouthpiece with a pressure of 40 mmHg for 15 s, and the ratio of the maximum RR interval after this maneuver to the minimum RR interval during the maneuver will be calculated. Orthostatic hypotension will be defined as a decrease of 30 mmHg in systolic blood pressure when the individual voluntarily changes from the supine to the standing position. Measurements will be obtained at minutes 1 and 3 after standing up. Sustained muscle contraction will be measured by a handgrip dynamometer. The

dynamometer will be first squeezed to maximum isometric contraction in the dominant hand. The highest of three measurements will be taken; then patients will be asked to hold at 30 % maximum for 3 min. A rise in diastolic blood pressure of<10 mmHg is considered an abnormal response.

Inspiratory muscle testing

Inspiratory muscle function testing will be performed using a pressure transducer (MVD-500 V.1.1 Microhard System; Globalmed, Porto Alegre, Brazil), connected to a system containing two one-way valves (DHD Inspiratory Muscle Trainer, Chicago, IL, USA) and equipped with a 2 mm diameter orifice to relieve facial muscle pressure. Patients will be instructed to remain seated, with elbows resting on the chair; a nasal clip will be used during all maneuvers. The PImax will be determined after patients take a deep breath at tidal volume, followed by slow exhalation to residual volume, and then quickly perform a maximal inspiration against the occluded circuit with a small air leak. The test will be repeated at least three times, with a 1 min interval between repetitions, to obtain six measurements with <10 % variation [29, 36, 37]. The values obtained from the PImax readings will be verified directly on the transducer. Predicted values will be corrected for age and sex.

Pulmonary function

Measurements of forced vital capacity, vital capacity, forced expiratory volume in 1 s, and their ratios (forced expiratory volume (1 s)/vital capacity and forced expiratory volume (1 s)/forced vital capacity) will be obtained using a computerized spirometer (Eric Jaeger GmbH, Würzburg, Germany), as recommended by the AmericanThoracic Society [38], and results will be expressed as a percentage of

predicted parameter. For maximum voluntary ventilation, subjects will be instructed to breathe as deeply and quickly as possible for 15 s; volume measurement will begin after the patient is able to achieve and maintain maximum effort [38].

Cardiopulmonary exercise testing

Maximal functional capacity will be assessed by means of an incremental exercise test to be performed on a treadmill (Inbramed 10200, Porto Alegre, Brazil), using a ramp protocol with a speed of 2–6 km/h and increasing slope of 4–10 % to reach exhaustion in about 10 min [36]. A 12-lead electrocardiogram tracing will be obtained every 1 min (Nihon Khoden Corp., Tokyo, Japan), and blood pressure will be measured every 2 min with a standard cuff sphygmomanometer. During the test, gas exchange variables will be measured breath-by-breath with a previously validated system (Metalyzer3B, CPX System; Cortex, Leipzig, Germany). Ventilatory and metabolic variables will be analyzed throughout the test. Peak oxygen uptake (VO2peak) will be defined as the highest value reached during the test.

Study intervention

After baseline evaluations, all patients will be instructed to present to the laboratory, three times per week for 2 weeks, for completion of the intervention stage of the study protocol, as follows:

Day 1: Placement of the continuous glucose monitoring system. Patients should arrive at 8:00 a.m. for device placement. Patients using beta blockers will be advised to discontinue their medication 24 hours before the next study visit.

Day 2: Acute inspiratory muscle exercise protocol (2 % or 60 % of PImax). Patients should arrive at 10:00 a.m. and will be instructed to rest in the supine position

for 10 min for collection of baseline glucose, continuous blood pressure, and heart rate measurements. Participants will then start controlled ventilation for 10 min. A 40-minute interval will be allowed to elapse before the start of acute inspiratory muscle loading. Pursuant to random group allocation, participants will receive loading to 2 % of PImax (low resistance, placebo load) for 10 min or to 60 % of PImax (high resistance, experimental load) until task failure. At the end of the exercise protocol, a 10-min recovery record will be obtained. Glucose levels will also be measured by capillary sample before and after the protocol reported above. Arterial oxygen saturation (SpO2) mean arterial pressure, end-tidal carbon dioxide, calf blood flow, and calf vascular conductance will be measured during all protocols.

Day 3: Continuous glucose monitoring system removal. Patients will be instructed to return to the laboratory at noon for removal of the device.

One week following these interventions, all subjects will return to the study facility to repeat these procedures, but using another randomized load for inspiratory muscle exercises. The experimental sessions will take place at the Hospital de Clínicas de Porto Alegre Exercise Pathophysiology Research Laboratory. All experiments will be performed in a temperature-controlled room and all subjects will be instructed to refrain from consuming caffeinated beverages and alcohol for at least 12 hours and from exercise for at least 24 hours prior to the evaluation. Continuous assessment of blood glucose levels and blood pressure will be performed before, during, and after all protocols. Patients will be asked to follow their habitual diet and keep a detailed food intake record across the 3 days of continuous glucose monitoring system use.

Glucose variability evaluation

Subjects will be admitted to the laboratory at 8:00 a.m., 24 hours before the acute inspiratory muscle exercise session, for placement of the continuous glucose monitoring system sensor (Enlite Glucose Sensor, Medtronic MiniMed Inc., Northridge, CA, USA). Glucose data are obtained every 5 min, providing a total of 288 readings per day. Individuals will be instructed to perform at least four capillary blood-glucose measurements per day, before each meal, for calibration. The monitoring will be performed by the patients themselves, with a digital glucometer (Accu-Check Performa, Roche Diagnostics, Mannheim, Germany). Along with the glucometer, diaries will be delivered to patients, in which they should record the glucose values at each measurement and also details of all meals ingested during the period of continuous glucose monitoring. Glucose variability will be evaluated using conventional and non-conventional analyses. Conventional analysis will be conducted using the statistical properties of the glycemic series classically used [16, 39, 40] and nonconventional analysis of glucose variability will be conducted by application of two methods to the glucose series, based on spectral analysis and symbolic dynamics. Spectral analysis is a linear method that allows quantification of the oscillatory components from a time series by autoregressive models, and is widely applied to heart rate and arterial pressure series [41]. Symbolic dynamics relies on the calculation of Shannon entropy of the distribution of patterns lasting three measures and the classification of frequent deterministic patterns lasting three measures, and distributes deterministic patterns of the group into four variations [42].

Cardiovascular autonomic control evaluation

Cardiovascular autonomic control will be measured continually before the protocol (during a 10min rest), during the controlled ventilation plus acute inspiratory

muscle exercise protocol (2 % or 60 % of PImax), and throughout the recovery period, using a noninvasive blood pressure device (NIBP100D system; Biopac, Santa Barbara, CA, USA) with a general purpose amplifier module (DA100; Biopac), according to methodological guidelines provided by Sherwood et al. [43]. This system acquires the blood pressure wave at a sampling frequency of 1000 Hz through a cuff placed on the middle phalanx of the third finger. Beat-to-beat time series of systolic blood pressure and pulse interval will be constructed from blood pressure recording data; their variances correspond to heart rate variability and blood pressure variability, respectively. In addition, spectral analysis, using an autoregressive model, will be applied to estimate the center frequency and power of each relevant oscillatory component [41]. The spectral bands for human beings are defined as: very low frequency, 0.0 to 0.04 Hz; low frequency, 0.04 to 0.15 Hz; and high frequency, 0.15 to 0.40 Hz, as described elsewhere [41].

Controlled ventilation

Individuals will be placed in the supine position for measurement of respiratory rate, heart rate, SpO2 by pulse oximetry, and mean arterial pressure, which will be measured using an automated sphygmomanometer (Dinamap 1846 SX/P, Critikon, Tampa, FL, USA) once per minute during the test. The glucose and blood pressure, calf blood flow (which will be measured by venous occlusion plethysmography (Hokanson TL-400, Bellevue, WA, USA)) will be recorded continuously.

Inspiratory volume will be assessed using a pneumotachograph (Pneumotach3700 series, Hans Rudolph, Kansas City, MO, USA). The end-tidal carbon dioxide concentration will be measured by oxycapnography (Takaoka Oxicap, São Paulo, Brazil) and maintained at eupneic levels during both protocols via addition of

CO2 to the inspiratory circuit. Participants will be instructed to maintain a breathing frequency of 15 breaths per min and a duty cycle (TI/TTot) of 0.7 by listening to an audio signal with distinct inspiratory and expiratory tones, and visual feedback will be displayed.

Acute inspiratory muscle exercise

This protocol was developed by Dempsey et al. [44] to reduce diaphragm blood flow and cause fatigue. During the protocol, individuals will breathe 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 (DHDInspiratory Muscle Trainer, Chicago, IL) or, for higher inspiratory pressures (60 % of PImax), to a POWERbreathe® inspiratory muscle trainer (Southam, UK). Inspiratory pressure will be continuously recorded and displayed on a computer screen shared through a television, providing visual feedback; the 10-point Borg scale [45] will be used to access inspiratory effort at task failure. Inability to maintain breathing will be defined as a reduction of PImax to less than 80 % of the prescribed value during three consecutive breaths [38]. For the placebo experiments conducted at 2 % of PImax, measurements will cease at 10 min. The sequence of experiments, induction of the inspiratory muscle metaboreflex (60 % of PImax), or allocation to the placebo experiment (2 % of PImax), will be randomized, and experiments will be conducted 1 week apart.

In both protocols, baseline data will be collected during 10 min of spontaneous breathing. After this period, a controlled ventilation protocol will be initiated, and individuals instructed to maintain a breathing frequency of 15 breaths per min and a duty cycle (TI/TTot) of 0.7 by listening to an audio signal with distinct inspiratory and

expiratory tones for 10 min. Calf blood flow will be measured by venous occlusion plethysmography (Hokanson, TL-400, Bellevue, WA, USA) every 10 s in the non-dominant lower limb. Calf vascular conductance will be calculated as calf blood flow divided by mean arterial pressure [46]. Blood pressure will be monitored once a minute using an automated monitor (Dinamap, USA), and heart rate will be monitored continuously throughout the protocol.

Ethics and data protection issues

Participation will be voluntary, and all ethical principles of confidentiality and data protection will be followed. All subjects will have to sign a standardized informed consent form prior to participating in the study. All procedures will be explained to participants, and information about the aim, design, potential risks and benefits, and all relevant details of the study will be provided in the informed consent form. The data obtained by the study will be available to the participant and to any other authorized person, and may be used anonymously for academic scientific purposes. The study protocol was approved by the Institutional Review Board of Hospital de Clínicas de Porto Alegre, Brazil (number 14–0194) and registered at ClinicalTrials.gov (NCT02292810).

Safety assessment

Data on all adverse events will be collected and included in medical reports, which will be forwarded to the health authorities. To ensure patient safety, if any adverse events occur at any time during the study, participants will be monitored for up to 4 weeks after study exit.

Statistical analysis

Data will be expressed as means and standard deviations or medians and interquartile ranges for nonparametric variables. Two-way analysis of variance (ANOVA) for repeated measures will be used to evaluate averages before and after acute inspiratory muscle exercise, comparing the placebo (2 % of PImax) and intervention (60 % of PImax) loads. Pearson correlation coefficients will be used to evaluate the association between components of cardiovascular autonomic control and glucose levels. For nonparametric variables, Spearman correlation will be used. The statistical power will be 80 % and differences will be considered significant for $P<0.05$. All analyses will be performed using SPSS Statistics for Windows, version 20.0 (IBM Corp., Armonk, NY, USA).

Discussion

Exercise programs have traditionally emphasized aerobic exercise, because of its well-known cardiovascular [47], metabolic [48], and autonomic benefits in type 2 diabetes [24, 49]. Resistance training also has favorable effects on muscle strength, abdominal fat, insulin sensitivity [22], and blood pressure [21]. Furthermore, combined exercise is also beneficial for metabolic parameters [19, 20] and glucose variability in subjects with type 2 diabetes [18]. However, as not all patients tolerate conventional exercise and some find it difficult to adhere to fitness programs, alternatives must be developed to enable these patients to obtain the benefits of physical exercise. Inspiratory muscle training is a novel exercise modality that has demonstrated impressive beneficial effects on inspiratory muscle strength and resistance [29, 50] and in reduction of glucose variability [30]. However, given the relatively small sample size of our preliminary study [30], we planned to examine this question in greater depth through a

new study with a larger sample of patients with type 2 diabetes, seeking to confirm that a single bout of inspiratory muscle exercise may provide a benefit similar to that achieved with aerobic exercise on glucose levels and glucose variability. In brief, this study will be conducted to evaluate glucose levels, glucose variability, and cardiovascular autonomic responses at rest, during, and after a session of acute inspiratory muscle exercise performed under two resistance loads (low, 2 % of PImax; high, 60 % of PImax).

Trial status

The trial is currently in the recruitment phase. Each patient is scheduled to attend nine hospital visits, and the total data collection time will be 10 months.

Abbreviations

ANOVA: analysis of variance; CONSORT: Consolidated Standards of Reporting Trials; HbA1c: glycated hemoglobin; PImax: maximal inspiratory pressure; SpO2: oxygen saturation; VO2peak: peak oxygen uptake.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

ASOS coordinated the recruitment. She participated in the sequence alignment, and drafted the manuscript. APSC participated in the sequence alignment, will perform the statistical analysis and drafted this manuscript. KRC participated in the sequence alignment and will participate in data analysis procedures. She reviewed and approved

this manuscript. BDS is the main investigator of the study. She participated in the coordination of all tasks, will participate in data analysis procedures, and reviewed and approved the manuscript. All authors read and approved the final manuscript.

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Table 1: Evaluated measures

Time Point	Data Collection	Measure	Outcomes
Pre-Evaluation	Blood / urinary collection	Glycemia, HbA1c, microalbuminuria/creatine	Metabolic responses
	Cardiovascular autonomic assessment	Ewing tests	Diagnosis of CAN
	Questionnaire IPAQ	Survey	Physical activity estimates
	Inspiratory muscle test	PImax	Inspiratory muscle strength
	Pulmonary function test	MVV, FVC, VC, FEV1, FEV1/VC and FEV1/FVC	Pulmonary flow and volume
	Rest electrocardiogram	12-lead ECG tracings	Electrical activity of the heart
	Cardiopulmonary exercise testing	VO_{2peak}, VCO_{2peak}, R_{peak}, VE_{peak}, VE/VO_{2peak}, VE/VCO_{2peak}	Ventilatory and metabolic variables
At Rest	Continuous glucose monitoring system		Glucose levels and variability
	Biopac System	Glucose Value	BP and HRV in time and frequency domains
		Continuous recording of BP	
Controlled Ventilation	Continuous glucose monitoring system	Glucose Value	Glucose variability
	Biopac System	Continuous recording of BP	BP and HRV in time and frequency domains

Inspiratory Muscle Exercise (2% or 60% of PImax)	Continuous glucose monitoring system	Glucose Value	Glucose levels and variability
	Biopac System	Continuous recording of BP	BP and HRV in time and frequency domains
Recuperation	Continuous glucose monitoring system	Glucose Value	Glucose levels and variability
	Biopac System	Continuous recording of BP	BP and HRV in time and frequency domains

HbA1c: glycated hemoglobin; IPAQ: International Physical Activity Questionnaire; PImax: inspiratory mouth pressure; CAN: cardiovascular autonomic neuropathy; MVV: maximum voluntary ventilation; FVC: forced vital capacity; VC: vital capacity, FEV1: forced expiratory volume in 1 s; FEV1/VC: relationships of forced expiratory volume in 1 s and vital capacity; FEV1/FVC: relationships of forced expiratory volume in 1 s and forced vital capacity; BP: blood pressure; HRV: heart rate variability; VO2 Peak: peak oxygen uptake; VCO2peak: carbon dioxide output; R: RER; VE: minute ventilation; VE peak: ; VE⁄VO2: ventilatory equivalent for oxygen; VE⁄VCO2: ventilatory equivalent for carbon dioxide.

ARTIGO 2

Acute inspiratory muscle exercise effect on glucose levels, glucose variability and autonomic control in patients with type 2 diabetes: A Crossover Randomized Trial

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Abstract

Inspiratory muscle exercise (IME) is an alternative that has demonstrated benefits in type 2 diabetes. This study aimed to evaluate the effect of IME on glucose levels, glucose variability, and autonomic cardiovascular control in patients with type 2 diabetes. Fourteen diabetic subjects were randomly assigned to IME with 2% maximal inspiratory pressure (PImax) or 60% PImax and they wore a continuous glucose monitoring system for three days. Glucose variability [glucose variance (VAR), glucose coefficient of variation (CV%), glucose standard deviation (SD), mean amplitude of glycemic excursions (MAGE)] were evaluated. Both protocols demonstrated a glucose reduction in 5 min (60% of PImax 33.2% and 2% of PImax 32%), 60min (60% of PImax 29.6% and 2% of PImax 31.4%) and 120 min (60% of PImax 21.4% and 2% of PImax 24%) after IME (*vs.*1h before the exercise), with no difference between loads. This reduction in glucose levels was observed in all moments of the IME protocol. Glucose variability was reduced after 12h and 18h of the IME (ΔCV: $P<0.001$, ΔSD: $P<0.001$ and ΔVAR: $P<0.001$) in both loads. No difference was found in MAGE ($P=0.594$) after IME. Moreover, the vagal modulation was lower during exercise session with 60% of PImax while blood pressure variability was increased. Inspiratory muscle exercise with 60% of PImax fails to demonstrate improvement in glucose levels and glycemic variability, when compared to 2% of PImax exercise session. Only the exercise session with 60% of PImax determined changes in autonomic modulation.

Keywords: breathing exercises, exercise, glucose, type 2 diabetes

Introduction

Diabetes mellitus is a chronic metabolic illness with expanding rates over the last decades. Currently, the worldwide prevalence of this disease is 425 million people and predictions indicate an increase in incidence of 63.1% until 2045 [1] . The cornerstone of diabetes management relies on accurate glycemic control, which is usually monitored through glycated hemoglobin (HbA1c) levels in order to prevent chronic complications. Another component that could be involved in the pathogenesis of diabetic chronic complications is glucose level fluctuation [2]. This relation was also suggested in clinical studies, linking higher glucose fluctuations to the development of microvascular diabetic complications [3-5]. Moreover, blood glucose variability was associated with increased risks of microvascular events, macrovascular events and all-cause death [6]. However, other reports showed that blood glucose variability does not appear to be an additional factor in the development of microvascular [7] or macrovascular [8, 9] complications.

Exercise training, consisting of aerobic exercise, resistance exercise, or a combination of both is a useful tool for reducing HbA1c levels in type 2 diabetes [10]. Other benefits, such as improved blood pressure control [11], and improved cardiovascular autonomic function [12] are expected. Moreover, we and others have shown that aerobic or resistance exercise are associated with lower glucose variability [13-15] which could be beneficial in the long term treatment of patients.

Notwithstanding many evidences indicating that structured exercise has an important role for type 2 diabetes treatment, not all patients tolerate conventional exercise, or even present difficulty in adhering to exercise programs. Inspiratory muscle training is an exercise alternative that has demonstrated to increase strength and endurance of inspiratory muscles [16, 17] in type 2 diabetes. Ahmad et al showed that

glucose levels did not change after inspiratory muscle training to 30% of maximal inspiratory pressure (PImax) [18]. On the other hand, this exercise modality with inspiratory load of 40% of PImax promoted decreased fasting glucose in older adults with fasting hyperglycemia [19] and improvement in insulin sensitivity in individuals with insulin resistance [20]. Preliminary data of our group indicated that acute inspiratory muscle exercise (IME) with load 60% of PImax could decrease glucose levels and glucose variability in a similar magnitude as aerobic exercise in type 2 diabetes [21]. We hypothesized that IME reduces blood glucose in patients with DM, this effect may possibly be linked to changes in cardiovascular autonomic control induced by the exercise. Therefore, the purpose of this study is to evaluate the effect of acute inspiratory muscle exercise on glucose levels, glucose variability, and autonomic cardiovascular control in patients with type 2 diabetes.

Methods

Study design/Participants

This study is a double blind crossover randomized clinical trial previously described in detail [22]. Type 2 diabetes patients were recruited from the Endocrinology Outpatient Clinic at Hospital de Clínicas de Porto Alegre, Brazil and through website posting. Subjects were included if they at least were 30 years old, and had an HbA1c higher than 7.5% but lower than 10%. Individuals were excluded if presented pulmonary disease (history of exercise induced asthma), cardiac arrhythmias, chronic kidney disease (glomerular filtration rate <30 ml/min), insulin treatment, pregnancy, exclusive use of beta-blockers as antihypertensive therapy, current smoking, varicose veins, and musculoskeletal conditions that would hinder the safe completion of the proposed exercise protocols. The study was approved by the Ethics Committee of Hospital de Clínicas de Porto Alegre (Brazil), and was registered at clinicalTrials.gov

under the identifier NCT02292810. All patients that accepted to participate in the study signed an informed consent before beginning the study.

Data collection

Participants were submitted to baseline evaluations on three different days. Clinical characteristics, Ewing tests, usual physical activity (International Physical Activity Questionnaire IPAQ), fasting blood samples (HbA1c, glucose and creatinine) and urine collection (albuminuria) were done on the first day: HbA1c was analyzed by ion-exchange HPLC (Merck-Hitachi L-9100 HbA1c analyzer; Merck, Darmstadt, Germany) and plasma glucose was analyzed by the glucose oxidase method. Glomerular filtration rate was calculated using the Modification of Diet in Renal Disease equation (MDRD), and serum creatinine by Jaffé's reaction. Urinary albumin excretion was measured by immunoturbidimetry (MICROALB-AMES Kit, CA, USA). Albuminuria was defined as values higher than 17 mg/dl of albumin in urine. On the second day, resting electrocardiogram, inspiratory muscle and pulmonary function tests were evaluated. On the third day, individuals underwent cardiopulmonary exercise testing.

Autonomic neuropathy evaluation (Ewing test)

Diabetic cardiovascular autonomic neuropathy was assessed by five noninvasive cardiovascular reflex tests: deep breathing, stand up (30:15 ratio), Valsalva maneuver, orthostatic hypotension and sustained handgrip, as proposed by Ewing and standardized in our institution. All tests were evaluated using a digital electrocardiography with a specific software (VNS-Rhythm Neurosoft, Ivanovo, Russia) and diagnostic of cardiovascular autonomic neuropathy (NAC) was defined when two or more abnormal tests were found [23, 24].

Inspiratory muscle testing

The PImax was assessed by a pressure transducer during deep breath from residual volume against an occluded airway with a minimum air lack (2mm) to relieve facial muscle pressure. PImax was determined by the highest value among six measurements with <10% variation [25, 26].

Pulmonary function

Pulmonary function was measured with a computerized spirometer (Eric Jaeger GmbH, Würzburg, Germany); the measurement of forced vital capacity (FVC), vital capacity (VC), forced expiratory volume in 1 s (FEV_1) and maximal voluntary ventilation (MVV) were obtained according to recommendations of the American Thoracic Society [27].

Cardiopulmonary exercise testing

Maximal functional capacity was evaluated on a treadmill (Inbramed 10200, Porto Alegre, Brazil) with incremental exercise test using a ramp protocol. The electrocardiogram (Nihon Khoden Corp, Tokyo, Japan) was assessed every 1 minute, and blood pressure values were obtained every 2 minutes. Gas exchange variables were assessed breath-by-breath by a previously validated system (Metalyzer 3B, CPX System; Cortex, Leipzig, Germany) [25].

Study intervention

The intervention was performed during three consecutive days on the first week, it was repeated following week, as follows: day 1: placement of the glucose sensor to start evaluation at 9:10 a.m (CGMS), day 2: IME with placebo load (2% of PImax) or experimental load (60% of PImax) to start at 9:52 a.m and day 3: glucose sensor removal.

In the study intervention patients were submitted to rest for 10 min. Then, patients started controlled ventilation for 10 min and they breathed at 15 breaths per min with a duty cycle (TI/TTot) of 0.7. Shortly after, there was a 40-minute interval, when the

individuals started exercise breathing in a two-way valve (Hans Rudolph, 2600 series, Shawnee, KS, USA) against a 2% of PImax through threshold inspiratory muscle trainer (DHDInspiratory Muscle Trainer, Chicago, IL) for 10 min or 60% of PImax through a POWERbreathe® inspiratory muscle trainer (Southam, UK) until task failure (approximately 7.5 min). During both IME, patients maintained the breathing pattern (15 breath per min and TI/TTot: 0.7) and were guided by audio and visual feedback. At the end of the IME protocol, a 10-min recovery record was obtained. The respiratory and hemodynamics parameters were evaluated during the entire intervention; measurement of arterial oxygen saturation (SpO2), respiratory rate (f_b), heart rate and end-tidal partial pressure of CO2 (PetCO2) were obtained through oxycapnography (Takaoka Oxicap, São Paulo, Brazil). Glucose levels, continuous blood pressure, mean arterial blood pressure (MAP) (Dinamap, USA) and calf blood flow (CBF) were also evaluated (venous occlusion plethysmography; Hokanson, TL-400, Bellevue, WA,USA). Calf blood resistance (CVR) was calculated as MAP/CBF.

Glucose variability evaluation

All patients were monitored by CGMS (Medtronic MiniMed, Northridge, CA 91325, USA) for three days and they were masked of the glucose values in real time. Individuals were instructed to accomplish, at least four capillary blood glucose measurements per day for calibration. Glucose variability was assessed using conventional analysis. Parameters of glycemic variability included the standard deviation of glucose (SD) [28], variation of glucose (VAR), coefficient of variation of glucose (CV%) and mean amplitude of glucose excursion (MAGE) [29].

Cardiovascular autonomic control evaluation

For the autonomic evaluation protocol, participants were given specific information about the test and were asked to avoid consuming beverages containing

caffeine, smoking, and performing physical and vigorous activities 24 hours before the evaluation. Light and temperature in the room were controlled for the evaluation.

Cardiovascular autonomic control was assessed using a noninvasive blood pressure device (NIBP100D system; Biopac, Santa Barbara, CA, USA) calibrated and connected to an amplifier (DA100; Biopac) during all intervention protocol. A finger cuff was placed on the patient's finger in order to evaluate the autonomic control in four different conditions: rest condition, controlled ventilation, inspiratory muscle exercise and recovery. For the analysis of blood pressure variability (BPV) and heart rate variability (HRV), temporal series of beat-to-beat of the systolic blood pressure and pulse interval were obtained from continuous blood pressure signal (sample 1000Hz). Frequency domain analysis was assessed through spectral analysis determining components, such as: very low frequency (VLF), low frequency (LF) and high frequency (HF) [30].

Randomization

Patients were randomized to IME with load 2% of PImax or 60% of PImax, which were separated by at least seven days. A randomization sequence was generated by software R (x64 version 3.1.1) by an independent investigator.

Statistical analysis

Data were expressed as mean ± SE or medians and interquartile ranges for nonparametric variables. Categorical variables were expressed as number and percentage. The data normality was assessed by Shapiro-Wilk test. The effect of both IME (2% or 60%) was estimated by generalized estimating equations (GEE) followed by Bonferroni's post-hoc test. Statistical significance was considered for $p<0.05$. All data were analyzed by Statistical Package for Social Sciences (SPSS- version 20.0).

Results

Seventeen patients participated in the study and fourteen finished both protocols. Three patients were excluded, because they did not want to continue in the study (figure 1).

Clinical characteristics of the participants are shown in table 1. They were 53.6 ± 1.9 years old, predominantly men (57.1%), being overweight (29.6± 0.9) and they had a median duration of 6.5 (2.75 – 11.00) years of diabetes. In addition, just one patient was diagnosed with autonomic neuropathy. The patients were poorly controlling their diabetes (HbA1c: 8.8 ± 0.2 and fasting plasma glucose: 196.8 ± 9.6) with renal function (microalbuminuria: 12.3 (8 - 35) and creatinine (0.78 ± 0.05)), pulmonary function and inspiratory muscle strength preserved. Most of the patients were sufficiently active (57.1%), as defined by the IPAQ questionnaire, with a low VO2 peak (21.71 (20 - 33)).

In figure 2, panel A it shows glucose levels 4 hours before and 6 hours after IME with 2% of PImax or 60% of PImax. There were reductions in glucose levels only in relation to time ($p < 0.001$), but there was no difference between the groups ($p = 0.741$). After 5 min of the IME the glucose decreased 33.2% in the EMI with a load of 60% of PImax and 32% with a load of 2% of PImax. Reductions of 29.6% and 31.4% were observed after 60 min of IME with a load of 60% of PImax and with a load of 2% of PImax, respectively. At 120 min after the IME with a load of 60% of PImax, glucose levels dropped by 21.4%, while with a load of 2% of PImax the reduction was 24% (*vs.* 1h before exercise). The same result was demonstrated when comparing the glucose level during the protocol (Figure 1 Panel B). There was a decrease in the level of glucose in controlled ventilation (60% of PImax 2.7% and 2% of PI

max 2.9%), interval (60% of PImax 12.1% and 2% of PImax 14.5%), EMI (60% of PImax 17.2% and 2% of PImax 17.6%) and recovery (60% of PImax 18.5% and 2%

of PImax 18.2%) when compared to the baseline, with no difference between the proposed protocols (time <0.001 and group p = 0.912).

A sub analysis was performed dividing the groups of patients taking only metformin and patients taking metformin and sulfonylureas. It was demonstrated that glucose level had a reduction throughout the time of the protocol (P<0.001), but no differences were found between antidiabetic groups (P=0.717) (data not show).

The glycemic variability was evaluated 0-6h, 6-12h and 12-18h after EMI (Figure 3). The delta of the glycemic variability was reduced after 12h (ΔCV: - 4.21%, ΔDP: -8.39 mg / dL and ΔVAR: -657.95 mg / dL2) and 18h (ΔCV: -5.65%, Δ SD: -14.37 mg / dL and ΔVAR: -872.35 mg / dL2) of the IME (vs. after 6h IME) regardless of the loading used. When comparing groups, regardless of time, CV was 7.6 times higher in IME with a load of 60% of PImax than in EMI with a load of 2% of PImax, SD was 8.6 times greater than in IME with a load of 2% of PImax and VAR was 4.5 times higher in IME with a load of 60% of PImax. MAGE was evaluated after IME with a load of 60% of PImax or 2% of PImax and no difference was observed in MAGE in both loads (60% of PImax 97.3 (61.3 – 160.1) mg/dL and 2% of PImax 103.1 (61.9 – 121.4) mg/dL).

Autonomic control was assessed in ten individuals, throughout protocol and the data is shown in table 2. Four patients were excluded because the continuous blood pressure signal could not be analyzed due to the presence of artifacts. HRV (P = 0.590) was not different in the IME with 60% of PImax and with a load of 2% of PImax, as well as some power spectral components of HRV [LF (nu) P = 0.073, LF (ms^2) P = 0.351, HF (ms^2) P = 0.952, LF/HF P = 0.481). Although HF (nu) of HRV was lower in the exercise session with 60% of PImax compared to the 2% of PImax exercise session (60% of PImax 25.0 ± 5.2 and 2% of PImax 45.2 ± 7.1). The BPV was higher in

exercise with a load of 60% of PImax than in exercise with 2% of PImax (60% of PImax 36.9 ± 9.8 and 2% of PImax 12.7 ± 2.9). There was no difference in the LF component ($mmHg^2$) (P = 0.116) but the HF component ($mmHg^2$) of BPV spectrum was higher in exercise with load 60% of PImax comparing with a load of 2% of PImax.

In figure 4, it was demonstrated hemodynamic effects and respiratory variables in baseline and during of the IME with a load of 2% of PImax or 60% of PImax, in twelve patients. Two patients had signal loss in some variables. A higher MAP was observed in the IME with 60% of PImax than the session exercise with 2% of PImax in the second minute (load 60% of PImax 121.4±3.6 and 2% of PImax 109.6±2.8) and in last minute of the exercise (load 60% of PImax 131.7±6.0 and 2% of PImax 109.1±3.8). Similar results were show in the HR, when comparing the groups, the 60% of PImax was greater in the first (load 60% of PImax 84.7±4.2 and 2% of PImax 69.3±3.8), second (load 60% of PImax 87.2±3.7 and 2% of PImax 69±3.8) and last (load 60% of PImax 85.9±4.4 and 2% of PImax 70.2±3.3) minutes of the exercise. There were no significant changes in the CBF (P=0.921), CRV (p=0.116), SpO2 (P=0.431) and f_b (P=0.124) between loads. However, there was a difference in PetCO2 between the groups in the end of the exercise session (load 60% of PImax 43.3±2.3 and 2% of PImax 33.2±1.5).

Discussion

To our knowledge, this is the first prospective crossover randomized clinical trial assessing the effect of acute inspiratory muscle exercise on glucose levels and glucose variability in patients with type 2 diabetes. Inspiratory muscle exercise with a load of 60% of PImax fails to demonstrate improvement in glucose levels and glycemic variability, when compared with a load of 2% of PImax. Only the exercise session with a load of 60% of PImax, however, determined changes in autonomic modulation (lower

vagal modulation and higher BPV), as well as higher MAP, HR and PetCO2, as expected.

Reduction of glucose levels after IME in approximately 29.6%, 31.4% (1 hour) and 21.4%, 24% (2 hours) was observed with 60% of PImax and 2% of PImax loads respectively. As expected, the higher glucose levels (1h before IME) were observed immediately after breakfast, followed by a gradual reduction. This decrease in glucose levels probably occurred in response to rises in insulin induced by the meal and by the effect of the antidiabetics that the patients used in the morning before starting IME. In contrast, other authors reported post-breakfast exercise lowered glucose during the exercise bout, although this effect was not sustained at later meals. Clearly, the difference between these results and ours was the higher intensity and duration of exercise and also the type (treadmill walking for 30 min at 50% of estimated maximal oxygen uptake) [31].

The present results are not in accordance with preliminary data in six patients that we have published [32]. Possible explanations for this discrepancy could be the small sample size evaluated previously, and different protocols. Moreover, in the previous report most patients that we included had cardiovascular autonomic neuropathy [32], which is frequently associated with inspiratory muscle weakness [33]. Challenging weaker muscles could result in more important glucose reductions than inspiratory loading applied to healthy/strong muscles.

Our results showed glucose variability reduction after both exercise loads (60% of PImax and 2% of PImax), and this reduction was not due effect of the IME. Considering that glucose variability could change because of facts occurring during the day or the night, or perhaps because it could have a circadian rhythm, we evaluated possible differences from day to night variability, but no differences were observed

between diurnal and nocturnal glucose variability (data not show). In contrast with our results, other studies have reported reduction in glucose variability after aerobic exercise [14, 15, 34] and combined exercise [13] in patients with diabetes. Additionally, in the preliminary data of our group, IME with a load of 60% of PImax and aerobic exercise showed comparable reductions in glucose variability [21].

In relation to autonomic control, we found a vagal modulation decreased and blood pressure variability increased during inspiratory exercise with a load of 60% of PImax, and not during inspiratory exercise with a load of 2% of PImax. Moreover, the HF component of BPV was higher in the exercise with a load of 60% of PImax when compared to the 2% Plmax load exercise. Findings from previous studies demonstrated that an inspiratory muscle exercise session improves the autonomic control by increasing vagal modulation and reducing sympathetic modulation in young smokers [35] and in healthy elderly individuals [36]. The same result was observed after seven days of IME in elderly women with metabolic syndrome [37]. This benefit in the autonomic control was observed when exercise was performed with a lower inspiratory load intensity, and authors suggest that these autonomic adjustments may be influenced by respiration, which is a modulator of heart rate variability [38] that increased vagal modulation and reduced sympathetic modulation. In addition, increasing tidal volume during exercise improves oxygenation and, consequently, decreases chemoreflex activity, which is associated with a reduction in sympathetic nerve activity [35, 39].

On the other hand, the induction of inspiratory muscle fatigue increases sympathetic activity leading to vasoconstriction and reduction of blood flow of limb locomotor muscle [40, 41]. Accordingly, our results showed lower values in the percentage of vagal cardiac modulation and greater variability of blood pressure, indicating a sympathetic predominance induced by the intervention of experimental load. In fact,

HR and MAP were increased during the session of inspiratory exercise with the experimental load. Our results were similar to those of McConell et al, who studied acute cardiorespiratory responses to differences inspiratory loadings and reported that the load of 60% of PImax determined a sustained increase in HR and MAP [42]. Although an exacerbation of the inspiratory muscle metaboreflex was previously observed in diabetic patients [32], the present data did not disclose changes in CBF and CVR. Thus, it is possible that the MAP increase was induced by changes of cardiac output instead of activation of the inspiratory metaboreflex.

The present study has some limitations that should be acknowledged. Firstly, we evaluated CBF by venous occlusion plethysmography instead of doppler ultrasonography, wich is the gold standard method. Secondly, the small sample evaluated could not disclose some results, as it was calculated for the main outcome (glucose). Third, there was no standardization of nutrition although patients were instructed to maintain their usual diet.

In conclusion, the effect of an inspiratory muscle exercise session with 60% loading of PImax was not able to improve glucose levels and glycemic variability, comparing to the 2% Plmax exercise session, in subjects with type 2 diabetes. The evaluation of the autonomic control during the inspiratory muscle exercise with a load of 60% of PImax showed lower values in the percentage of cardiac vagal modulation and greater variability of the arterial pressure, indicating a sympathetic cardiac predominance. These results are expected during physical exercise, especially in the case of a fatigue induction protocol. The high intensity load characterized the autonomic response as an active sympathetic, different from that indicated in studies with lower intensity (30% of PImax) that demonstrated an increase in vagal modulation after exercise.

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Legends

Figure 1: Flow diagram of patient's selection. IPAQ, International Physical Activity Questionnaire; PImax, maximal inspiratory pressure; HbA1c, glycated hemoglobina; CGMS, e continuous glucose monitoring system.

Figure 2: Panel A Glucose levels 4 hours before and 6 hours after acute inspiratory muscle exercise protocol with placebo load (2% of PImax) or experimental load (60% of PImax). Pimáx, maximal inspiratory pressure. Data are expressed as mean ± SEM. Statistics: Generalized estimating equations (GEE). Time ($p < 0.001$), group ($p=0.741$) and interaction ($p= 0.343$). *$p<0.05$ *vs* 1h before exercise. Panel B: Detail from intervention (baseline, controlled ventilation, interval, inspiratory exercise, recovery). Data are expressed as mean ± SEM. Statistics: GEE. Time ($p < 0.001$), load ($p=0.912$) and interaction ($p= 0.177$). *$p<0.05$ *vs* 5 min baseline.

Figure 3: Delta of glucose variability (all values Δ from -0 to -6h before exercise) assessed 0-6h, 6-12h and 12-18h after acute inspiratory muscle exercise protocol with placebo load (2% of PImax) or experimental load (60% of PImax). (A): glucose coefficient of variation, (B): glucose standard deviation, (C): glucose variance, PImax: maximal inspiratory pressure. Data expressed as mean ± SEM. Statistics: Generalized estimating equations (GEE). *$p<0.05$ *vs* 0h-6h.**Figure 4:**

Figure 4: Hemodynamic effects and respiratory variables in baseline and during acute inspiratory muscle exercise protocol with placebo load (2% of PImax) or experimental load (60% of PImax). MAP, mean arterial pressure; HR, heart rate; CBF, calf blood flow; CVR, calf blood resistance. Data are expressed as mean ± SEM. Statistics: Generalized estimating equations (GEE), followed by Bonferroni's *post hoc* test.*$p<0.05$ *vs.* 2%PImax.

TABLES

Table 1: Clinical characteristics of the participants studied

Characteristics	Total Sample (n=14)
Age	53.6 ± 1.9
Male gender	8 (57.1)
Duration of diabetes (years)	6.5 (2.75 – 11.00)
BMI (Kg/m²)	29,6 ± 0.9
Office SBP (mmHg)	131.6 ± 2.9
Office DBP (mmHg)	83.3 ± 2.6
HbA1c (%)	8.8 ± 0.2
Fasting plasma glucose (mg/dL)	196.8 ± 9.6
High albuminuria*	12.3 (8 - 35)
Creatinine (mg/ dL)	0.78 ± 0.05
GFR (mL/min/ 1.73m²)	96.3 ± 3.8
Pulmonary function	
FEV1, % predicted	101.9 ± 4.5
FVC, % predicted	102.8 ± 3.7
MVV, % predicted	111.4 ± 7.0
Inspiratory muscle function	
PImax, cmH2O	94.1 ± 10.4
PImax, % predicted	106.9 (82.8 – 115.5)
Physical activity level (IPAQ)	
Insufficiently active	1 (7.1)
Sufficiently active	8 (57.1)
Very active	5 (35.7)
Cardiopulmonary exercise test	
VO2 peak (mL.Kg.min^{-1})	21.71 (20 - 33)
Heart rate peak (bpm)	163 (154.2 – 174.2)
Rpeak	1.2 (1.08- 1.24)
VE/VCO2 slope, mL/min L/min	32.0 ± 4.9
OUES (L/min)	1.9 ± 0.7
Heart rate recovery (bpm)	20 ± 7.9
Medications	
Metformin	14 (100)
Sulfonylureas	6 (42.9)
Other antidiabetic drugs	2 (14.3)
Calcium channel blockers	2 (14.3)
Angiotensin II receptor blockers	4 (28.6)
Angiotensin-converting enzyme inhibitors	3 (21.4)
Diuretics	6 (42.9)
β-blockers	3 (21.4)
Antiplatelet	1 (7.1)
Statins	3 (21.4)

Continuous variables are expressed as mean ± SE or median (interquartile range (p25-p75)). Categorical variables are expressed as number (%).

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, glycated hemoglobin; GFR, estimated glomerular filtration rate calculated by the MDRD equation; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; MVV, maximal voluntary ventilation; PImax, maximal inspiratory pressure; VO2 peak, peak oxygen uptake; R, respiratory exchange ratio; VE/VCO2 slope, minute ventilation/carbon dioxide production slope; OUES, oxygen uptake efficiency slope; IPAQ, International Physical Activity Questionnaire.

*High albuminuria, defined by values > 17 mg/L.

Table 2: Spectral analysis of heart rate variability components and blood pressure variability components during acute inspiratory muscle exercise protocol with placebo load (2% of PImax) or experimental load (60% of PImax)

	Acute inspiratory muscle exercise protocol with placebo load (2% of PImax) (n= 10)				**Acute inspiratory muscle exercise protocol with experimental load (60% of PImax) (n= 10)**				**P Interaction**
	Baseline	**Controled Ventilation**	**Inspiratory Exercise**	**Recovery**	**Baseline**	**Controled Ventilation**	**Inspiratory Exercise**	**Recovery**	
HRV (ms)	1552.9±359.9	1638.5±382.5	1116.6±156.9	1586.9±237.2	1696.7±352.2	1353.7±262.0	1424.5±288.9	1862.6±566.6.	0.590
LF (nu)	31.5±7.4	24.6±4.4	17.7±4.1	27.7±6.5	37.1±8.9	20.6±4.1	27.5±4.8	24.4±5.6	0.073
LF (ms^2)	548.5±188.3	352.2±126.9	199.9±69.8	530.9±162.6	669.7±264.2	265.4±112.9	265.4±84.0	469.1±190.7	0.351
HF (nu)	26.9±5.7	32.9±5.1	45.2±7.1	31.8±3.8	26.2±5.0	33.6±3.3	25.0±5.2*	36.2±4.0	0.010
HF (ms^2)	349.8±107.4	463.1±138.5	424.3±97.7	490.4±93.1	299.3±107.4	436.2±120.6	344.4±49.7	487.4±117.8	0.952
LF/HF	1.8±0.6	0.6±0.5	0.6±0.2	0.8±0.2	2.8±1.0	0.8±0.2	0.6±0.3	0.7±0.2	0.481
BPV (mmHg)	11.9±2.3	16.4±4.7	12.7±2.9	8.8±1.9	18.3±4.5	18.6±5.6	36.9±9.8*	11.9±2.7	0.019
LF BPV ($mmHg^2$)	2.4±0.8	2.8±0.9	2.1±0.8	0.9±0.2	4.6±1.3	2.3±0.8	5.7±1.8	2.0±0.9	0.116
HF BPV($mmHg^2$)	3.3±0.7	4.9±0.8	5.0±1.1	3.6±0.9	5.6±2.5	9.5±3.7	20.9±6.2*	4.7±1.5	0.001

HRV; Heart rate variability, LFms^2; power spectrum of low frequency band in absolute value, HFms^2; power spectrum of high frequency band in absolute value, LFnu; power spectrum of low frequency band in normalized units, HFnu; power spectrum of high frequency band in normalized units, LF/HF; sympathetic–vagal balance; BPV; Blood pressure variability, LF BPV$mmHg^2$;

power spectrum of low frequency band in absolute value, HF BPVmmHg²; power spectrum of high frequency band in absolute value. Data are expressed as mean ± SE. Results of generalized estimating equations (GEE) for repeated measures. *P<0.05 inspiratory exercise 60%PImax *vs* inspiratory exercise 2%PImax

Figure 1

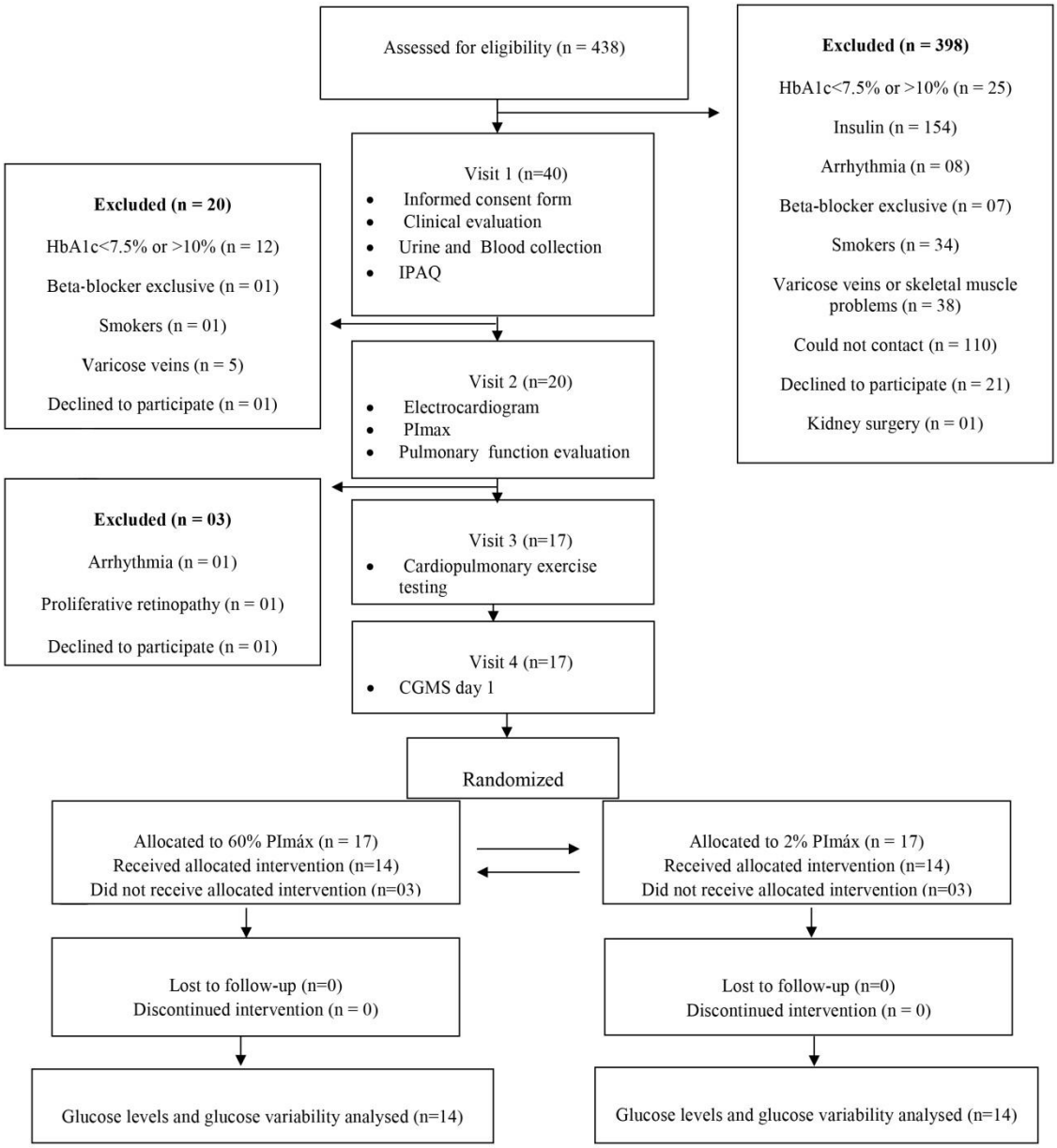

Assessed for eligibility (n = 438)
Excluded (n = 398)
HbA1c<7.5% or >10% (n = 25)
Insulin (n = 154)
Arrhythmia (n = 08)
Beta-blocker exclusive (n = 07)
Smokers (n = 34)
Varicose veins or skeletal muscle problems (n = 38)
Could not contact (n = 110)
Declined to participate (n = 21)
Kidney surgery (n = 01)
Visit 1 (n=40)
Informed consent form
Clinical evaluation
Urine and Blood collection
IPAQ
Excluded (n = 20)
HbA1c<7.5% or >10% (n = 12)
Beta-blocker exclusive (n = 01)
Smokers (n = 01)
Varicose veins (n = 5)
Declined to participate (n = 01)
Visit 2 (n=20)
Electrocardiogram
PImax
Pulmonary function evaluation
Excluded (n = 03)
Arrhythmia (n = 01)
Proliferative retinopathy (n = 01)
Declined to participate (n = 01)
Visit 3 (n=17)
Cardiopulmonary exercise testing
Visit 4 (n=17)
CGMS day 1
Randomized
Allocated to 60% PImáx (n = 17)
Received allocated intervention (n=14)
Did not receive allocated intervention (n=03)
Allocated to 2% PImáx (n = 17)
Received allocated intervention (n=14)
Did not receive allocated intervention (n=03)
Lost to follow-up (n=0)
Discontinued intervention (n = 0)
Lost to follow-up (n=0)
Discontinued intervention (n = 0)
Glucose levels and glucose variability analysed (n=14)
Glucose levels and glucose variability analysed (n=14)

Figure 2

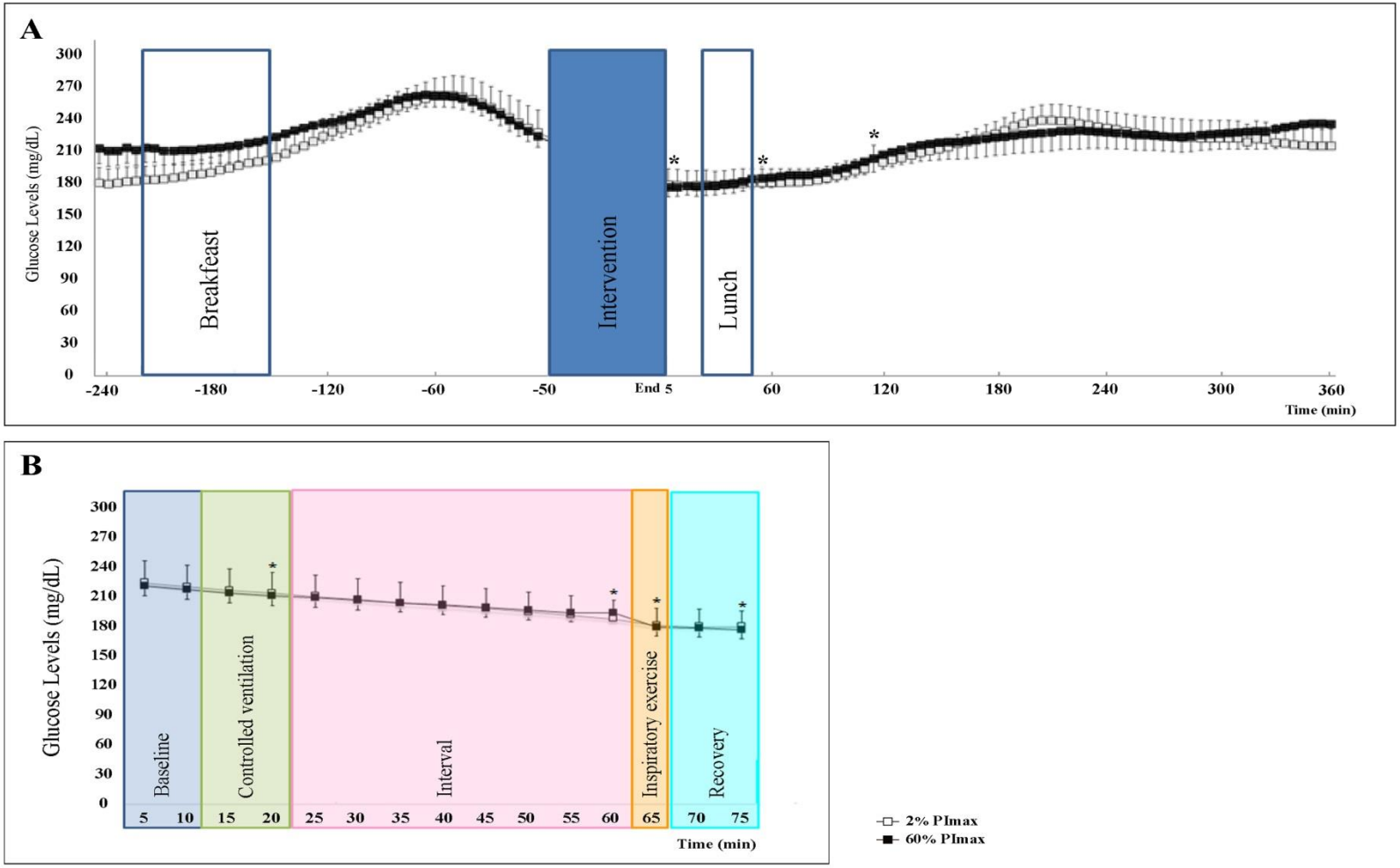

A
Glucose Levels (mg/dL)
300
270
240
210
180
150
120
90
60
30
0
Breakfeast
Intervention
Lunch
*
*
*
-240
-180
-120
-60
-50
End 5
60
120
180
240
300
360
Time (min)
B
Glucose Levels (mg/dL)
300
270
240
210
180
150
120
90
60
30
0
Baseline
Controlled ventilation
Interval
Inspiratory exercise
Recovery
*
*
*
*
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
Time (min)
2% PImax
60% PImax

Figure 3

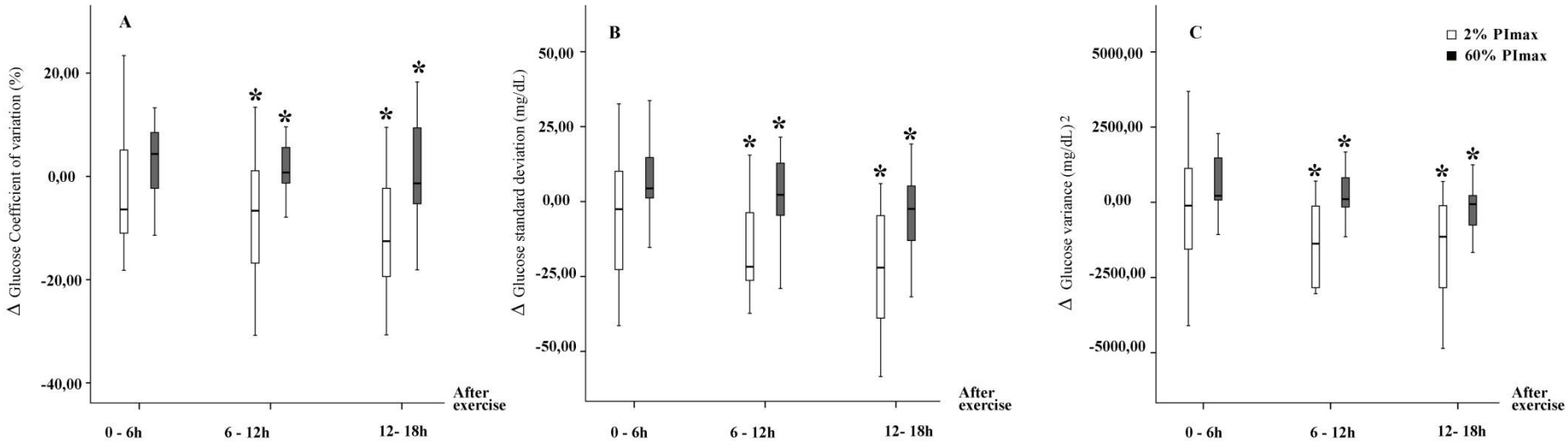
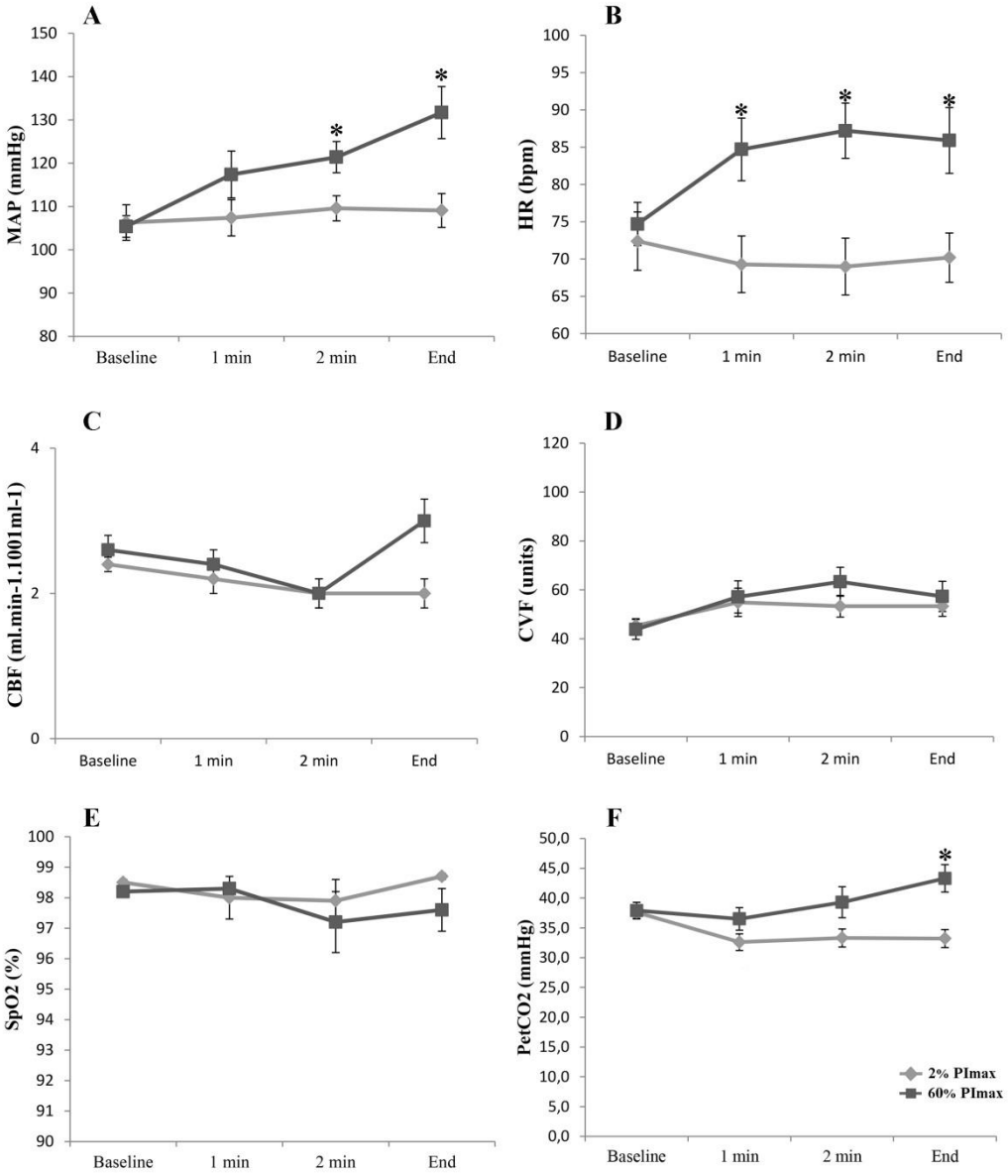

A
Δ Glucose Coefficient of variation (%)
20,00
0,00
-20,00
-40,00
0 - 6h
6 - 12h
12- 18h
After exercise
B
Δ Glucose standard deviation (mg/dL)
50,00
25,00
0,00
-25,00
-50,00
0 - 6h
6 - 12h
12- 18h
After exercise
C
Δ Glucose variance (mg/dL)2
5000,00
2500,00
0,00
-2500,00
-5000,00
0 - 6h
6 - 12h
12- 18h
After exercise
2% PImax
60% PImax

Figure 4


A
MAP (mmHg)
150
140
130
120
110
100
90
80
Baseline
1 min
2 min
End
*
*
*
B
HR (bpm)
100
95
90
85
80
75
70
65
60
Baseline
1 min
2 min
End
*
*
*
C
CBF (ml.min-1.100ml-1)
4
2
0
Baseline
1 min
2 min
End
D
CVF (units)
120
100
80
60
40
20
0
Baseline
1 min
2 min
End
E
SpO2 (%)
100
99
98
97
96
95
94
93
92
91
90
Baseline
1 min
2 min
End
F
PetCO2 (mmHg)
50,0
45,0
40,0
35,0
30,0
25,0
20,0
15,0
10,0
5,0
0,0
Baseline
1 min
2 min
End
*
2% PImax
60% PImax

CONCLUSÕES E CONSIDERAÇÕES FINAIS

Uma sessão de exercício muscular inspiratório com carga de 60% não foi capaz de demonstrar melhoras nos níveis de glicose e variabilidade glicêmica, quando comparado ao exercício muscular inspiratório com carga de 2% em indivíduos com DM tipo 2. A literatura é controversa, não há padronização em relação aos protocolos de exercício muscular inspiratório e nem quanto o tipo de intensidade ideal a ser utilizada durante o exercício. É necessária a elaboração de mais estudos para estabelecer se realmente o exercício da musculatura inspiratória tem ou não efeitos benéficos no controle glicêmico.

O controle autonômico durante o exercício muscular inspiratório demonstrou respostas esperadas para um protocolo de exaustão muscular com intensidade alta de carga. O exercício agudo com baixas intensidades demonstrou benefício sobre o sistema nervoso autônomo, já com o treinamento inspiratório crônico foram observados resultados contraditórios e o efeito desse tipo de exercício no controle autonômico permanece inconsistente.