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CARACTERIZAÇÃO DA VARIABILIDADE GLICÊMICA DURANTE E APÓS DIFERENTES PROTOCOLOS DE EXERCÍCIO AGUDO ATRAVÉS DE FERRAMENTAS MATEMÁTICAS LINEARES E NÃO LINEARES EM INDIVÍDUOS SAUDÁVEIS E COM DIABETES MELLITUS

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SUMÁRIO

LISTA DE ABREVIATURAS

CGMS: Monitorização Contínua de Glicose

CONGA: Continuous Overlapping Net Glycemic Action

CV%: Glucose Coefficient of Variation

DFA: Detrended Fluctuation Analysis

DM: Diabetes Mellitus

DM1: Diabetes Mellitus do tipo 1

DM2: Diabetes Mellitus do tipo 2

FMD: Dilatação Mediada pelo Fluxo

HbA1c: Hemoglobina Glicada

IDF: International Diabetes Federation

IL-6: Interleucina 6

IL-10: Interleucina 10

MAGE: Amplitude Média de Excursões Glicêmicas

MODD: Absolute Means of Daily Differences

MPPGE: Mean Post Prandial Glycemic Excursions

PAD: Pressão Arterial Diastólica

PAS: Pressão Arterial Sistólica

PGF2: Prostaglandina 8-iso F2

SD: Glucose Standard Deviation

SM: Síndrome Metabólica

TGF-β1: Fator de Transformação do Crescimento Beta

VAR: Gucose Variance

VO_{2max}: Consumo Máximo de Oxigênio

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CAPÍTULO II

RESUMO

Objetivo: O objetivo desta tese foi avaliar a variabilidade glicêmica (VG) utilizando ferramentas matemáticas lineares e não lineares, em situação basal e em resposta a protocolos específicos de exercício físico agudo em indivíduos hígidos e em pacientes com diabetes tipo 2 diabetes. **Métodos.** Foram desenvolvidos dois artigos originais. No primeiro estudo, utilizamos o sistema de monitorização contínua de glicose (CGMS) para avaliar a VG por um período de 3 dias. Os voluntários hígidos foram randomizados para realizar duas sessões de exercícios, aeróbico (AER) (40 min. de biclicleta ergométrica a 70% de VO_2pico) e excêntrico (EX) (40 min. de pressão de pernas, 6 séries de 10 repetições a 120% de 1RM), com um intervalo de 7 dias entre as sessões. Amostras de sangue foram coletas pré e após ambas as sessões de exercício para análises de inflamação e estresse oxitativo. A VG foi avaliada através do desvio padrão da glicose, variância da glicose, coeficiente de variação e variância da glicose normalizada, além de análise espectral e simbólica. No segundo estudo, os pacientes com diabetes tipo 2 foram submetidos a três diferentes tipos de exercícios, AER (40 min. de biclicleta ergométrica a 70 % de VO_2pico) e combiando (COMB) (20 min. de biclicleta ergométrica a 70 % de VO_2pico + 20min de exercício de força composto por 12 repetições a 65% de 1RM de pressão de pernas, extensão de pernas, biceps rosca e supino reto) e duas sessões de carga muscular inspiratória em alta resistência (60% da pressão muscular inspiratória (PImax)) ou baixa resistência (2% da PImax), com intrervalo de 7 dias entres as sessões. A glicose foi avaliada utilizando o CGMS, e foram consideradas para análises medidas obtidas 30 min antes, durante e 30 min após as sessões de exercícos. Desvio padrão da glicose, variância da glicose e coeficente de variação foram calculados para cada séries, antes e após as sessões de exercícios. **Resultados.** Concluímos que em indivíduos hígidos é possível caracterizar a

variabilidade glicêmcia através de métodos convencionais e não convencionais como análise espectral e simbólica. Além disso, uma sessão aguda de exercício AER e EX reduzem a VG nestes individuos, existindo uma correlação de marcadores de inflamação e de estresse oxidative com indices de análise convencional e não convencional. Também mostramos que o exercício AER e o exercício de carga muscular inspiratória a 60% da PImáx promovem reduções similares da variabilidade glicêmica, contudo sem alterações com exercício COMB e quando a musculatura inspiratória é exercitada a 2% da PImáx.

Palavras chaves: Variabilidade glicêmica; estresse oxidativo; inflamação e exercício

CAPÍTULO I

1. REVISÃO DE LITERATURA

De acordo com a *International Diabetes Federation* (IDF) o número de pessoas com diabetes mellitus (DM) aumenta a cada ano. Estima-se que em 2015, mais de 415 milhões de pessoas foram diagnosticadas com DM e a expectativa é que este número aumente para 642 milhões até 2040 (1). Estudo multicêntrico sobre a prevalência de diabetes no Brasil, realizado entre 1986 e 1989, mostrou que 7,6% da população com idade entre 30 e 69 anos tinham DM, sendo que 50% das pessoas não conheciam o diagnóstico (2). Mais recentemente, dados de um estudo realizado entre 2008 e 2010 onde foram avaliados funcionários públicos de 35 a 74 anos, de 6 capitais brasileiras, mostraram prevalência de DM de 19.7% nestes trabalhadores. Destes, 50,4% já tinham diagnóstico prévio (3).

O tratamento do DM no Brasil tem refletido em aumento dos custos para o Sistema Único de Saúde e para a sociedade. O custo total com a doença no ano de 2007 em atendimento ambulatorial foi US$ 2108 por paciente, e estes custos aumentaram de acordo com o nível de doença, complicações crônicas e nível de atendimento, o que indica necessidade de realocação de recursos da saúde com foco na sua prevenção e de suas complicações (4). Mais recentemente, dados do UKPDS 86 (2014) mostrou que as complicações do DM estão associadas com custos substanciais de saúde imediatos e de longo prazo. Na Inglaterra no período de 1997 a 2007, os custos com cuidados de internação foram de £2012 (libras esterlinas) por paciente ao ano. Estes custos aumentaram de acordo com outros eventos como amputação, cardiopatia isquêmica, insuficiência cardíaca, cegueira, acidente vascular cerebral e infarto do miocárdio. Nos anos seguintes, os custos com internação devido a estes eventos variaram entre £1060 e £2943, mas também existiu um custo alto com amputação e doença isquêmica do

coração sem internação que variou entre £1193 e £2116 (5). Não só no Brasil, mas também em outros países é muito importante o trabalho na prevenção das complicações do DM para diminuir custos que só tendem a aumentar com a evolução da doença.

Além da hiperglicemia crônica que classicamente caracteriza o DM, flutuações agudas de glicemia também são observadas nestes indivíduos (6). Em longo prazo as conseqüências dos danos locais e sistêmicos da hiperglicemia sustentada são a ocorrência de complicações micro e macrovasculares (7-8), responsáveis pela elevada morbi-mortalidade associada à doença (9-10). Para prevenir as complicações agudas e crônicas, bem como diminuir sintomas associados ao DM, é necessário controle glicêmico intensivo (7, 11-12). A hemoglobina glicada (HbA1c), glicemia de jejum e medidas capilares de glicose, isolados ou em combinação são utilizados na avaliação do controle glicêmico (13). No entanto, embora menos comumente, as complicações crônicas podem ocorrer em indivíduos com HbA1c dentro dos parâmetros recomendados, de forma que outros componentes da disglicemia característica do diabetes, tais como glicemia pós-prandial e variabilidade glicêmica, têm sido buscados recentemente como possíveis alvos terapêuticos (14-17).

Flutuações glicêmicas e as complicações crônicas do Diabetes Mellitus

O reconhecimento de que a hiperglicemia sustentada é preditora de complicações microvasculares e macrovasculares em pacientes com diabetes mellitus do tipo 1 (DM1) (12, 18) e diabetes mellitus do tipo 2 (DM2) (7, 11, 19) é bem estabelecido. Porém, há questionamentos sobre o papel da variabilidade glicêmica em relação a ser ou não preditora de complicações crônicas do DM, assim como qual seria o papel de focar o tratamento da doença em reduzir a variabilidade glicêmica (20-22).

Estudo que avaliou a relação entre a disfunção endotelial macrovascular e flutuações de glicose, mostrou maior grau de flutuação glicêmica em pacientes que apresentavam DM2 com doença arterial coronariana quando comparada à DM2 sem a doença arterial coronariana (MAGE: 4,95 ± 1,38 mmol/L *vs* 3,52 ± 1,12 mmol/L, P <0,001, respectivamente). Os pacientes com DM2 e doença arterial coronariana também apresentaram uma dilatação mediada pelo fluxo (FMD) menor comparados aos DM2 sem doença arterial coronariana (FMD: 4,71± 1,13 % *vs* 7,12 ± 1,22%, respectivamente), além disso, a FMD se correlacionou inversamente com a flutuação glicêmica no grupo com DM2 e doença arterial coronariana (r= -0,520 P=0,003). Para verificar os fatores que afetaram a FMD neste grupo de pacientes, foi feita uma analise de regressão multivariada onde foi observada uma forte correlação entre HOMA-IR (R^2= -0,877; P= 0,039), pressão arterial (R^2= -0,030; P= 0,006) e amplitude média de excursões glicêmicas (MAGE) (R^2= 0,303; P= 0,028). Sugerindo que um controle efetivo da pressão arterial e a atenuação da flutuação da glicose podem proteger pacientes com DM2 e doença coronariana de uma disfunção endotelial vascular (23).

Em estudo similar ao citado anteriormente, também foi observado uma maior flutuação glicêmica em pacientes com DM2 que apresentavam neuropatia periférica (bem controlados com HbA1c menor do que 7,0%), quando comparados com DM2 bem controlados e sem neuropatia periférica (MAGE: 5,8 ± 1,6 mmol/L *vs* 4,5 ± 0,9 mmol/L; P <0,001, respectivamente). Além disso, o MAGE numa analise de regressão multivariada, foi significativamente associado com neuropatia periférica diabética (OR 2,05, CI 1,36–3,09, P = 0,001), sugerindo que o aumento das flutuações glicêmicas, demonstrada pelo aumento do MAGE, pode resultar em aumento de risco para complicações microvasculares e macrovasculares (24).

A relação entre parâmetros ligados ao risco cardiovascular e as oscilações presentes em curvas glicêmicas e níveis de HbA1c foi investigada em indivíduos saudáveis e pacientes com DM2. Para isso, os voluntários saudáveis e pacientes DM2 participaram de um experimento onde a sua glicemia foi normalizada através de insulina e/ou 5% de glicose para manter os níveis de glicemia entre 4 e 6 mmol/L. Após foram randomizados para três protocolos feitos num período de 24h: (Protocolo 1- Oscilação de glicose): a glicemia foi aumentada à 15 mmol/L a cada 6h e normalizada para as próximas 6h; (Protocolo 2- Hiperglicemia sustentada): mantida a glicemia à 15 mmol/L; (Protocolo 3- Glicose constante): mantida a glicemia à 10mmol/L durante 24h (média dos valores do protocolo 1). Foi observado que as oscilações de glicose são associadas a maiores valores de estresse oxidativo, no qual foi mensurado pela taxa de excreção urinária de prostaglandina 8-iso F2 (PGF2) de 24h. Tanto nos indivíduos saudáveis, quanto nos pacientes com DM2, a taxa de excreção urinária de PGF2 foi maior nos grupos que apresentaram maiores oscilações glicêmicas quando comparada aos grupos com hiperglicemia sustentada (DM2: 536 ± 51 vs. 476 ± 48 pg/mg creatinina [$P < 0,05$] e saudáveis: 342 ± 52 vs. 271 ± 54 pg/mg creatinina [$P < 0,05$], respectivamente). A disfunção endotelial também foi avaliada nestes indivíduos através da FMD. Observou-se que a disfunção endotelial aumentou nos dois grupos: saudáveis ($P <0,01$) e DM2 ($P <0,05$) com ambos os protocolos de hiperglicemia sustentada e glicose constante. No entanto, a oscilação glicêmica foi a que resultou em maior disfunção endotelial nos dois grupos avaliados (saudáveis $P <0,01$; DM2 $P <0,01$). Estes achados podem se relacionar às fases iniciais do processo aterosclerótico (25).

Experimento *in vitro* avaliou células incubadas em cinco diferentes condições de estado de glicose: (1) glicose normal (3 semanas glicose a 5 mmol/l); (2) glicose alta (3 semanas glicose a 25 mmol/l); (3) glicose oscilando (3 semanas de 24h de glicose média

a 25 mmol/l seguida de 24h de glicose a 5 mmol/l); (4) condição de memória de oscilação de glicose (1 semana de glicose normal a 5 mmol/l após 2 semanas de oscilação de glicose alta [24h de glicose a 25 mmol/l seguido de 24h de glicose a 5 mmol/l]) e (5) condição de memória de glicose alta (1 semana de glicose normal a 5 mmol/l após exposição por 2 semanas de glicose alta contínua a 25 mmol/l). Na condição 2 (exposição constante a altos valores de glicose) e na condição 3 (oscilações glicêmicas) foi observada maior produção de espécies reativas de oxigênio, estresse oxidativo e dano ao DNA (14). Neste contexto, as oscilações da glicemia parecem conter informações adicionais sobre os processos diretamente envolvidos no quadro da hiperglicemia do DM, sendo esta análise complementar às demais medidas usuais, caracterizando uma potencial ferramenta de investigação e avaliação nesses pacientes (14-15, 26). Além disso, a avaliação da variabilidade das oscilações glicêmicas pode inclusive auxiliar na compreensão do efeito de intervenções específicas utilizadas nesta doença.

Variabilidade glicêmica e os métodos de avaliação da glicose

A literatura contém muitos conceitos diferentes sobre o mesmo termo "variabilidade glicêmica". Podemos ver conceitos referindo-se à variabilidade da glicemia de jejum, picos de glicemia pós-prandial, variabilidade da HbA1c ao longo do tempo, episódios de hipoglicemias, e, finalmente, o mais comum, inclui a variabilidade glicêmica intra-dia que é por sua vez, avaliada por meio de medidas obtidas pela glicemia capilar ou pelo Sistema de Monitorização Contínua de Glicose (CGMS - *"Continuous Glucose Monitoring System"*) (27). Alguns estudos utilizam medidas de glicemia capilar com um método para avaliação da variabilidade glicêmica, contudo este método considera várias medidas em um período de tempo específico, geralmente

utilizando apenas 7 a 8 pontos de medidas de glicemia (20, 28-29) e geralmente produzindo poucas informações sobre o período noturno (30).

Entre os métodos disponíveis para avaliar o comportamento glicêmico existem dispositivos que permitem a monitorização glicêmica domiciliar, tais como o CGMS que vem sendo utilizado para auxiliar no monitoramento de pacientes, especialmente em condições clínicas que cursam com oscilações dos níveis de glicemia, permitindo que profissional especializado possa adequar melhor a terapêutica idealizando melhor controle glicêmico do paciente com DM (31). O CGMS é constituído de um sensor instalado no tecido subcutâneo, que capta a medida da glicose com base na reação eletroquímica da enzima glicose-oxidase com a glicose do fluido intersticial, identificando valores de 40 a 400mg/dl, a cada 10 segundos, com o registro da média desses valores a cada 5 minutos, em um total de 288 medidas ao dia, que pode ser usado por até 72hs (32-33).

Este sistema de monitorização tem mostrado ser sensível e fornecer medidas suficientemente estáveis, mostrando eficácia no registro de mudanças nos níveis de glicose em atividades usuais, registrando episódios de hipoglicemia noturna e hiperglicemia pós-prandial que não são evidenciados no monitoramento de rotina (31-32). Em estudo realizado por nosso grupo em pacientes com DM2, mostramos que durante uma sessão de exercício aeróbico, bem como numa sessão de exercício combinado (aeróbico + exercício de força) as medidas do CGMS estavam 100% dentro dos limites aceitáveis de acordo com os critérios da Organização Internacional de Padronização (ISO) para a avaliação da acurácia de medidas de glicose (34).

Em indivíduos saudáveis o CGMS pode ser eficaz em estudos investigativos para avaliar e caracterizar a glicose e suas variações. Estudo realizado para verficar a relação entre situação de estresse e o controle glicêmico de 24h, avaliou 17 idosos

saudáveis submetidos a uma sessão controle (30 min lendo ou ouvindo música) e uma sessão em situação de estresse (30 min. de teste psicopfisico contendo medidas de desempenho em séries computadorizados avaliando tempo de reação, escolha de reação, acuidade dinâmica e tática, processamento de informações, além de acompanhamento visual), com intervalo de 2 à 4 semanas entre as sessões. Observou-se um pequeno atraso de resposta de pico de glicose de 30 a 40 min após o teste psicofisico (condição de estresse). Além disso, os níveis de glicose foram mais elevados na condição de estresse em relação à situação controle nas seis horas após. No entanto, não houve diferença nas concentrações médias de glicose de 24 h nas diferentes condições, controle e sessão de estresse (111,7 ± 12,3 mg·dL $^{-1}$ *vs* 114,0 ± 24,0 mg·dL $^{-1}$, respctivamente) (35).

A variabilidade glicêmica também sofre influência de alterações no estresse oxidativo (36-37) provavelmente relacionadas às alterações agudas induzidas pelo exercício físico (38-39). No intuito de verificar a influência de exercício físico menos exaustivo e, desta forma de prescrição mais ampla aos pacientes DM, nosso grupo avaliou a influência do treinamento da musculatura inspiratória sobre a variabilidade glicêmica de pacientes DM2. Dados prévios deste estudo demonstraram que assim como as sessões de exercícios aeróbico e de força, uma única sessão de treinamento da musculatura inspiratória foi capaz de reduzir os valores glicêmicos e alterar a variabilidade das curvas glicêmicas (Desvio Padrão da Glicose: 13,09 ± 8,15 mg/dL *vs*. 1,57 ±1,15 mg/dL; P= <0,001) (40). Esses achados apontam a possibilidade de novas terapias para melhora do perfil glicêmico, complementares às convencionais, e instigam a investigação sobre os mecanismos fisiológicos envolvidos nas características dos sinais glicemicos extraídos por CGMS.

Diversos índices de avaliação da variabilidade glicêmica podem ser calculados a partir das múltiplas medidas de glicose que são obtidas de curvas glicêmicas. O CGMS, sendo um método que apresenta medidas contínuas de glicose ao longo do dia, permite uma avaliação mais precisa da variação da glicose em curto prazo, dentro do dia e entre os dias, e por isso vem sendo amplamente utilizado por vários estudos que apresentam diferentes índices de avaliação da variabilidade glicêmica (15, 23, 41)

Apesar de existirem muitos índices de avaliação da variabilidade glicêmica, atualmente há pouco consenso sobre qual índice oferece a avaliação mais significativa e a quais parâmetros fisiológicos cada um está relacionado. Dentre os vários índices propostos para quantificar características estatísticas das variações glicêmicas, estão incluídos: *mean post prandial glycemic excursions* (MPPGE), *absolute means of daily differences* (MODD), *means of detrended fluctuation analysis* (DFA), *continuous overlapping net glycemic action* (CONGA) (15, 23, 410, *glucose variance* (VAR), *glucose coefficient of variation* (CV%), *glucose standard deviation* (SD) e (MAGE). Representação gráfica destes índices pode ser visualizada na figura 1. Mais detalhes sobre a metodologia para cada um dos métodos mencionados são fornecidos na revisão de Service (2013) (42).

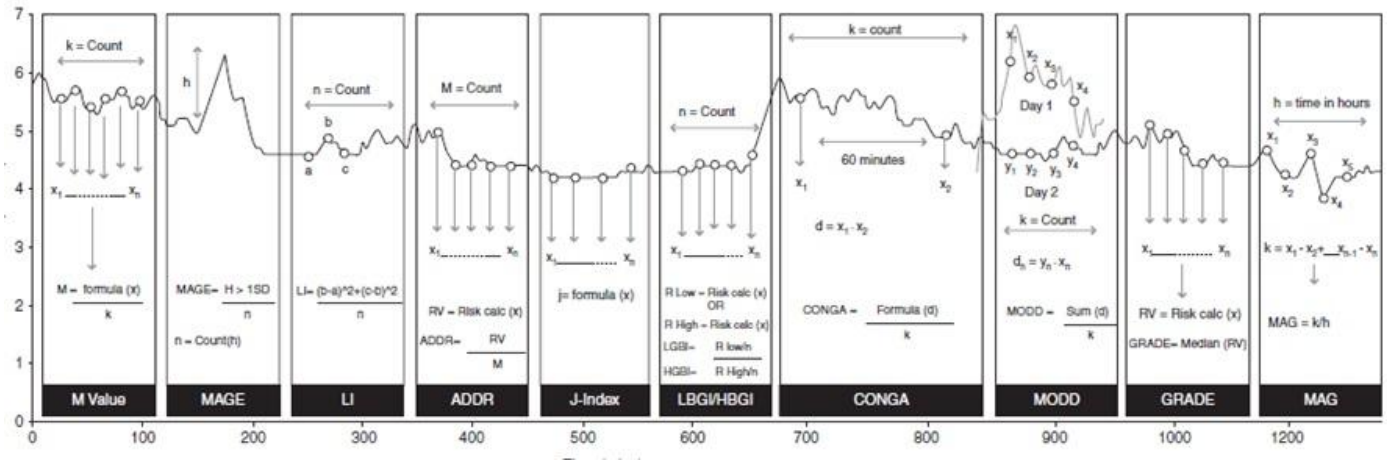

Figura1. Ilustração gráfica de 10 métodos de avaliação da variabilidade glicêmica calculada a partir do sinal captado pelo CGMS (15).

O MAGE é o primeiro índice de variabilidade glicêmica (37) que foi introduzido por Service e colaboradores em 1970 e ainda hoje é um dos índices mais utilizados nesta avaliação (43-44). No entanto, este índice pode não ser sensível na detecção ou identificação de características específicas intrínsecas de cada patologia.

Estudo realizado por Churruca e colaboradores (2008), avaliou a variabilidade glicêmica por medidas convencionais e aplicando um método não linear, baseado no *detrended fluctuation analysis* (DFA), para quantificar a complexidade das variações glicêmicas em grupos diferentes de pacientes: com síndrome metabólica (SM), DM2 e controles saudáveis. O índice MAGE detectou as variações glicêmicas nos grupos: (controles saudáveis [30,0 ± 14,3]; MS [45,5 ± 17,2] e DM2 [62,7 ± 27,5]), mas este método não mostrou ser suficientemente sensível para diferenciar a variabilidade glicêmica entre controles e SM ou entre SM e DM2. O método DFA, por sua vez, identificou nas curvas glicêmicas de indivíduos saudáveis um perfil mais complexo do que pacientes com DM2 e SM: diferenciando os pacientes com SM ($p = 0,006$) e DM2 ($p = 0,001$) do grupo controle (45). Tais achados demonstram que o estudo das curvas glicêmicas e extração de medidas que caracterizem suas variabilidades pode auxiliar na identificação e diagnóstico de doenças, permitir um maior entendimento das mesmas e a indicação de intervenções mais direcionadas no controle do perfil glicêmico.

Desta forma, apesar do grande número de estudos recentes buscando a caracterização da variabilidade glicêmica em situações patológicas, ainda existem lacunas entre os índices e suas relações aos mecanismos fisiológicos envolvidos na manutenção do padrão glicêmico. A análise de sinais biológicos exige um estudo sistemático e criterioso, primeiramente em condições fisiológicas, para que seja ajustado

o modo mais apropriado para as análises. Além disso, levar em consideração o comportamento do sinal apresentado é muito importante, conforme ilustrado na figura 2. A média e o desvio padrão de dois perfis de glicose apresentados são iguais, mas ao observarmos o comportamento dos sinais, eles se diferem (46).

Situações como esta apontam para a necessidade de investigação criteriosa e desenvolvimento de novas ferramentas matemáticas e estatísticas com intuito de extrair as informações adicionais presentes nestes sinais, ligadas aos mecanismos fisiológicos que controlam as oscilações glicêmicas (46).

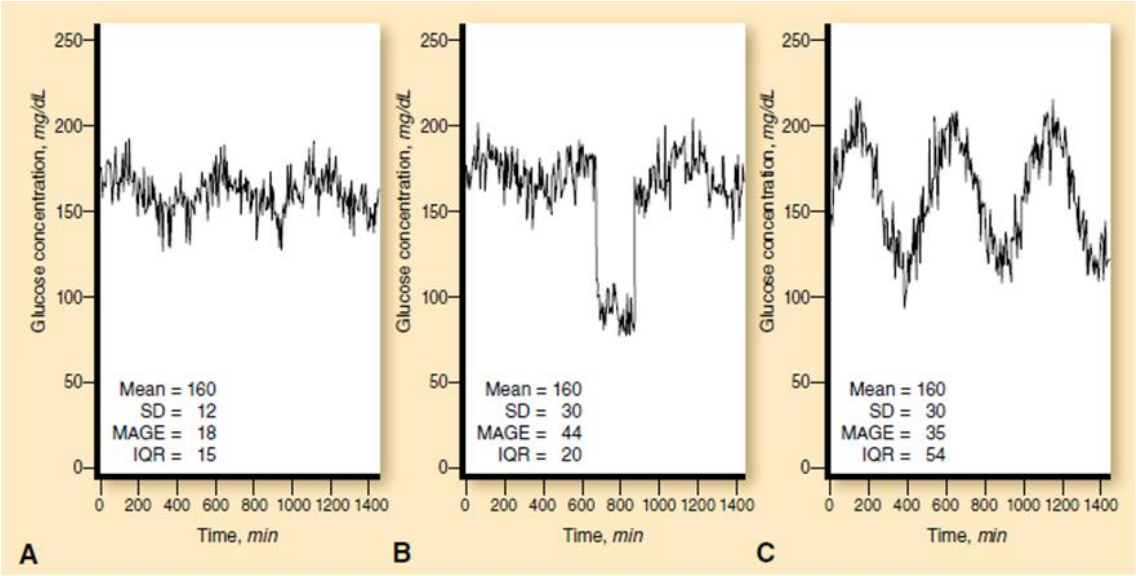

Figura 2. Ilustração gráfica de três exemplos de perfil de glicose sanguínea de 24 h avaliadas pelo CGMS. IQP- Intervalo Interquartil; MAGE- Amplitude Média de Excursões de Glicemia; DP- Desvio Padrão (46).

Variabilidade glicêmica, ativação de estresse oxidativo e o processo inflamatório

Muitas das investigações envolvendo a análise de variabilidade do sinal glicêmico têm buscado quantificar variáveis ligadas ao estresse oxidativo, sensibilidade insulínica e marcadores inflamatórios com o intuito de correlacionar mecanismos

fisiológicos específicos às características matemáticas das variações glicêmicas (14, 32, 33). De acordo com Brownlee (2005) a liberação de radicais livres e a ativação do estresse oxidativo são mecanismos que contribuem para o desenvolvimento de grande parte das complicações crônicas na evolução do DM (36). Estudos mostram que a hiperglicemia do DM está associada com aumento da formação e excreção urinária de (PGF2) (47), importante marcador de estresse oxidativo (48) que está relacionado com a variabilidade glicêmica (47, 49) e também associado à disfunção endotelial (25), bem como um aumento da espessura íntima-média da carótida e massa ventricular (50). No entanto, o mesmo resultado não foi mostrado em DM1 (51). É importante entender que a insulina devido à sua ação antioxidante pode exercer um efeito inibidor sobre o estresse oxidativo, e a correlação entre a variabilidade da glicose e os índices de estresse oxidativo pode não ocorrer (52).

O processo inflamatório também parece interferir na variabilidade glicêmica. Em estudo realizado em indivíduos com obesidade e SM e DM com SM, marcadores inflamatórios como a interleucina (IL-6) relacionaram-se com a variabilidade glicêmica destes pacientes ($R(2)= 0,35$ beta$= 0,13$; $P < 0,05$) independentemente do índice de massa corporal (IMC), circunferência da cintura, concentrações de adiponectina e insulinemia (53). Em células de rim humano *in vitro*, a produção de citocinas inflamatórias, avaliadas através do fator de transformação do crescimento beta (TGF-β1) foi maior quando as células foram expostas a concentrações variáveis de glicose (353%, $P <0,0001$) do que quando expostas a estados hiperglicêmicos constantes (230%, $P <0,05$). Os autores concluíram que, embora a manutenção de níveis normais de glicose no sangue resultaria em menor grau de estresse oxidativo e inflamação no túbulo-intersticial, o controle glicêmico variável provavelmente seria ainda mais prejudicial do que a hiperglicemia constante (54).

Um possível mecanismo através do qual a glicose oscilante pode ter um efeito mais prejudicial do que a glicose constantemente alta é a defesa antioxidante inadequada de células com a glicose oscilante (55), uma condição que poderia favorecer o desenvolvimento de complicações do DM (56). Em suma, a fisiopatologia de complicações do DM pode ser considerada o resultado de duas grandes alterações metabólicas deletérias (excessiva glicação e geração de extresse oxidativo) que são ativados por três principais distúrbios glicêmicos: hiperglicemia de jejum, hiperglicemia durante períodos pós-prandial e flutuações de glicose. De forma mais geral, flutuações agudas da glicose em torno do valor médio da glicose ativam estresse oxidativo e o resultado disso é o aumento do risco de complicações do DM (16). Dentre as várias medidas para prevenção e tratamento do DM, o exercício físico tem contribuído com o controle glicêmico e em reduzir risco de desenvolvimento de DM (57-60).

O exercício físico no manejo do Diabetes Mellitus

A redução da glicemia induzida de forma aguda pelo exercício é resultante da elevada captação de glicose pelo músculo determinada pelo aumento da sensibilidade à insulina induzida pela contração muscular durante o exercício (61), efeito que pode durar até 72 h (62). Sabe-se que o aumento da sensibilidade à insulina determinada pelo exercício é decorrente do aumento da proteína GLUT4 (61, 63), que por sua vez é determinado pela maior atividade muscular esquelética da proteína quinase ativada pelo monofosfato de adenosina (AMPK) (61). Essas mudanças ocorrem muito rapidamente em resposta a uma única sessão de exercício físico sem metformina e podem durar 16 horas após a intervenção (63).

Em ensaio clínico com indivíduos com tolerância diminuída à glicose, mudanças de estilo de vida (150 minutos de caminhada/semana e perda de 5% de peso) foram

capazes de reduzir risco de desenvolvimento de DM2 em 58% de indivíduos com tolerância diminuída à glicose. Metformina também se mostrou eficaz, porém com efeito menor (31%) do que o da mudança de estilo de vida e não duradouro após suspensão da intervenção (58).

Já em relação à busca por um bom controle glicêmico no DM através do exercício físico, considerando-se a modalidade a ser empregada, melhor controle glicêmico é igualmente obtido com quaisquer das práticas usuais (aeróbico, de força e a combinação de ambos). Revisão sistemática com o objetivo de avaliar a associação de treinamento de exercício estruturado (aeróbico, de resistência, ou ambos) e do aconselhamento para atividade física, com ou sem co-intervenção dietética sobre as mudanças nos níveis de HbA1c em pacientes com DM2, mostrou que exercícios estruturados reduzem os valores HbA1c em aproximadamente 0,67%, mas um importante achado está em relação ao tempo de exercício estruturado. A duração de mais de 150 minutos por semana foi associada a uma redução de HbA1c de 0,89% e a duração de 150 minutos ou menos por semana foi associada com reduções de HbA1c de 0,36%. A intervenção de aconselhamento para atividade física foi associada com redução da HbA1c quando associada com intervenção sobre a dieta. Este resultado de queda da HbA1c foi menor do que o observado para qualquer modalidade de exercício físico estruturado (60, 64).

Estudos também têm mostrado que tanto o exercício físico estruturado como a orientação de atividade física pode reduzir a pressão arterial em pacientes com DM. Três metanálises (65-67) avaliaram os efeitos de diferentes tipos de treinamento de exercício estruturado sobre a pressão arterial, apresentando resultados controversos em ambas, pressão arterial sistólica (PAS) e diastólica (PAD). Chudyk e Petrella (2011) mostram reduções na PAS somente com o exercício aeróbico (-6,8mmHg) e combinado

de (-3,59mmHg), sem modificações na PAD, enquanto que o exercício de força não apresentou nenhuma modificação em ambas PAS e PAD. Já o estudo de Snowling e Hopkins (2006) apresentou reduções na pressão arterial com os três diferentes tipos de exercícios físicos, mas pouco significativas (aeróbio [PAS: -0,22mmHg e PAD: -0,21 mmHg]; força [PAS: -0,08mmHg e PAD: -0,15mmHg] e combinado [PAS: -0,35mmHg e PAD: -0,63mmHg]). Quando avaliado o efeito do exercício estruturado (aeróbico, força e combinado), todas as intervenções juntas, foi observado reduções em ambas PAS (-2,42mmHg) e PAD (-2,23mmHg) (67). No entanto, as revisões citadas acima, não exploraram a heterogeindade dos estudos, pois não avaliaram a atividade física como uma intervenção independente, sendo avaliada com os demais exercícios estruturados.

Estudo que nos permite entender melhor os efeitos da atividade física sobre a pressão arterial, isoladamente dos exercícios estruturados, foi realizado com DM2. O objetivo do estudo foi avaliar um programa de atividade física prescrito para atingir gradualmente uma meta de 175 minutos por semana de atividade física de intensidade moderada realizado em casa, avaliado em conjunto com outras mudanças no estilo de vida como co-intervenção. Foi possível verificar que a atividade física aliada a mudança de estilo de vida também pode resultar em reduções da PAS em (-4,0 mmHg) e PAD em (-1,2 mmHg) (68), podendo a chegar a valores mais elevados que os estudos que não tomaram o cuidado de isolar a atividade físca dos exercícios estruturados.

Além do exercíco físico contribuir no controle glicêmico através de reduções de níveis de HbA1c e reduzir a pressão arterial em pessoas com DM, ele têm se mostrado efetivo também em reduzir níveis elevados de glicose pós prandial (69) que também é considerada influência para maior risco de doenças cardiovasculares (70). Estudo realizado em indivíduos que apresentavam obesidade e DM2 que realizaram sessões de

exercício físico de força antes e após a injestão alimentar (injestão notuna), mostrou que realizar exercício de força antes injestão alimentar reduz as concentrações de glicose pós-prandial em torno de 35%, mas realizando o exercício 45min. após a injestão, além de reduzir as concentrações de glicose em torno de 48% também pode reduzir concentrações de triacilglicerol. Os dados indicam que o exercício de força realizado após a injestão alimentar noturna poderia reduzir o risco de doença cardiovascular de forma mais eficaz em pacientes com DM (69).

Diferentes protocolos de exercício podem evocar respostas fisiológicas completamente diversas fazendo com que a escolha e o ajuste de parâmetros específicos, como intensidade e tipos de exercícios, sejam fundamentais (71), inclusive em situações patológicas como é o DM (72). O exercício excêntrico, por exemplo, aumenta a secreção de insulina (62) e de forma aguda, devido ao dano muscular causado, evoca resposta inflamatória (73) e aumento do estresse oxidativo (74).

Avaliando o dano muscular através da força isométrica, amplitude de movimento, dor muscular e circunferência do braço após uma sessão de exercício excêntrico, em mulheres jovens e mulheres pós-menopausa que não faziam uso da reposição hormonal, não foi observado diferença em dano muscular entre os grupos, contudo, dentro dos grupos ao longo do tempo ocorreram modificações significativas ($P < 0.05$). Já os níveis de marcadores inflamatórios como interleucina 6 (IL-6) pré exercício excêntrico foram maiores no grupo das mulheres pós-menopausa comparadas as mulheres jovens (2,09 ± 1,12 *vs*. 0,58 ± 0,33 P= 0,031, respectivamente). A correlação positiva entre idade e citocinas (IL6= r: 0,75 P=0,0001; TNFα= r: 0,79 P=0,001) pode justificar os níveis de IL-6 basais maiores em mulheres na menopausa, pois marcadores inflamatórios aumentam conforme a idade. O estudo também nos mostra que mulheres jovens não têm dano muscular atenuado em relação a mulheres na

menopausa sem uso de reposição hormonal, contudo, mulheres jovens têm uma maior resposta anti-inflamatória após exercício excêntrico em relação às na menopausa (IL6: 2,50 ± 1,27 *vs.* 2,14 ± 1,52; IL10: 2,34 ± 1,20 *vs.* 1,04 ± 0,72, respectivamente). O aumento da produção de IL-6 72h após exercício excêntrico pode estar relacionado com uma ação anti-inflamatória que concomitantemente também aumentou a produção de interleucina 10 (IL-10), porém, neste estudo o dano muscular não foi associado ao processo inflamatório (75).

O exercício aeróbico também pode causar mudanças em marcadores inflamatórios. Quando indivíduos treinados realizaram exercício em esteira durante 45 min. a 60% do seu consumo máximo de oxigênio (VO_{2max}), observou-se um aumento de 410% nos valores IL-6 1h após a sessão do exercício em relação aos valores prévios (76). Desta forma, o uso de diferentes protocolos, padronizados adequadamente, pode servir não apenas como intervenção sobre determinadas variáveis, mas também como ferramenta experimental para análise de respostas a determinados estímulos.

O papel do exercício físico na variabilidade glicêmica

Tendo em vista que a variabilidade glicêmica atualmente pode ser um alvo terapêutico no DM, o exercício físico também é aliado para atuar sobre as variações da glicemia (39, 77-78). Estudo realizado em mulheres com DM1 submetidas a uma avaliação do nível de atividade física através de acelerômetros e glicemia monitorada pelo CGMS, propôs para as voluntárias alcançarem um tempo de caminhada livre de 5-6h por dia durante um período de avaliação de três dias. Para atingir o tempo de caminhada diária as participantes tiveram que distribuir ao longo do dia sessões de caminhadas com tempo de 33,5 min. numa velocidade de 1,9km/h seguido de 26,5 min sentado, totalizando 3,5 à 4,2 km/h percorridos durante um período de 24h. O tempo de

atividade física avaliado foi de 30 min. antes da refeição até 270 min. após. O estudo mostrou que caminhadas livres, mesmo com intensidades muito baixas (1,9 Km/h), podem reduzir as variações da glicemia pós-prandial (77).

Não muito diferente destes resultados, em estudo que avaliou mulheres grávidas com DM1 submetidas à atividade física (orientação para realizar 20 min. de caminhada após o café da manhã, almoço e janta) e duas sessões de exercício físico estruturado associado à dieta (50 min. de caminhada em esteira na velocidade de 3,0 Km/h no turno da manhã e 50 min. de caminhada em esteira na velocidade de 2,6 Km/h à 4,8 no turno da tarde). Observou-se reduções bastante significativas na variabilidade da glicose no período noturno destas pacientes (desvio padrão da glicose (DP) 1,3 *vs.* 0,7 mmol/L, P = 0,022) após exercício físico associado à dieta (78). Porém, o uso de insulina, que é tratamento padrão no DM1, pode contribuir muito para variações grandes na glicemia e variabilidade glicêmica nestes estudos. Esta é uma limitação inerente aos resultados de estudos de variabilidade glicêmica em pacientes com DM1.

Em relação aos efeitos do exercício físico aeróbico ou a combinação de aeróbico/força em pacientes com DM2 sobre a variabilidade glicêmica, poucos estudos são encontrados na literatura. Com a finalidade de buscar entender melhor os efeitos destes tipos de exercícios físicos, nosso grupo avaliou em pacientes com DM2 os níveis de glicose intersticial e variabilidade glicêmica (CGMS) após uma sessão aguda de exercício físico aeróbico e combinado (aeróbico/força) (39). A análise das curvas de múltiplas medições de glicose usando o CGMS mostrou redução da glicemia ~16% logo após a intervenção, mas seu efeito não foi sustentado por mais de 6 horas após o protocolo de exercício. Entretanto, mesmo sem alteração sustentada da glicemia, a variabilidade glicêmica reduziu e foi mantida até 24h após o exercício. A redução foi similar nas diferentes modalidades de exercício físico e pode ser vista em dois índices

de variabilidade glicêmica: variância da glicose (exercício aeróbico: 287,06 ± 66,18 mg^2/dL^2; exercício combinado: 421,45 ± 85,72 mg^2/dL^2, P= 0,389) e coeficiente de variação da glicose (exercício Aeróbico: 13,13 ± 1,93 %, exercício combinado: 14,29 ± 1,25%, P = 0,531). Tal resultado aponta para uma possível alternativa de intervenção não-medicamentosa sobre a variabilidade glicêmica em pacientes com DM.

Diferentemente dos outros estudos que avaliaram a variabilidade glicêmica em relação ao exercício, nosso estudo prévio (39) utilizou apenas uma única sessão de exercício físico e evidenciou que esta uma única sessão pode alterar a variabilidade glicêmica em DM2, sugerindo que nossos protocolos poderiam ser indicados para acessar mecanismos alterados nesta patologia. No entanto, mesmo que alguns mecanismos fisiológicos já mencionados (16, 61, 63) possam agir sobre a sensibilidade à insulina em resposta ao exercício e explicar as alterações que ocorrem com a variabilidade glicêmica, nosso estudo não investigou estes possíveis mecanismos e nem os diversos mecanismos locais e sistêmicos que podem estar modulando a variabilidade glicêmica e interferindo sobre tais alterações.

Considerando que a variabilidade glicêmica em pacientes com DM2 foi reduzida após sessão aguda de exercício aeróbio e combinando, e o potencial de informação contida na variabilidade glicêmica, justifica-se entender melhor os parâmetros relacionados a estas variáveis utilizando métodos matemáticos específicos já amplamente utilizados na análise de sinais biológicos. Estes métodos incluem análises espectral e simbólica. Análises de correlação dos parâmetros encontrados às medidas específicas ligadas aos mecanismos fisiológicos atuantes sobre a variabilidade glicêmica, como marcadores de estresse oxidativo e inflamação seria o passo a seguir (16), em indivíduos hígidos submetidos aos dois protocolos de exercício agudo, um que conhecidamente causa aumento de sensibilidade insulínica e redução glicêmica

(exercício aeróbio) e outro que evoca resposta predominantemente inflamatória (exercício excêntrico). Poucos estudos analisaram sinais obtidos por CGMS em indivíduos saudáveis e suas características estatísticas associadas a parâmetros fisiológicos (14-15, 45). Para a caracterização ideal de um sinal biológico, como a variabilidade glicêmica obtida por CGMS, análises investigativas de associação entre características matemáticas e mecanismos fisiológicos específicos, é necessário, primeiramente, o estudo em indivíduos hígidos para que seja descrita a presença de tal condição fisiológica.

2. OBJETIVOS

2.1 Objetivo Geral

Caracterizar da variabilidade glicêmica durante e após diferentes protocolos de exercício agudo (aeróbico e excêntrico, aeróbico e aeróbico + força e exercício de carga muscular inspiratória), através de ferramentas matemáticas lineares e não lineares em indivíduos saudáveis e com diabetes mellitus.

2.1.2 Objetivos Específicos

Avaliar de forma aguda em indivíduos hígidos:

(a) a variabilidade glicêmica utilizando índices já estabelecidos na literatura e aplicar as análises espectral e simbólica, para caracterização do sinal;

(b) o estresse oxidativo sistêmico através da avaliação das proteinas (carbonilas) e antioxidantes ácido úrico e sulfidril.

(c) o quadro inflamatório através das medidas de IL-6.

(d) correlacionar as variáveis matemáticas às medidas bioquímicas.

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CAPITULO II

ARTIGO ORIGINAL

Exercise as a tool for studying the mechanisms of glucose variability in healthy subjects: Crossover Randomized Trial

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Running title: Exercise and of glucose variability in healthy subjects

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Abstract

Purpose: To characterize glucose variability using linear and nonlinear mathematical tools, under basal conditions and in response to specific protocols acute, aerobic exercise (AER) or eccentric exercise (ECC) that evoke oxidative stress and inflammation in healthy subjects.

Methods: Sixteen healthy subjects (32 ± 12 years) wore a continuous glucose monitoring system (CGMS) during 3 days. Participants randomly performed AER and ECC sessions, both in the morning (24 h after CGMS placement), and at least 7 days apart. Glucose variability was evaluated by glucose standard deviation, glucose variance, glucose coefficient of variation and glucose variance normalized (conventional methods) as well as by spectral and symbolic analysis (non-conventional methods).

Results: Baseline fasting glycemia was 84±8 mg/dL and HbA1c 5± 0.3%. Comparing the two exercise modalities, responses over a 24-h period after the sessions were similar for glucose levels, glucose variance and glucose, glucose variance normalized, and coefficient of variation, but the glucose variability, as evaluated by glucose standard deviation reduced significantly after AER (17.2 ± 6.0 mg/dL *vs* 15.5 ± 4.9 mg/dL; $P < 0.001$) and ECC exercise sessions (19.3 ± 8.4 mg/dL *vs* 14.8 ± 6.3; $P < 0.001$) and this change was different between types of exercises ($P < 0.001$). For non-conventional analysis of glucose variability (spectral and symbolic analysis) the results obtained no reported differences after both exercise sessions. IL-6 increased after AER exercises (0.889 ± 0.530 pg/mL *vs* 1.505 ± 0.846 pg/mL; $P < 0.001$), with increased no significant after ECC exercise (1.333 ± 1.660 pg/mL *vs* 1.708 ± 2.798 pg/mL; $P > 0.05$). Delta of IL-6 presented correlation with delta of 0V ($r = 0.643$; $P < 0.05$), 1V ($r = -0.608$; $P < 0.05$) and 2LV ($r = -0.594$; $P < 0.05$) in ECC exercise, no correlation in AER exercise. The delta of protein carbonyl correlated with index of conventional analysis (glucose variance, coefficient of variation, glucose standard deviation and glucose variance normalized) in AER exercise, no correlation in ECC. The antioxidants represented a correlation with the patterns of symbolic analysis. The total protein sulfhydryl represent a correlation with delta of 0V ($r = -0.732$; $P < 0.05$) and 1V ($r = -0.776$; $P < 0.001$) in ECC exercise, and delta 2UV in AER exercise ($r = 0.761$; $P < 0.05$) and the delta of uric acid showed a correlation with delta of 2LV in both exercise sessions, AER ($r = 0.852$; $P < 0.001$) and ECC ($r = 0.657$; $P < 0.05$).

Conclusions: Acute AER and ECC modalities reduce glucose variability evaluated by glucose standard deviation (conventional analysis) in healthy individuals, no change with spectral and symbolic analysis (no-conventional analysis). However, the correlation of inflammatory and oxidative stress markers with index of glucose variability by conventional and no-conventional analysis shows an influence of the physiological mechanisms in glycemic behavior.

Keywords: Glucose variability; oxidative stress; inflammation; exercise.

INTRODUCTION

Diabetes mellitus is characterized by chronic hyperglycemia, which is the main target of its treatment, mainly for chronic complications prevention (1-2). Glucose variability may futurely change the insight in the direction of the treatment for patients with diabetes, as it can possibly be related to the development of chronic diabetic complications (3-5). Glucose variability refers to short-term fluctuations in glycemia, such as within-day variability, variability between daily means, or within-series (6). Many of the investigations involving the analysis of the signal of glucose variability have sought to quantify variables linked to oxidative stress, insulin sensitivity and inflammatory markers in order to correlate specific physiological mechanisms to mathematical characteristics of glucose variations (7-9). The release of free radicals and the activation of oxidative stress are mechanisms that contribute to the development of much of the complications in the evolution of diabetes (10). Clinically this relationship could be observed by the association of hyperglycemia of diabetes to increased formation and excretion of 8-iso-PGF2a (11), which is correlated to the variability glucose (7).

Fluctuations of glucose appear to contain additional information about processes directly involved in frame of diabetes hyperglycemia, being complementary to the other usual measures, featuring a potential tool of research and evaluation in these patients (12-14). These glucose fluctuations may also be auxiliary to the understanding of effect of specific interventions used in this disease. Exercise is a non pharmacological measure extremely efficient in preventing (15-16) and treating diabetes (17). It has also shown positive effects on glucose variability (18). In patients with type 2 diabetes, glucose variability, measured by variance and the coefficient of variation (conventional methods) was reduced and maintained until 24 hours after a single session of AER and

AER/resistance exercise. The application of specific methods of heart rate series was also used in this study (non-conventional methods: symbolic analysis and spectral analysis). Glucose variability was evaluated by symbolic analysis induced an increase of 0 V pattern, accompanied by a reduction in the 1 V pattern. These changes, favoring 0 V pattern, represent no variation, indicating a decrease in glucose variability when evaluated on three consecutive measures. Moreover, spectral analysis indicated the presence of a slow physiological mechanism acting within a period of around 8 minutes (predominant band at around 0.002 Hz) (18). These results obtained with non-conventional methods of glucose variability evaluation showed that complementary information on glucose oscillation could be provided. However, the study did not investigate the possible mechanisms involved in the response of glucose variability to the exercise protocols tested.

Symbolic analysis is an approach based on quantification of complexity that allows an advanced characterization of glucose variability series and the identification of experimental conditions known to differently perturb glucose oscillations (18). Spectral analysis is a linear method that allows quantifying the oscillatory components from time series, by autoregressive model, widely applied to heart rate and arterial pressure series (19). A peak detector algorithm is used to determine possible predominant bands in the frequency power spectrum (18). The methodology was applied to 10 surrogate signals, generated from each segment, to test false spectral results (20).

Despite large number of recent studies have attempted to characterize glucose variability in pathological situations, there are still gaps between the indexes and their relationship to the physiological mechanisms involved in the maintenance of glucose pattern. Oxidative stress and inflammation have previously been related to changes in

glucose variability (7). Considering that different exercise protocols would evoke responses and that aerobic exercise (AER) induces increase of oxidative stresss (21) and eccentric exercise (ECC) increase of inflammation (22), in the present study we aimed to characterize glucose variability using linear and nonlinear mathematical tools, under basal conditions and in response to specific subacute exercise protocols, AER or ECC in healthy subjects. Glucose variability was evaluated by conventional (glucose standard deviation, glucose variance, glucose variance normalized and glucose coefficient of variation) and non-conventional methods (spectral and symbolic analysis).

METHODS

Research Design and Participants. Sixteen healthy subjects participated in the experiments using a crossover randomized design. Exclusion criteria were regular practice of exercise, or having any chronic disease, mainly diabetes mellitus, hypertension, heart failure and cancer. The protocol was approved by the Ethics in Research Committee at Hospital de Clínicas de Porto Alegre (Number: 120148) and all patients provided their written informed consent before the participation. At the entry of the study, clinical characteristics, usual physical activity (International Physical Activity Questionnaire - IPAQ) (23), anthropometric evaluation, as well as a 12-h fasting blood sample (glucose, HbA1c) were obtained for each subject**.** One week prior to the experimental exercise session, subjects underwent a maximal cardiopulmonary exercise testing and a maximal strength testing.

Maximal Cardiopulmonary Exercise Testing. The maximal incremental exercise test was performed on an electrically braked cycle ergometer (ER-900, Jaeger, Würzburg, Germany) with increments of 20W/minute, as previously described (24). During the test, gas exchange variables were measured by a previously validated system (Oxycon

Delta, VIASYS, Healthcare GmbH, Jaeger, Germany). Heart rate was continuously monitored by a 12-lead electrocardiogram (Nihon Kohden Corporation, Japan) and blood pressure was measured with an automatic oscillometric device every 2 min.

Strength Testing. Strength was measured by 1 repetition maximum (1-RM), which was preceded by exercises at mild intensity for movement familiarization and warm-up. Proper technique was demonstrated and practiced for leg press exercise (Sculptor, Porto Alegre Brazil).When new attempts were needed, a 5-min resting period was allowed between subsequent attempts.

CGMS Measurements. Subjects were admitted to the laboratory in the morning at approximately 9:00 a.m., 24 h before the exercise session, when the glucose sensor (Sof-Sensor™, Guardian® REAL-Time System/Medtronic, Northridge, USA) was inserted subcutaneously. This method was fully described previously (18). Glucose profiles were collected the day before (day 1), the day of (day 2), and the day following (day 3) the single 1-h bout of exercise. Each sensor was used continuously for up to 72 h. Subjects were oriented to avoid physical exercise except for the protocol.

Glucose Variability Evaluation: Glucose variability was assessed from series of absolute values of glucose, obtained by CGMS, sampled every 5 minutes. The first set period for the analysis was the one obtained 17h before each exercise, which was compared to the one obtained 17h after the AER and ECC exercise sessions. Glucose variability was evaluated using conventional analysis and other mathematical methods, here nominated as non-conventional analysis of glucose variability.

Conventional analysis of glucose variability was constructed from the statistical properties of the series, obtaining the following indices: glucose variance (VAR), glucose coefficient of variation (CV%), and glucose standard deviation (SD), and

glucose variance normalized (VarN), all normalized by the mean blood glucose on each period (14, 25-26).

Non-conventional analysis of glucose variability was conducted using two methods applied to the glucose series: a linear method based on spectral analysis and an integrated nonlinear approach to the complexity analysis, symbolic analysis. Spectral analysis is a linear method that allows quantifying the oscillatory components from time series, by autoregressive model. A peak detector algorithm was used to determine possible predominant bands in the frequency power spectrum (18).

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 (27). Symbolic analysis is an approach based on quantification of complexity that allows an advanced characterization of glucose variability series and the identification of experimental conditions known to differently perturb glucose oscillations. This method was described and validated previously in glucose variability series (18). Each subject and each experimental condition had its own range of glucose variations. Therefore, the full range of the sequences was uniformly spread on 6 levels (from 0 to 5), and presence of patterns was quantified. A redundancy reduction criterion was proposed to distribute deterministic patterns of the group in four categories according to the number and type of glucose changes: 1) no variation (0V); 2) one variation (1V); and 3) two like variations (2LV); 4) two unlike variations (2UV) (18).

The surrogate signals were generated by routines implemented in Matlab.

Biochemical analysis: Approximately 9 ml of blood was collected from an antecubital vein using a disposable needle and vacutainer containing sodium heparin or ethylene diamine tetra-acetic acid (EDTA). Venous blood was drawn 10 min. before and

immediately after each exercise bout. The blood was centrifuged for 10 min to separate plasma, and then was stored at -80 °C until further analysis to evaluate the markers as follows:

Total Protein Sulfhydryl: Briefly, 45 µL of plasma was mixed with 120 µL of PBS and 35 µL of 30 mM Tris/3 mM EDTA (pH 8) in a microplate well. After reading baseline absorbances (412nm), samples were reacted with 10 µL of 5,5'-dithiobis-(2-nitrobenzoic acid) (10 mM in ethanol) for 1 hour. Samples were read again (412nm) and baseline absorbances were discounted. The obtained values were compared with those obtained with a cysteine standard curve and results were expressed as nmol –SH/mg protein, as described before (28).

Protein carbonyl: Briefly, protein content in samples was determined with Bradford method, using a commercial kit (BIORAD, cod. 500-0001). After, a volume of sample containing 1 mg of protein was reacted with 10 mM 2, 4-dinitrophenylhydrazine for 30 min and subjected to protein precipitation with 10% TCA followed by centrifugation (11,000 *xg*, 3 min, 4ºC). The pellet was washed with ethanol: ethyl acetate (1:1) and centrifuged (11,000 xg, 3 min, 4ºC) three times. After, the pellets were suspended in 6 M guanidine hydrochlorie (in 20 mM KH_2PO_4, pH 2.4). Blank samples reacted with 2 M HCl instead of 10 mM 2, 4-dinitrophenylhydrazine and were run in parallel. Results were expressed as nmol carbonyl/mg protein, as described before (29).

Interleukin-6 (IL-6): For the measurement of IL-6, commercially available Human Ultrasensitive IL-6 Magnetic Bead Kit (Carlsbad, United States). The kit was used according to the manufacturers' instructions (Life Tecnologies ^{TM,} Catalog number: LHC0063M, Carlsbad, United States) and data were collected using the Luminex (Carlsbad, United States).

Uric Acid: The measurement of uric acid was determined according to Roche/Hitachi Cobas c 311, Cobas c 501/502 (Mannheim, Germany) in plasma.

Randomization. Subjects participated in the experimental sessions according to a computer-generated randomization. Such process occurred once before subjects were recruited in the study, indicating the order of the two 40-min sessions of either aerobic exercise (AER, n = 15) or eccentric (ECC, n = 15) exercise, which were separated by at least 7 days. A researcher who was not part of the interventions handled the randomization.

Exercise Protocols. Two exercise sessions, consisting of either AER or ECC exercise protocols, were carried out in a randomized order. Exercise intensity was recorded for each individual by a heart rate monitor (Polar F1 TM, Polar Electro Oy, Helsinki, Finland), and a Borg 0-10 scale was used to register individuals' perceived exertion every 5 minutes throughout the experimental sessions. For the AER session, subjects exercised on a cycle ergometer (Embreex 360, Brusque, Brazil**)**. Each session included a 5-min warm-up at 20 watts, followed by 40 min at 70% of the peak heart rate, as determined in the incremental exercise test, and 5-min of stretching exercise as cool down. In the ECC session, subjects initially performed a specific warm-up of 15 repetitions on leg press. Thereafter, the main part of the ECC session was conducted during 40 min, in which subjects completed 6 eccentric sets of 10 repetitions at 120% of 1-RM for each leg (the exercise was one-sided), with completely unloaded concentric phase (lasting approximately 2 seconds), and 2-second eccentric phase. Resting between sets and exercises lasted 2 min.

Statistical Analysis. SPSS Statistical software was used for statistical analysis. Descriptive data are presented as mean and SEM or median and interquartile range (P25-P75). Deltas values represented the difference between pre and post situation on

each exercise session. The non-normal data were parametrically transformed into a logarithmic scale before analysis. The Spearman correlation was used to non-normal distribution data. The effects of the interventions were compared by two-way analysis of variance for repeated measures (ANOVA), and multiple comparisons were performed with the Bonferroni correction. Statistical significance was accepted when $P<0.05$.

RESULTS

Twenty healthy subjects were screened for participation; 16 were included to participate in the protocols. Demographic and clinical characteristics of participants are shown in Table 1. Patients were 32 ± 12 years old, predominantly women. Regarding the physical activity level, eight subjects were classified as insufficiently active, four were sufficiently active, while three were classified as very active. A flow diagram of the sample selection and study conduction is shown in figure 1.

All participants completed both exercise interventions (AER and ECC). Glucose levels (CGMS, first measure, representing a 5 min average glucose), heart rate, systolic and diastolic blood pressure measured before and at the end of the exercise sessions are presented in Table 2. Both exercise modalities elicited increases in heart rate and systolic blood pressure, however no changes in glucose levels were observed.

Table 3 shows the results of glucose variability analysis. Glucose variability, as evaluated by glucose standard deviation, was reduced after both AER and ECC exercise sessions. Glucose variance, glucose coefficient of variation, and glucose variance normalized did not no change after neither exercise sessions. The same was observed in spectral analysis results. The predominant band, centered at 0.002 Hz, was present in all

analyzed series and similar spectrum parameters were presented after exercise for the two exercise modalities. In the patterns of symbolic analysis, the results obtained appointed no differences between exercise sessions.

Biochemical analyses are presented in figure 2. Interleukin 6 levels increased after the AER session, and did not change after the ECC session. Uric acid levels were increased after both AER and ECC sessions. Protein damage, evaluated by protein carbonyl quantification, did not differ between after and before any of the exercise sessions. Similar findings were obtained for total protein sulfhydryl after both exercise sessions.

The correlation between inflammatory and oxidative stress markers with glucose variability evoked by AER and ECC sessions are presented in figure 3. This figure illustrates only one variable of each marker. The IL-6 showed a positive correlation with the index of glucose variability (no-conventional analysis), represented by the correlation of IL-6 with 0V pattern after ECC, whereas oxidative stress showed a positive correlation with the index of glucose variability (conventional analysis) represented by the correlation of carbonyl with glucose variance normalized after AER.

Tables 4 and 5 show all the results of correlation analyses between delta indeces of glucose variability (conventional and no-conventional analysis) with inflammatory and oxidative stress markers.

DISCUSSION

In the present study, we showed that acute sessions of AER and ECC promote similar reductions in glucose variability as evaluated by glucose standard deviation in healthy individuals. However, other parameters of glucose variability (glucose variance, glucose coefficient of variation, glucose variance normalized, spectral and symbolic

analysis) did not change after exercise sessions. Interestingly, inflammatory markers correlated positively with the index of glucose variability by symbolic analysis in ECC, whereas oxidative stress markers correlated postively with the índex of glucose variability by conventional analysis and symbolic analysis in AER and ECC.

It is well-known that aerobic exercise evokes an inflammatory response (30), and this was shown here by the IL-6 increments observed after the AER session. In healthy subjects with 23.6 ± 0.4 years one hour session of AER on cycle ergometer at 60% VO_{2max} determined an increase in IL-6 levels 30 min after the session. These levels were maintained high for approximately one hour (31). Another study in healthy subjects using a similar intensity (55% VO_{2max}), also reported an increase in IL-6 from 30 min on after AER (32). Our study used a higher intensity (70% of VO_{2peak}) in a population with the same characteristics of these previous studies, however, we performed only one evaluation of IL-6 immediately after exercise. More measurement points could confirm the prolonged effect of intensity of exercise on the IL-6 in this study. Our findings are in agreement with the study of Huang (30) that showed that a single AER session with duration of 30 min at 75% VO_{2max} induces an elevation of IL-6 immediately after AER in healthy subjects.

We did not find increased IL-6 levels after the ECC sessions. The use of large muscle groups during resistance exercise as used in our exercise protocols was previously shown in literature to induce increased IL-6 levels. However, the amount of muscle mass involved in the exercise is important for the increased systemic inflammatory response (33). Some studies that also evaluated exercise using large muscle groups have not shown inflammation as evaluated systemically. One study that compared four different exercise intensities (50% 1-RM, 75% 1-RM, 90% 1-RM, 110% 1-RM) showed that any of these intensities were able to elicit inflammation in a healthy

population (34). Using a similar exercise intensity in the ECC session (120% 1-RM), our findings were similar in relation to IL-6. Also studies that identified delayed-onset muscle soreness after ECC exercise (35) and muscle damage (36-37), did not produce a sustained systemic inflammatory reaction.

On the other hand, when the inflammatory process is investigated through an analysis of the exercised muscle tissue by muscle biopsies obtained from the vastus lateralis of the dominant leg, inflammation appears to be evident. Comparing the inflammatory response by genetic and systemic expression after performing strength exercises of the lower limbs, it was observed that circulating levels of IL-6 and TNF-α cytokines did not change systemically, although there was an increase in mRNA of these cytokines in muscle (38). Moreover, one study did not show an increase in IL-6 after 15 min of one-legged eccentric knee extensor exercise, but showed approximate 2 fold increase 45 and 90 min after the session, that persisted for 2–4 days after the exercise bout (39). This study of Kellsten et al. (1997) the ECC was performed in five sessions each lasting 5 min with 4 min rest between sessions and IL-6 was not evaluated immediately after the session. In non-weight-trained individuals that completed ECC (3 x 15 maximal eccentric elbow flexor actions using 1 arm) plasma IL-6 increased 4 h after the session, showing a second peak between 8 and 12 h after the ECC session (40). Both studies previously mentioned have shown increases in IL-6 after ECC using more points of measures after the sessions, which would have been missed in the present study, because we assessed only immediately after exercise, being one of the limitations of our study. Moreover, differently from our protocol, that used dynamic ECC, these studies cited used isometric ECC, which also may have influenced the different IL-6 responses.

Considering oxidative stress, after both AER and ECC sessions carbonyl have decreased, and the anti-oxidants protein sulfhydryl and uric acid have increased, but only the uric acid increases were statistically significant. During high-intensity AER (ergometric cycle) uric acid reacts with oxygen-derived free radicals and becomes oxidized in skeletal muscle (41); after exercise intracellular uric acid concentrations are rapidly replenished by uptake from plasma (42). The effects of raising uric acid concentration were studied during acute AER (20 min in ergometric cycle) in healthy young subjects. It was shown that uric acid has a scavenger effect upon free radicals. The administration of uric acid, temporarily increased uric acid concentration has shown to reduce exercise-induced oxidative stress (43). These findings point out the importance of uric acid evaluation as a marker of oxidative stress, as it has a protector effect against oxidative stress.

The variation of glucose variance normalized reflects the mechanism the oxidative stress represented by correlation with the carbonyl in AER exercise, which does not happen after the ECC exercise. However, the variation of 0V may also be represented by the variation of the delta of total protein sulfhydryl in ECC exercise, suggesting that the variation in this parameter can have influences on mechanism anti-oxidant of sulfhydryl . Studies related a correlation of the inflammatory and oxidative stress with conventional methods of assessment of glucose variability, such as standard deviation, the coefficient of variability and the mean amplitude of glycemic excursions (MAGE) (7, 9). This correlation under physiological situations may indicate a possible influence of these mechanisms on these variables (7, 44). Inadequate antioxidant defense with oscillating glucose may have a more damaging effect than a constantly high glucose (45), a condition that could possibly favor the development of diabetes complications (46-48).

Uric acid showed a correlation with the delta 2LV after both exercise sessions (AER and ECC), suggesting that the variation of uric acid, an anti-oxidant, can represent the variation of 2LV. The acute increase of uric acid may have influenced the small increase in oxidative stress after the exercises in our study, as well as other factors such as the exercise intensity and insulin secretion. Insulin, due to its antioxidant action, could affect the generation of oxidative stress and therefore conceal the correlation between glucose variability and oxidative stress indices (49).

The relationship between IL-6 responses after AER protocol, expressed by delta index did not show any correlation with the glycemic variability indexes. However, in ECC exercise the IL-6 delta index was correlated with 0V, 1V and 2LV pattern of glucose variability. Despite the observation that IL-6 levels were not increased after the ECC session, the relation between IL-6 responses and 0V, 1V and 2LV variations may be reflecting an important relation between inflammatory mechanisms and theses patterns of glucose variability.

LIMITATIONS

The small sample size may have influenced the results of glucose variability. Besides, was carried out only measurements of blood before and immediately after both exercise sessions to observe changes in markers of inflammation and oxidative stress which would have been missed in the present study.

CONCLUSION

In conclusion, both AER and ECC modalities were associated with reductions in glucose variability evaluated by glucose standard deviation (conventional analysis) in healthy individuals, although no change with spectral and symbolic analysis (no-

conventional analysis) were observed. However, the correlation of inflammatory and oxidative stress markers with indeces of glucose variability by conventional and non-conventional analysis suggest an influence of these physiologic changes in glycemic behavior.

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Conflict of interest

All other authors have no conflict of interest to declare.

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Legends

Figure 1. Flow Diagram

Figure 2. Inflammatory markers and oxidative stress before and after aerobic or eccentric exercise: Panel A, total protein sulfhydryl (n=14); Panel B, protein carbonyl (n=15); Panel C, uric acid (n=12) and Panel D, interleukin-6 (n=15). Data are reported as median and interquartile range (P25-P75). *P <0.01 vs. pre-exercise values (Aerobic); **P <0.05 vs. pre-exercise values (Eccentric). Two-way analysis of variance for repeated measures (ANOVA); Bonferroni correction.

Figure 3. Scatter plot to illustrate the degree of correlation between delta protein carbonyl and delta glucose variance normalized during aerobic exercise (Panel A); delta interleukin-6 and delta 0V pattern during aerobic exercise (Panel B); delta protein carbonyl and delta glucose variance normalized during eccentric exercise (Panel C) and delta interleukin-6 and delta 0V pattern during eccentric exercise (Panel D).

TABLES

Table 1. Baseline characteristics of the patients studied.

Characteristics	
No. of men/women	6/9
Age (yr)	32 ± 12
Anthropometrics	
Body weight (kg)	69 ± 9.7
Body mass index ($kg.m^{-2}$)	24 ± 2.8
Waist circumference (cm)	85 ± 11
Blood pressure (mmHg)	
Systolic	113 ± 15
Diastolic	68 ± 10
Heart rate (bpm)	75 ±12
HbAlc (%)	5 ± 0.3
Fasting plasma glucose (mg/dl)	84 ± 8.5
Physical activity level (IPAQ) n (%)	
Insufficiently active	8 (53)
Sufficiently active	4 (27)
Very active	3 (20)
VO_2 peak ($mL.Kg^{-1}.min^{-1}$)	30 ± 5.6
Heart rate peak (bpm)	175 ± 13
$R_{E\text{-}peak}$	1.2 ± 0.0
Maximal strength testing (1-RM) Kg	
Leg extension (right)	60 ± 15
Leg extension (left)	59 ± 14

HbA1c: glycated hemoglobin; VO2peak: peak oxygen uptake per kilogram of body weight/fat-free mass; Rpeak peak respiratory exchange ratio of peak.
**Data are expressed as mean ± SEM, Categorical variables are presented as numbers
(%).

Table 2. Cardiovascular and metabolic responses to exercise

	AER			ECC			Time	Interaction
	Pre	**Post**	**Delta**	**Pre**	**Post**	**Delta**	**P**	**P**
Heart rate (bpm)	78 ± 12	131 ± 14	- 51 ± 16	77 ± 12	91 ± 20	-13 ± 16	0.001*	0.001
Systolic blood pressure (mmHg)	110 ± 10	129 ± 21	-18 ± 14	115 ± 14	126 ± 24	-11 ± 23	0.001*	0.009
Glucose (CGMS; mg/dl)	96 ± 17	94 ± 31	-2 ± 24	101 ± 17	102 ± 20	-1 ± 19	0.772	0.529

P: Two-way analysis of variance for repeated measures (ANOVA). Data are expressed as mean ± SEM. *P < 0.01 vs. pre-exercise values (AER). Glucose (CGMS), measure represents one point (5 min average glucose pre and post exercise). O other variables the measures were taken immediately pre and after exercise session.

Table 3. Glucose variability evaluated by conventional analysis and non-conventional analysis before and after AER or ECC exercise

	AER			ECC			Time	Interaction
	Pre	**Post**	**Delta**	**Pre**	**Post**	**Delta**	**P**	**P**
Conventional								
- Mean glucose (CGMS; mg/dL)	109.1 ± 13.3	111.0 ± 19.6	-1.8 ± 15.0	114.8 ± 21.9	108.1 ± 13.9	6.5 ± 27.5	0.798	0.874
- Glucose variance (mg^2/dL^2)	331.8 ± 247.1	263.3 ± 169.0	68.4 ± 279.7	437.3 ± 449.3	255.3 ± 219.7	182.0 ± 500. 8	0.193	0.335
- Coefficient of variation (%)	6.9 ± 2.1	7.8 ± 2.4	0.7 ± 2.5	6.6 ± 2.2	8.5 ± 3.9	1.9 ± 3.7	0.268	0.264
- Glucose standard deviation (mg/dL)	17.2 ± 6.0	15.5 ± 4.9	1.7 ± 6.7	19.3 ± 8.4	14.8 ± 6.3	4.5 ± 10.2	0.001*	0.001*
- Glucose variance normalized	2.9 ± 2.0	2.4 ± 1.5	0.6 ± 2.3	3.7 ± 3.2	2.3 ± 1.9	1.4 ± 3.4	0.213	0.301
Non-conventional								
Spectral analysis								
- Predominant band (peak at 0.002Hz) (mg^2/dL^2)	61.5 ± 15.6	60.1 ± 20.4	1.5 ± 24.4	61.8 ± 18.4	48.7 ± 17.7	13.1 ± 20.1	0.128	0.109
Symbolic analysis								
- 0V pattern (%)	68.2 ± 7.8	66.5 ± 7.3	1.7 ± 9.4	67.7 ± 9.1	67.4 ± 9.1	0.3 ± 11.7	0.131	0.887
- 1V pattern (%)	28.3 ± 6.6	29.9 ± 6.1	1.5 ± 88.9	28.8 ± 7.7	28.6 ± 7.6	0.2 ± 9.9	0.924	0.815
- 2LV pattern (%)	1.9 ± 1.4	2.2 ± 1.5	-0.3 ± 1.8	1.7 ± 1.2	1.8 ± 1.4	0.1 ± 1.9	0.785	0.839
- 2UV pattern (%)	1.6 ± 0.9	1.4 ± 0.8	0.2 ± 0.7	1.7 ± 1.2	2.2 ± 1.2	-0.5 ± 1.4	0.062	0.153

Occurrence percentage of each pattern: 0 V (no variation), 1 V (one variation), 2 LV (two low variations) and 2 UV (two up variations). Data are reported as mean ± SEM. *P < 0.01 vs. pre-exercise values (AER and ECC) and interaction between exercises. Two way repeated measures ANOVA. The variables here presented were obtained from 17 hours' CMS records before and after the exercise sessions.

Table 4. The correlation between delta glucose variability by conventional analysis and delta measurements biochemical in AER and ECC

		IL-6 AER	IL-6 ECC	Sulfhydryl AER	Sulfhydryl ECC	Carbonyl AER	Carbonyl ECC	Uric Acid AER	Uric Acid ECC
Conventional analysis									
Mean glucose (mg/dL) AER	*R*	-0.325	-0.129	-0.231	0.042	-0.261	0.275	-0.169	0.654*
	P	0.237	0.648	0.427	0.887	0.248	0.321	0.599	0.021
Mean glucose (mg/dL) ECC	*R*	0.043	0.200	-0.051	-0.385	0.036	-0.286	0.155	-0.383
	P	0.879	0.475	0.864	0.175	0.899	0.302	0.631	0.219
Glucose variance (mg^2/dL^2) AER	*r*	-0.196	0.018	0.086	-0.095	0.625*	0.304	0.268	0.049
	P	0.483	0.950	0.771	0.748	0.013	0.271	0.400	0.879
Glucose variance (mg^2/dL^2) ECC	*r*	0.132	-0.011	-0.187	-0.371	0.093	0.018	0.004	0.253
	P	0.639	0.970	0.523	0.191	0.742	0.950	0.991	0.427
Coefficient of variation (%) AER	*r*	-0.100	-0.154	-0.442	0.200	-0.682*	-0.136	-0.268	-0.021
	P	0.723	0.586	0.114	0.493	0.005	0.630	0.400	0.948
Coefficient of variation (%) ECC	*r*	0.100	0.021	0.231	0.398	-0.046	-0.139	0.271	-0.359
	P	0.723	0.940	0.427	0.159	0.869	0.621	0.394	0.252
Glucose standard deviation (mg/dL) AER	*r*	-0.096	0.034	0.200	-0.305	0.564*	0.125	-0.239	0.067
	P	0.733	0.903	0.491	0.288	0.028	0.657	0.454	0.837
Glucose standard deviation (mg/dL) ECC	*r*	-0.015	-0.076	-0.169	0.424	0.264	-0.046	-0.095	0.190
	P	0.958	0.788	0.563	0.131	0.341	0.869	0.769	0.555
Glucose variance normalized AER	r	-0.086	0.154	0.367	-0.266	0.707*	0.189	-0.306	-0.060
	P	0.761	0.585	0.197	0.358	0.003	0.499	0.333	0.854
Glucose variance normalized ECC	r	0.079	-0.243	-0.130	-0.064	0.143	0.064	-0.489	0.330
	P	0.781	0.381	0.659	0.829	0.612	0.820	0.106	0.294

Deltas values represented the difference between pre and post situation on each exercise session. The variables here presented were obtained from 17 hours' CMS records before and after the exercise sessions.

Table 5. The correlation between delta glucose variability by spectral and symbolic analysis and delta measurements biochemical in AER and ECC

		IL-6 AER	IL-6 ECC	Sulfhydryl AER	Sulfhydryl ECC	Carbonyl AER	Carbonyl ECC	Uric Acid AER	Uric Acid ECC
Spectral analysis									
Predominant band (peak at 0.002Hz) (mg^2/dL^2) AER	*r*	0.011	-0.482	0.138	0.165	0.411	0.293	-0.257	-0.158
	P	0.970	0.069	0.637	0.573	0.128	0.289	0.420	0.623
Predominant band (peak at 0.002Hz) (mg^2/dL^2) ECC	*r*	-0.282	0.011	0.029	-0.358	0.075	0.064	0.436	-0.320
	P	0.308	0.970	0.923	0.208	0.791	0.820	0.157	0.311
Symbolic analysis									
0V pattern (%) AER	*r*	0.246	0.404	0.341	-0.218	0.129	0.025	0.042	0.084
	P	0.376	0.136	0.233	0455	0.648	0.930	0.896	0.794
0V pattern (%) ECC	*r*	-0.413	0.643*	-0.130	-0.732*	0.132	-0.346	0.106	-0.351
	P	0.183	0.024	0.659	0.003	0.639	0.206	0.744	0.261
1V pattern (%) AER	*r*	-0.091	-0.260	-0.393	0.222	-0.129	-0.146	-0.106	0.035
	P	0.779	0.404	0.185	0.446	0.648	0.603	0.744	0.914
1V pattern (%) ECC	*r*	0.392	-0.608*	0.130	0.776*	-0.061	0.332	-0.007	0.271
	P	0.208	0.036	0.659	0.001	0.830	0.226	0.983	0.395
2LV pattern (%) AER	*r*	0.196	0.098	-0.196	0.231	-0.086	0.121	0.852*	-0.141
	P	0.542	0.762	0.503	0.427	0.761	0.666	0.001	0.663
2LV pattern (%) ECC	*r*	0.217	-0.594*	-0.174	0.332	0.236	0.393	-0.081	0.657*
	P	0.499	0.042	0.553	0.246	0.398	0.147	0.802	0.020
2UV pattern (%) AER	*r*	0.196	-0.235	0,761*	0.214	0.369	-0.129	-0.323	-0.572
	P	0.542	0.463	0.002	0.463	0.177	0.647	0.306	0.052
2UV pattern (%) ECC	*r*	0.393	-0.126	0.497	0.452	-0.281	0.367	0.216	0.183
	P	0.206	0.696	0.070	0.105	0.311	0.179	0.501	0.568

Deltas values represented the difference between pre and post situation on each exercise session. The variables here presented were obtained from 17 hours' CMS records before and after the exercise sessions.

Figures
Figure 1.

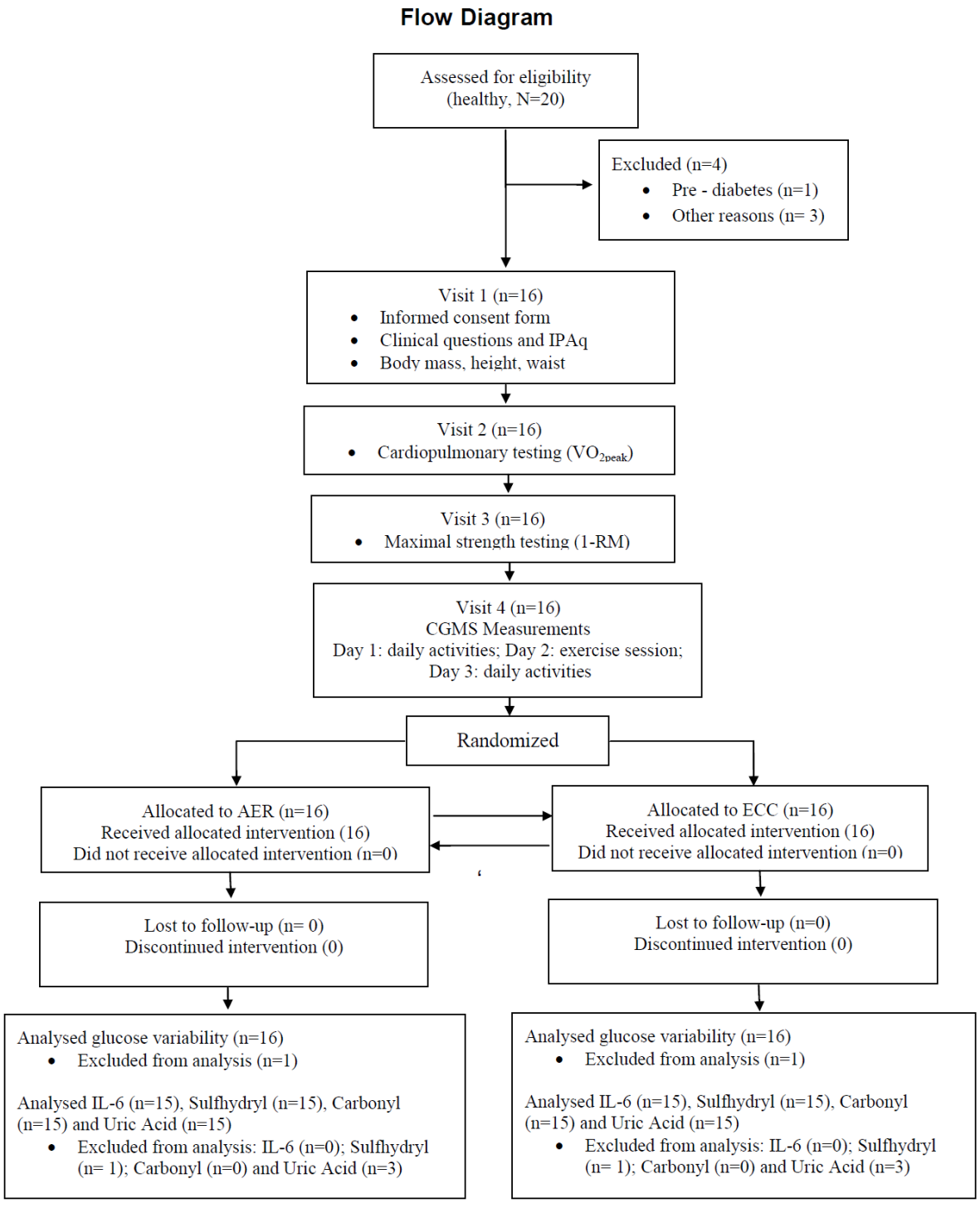


Figure 2.

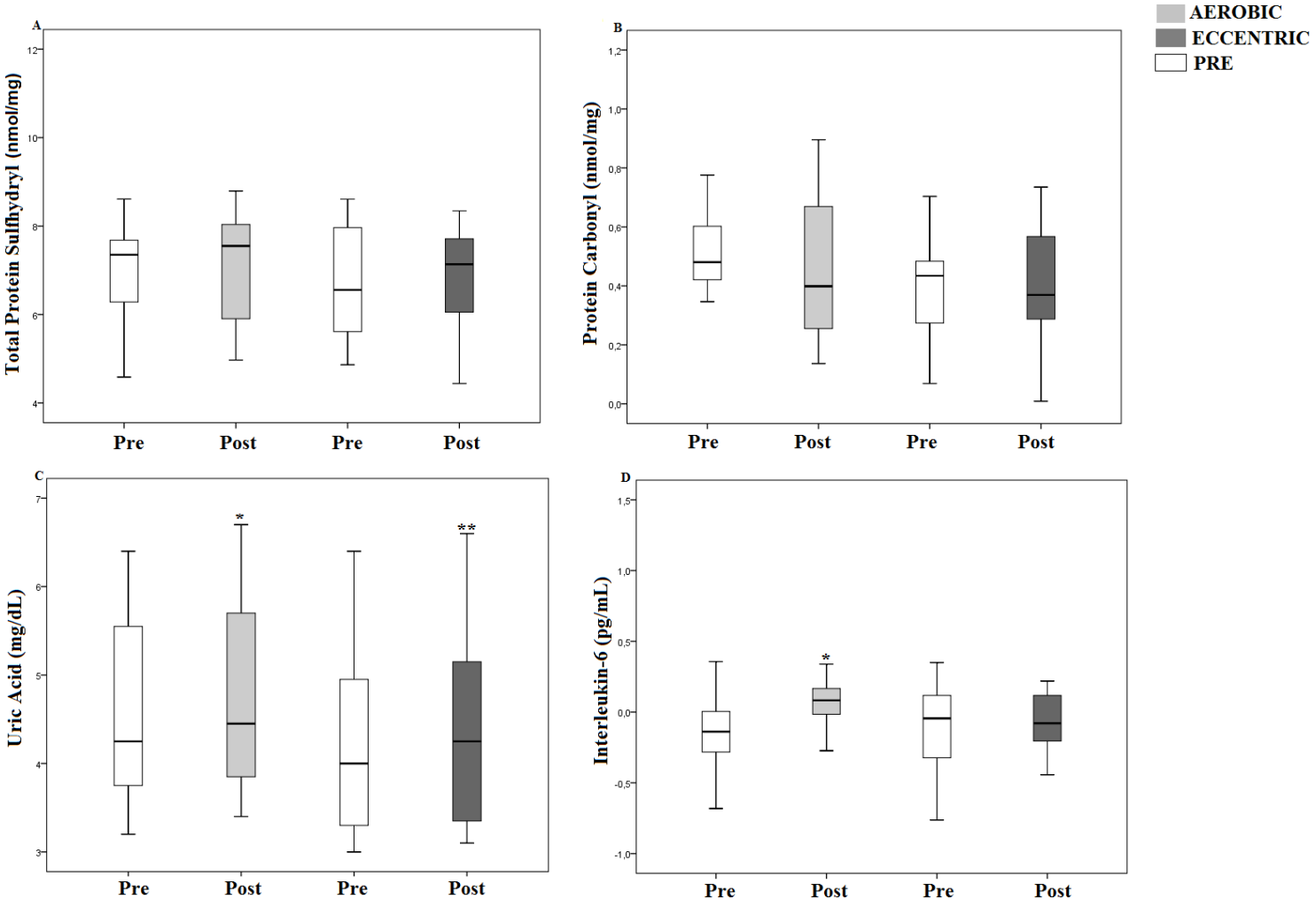
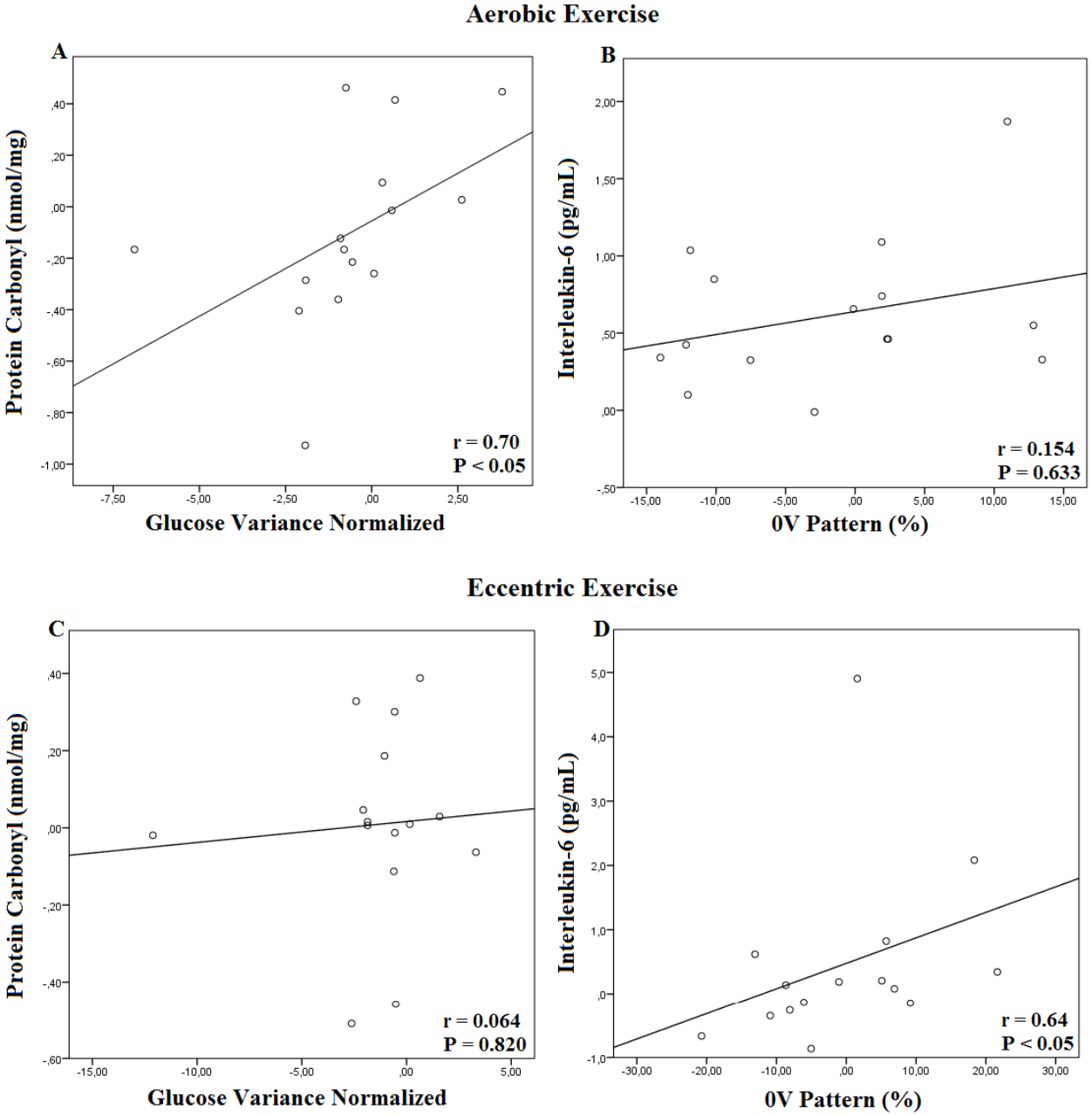

AEROBIC
ECCENTRIC
PRE
A
Total Protein Sulfhydryl (nmol/mg)
B
Protein Carbonyl (nmol/mg)
C
Uric Acid (mg/dL)
D
Interleukin-6 (pg/mL)
Pre
Post
Pre
Post
*
**
*

Figure 3.


Aerobic Exercise
A
Protein Carbonyl (nmol/mg)
r = 0.70
P < 0.05
Glucose Variance Normalized
B
Interleukin-6 (pg/mL)
r = 0.154
P = 0.633
0V Pattern (%)
Eccentric Exercise
C
Protein Carbonyl (nmol/mg)
r = 0.064
P = 0.820
Glucose Variance Normalized
D
Interleukin-6 (pg/mL)
r = 0.64
P < 0.05
0V Pattern (%)

CAPITULO III

ARTIGO ORIGINAL

Inspiratory muscle loading: a new approach for lowering glucose levels and glucose variability in patients with Type 2 diabetes

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

Glucose variability reflects glycaemic excursions that ultimately contribute to increased levels of protein glycation and oxidative stress [1]; therefore, analysis of glucose variability may be promising as a target for intervention to reduce organ damage in patients with Type 2 diabetes.

Physical exercise reduces glycaemia and glucose variability in diabetes [2], but the effects of diaphragm muscle contractions induced by inspiratory loading are still unknown. Accordingly, we aimed to compare glucose levels and glucose variability after aerobic, combined exercise and inspiratory muscle-loading exercise in patients with Type 2 diabetes.

Fourteen patients with Type 2 diabetes, treated with metformin participated in a

crossover trial from two other studies previously approved by the institutional review board; all participants gave their written informed consent. The participants underwent two conventional exercise protocols (aerobic or combined exercise) in a randomized order. For the aerobic session, participants exercised on a cycle ergometer (Embreex 360, Brusque, Brazil) for 40 min at 70% of peak heart rate. In the combined session, the same aerobic protocol was performed for 20 min, complemented by four resistance exercises (leg press, leg extension, bench press and biceps curl), with three sets of 12 repetitions at 65% of one repetition maximum (1 RM). Six of 14 participants Who performed aerobic and combined exercise were also randomly assigned to two sessions of inspiratory muscle-loading exercise [high resistance, 60% of maximum inspiratory mouth pressure (PImax) or low resistance, 2% of PImax]. The inspiratory muscle-loading exercise at 60% of PImax protocol was designed to cause fatigue of the diaphragm at intensities >80% of maximal oxygen consumption (VO2max) [3]. The participants breathed through a two-way valve (Hans Rudolph, 2600 series; Shawnee, KS, USA), with high resistance of inspiratory muscle at 60% of PImax, connected to a POWERbreathe® inspiratory muscle trainer (HaB International, Southam, UK) or to a threshold inspiratory muscle trainer (DHD, Chicago, IL, USA) for low resistance

of inspiratory muscle at 2% of PImax. In both protocols, the baseline data were collected during 5 min of spontaneous breathing. Thereafter, distinct inspiratory and expiratory audio tones were used so that participants could maintain a breathing frequency of 15 breaths per min and a duty cycle of 0.7. Inspiratory pressure was continuously recorded and displayed on a computer screen and a 10-point Borg scale was used to assess inspiratory effort at task failure. Inability to maintain breathing was defined as a reduction of PImax to < 80% of the prescription during three consecutive breaths. As the low resistance experiments do not induce task failure (inspiratory muscle-loading exercise, 2% of PImax), measurements were recorded for 5 min. Thus, in the high resistance session at 60% of PImax (inspiratory muscle-loading exercise), the participants performed breath control/3 min; 60% loading/~5 min and, in the low resistance session at 2% of PImax (inspiratory muscle-loading exercise), they performed breath control/3 min; 2% loading/5 min. For all exercise protocols, participants wore a continuous glucose monitoring system. The four experimental sessions took place 7 days apart. Glucose measurements were obtained every 10 s and averaged every 5 min; these profiles were obtained for 30 min before, during and for 30 min after exercise sessions. Glucose standard deviation, glucose variance and glucose coefficient of variation were calculated. Two-way ANOVA and multiple comparison tests (Student--Newman--Keuls) were performed. Statistical significance was accepted at a P value ≤ 0.05.

Participants were aged 56 ± 7 years, had a BMI 30 ± 4 kg.m-2 and HbA1c levels 61 ± 5 mmol/mol (7.9 ± 0.6 %), characteristics that were similar between those who performed aerobic, combined and inspiratory muscle loading exercise (n=6) and those how performed only aerobic and combined exercise (n=8). Figure 1a shows glucose levels determined by the continuous glucose monitoring system, which were reduced after aerobic (25%), combined (11%) and inspiratory muscle-loading exercise at 60% of PImax (24%). Glucose variability evaluated by glucose standard deviation reduced after exercise aerobic and inspiratory

muscle-loading exercise at 60% of PImax sessions, but not by combined or inspiratory muscle-loading exercise at 2% of PImax (Fig. 1b).

Similar findings were obtained for glucose variability evaluated by glucose variance (Fig. 1c). Glucose coefficient of variation decreased only after inspiratory muscleloading exercise at 60% of PImax session (Fig. 1d). The low resistance of inspiratory muscle-loading exercise at 2% of PImax did not cause any change in glucose levels.

In the present paper, we report for the first time that high resistance of inspiratory muscle exercise at 60% of PImax reduces glucose levels immediately after na acute session, similarly to aerobic and combined sessions. Moreover, aerobic and inspiratory muscle exercise at 60% of PImax promote similar reductions in glucose variability, which were not observed after a single combined session or inspiratory muscle exercise at 2% of PImax in these participants. Conventional exercise can acutely reduce glucose levels in people with diabetes, as it induces acute increases in insulin sensitivity and high muscle glucose uptake [4]; similar responses were expected after non-conventional exercises of inspiratory muscle at 60% of PImax. In an experimental model of muscle fatigue induced by acute respiratory loading, glycogen content decreases significantly, suggesting that the diaphragm may be more dependent on blood glucose for ATP production as compared with limb muscles [5]. This is consistent with findings of increased GLUT4 protein content in the sheep diaphragm induced by chronic inspiratory resistive flow [6]. Repetitive inspiratory muscle-loading exercise at 60% of PImax sessions may provide a new therapeutic avenue for glucose-lowering in patients with Type 2 diabetes; this finding has clinical implications when patients have poor exercise tolerance or are completely unable to perform limb exercises.

Combined aerobic exercises did not elicit glucose variability reductions, probably because of low statistical power for this comparison; however, this small sample size was able

to show great differences in glucose levels and glucose variability after the inspiratory muscle-loading exercise at 60% of PImax and after the aerobic sessions.

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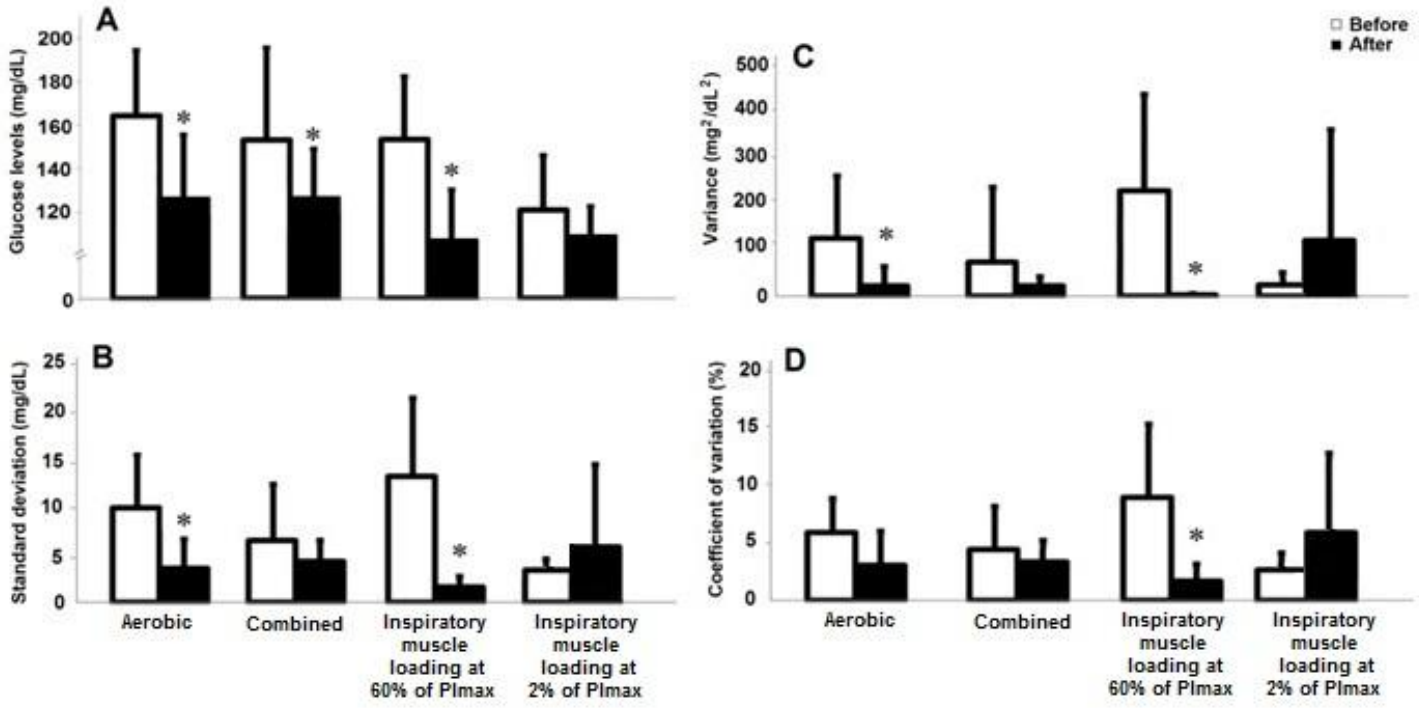
FIGURE 1. Glucose levels and glucose variability in type 2 diabetes. Glucose levels (a), glucose standard deviation (b), glucose variance (c) and glucose coefficient of variation (d) after aerobic, combined, inspiratory muscle loading exercise at 2% of maximum inspiratory mouth pressure (PI_{max}), and inspiratory muscle-loading exercise at 60% of PI_{max} exercise sessions. Data expressed as mean ± SD.



CAPITULO IV

CONCLUSÕES

1. Em indivíduos hígidos é possível caracterizar a variabilidade glicêmica através de métodos convencionais e não convencionais (análise espectral e simbólica). E uma sessão aguda de exercício aeróbico e excêntrico reduzem a variabilidade glicêmica avaliada através do índice de análise convencional, desvio padrão da glicose em indivíduos saudáveis, contudo sem mudanças com análise espectral e simbólica (análise não convencional).

2. A correlação de marcadores de inflamação e de estresse oxidative com índices de variabilidade glicêmica, convencional e não convencional, sugerem uma influência dessas alterações fisiológicas no comportamento glicêmico de indivíduos saudáveis.

3. Uma sessão aguda de exercício aeróbico e exercício de carga muscular inspiratória a 60% da PImáx promovem reduções similares da variabilidade glicêmica, contudo sem alterações com exercício combinado e quando a musculatura inspiratória é exercitada a 2% da PImáx.

ANEXOS

ARTIGO ORIGINAL

Association Between Physical Activity Advice Only or Structured Exercise Training With Blood Pressure Levels in Patients With Type 2 Diabetes: A Systematic Review and Meta-Analysis

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Short title: Exercise and blood pressure in diabetes

Manuscript: tables: 3; figure: 3

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Key points:

1. Structured exercise training and physical activity advice only improve glucose control in patients with type 2 diabetes, but their effect on blood pressure (BP) has not yet been evaluated in a well-conducted systematic review and meta-analysis.

2. Different types of structured exercise (aerobic, resistance, and combined training) and also physical activity advice only are associated with BP reduction in patients with type 2 diabetes, especially when higher levels of intensity are applied across any of the types of exercise training, and a training volume superior than 150min/wk is used.

Abstract

Background: Diabetes is associated with marked cardiovascular morbidity and mortality. However, the association between different types of exercise training and blood pressure (BP) changes is not fully clear in type 2 diabetes.

Objective: The aim of this systematic review and meta-analysis of randomized controlled clinical trials (RCTs) was to determine the effects of structured exercise training [aerobic (AER), resistance (RES), or combined (COMB)] and physical activity advice only (PA advice) on BP changes in patients with type 2 diabetes.

Methods: Searches in five electronic databases were conducted to retrieve studies published from 1980 to 2013. Eligible studies were RCTs consisting of structured exercise training or PA advice *vs.* no intervention in patients with type 2 diabetes. We used random effect models to derive weighted mean differences (WMD) of exercises on absolute changes in systolic BP (SBP) and diastolic BP (DBP).

Results: Thirty RCTs of structured training (2217 patients) and 21 of PA advice (7323 patients) were included. Data were extracted independently in duplicate. Structured exercise was associated with reductions in SBP (WMD -4.22 mmHg; 95% CI: -5.89 to -2.56) and DBP (WMD -2.07 mmHg; 95% CI: -3.03 to -1.11) *vs.* controls. In structured exercise interventions, AER and RES were associated with declines in BP, and COMB was not associated with BP changes. However, in sensitivity analysis, a high intensity protocol within COMB was associated with declines in SBP (WMD -3.30mmHg; 95% CI: -4.71 to -1.89). Structured exercise longer than 150min/wk was associated with greater BP reductions. Physical activity advice only was associated with reduction in SBP (WMD -2.97 mmHg; 95% CI: -4.52 to -1.43) and DBP (WMD -1.41 mmHg; 95% CI: -1.94 to -0.88) *vs.* controls.

Conclusions: Aerobic, resistance, and high-intensity combined training are associated with BP reduction in patients with type 2 diabetes, especially in exercise programs lasting more than 150min/wk. Physical activity advice only is also associated with lower BP levels.

1. Introduction

Cardiovascular disease is a major cause of morbidity and mortality [1,2] in patients with diabetes mellitus, increasing direct and indirect costs related to the disease [2]. Cardiovascular risk factors, such as dyslipidemia and hypertension, when present in patients with type 2 diabetes, magnify its inherent high cardiovascular risk [3]. Hypertension is a frequent comorbidity of diabetes, affecting the majority of patients and impacting on both cardiovascular disease and microvascular complications [4]. Tight blood pressure (BP) control in patients with hypertension and type 2 diabetes is associated with reduction in the risk of deaths related to diabetes, heart failure, chronic complications [5] and stroke [6].

Nonpharmacological therapy is feasible in diabetic individuals with mildly elevated BP [7], whereas pharmacological therapy should be added for patients with higher BP levels. Lifestyle intervention consists in reducing sodium intake and body weight, increasing consumption of fruits, vegetables, and low-fat dairy products [8], avoiding excessive alcohol consumption, and increasing physical activity [9,10]. However, there is no systematic review of trials which analysed the effects of physical activity advice only on BP specifically in patients with diabetes.

Aerobic and resistance exercise, but not combined exercise, are associated with BP lowering in nondiabetic individuals [11,12]. However, the precise effect of these different modalities of exercise on BP control in patients with type 2 diabetes has been recently questioned. Four meta-analyses [13-16], assessed the effect of several cardiovascular variables in patients with diabetes, which yielded controversial results in both systolic BP (SBP) and diastolic BP (DBP) changes after different types of structured exercise training. These reviews did not explore heterogeneity of studies, and also did not address physical activity advice only as an independent intervention, which raises the need for a systematic

review addressing specifically the association between BP and different modalities of exercise training in type 2 diabetes.

Structured exercise programs are not available for all patients with diabetes, and most of them will receive only the advice to increase physical activity from their primary care physician. A home-based physical activity program, prescribed to gradually achieve a goal of 175 minutes of moderate-intensity physical activity/wk, was evaluated together with other changes in lifestyle as co-intervention, and resulted in benefits in SBP (-4.0mmHg) and DBP (-1.2mmHg) [17]. However, studies reporting potential effects of physical activity advice only on BP changes have never so far been included in a systematic review.

The aim of this systematic review and meta-analysis of randomized controlled clinical trials (RCTs) was to compare the effects of structured exercise training (aerobic, resistance, or a combination of aerobic and resistance training) and physical activity advice only on changes in BP in patients with type 2 diabetes.

2. Methods

2.1. Search Strategy and Study Selection

The searches were conducted in different databases: MEDLINE, Cochrane CENTRAL, Embase, ClinicalTrials.gov and LILACS in order to retrieve studies published from January 1980 to May 2013. Although no language restrictions were used during the searches, only eligible full texts in English, Portuguese or Spanish were considered for review, regardless of the primary outcome or intervention (supervised exercise training or physical activity advice only). Electronic databases were searched using similar search strategies focusing on the terms “diabetes mellitus type 2”, “exercise”, “physical activity”, and related entry terms, associated with a high sensitivity strategy for the search of RCTs [18]. The complete search strategy used for the PubMed database is shown in Electronic Supplementary Material Table

S1. This systematic review and meta-analysis is reported in accordance with the PRISMA statement [19].

2.2. Eligibility Criteria

Studies including individuals with type 2 diabetes, aged ≥18 years old, in which treatment allocation was randomized, and the control group was not prescribed exercise training as part of the intervention were considered for further eligibility assessment. The RCTs were required to have at least one intervention arm of either *supervised exercise training* (aerobic, resistance, or a combination of both) in which patients were engaged in planned and supervised exercise programs, or *physical activity advice only* in which patients received a prescription for the gradual increase in the frequency of moderate to vigorous physical activity with a goal of 150 minutes per week. For inclusion, studies were required to provide before and after-intervention absolute values of SBP and DBP or differences between means and dispersion values. We excluded studies that had criteria summarized as follows: (1) study samples including only patients with type 1 diabetes or gestational diabetes; (2) RCTs that did not provide information regarding the effects of intervention, either in the experimental or control groups; (3) duplicate publications or sub studies of included trials; (4) intervention/program duration shorter than 6 weeks; (5) dietary cointervention for control group in studies with supervised exercise training.

2.3. Assessment of Risk of Bias

Study quality assessment was conducted independently by two investigators and disagreements were solved by consensus or by a third investigator. The kappa agreement rate between reviewers was k=0.94 for quality assessment. The criteria for methodological quality of studies included were as follows: adequate sequence generation, allocation concealment, blinding of outcomes assessors, use of intention-to-treat analysis, and description of losses/exclusions in each trial. Studies without a clear description of an adequate sequence

generation were considered not to have fulfilled this criterion. Lack of a description of how the allocation list was concealed was judged to characterize absence of allocation concealment. Whenever the use of intention-to-treat analysis was not clearly stated in studies, evaluation was conducted by confirming if the number of participants initially randomized and the number analyzed at the end of the interventions were identical. Studies without this information were considered not to have fulfilled this criterion. Risk of bias in RCTs was evaluated according to the Cochrane handbook [20].

2.4. Summary of Evidence: GRADE-criteria

The GRADE criteria were used to assess overall quality of evidence. We evaluated the quality for each specific outcome (SBP and DBP) in each type of exercise (aerobic, resistance or combine structured exercise; and physical activity advice only). The quality of evidence was based on five factors: limitations of the study design, consistency of results, directness, precision, and potential for publication bias. The quality of evidence was reduced by one or two levels for each factor not met. The GRADE approach categorizes quality of evidence into one of four levels: high, moderate, low, and very low [21].

2.4. Data Extraction

Two investigators independently assessed titles and abstracts of all retrieved articles. Abstracts that did not provide enough information regarding the eligibility criteria were retrieved for full-text evaluation. Reviewers independently evaluated and selected full-text articles in accordance with the eligibility criteria. Disagreements were solved through consensus and discussion with a third reviewer. Corresponding authors of potentially eligible studies were contacted if the required data could not be located in the published report.

Data extraction was independently performed in duplicate, both for studies with structured exercise training as well as for studies with physical activity advice only. By using standardized forms, reviewers extracted methodological characteristics of the studies, number

of participants, age, sex, types of interventions and associated variables, outcomes, trial duration (in weeks), and assessment technique of BP used in each study. Information on adherence to protocols and dropout rates was also extracted.

3. Data Analyses

Analyses were conducted using Stata 12.1 software (Stata, College Station, TX, USA). Absolute changes in BP were reported as differences between arithmetic means before and after interventions. Whenever possible, data from intention-to-treat analyses were used in the meta-analysis. Pooled-effect estimates were obtained by comparing the least squares mean absolute change from baseline to the end of the study for each group, and were expressed as weighted mean differences (WMD) between groups. Calculations were performed using a random-effect model, with the DerSimonian-Laird method. Heterogeneity was assessed by the Cochran's Q test, with a threshold *p* value of 0.1 considered statistically significant, and the inconsistency I^2 test, in which values above 50% were considered indicative of high heterogeneity [20]. Meta-analyses comprised the comparisons of structured aerobic exercise training *vs*. no intervention (control), structured resistance exercise training *vs.* no intervention (control), structured combined aerobic/resistance exercise training *vs.* no intervention (control), and physical activity advice only *vs.* no intervention (control). An α value = 0.05 was considered statistically significant.

Some studies compared multiple exercise interventions with a single control group, therefore, we split this 'shared' group into two or more groups, with smaller sample sizes weighted in relation to different exercise interventions. This approach was applied in order to have reasonably independent comparisons and avoid a unit-of-analysis error for studies that could contribute to multiple and correlated comparisons [20]. Imputation and/or transformation methods were used for the few studies that showed results as confidence intervals or interquartile range [20]. We also performed a stratified analysis to evaluate if the

effect of the interventions was different for normotensive *vs.* hypertensive population. The studies were considered with a hypertensive sample if they reported more than 70% of hypertensive individuals and those with baseline BP levels higher than 140/90 mmHg [22]. Sensitivity analyzes considering the volume and intensity of interventions involving structured exercise training were also performed. In these analyses we use the median for dichotomizing the interventions.

We constructed four multivariate models to assess the heterogeneity observed in each meta-analysis. We included in the models those variables that were well-known confounders and those with P value ≤ 0.2 in the univariate analysis for each intervention. Therefore, variables entered in the multivariate analyses were: body mass index (BMI) change + weekly volume (model 1: aerobic exercise training); baseline SBP + weekly volume (repetitions/wk) (model 2: resistance exercise training); baseline SBP + aerobic volume (model 3: combined exercise training); baseline SBP + encouragement by phone + recommended duration (model 4: physical activity advice). We evaluated the goodness of fit of each model by the adjusted R^2 which denotes the proportion of between-study variation explained by the covariates [23,24].

In addition, we generated correlations to test the association between changes in SBP and variables that explained heterogeneity in meta-regression analyses, such as BMI. Some key exercise variables (i.e, exercise volume) were also tested based on clinical judgment of their relevance. All correlation analyses were weighted by the inverse of the variance of each observation, and scatter 'bubble' plots were constructed to graphically display the proportional weights of the different trials.

For the assessment of publication bias, we used a contour-enhanced funnel plot of each trial's effect size against the standard error [25]. Funnel plot asymmetry was evaluated by Begg's and Egger's tests, and a significant publication bias was considered if the P value

was < 0.01. The trim-and-fill computation was used to estimate the effect of publication bias on the interpretation of results [26,27].

4. Results

4.1. Description of Studies

In the initial search of databases, 4454 potentially relevant citations were retrieved, from which 51 articles met the inclusion criteria: 30 were RCTs of structured exercise training (including all arms n=39) and 21 were RCTs of physical activity advice only. A flow diagram of the search and study selection is shown in Figure 1. Included studies contributed to combined samples of 2217 and 7323 patients for the structured exercise training and physical activity advice only meta-analyses, respectively. The intervention characteristics for structured exercise training and physical activity advice only are shown in Tables 1 and 2, respectively. Briefly, structured exercise training mean frequency was three sessions per week (range: 2-5) and mean duration of interventions was 20 weeks (range: 6-48); physical activity advice only mean recommended frequency was four exercise practices per week (range: 3-5), and mean duration of interventions was of 32 weeks (range: 6-48).

4.2. Quality assessment (risk of bias)

Among the included studies, 60.8% presented adequate sequence generation (31 of 51), 27.4% reported allocation concealment (14 of 51), 19.6% had blinded assessment of outcomes (10 of 51), 100% described losses to follow-up and exclusions (51 of 51), and 35.3% used the intention-to-treat principle for statistical analyses (18 of 51). Risk of bias in RCTs of structured exercise training and physical activity advice only is described in Electronic Supplementary Material Table S2 and Electronic Supplementary Material Table S3, respectively. Contour-enhanced funnel plots and Egger regression test (Electronic Supplementary Material Figure S1) did not show publication bias for resistance (*P*=.94), combined exercise training (*P*=.14), and for physical activity advice only studies (*P*=.87), but

suggested an asymmetry in the analysis of aerobic exercise training (*P*=.07). However, the trim-and-fill computation revealed that publication bias did not interfere with the interpretation of results (Electronic Supplementary Material Figure S2). Summary of quality of the evidence (GRADE) is reported for each intervention and respective outcomes (SBP and DBP) in Table 3.

4.3. Association of Interventions with SBP and DBP:

The relationships between the different types of structured exercise training programs (aerobic, resistance or combined) and changes in SBP and DBP are shown in Figure 2 and Electronic Supplementary Material Figure S3, respectively. The overall association of any structured exercise training *vs.* control with absolute SBP levels reduction was WMD -4.22 mmHg (95% CI: -5.89 to -2.56; I^2, 86.7%; *P* for heterogeneity <.001) and DBP levels reduction was WMD -2.07 mmHg (95% CI: -3.03 to -1.11; I^2, 87.1%; *P* for heterogeneity <.001). Aerobic exercise training (21 interventions, 836 patients) was associated with an absolute reduction in SBP (WMD -4.57 mmHg; 95% CI; -6.90 to -2.25; I^2, 80.3%; *P* for heterogeneity <.001) and in DBP (WMD -2.06 mmHg; 95% CI; -3.28 to -0.84; I^2, 73.9%; *P* for heterogeneity <.001), as compared with controls. Resistance exercise training (10 interventions, 403 patients) was associated with reduction in SBP (WMD -4.44 mmHg; 95% CI; -6.76 to -2.11; I^2, 55.7%; *P* for heterogeneity=.02) and in DBP (WMD -2.84 mmHg; 95% CI; -3.88 to -1.81; I^2, 30.3%; *P* for heterogeneity=.17), as compared with controls. Combined exercise training (8 interventions, 915 patients) was neither associated with reduction in SBP (WMD -2.64mmHg; 95% CI; -7.64 to 2.56; I^2, 94.4%; *P* for heterogeneity <.001) nor with changes in DBP (WMD -1.40 mmHg; 95% CI; -3.61 to 0.81; I^2, 92.4%; *P* for heterogeneity <.001), as compared with controls.

Importantly, 21 studies (7323 patients) showed that the advice to perform physical activity only was associated with a SBP reduction (WMD -2.97 mmHg; 95% CI; -4.52 to -

1.43; I^2, 79.5%; *P* for heterogeneity <.001), as well as with DBP reduction (WMD -1.41 mmHg; 95% CI; -1.41 to -0.88; I^2, 27.0%; *P* for heterogeneity=.12), as compared with control (Figure 3).

4.4. Sensitivity Analyses and Exploration of Heterogeneity

Absolute changes in SBP and DBP of studies of structured exercise training and physical activity advice only *vs.* no intervention were also analyzed according to the presence or absence of hypertension at baseline (Electronic Supplementary Material Table S4). Overall, a previous diagnosis of hypertension is associated with greater SBP lowering effect by aerobic training. Likewise, resistance training caused a major decrease both of SBP and DBP in hypertensive patients, but was not associated with lower SBP and DBP in studies using combined training. Physical activity advice showed a negligible reduction in both SBP and DBP in hypertensive patients. Among the studies included, diuretics, angiotensin-converting enzyme inhibitors, angiotensin receptor antagonists and beta-blockers were the four most prescribed drugs; no differences in their prescription were reported between study groups.

Analyses of data according to the weekly amount of exercise (Electronic Supplementary Material Figure S4) showed that structured exercise consisting of more than 150 min/wk (15 interventions, 686 patients) was associated with a higher reduction in SBP (WMD -6.17 mmHg; 95% CI; -8.83 to -3.51; I^2, 87.1%; *P* for heterogeneity <.001), when compared to programs of less or equal than 150 min/wk (14 interventions, 1075 patients; WMD -2.80 mmHg; 95% CI; -3.86 to -1.74; I^2, 0.0%; *P* for heterogeneity=.531).

Analyses of data according to volume and intensity of absolute changes in SBP (Electronic Supplementary Material Table S5) showed that aerobic training of higher intensities (8 interventions, 333 patients) was associated with reductions in SBP (WMD -5.47 mmHg; 95% CI:-7.94 to -3.00 I^2, 67.9%; *P* for heterogeneity=.003), as well as resistance training of higher intensities (5 interventions, 194 patients; WMD -3.99 mmHg; 95% CI:-6.66

to -1.32 I^2, 0.0%; *P* for heterogeneity=.644), and combined training of higher intensities (2 interventions, 596 patients; WMD -3.30 mmHg; 95% CI:-4.71 to -1.89 I^2, 0%; *P* for heterogeneity=.331), compared with control. For structured exercise of high volume, only aerobic training (11 interventions, 459 patients; WMD -7.05 mmHg; 95% CI:-9.58 to -4.53 I^2, 76.1%; *P* for heterogeneity=.001) and resistance training (5 interventions, 215 patients; WMD -3.14 mmHg; 95% CI:-5.95 to -0.34 I^2, 34.9%; *P* for heterogeneity=.188) were associated with reduction in SBP. Combined training of high volume was not associated with reduction in SBP (2 interventions, 142 patients WMD -6.69 mmHg; 95% CI:-20.21 to 6.83 I^2, 98%; *P* for heterogeneity=.001).

Covariates used in univariate analysis explained heterogeneity in aerobic training (weekly volume) and physical activity advice only (baseline SBP), but did not explain it in resistance and combined exercise training (Electronic Supplementary Material Table S6). A multivariate meta-regression using BMI change and weekly volume (model 1) as covariates explained the between studies variance of aerobic exercise training (overall, R^2=91.25%; *P*<.001). For resistance exercise training, a model using as covariates baseline SBP and weekly volume (repetitions/wk) (model 2) did not explain the between studies variance (overall, *P*=.09). In combined exercise training, a model using baseline SBP and aerobic volume (model 3) as covariates explained the between studies variance (overall, R^2=100%; *P*<.001). For physical activity advice only, a model with baseline SBP, encouragement by phone and recommended duration (model 4) as covariates explained the between studies variance (overall, R^2=97.42%; *P*=.007).

The association between changes in BMI and changes in SBP are depicted in Electronic Supplementary Material Figure S5. The decrease in SBP was associated with greater decrease in BMI only for structured exercise and not for physical advice alone. Association between exercise volume with changes in SBP in studies of aerobic training

(panel A), resistance training (panel B), aerobic component (panel C) and resistance component (panel D) in combined training are shown in Electronic Supplementary Material Figure S6 . In this case, a higher amount of exercise volume was associated with a greater decrease in SBP only in aerobic training.

5. Discussion

The findings of this systematic review with meta-analysis demonstrate that in patients with type 2 diabetes structured exercise training is associated with SBP and DBP reductions of -4.22 mmHg and -2.07 mmHg, respectively, an effect that can be magnified when the training volume exceeds 150 min/week. Physical activity advice only is associated with a less pronounced, but also significant, BP reduction (SBP:-2.97 mmHg; DBP: -1.41 mmHg, respectively). Previous diagnosis of hypertension is associated with greater BP lowering effect by aerobic and resistance training, but not by combined exercise and physical activity advice only.

The clinical importance of BP reductions has been reported in large studies investigating morbidity and mortality outcomes, and even a small lowering of SBP and DBP was associated with reduction in cardiovascular events and mortality in the general population [28] and in patients with diabetes [29]. Since the association between BP and cardiovascular outcomes does not have a threshold point for risk, even BP reductions within the normal range may be clinically significant, especially in older subjects [28], the age group in which patients with type 2 diabetes are included.

The more pronounced effect of exercise on BP in hypertensive than in normotensive individuals was expected, and it is similar to data previously shown in nondiabetic patients [12]. Additionally, baseline BP levels were one of the variables explaining the heterogeneity among studies, supporting the notion that patients with higher BP levels or previous diagnosis of hypertension would achieve greater BP changes from either aerobic or resistance training [30]. We speculate that the same effect was not seen in meta-regressions of combined exercise and physical activity advice only because these modalities were associated with smaller BP reductions.

The results of this systematic review indicating that aerobic and resistance training, but not combined training (as a whole) improve BP control in type 2 diabetes are in agreement with those found in healthy individuals [11,12]. However, we identified that high-intensity combined training is also associated with BP benefits. Regarding the diabetic population, a previous systematic review [16] that also evaluated structured exercises reported that the overall effect of structured exercise on SBP and DBP were similar to those we observed. However, when exercise modalities were considered separately, results differed from those of the previous review: SBP did not reduce in association with aerobic and resistance training, but a significant reduction was associated with their combination [16]. It is important to point out methodological differences between our review and the previous one; for example, in our review we did not include studies with dietary co-intervention. Moreover, the present review was specifically designed to summarize the association between structured exercise training and physical activity interventions with BP in type 2 diabetes, therefore including trials with shorter intervention lengths, which are known to induce BP changes [31].

In order to gain insight on possible factors underlying the varied effects in the different meta-analyses presented in this review, we explored effect sizes and heterogeneity for each type of exercise individually. In this regard, we attempted to gain insight on possible factors underlying the lack of overall effect in the meta-analysis of combined exercise training studies. Using a sensitivity analysis stratified by intensity and volume of combined training, we observed significant SBP reduction by interventions having higher intensities in their aerobic and resistance parts (equal or greater than 70% in maximal heart rate and 1-repetition maximum, respectively). Therefore, from a physiological standpoint, these effects may indicate a dose-response relationship especially related to the amount of exercise in combined training, in which the fractionized aerobic and resistance stimuli could require at least moderate-to-high intensities to elicit changes in BP. Although there were wide variations in

the weekly volume of exercise across the analysed studies, we did not observe a clear trend for the amount of training to affect BP in interventions of combined exercise training. It is important to highlight that other physical components of the health spectrum, such as cardiorrespiratory fitness and muscular strength, are consistently improved by combined exercise training [32-34]. Thus, the practice of combined exercise training should not be discouraged in patients with type 2 diabetes based on our results. However, our observation may guide its prescription, emphasizing the need for increased intensity.

This is the first systematic review that evaluated the effects of physical activity advice only on BP levels in diabetes. Recently, regular physical activity was shown to be beneficial for reducing mortality in nondiabetic hypertensive patients [34], a result that was attributed not only to BP reduction, but also to improved glucose tolerance, and lower BMI. These are well-known benefits of lifestyle changes when physical activity is included in the daily habits of patients with diabetes [13,17,35,36]. Moreover, observational data showed that higher levels of physical activity were associated with lower mortality risk in individuals with diabetes [37].

Considering our results, we suggest that aerobic, resistance or high intensity combined structured exercises should be the first choice for patients with type 2 diabetes and hypertension, and the incentive to increase levels of physical activity can be provided. We are aware that structured exercise will not be available to all such patients, and in this situation physical activity advice alone should be encouraged. Also, physical activity can be recommended in order to prevent hypertension in patients with type 2 diabetes and borderline or normal levels of BP.

Since each modality of exercise is considered under particular clinical situations, available infra-structure or according to patients' preferences, the results of the present systematic review are substantial regarding the exercise prescription for diabetic patients.

Aerobic and resistance exercises are each associated with BP decreases, and the magnitude of BP reduction is similar across both exercise modalities. In addition, regression and correlation analyses indicated that the BP change is influenced by factors such as: BMI, weekly exercise volume (aerobic), baseline SBP, aerobic weekly exercise volume (within combined training), and intensive advice (physical activity advice only). Therefore, emphasizing body weight reduction (as indicated by BMI change), and increasing the amount of aerobic exercise (in aerobic and combined exercise programs) may effectively optimize BP reduction. We point out that heterogeneity of resistance training studies was slightly lower (I^2=55%) and was not explained by clinically plausible factors, such as baseline BP, training intensity, and variables of training amount (e.g., sets, repetitions) [11,38].

Finally, our findings demonstrate that structured exercise performed for more than 150 minutes/week is associated with greater declines in BP (-6.17 mmHg; 95% CI -8.83; -3.51) than structured exercise performed for less than 150 minutes/week (-2.80 mmHg; -3.86; -1.74) in patients with type 2 diabetes. This is an interesting finding, because it supports the current guidelines that recommend exercise duration for at least 150 minutes per week in this population [39]. Moreover, as BP lowering and glucose control are known to be additive in reducing chronic complications of diabetes [4,40,41] and both are substantially better controlled with higher volumes of exercise [36], this synergic effect should be taken into account when planning and delivering exercise interventions.

This review has limitations. Data extraction was not blinded, a potential source of bias. Although heterogeneity was identified in all the meta-analyses performed, it was fully explained by expected variables, such as baseline BP, associated BMI change, exercise volume and intensity of advice, except for resistance exercise. Regarding physical activity advice only, it was difficult to conduct full heterogeneity exploration because methodological characteristics were not clearly described across studies. Overall, general quality of the

studies was low, reflecting increased risk of bias in many of the included studies. Furthermore, patients in exercise training groups are likely to experience increased care and may improve the adherence to pharmacological therapy. Although it is intrinsic to exercise interventions, 52% (n = 27) of the studies reported that the control group had non-exercise intervention that could reduce control group bias. Finally, most effect sizes are derived from measurements of office BP, whereas 24h ambulatory BP should be targeted as outcome in future RCTs due to its better association with important outcomes.

6. Conclusions

Structured exercise, either aerobic, resistance, and their combination (in high-intensity), for at least 6 weeks, is associated with lowering SBP and DBP in type 2 diabetic patients, especially in hypertensive individuals, an effect that is magnified when performed for more than 150 min/week. Physical activity advice only is also beneficial, to a lesser degree.

Considering that worldwide, 31.1% of adults are physically inactive [42], the present evidence indicates that patients with diabetes would derive much benefit in terms of control of BP by engaging in regular structured exercise or physical activity, which is in accordance with current views that such programs may reduce the burden of the disease. The present data have practical applications. All types of structured exercise, as well as physical activity advice only, are associated with BP reductions in people with diabetes. Furthermore, as we have previously shown for glucose control [38], exercising 150 min/week or more is likely to be advantageous.

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Legends

Figure 1. Identification and selection of articles included in the meta-analysis.

Figure 2. Absolute changes in systolic blood pressure (SBP) in individual studies of structured exercise training *vs.* no intervention in patients with type 2 diabetes. Squares represent study-specific estimates; diamonds represent pooled estimates of random-effects meta-analyses.

Figure 3. Absolute changes in (a) systolic blood pressure (SBP) and (b) diastolic blood pressure (DBP) in individual studies of physical activity advice only *vs.* no intervention in patients with type 2 diabetes. Squares represent study-specific estimates; diamonds represent pooled estimates of random-effects meta-analyses.

Table 1. Characteristics of the structured exercise studies included in the meta-analysis

Source	Age (y)	DM duration (y)	Systolic BP (mmHg)	Diastolic BP (mmHg)	Hypertension (%)	Frequency (sessions/wk)	Session duration	Program duration (weeks)	Dropouts (%)
Aerobic training									
Arora et al, 2009[43]	52± 9	5±2	132±8	84±5	NR	3	30 min	8	0
Balducci et al, 2010[44]	64±8	9±6	140±3	82±1	65	2	60 min	48	0
Belli et al, 2011[45]	55±2	4±1	134±6	84±3	NR	3	60 min	12	I: 11.1 C: 10
Bjorgaas et al, 2005[46]	57±8	2±8	147±19	89±11	30	2	45 min	12	0
Gram et al, 2010[47]	62±10	NR	153±25	88±11	NR	2	30 min	16	I: 4.76 C: 0
Jorge et al, 2011[48]	52±9	5±4	139±13	87±12	58	3	60 min	12	NR
Kadoglou et al, 2007[49]	62±6	7±4	141±16	82±8	I: 68 C: 61.5	4	45 min	24	I: 3.3 C: 10
Kadoglou et al, 2007 [50]	59±8	7±5	137±18	80±9	NR	4	60 min	16	I: 6.5 C: 13
Kadoglou et al, 2010[51]	59±8	6±3	138±16	79±9	66	4	45 min	16	I: 12 C: 16
Kadoglou et al, 2010[52]	59±8	7±5	142±17	82±9	NR	4	45 min	48	I: 8 C:5
Kadoglou et al, 2012[53]	60±5	5±2	136±15	81±11	66	4	60 min	12	I: 3.7 C: 0
Kadoglou et al, 2013[54]	58±5	8±2	131±21	79±8	I: 71 C: 75	4	60 min	24	I: 9.5 C: 4
Source	**Age (y)**	**DM duration**	**Systolic BP (mmHg)**	**Diastolic BP (mmHg)**	**Hypertension (%)**	**Frequency (sessions/wk)**	**Session duration**	**Program duration**	**Dropouts (%)**

Kurban et al, 2011[55]	54±5	6±5	127±13	78±9	NR	3	30 min	12	0
Leehey et al, 2009[56]	66	NR	140±22	75±20	NR	3	30 min	6	0
Madden et al, 2012[57]	69±1	8±1	144±3	82±2	100	3	60 min	24	0
Middlebrooke et al, 2006[58]	63±8	5±4	136±16	74±9	I: 59 C: 50	3	30 min	24	27.1
Monteiro et al, 2010[59]	61±9	NR	140±17	76±12	54.5	3	50 min	13	0
Negri et al, 2010[60]	66±5	11±7	133±15	78±6	NR	3	45 min	16	I: 20 C: 4.7
Shenoy et al, 2010[61]	52±4	5±1	126±13	86±12	NR	5	35 min	8	0
Sigal et al, 2007[62]	54±7	5±5	134±22	82±14	53	3	45 min	26	I: 20 C: 5
Yavari et al, 2010[63]	50±6	4±2	132±16	81±9	NR	3	60 min	48	I: 14.3 C: 0
Resistance training									
Arora et al, 2010[43]	*49±5*	*5±1*	*126±7*	*82± 3*	*NR*	*2*	*7 exercises*	*8*	*10*
Castaneda et al, 2002[64]	*67±5*	*8±7*	*146±17*	*77±8*	*69*	*3*	*9 exercises*	*24*	*6*
Dunstan et al, 1998[65]	*51±2*	*5±1*	*128±3*	*72±2*	*NR*	*3*	*10 exercises*	*8*	*26.6*
Source	**Age (y)**	**DM duration**	**Systolic BP (mmHg)**	**Diastolic BP (mmHg)**	**Hypertension (%)**	**Frequency (sessions/wk)**	**Session duration**	**Program duration**	**Dropouts (%)**
Dunstan et al, 2002[66]	*67±5*	*8±7*	*146±17*	*77±8*	*69*	*3*	*9 exercises*	*24*	*15.8*

Hameed et al, 2012[67]	*45±5*	*NR*	*130±10*	*79±6*	*NR*	*3*	*5 exercises*	*8*	*12.5*
Jorge et al, 2011[48]	*54±9*	*8±4*	*137±18*	*86±15*	*67*	*3*	*7 exercises*	*12*	*NR*
Kadoglou et al, 2012[68]	*63±5*	*6±2*	*133±13*	*77±10*	*I: 90*	*3*	*8 exercises*	*12*	*NR*
Kadoglou et al, 2013[54]	*56±5*	*7±3*	*130±10*	*81±8*	*74*	*4*	*8 exercises*	*24*	*4.3*
Plotnikoff et al, 2010[69]	*55±12*	*NR*	*124±13*	*75±8*	*NR*	*3*	*8 exercises*	*16*	*0*
Sigal et al, 2007[62]	*55±7*	*6±5*	*136±22*	*80±13*	*56*	*3*	*7 exercises*	*26*	*10.9*
Combined training									
Balducci et al, 2010[35]	*59±8*	*8 (4-10)[a]*	*141±18*	*84±10*	*I: 67*	*2*	*75 min*	*48*	*5*
Balducci et al, 2010[44]	*60±9*	*8±6*	*142±3*	*83±2*	*59*	*2*	*60 min*	*48*	*0*
Dobrosielski et al, 2012[70]	*56±6*	*NR*	*127±2*	*72±1*	*70*	*3*	*45 min*	*26*	*I: 27.2*
Gram et al, 2010[47]	*59±10*	*NR*	*152±19*	*85±10*	*NR*	*2*	*30 min*	*16*	*0*
Jorge et al, 2011[48]	*58±10*	*7±5*	*135±15*	*86±8*	*83*	*3*	*60 min*	*12*	*NR*
Source	**Age (y)**	**DM duration**	**Systolic BP (mmHg)**	**Diastolic BP (mmHg)**	**Hypertension (%)**	**Frequency (sessions/wk)**	**Session duration**	**Program duration**	**Dropouts (%)**
Kadoglou et al, 2013[54]	*58±6*	*5±2*	*138±16*	*82±12*	*68*	*4*	*60 min*	*24*	*0*
Okada et al, 2010[71]	*63±7*	*10±8*	*128±19*	*74±12*	*I:43* C:18	*5*	*60 min* Exercises NR	*12*	*0*

Sigal et al, 2007[62]	*53±7*	*5±5*	*131±22*	*79±13*	*55*	*3*	*45 min*	*26*	*12.5*

Data represent weighted mean (SD) for age, systolic BP, and diastolic BP between intervention and control groups. In studies with more than 2 interventions, data represent mean (SD) of each intervention group;[a]Duration of diabetes is expressed as median (95% CI). Abbreviations: NR, not reported; I, intervention; C, control; BP, blood pressure; DM, diabetes mellitus.

Table 2. Characteristics of the physical activity advice studies included in the meta-analysis

Source	Age (y)	DM duration (y)	Systolic BP (mmHg)	Diastolic BP (mmHg)	Hypertension (%)	Frequency (sessions/wk)	Session duration (min)	Program duration (weeks)	Dropouts (%)
Agurs-Collins et al, 1997[72]	62±6	NR	141±16	78±10	NR	3	30	24	15
Allen et al, 2008[73]	57±13	8±6	132±17	77±10	NR	5	30	8	8.5
Andrews et al, 2011[74]	60±10	1	134±14	79±8	I: 57 C: 59	NR	NR	48	I: 2.5 C: 6
Goldhaber-Fiebert et al, 2003[75]	58±9	NR	136±18	83±10	I: 51 C: 43	3	60	12	19
Hare et al, 2011[76]	55±10	6±6	132± 16	82± 9	NR	NR	NR	144	I: 15 C: 7
Pi-Sunyer, 2007[17]	59±7	NR	129±18	70±18	I: 75 C: 74	NR	175 min/wk	48	I: 3 C: 4.3
Schultz et al, 2011[77]	54±10	6±6	132±16	82±9	I: 41 C: 30	NR	NR	48	I: 13.4 C: 21
Sun et al, 2008[78]	51±1	4±0.3	132±2	88±2	I: 49 C: 54	NR	NR	24	2.6
Toobert et al, 2003[79]	NR	9±10	135±14	78±9	NR	5	60	24	I: 16 C: 7
Wing et al, 1988[80]	56±7	7±6	137±21	82±11	NR	3	60	10	I: 13.3 C:0
Araiza et al, 2006[81]	50±10	NR	139±20	79±11	0	5	NR	6	NR
Christian et al, 2008[82]	53±11	NR	132±17	77±10	NR	NR	NR	36	I: 9 C: 15
Source	**Age (y)**	**DM duration**	**Systolic BP (mmHg)**	**Diastolic BP (mmHg)**	**Hypertension (%)**	**Frequency (sessions/wk)**	**Session duration**	**Program duration**	**Dropouts (%)**

De Greef et al, 2010[83]	61±6	NR	152±23	83±10	NR	NR	NR	52	10
Diedrich et al, 2010[84]	56±12	NR	127±16	73±10	12	NR	NR	NR	38
Ferrer-Garcia et al, 2011[85]	67±8	9±6	137±15	78±11	57	3	NR	24	I: 9 C: 15
Kirk et al, 2003[86]	58±8	6±4	146±20	84±11	NR	5	30	24	I: 8.6 C: 11.4
Krousel-Wood et al, 2008[87]	57±10	NR	132±16	77±9	NR	5	30	12	I: 17.8 C: 20.4
Piette et al, 2011[88]	56±10	NR	135±17	79±11	NR	NR	NR	24	14
Plotnikoff et al, 2011[89]	60	6±10	134±17	76±9	I: 62 C: 71	NR	NR	24	I: 4 C: 12
Tudor-Locke et al, 2004[90]	53±5	3±3	134±17	80±9	55	NR	NR	16	I: 46.6 C: 26.7
Wisse et al, 2010[91]	53±1	NR	133±19	78±9	I: 66 C: 72	3	60	96	17.5

Data represent weighted mean (SD) for age, systolic BP, and diastolic BP between intervention and control groups. Abbreviations: NR, not reported; I, intervention; C, control. BP, blood pressure; DM, diabetes mellitus.

Table 3. Quality of evidence using the GRADE approach

Quality assessment							Number of participants		Effect size (WMD [95% CI])	Quality
Number of interventions	Design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Intervention	control		
Systolic blood pressure, mmHg (follow-up 6-48 weeks)										
Analysis 1: Quality of evidence for structured aerobic exercise training										
21	Randomized trials	Serious[a]	Serious[b]	No serious indirectness	No serious imprecision	Reporting bias[c]	461	375	-4.57 (-6.90; -2.25)	Low
Analysis 2: Quality of evidence for structured resistance exercise training										
10	Randomized trials	Serious[a]	Serious[b]	No serious indirectness	No serious imprecision	Reporting bias[c]	241	162	-4.44 (-6.76; -2.11)	Low
Analysis 3: Quality of evidence for structured combined exercise training										
9	Randomized trials	Serious[a]	Serious[b]	No serious indirectness	Serious[d]	Reporting bias[c]	504	411	-2.64 (-7.64; 2.36)	Very low
Analysis 4: Quality of evidence for physical activity advice										
21	Randomized trials	Serious[a]	Serious[b]	No serious indirectness	No serious imprecision	Reporting bias[c]	3805	3518	-2.97 (-4.52; -1.43)	Low
Diastolic blood pressure, mmHg (follow-up 6-48 weeks)										
Analysis 5: Quality of evidence for structured aerobic exercise training										
21	Randomized trials	Serious[a]	Serious[b]	No serious indirectness	No serious imprecision	Reporting bias[c]	461	375	-2.06 (-3.28; -0.84)	Low
Analysis 6: Quality of evidence for structured resistance exercise training										
10	Randomized trials	Serious[a]	No serious inconsistency[e]	No serious indirectness	No serious imprecision	Reporting bias[c]	241	162	-2.84 (-3.88; -1.81)	Moderate
Analysis 7: Quality of evidence for structured combined exercise training										
9	Randomized trials	Serious[a]	Serious[b]	No serious indirectness	No serious imprecision	Reporting bias[c]	504	411	-1.40 (-3.61; 0.81)	Low
Analysis 8: Quality of evidence for physical activity advice										
21	Randomized trials	Serious[a]	No serious inconsistency[e]	No serious indirectness	No serious imprecision	Reporting bias[c]	3805	3518	-1.41 (-1.94; -0.88)	Moderate

Figure 1.

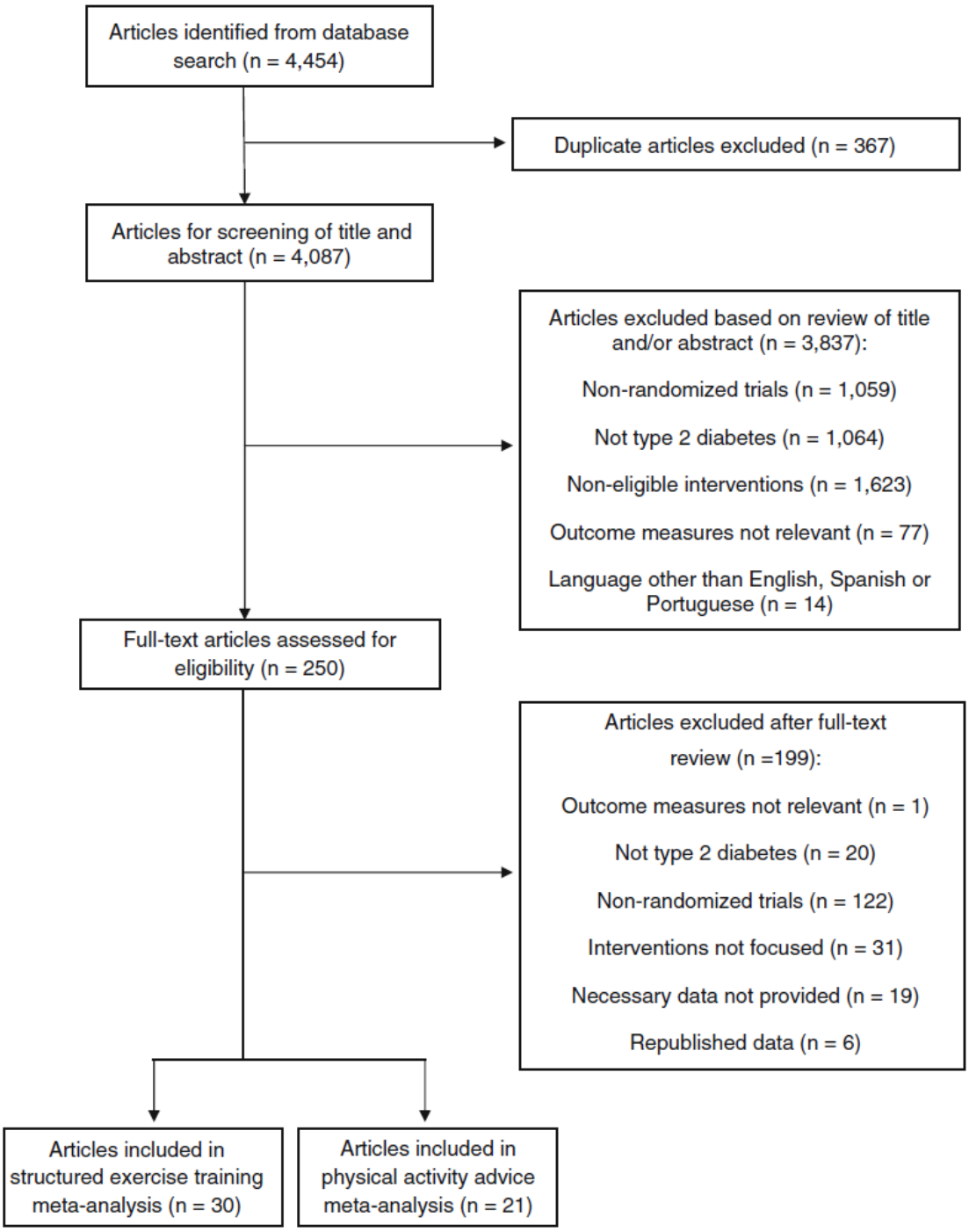

Articles identified from database search (n = 4,454)
Duplicate articles excluded (n = 367)
Articles for screening of title and abstract (n = 4,087)
Articles excluded based on review of title and/or abstract (n = 3,837):
Non-randomized trials (n = 1,059)
Not type 2 diabetes (n = 1,064)
Non-eligible interventions (n = 1,623)
Outcome measures not relevant (n = 77)
Language other than English, Spanish or Portuguese (n = 14)
Full-text articles assessed for eligibility (n = 250)
Articles excluded after full-text review (n =199):
Outcome measures not relevant (n = 1)
Not type 2 diabetes (n = 20)
Non-randomized trials (n = 122)
Interventions not focused (n = 31)
Necessary data not provided (n = 19)
Republished data (n = 6)
Articles included in structured exercise training meta-analysis (n = 30)
Articles included in physical activity advice meta-analysis (n = 21)

Figure 2.

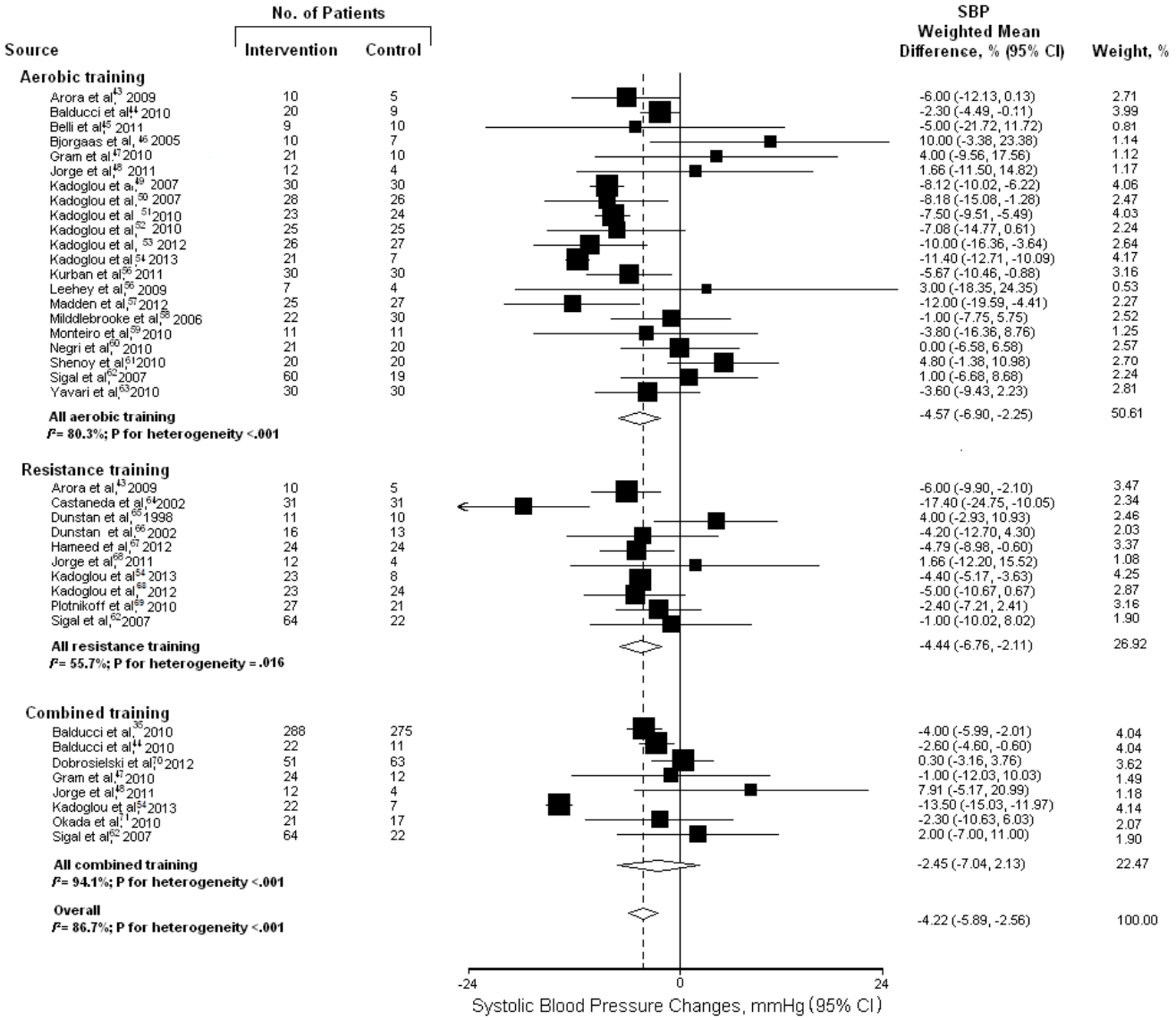
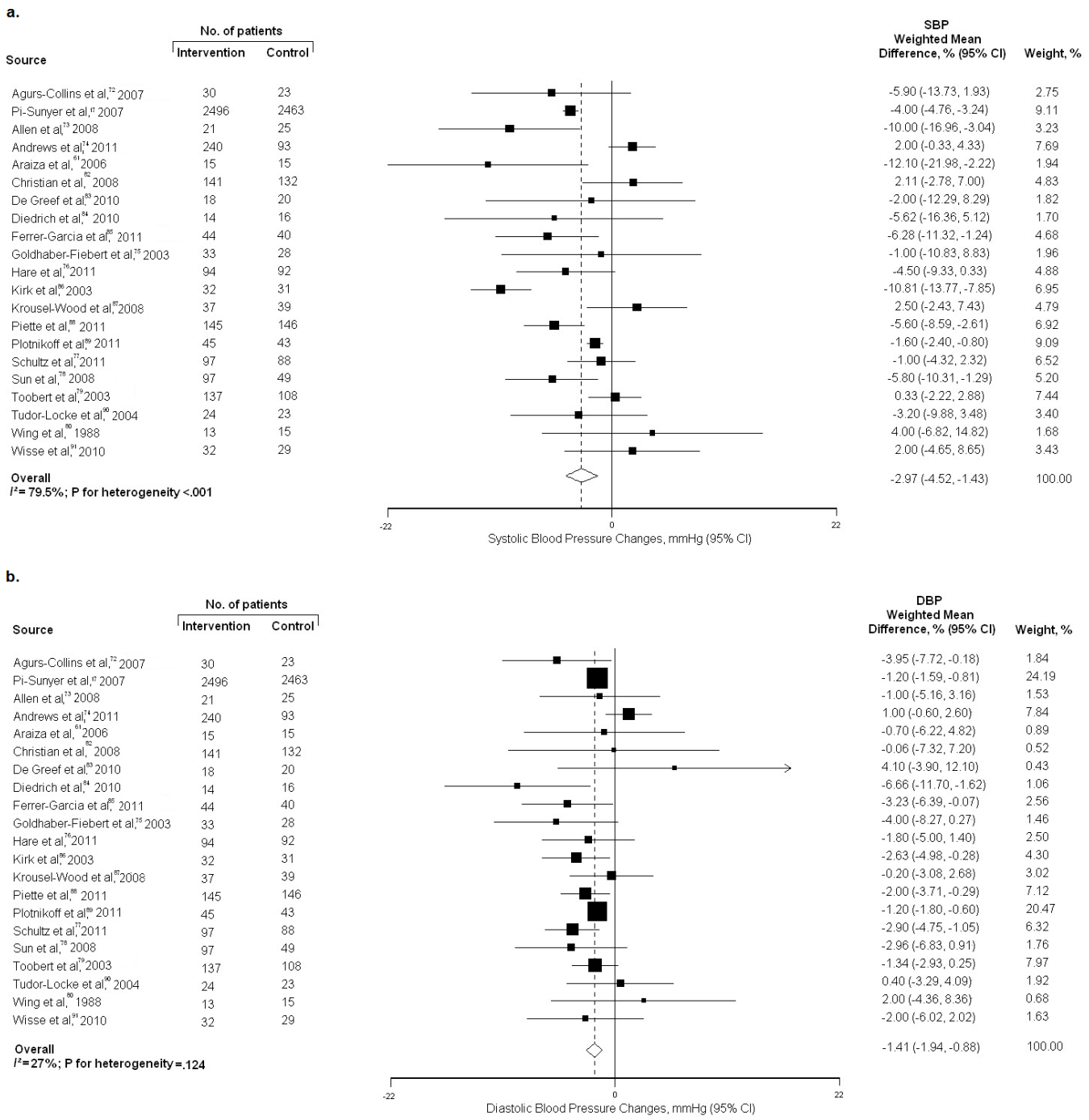

No. of Patients
Source
Intervention
Control
SBP
Weighted Mean
Difference, % (95% CI)
Weight, %
Aerobic training
Arora et al,[43] 2009 10 5 -6.00 (-12.13, 0.13) 2.71
Balducci et al[44] 2010 20 9 -2.30 (-4.49, -0.11) 3.99
Belli et al[45] 2011 9 10 -5.00 (-21.72, 11.72) 0.81
Bjorgaas et al, [46] 2005 10 7 10.00 (-3.38, 23.38) 1.14
Gram et al[47] 2010 21 10 4.00 (-9.56, 17.56) 1.12
Jorge et al,[48] 2011 12 4 1.66 (-11.50, 14.82) 1.17
Kadoglou et a,[49] 2007 30 30 -8.12 (-10.02, -6.22) 4.06
Kadoglou et al,[50] 2007 28 26 -8.18 (-15.08, -1.28) 2.47
Kadoglou et al ,[51]2010 23 24 -7.50 (-9.51, -5.49) 4.03
Kadoglou et al,[52] 2010 25 25 -7.08 (-14.77, 0.61) 2.24
Kadoglou et al, [53] 2012 26 27 -10.00 (-16.36, -3.64) 2.64
Kadoglou et al [54] 2013 21 7 -11.40 (-12.71, -10.09) 4.17
Kurban et al[55] 2011 30 30 -5.67 (-10.46, -0.88) 3.16
Leehey et al,[56] 2009 7 4 3.00 (-18.35, 24.35) 0.53
Madden et al,[57] 2012 25 27 -12.00 (-19.59, -4.41) 2.27
Middlebrooke et al,[58] 2006 22 30 -1.00 (-7.75, 5.75) 2.52
Monteiro et al,[59] 2010 11 11 -3.80 (-16.36, 8.76) 1.25
Negri et al,[60] 2010 21 20 0.00 (-6.58, 6.58) 2.57
Shenoy et al[61]2010 20 20 4.80 (-1.38, 10.98) 2.70
Sigal et al,[62] 2007 60 19 1.00 (-6.68, 8.68) 2.24
Yavari et al,[63]2010 30 30 -3.60 (-9.43, 2.23) 2.81
All aerobic training -4.57 (-6.90, -2.25) 50.61
I² = 80.3%; P for heterogeneity <.001
Resistance training
Arora et al,[43] 2009 10 5 -6.00 (-9.90, -2.10) 3.47
Castaneda et al,[64]2002 31 31 -17.40 (-24.75, -10.05) 2.34
Dunstan et al,[65] 1998 11 10 4.00 (-2.93, 10.93) 2.46
Dunstan et al,[66] 2002 16 13 -4.20 (-12.70, 4.30) 2.03
Hameed et al,[67] 2012 24 24 -4.79 (-8.98, -0.60) 3.37
Jorge et al[65] 2011 12 4 1.66 (-12.20, 15.52) 1.08
Kadoglou et al[54] 2013 23 8 -4.40 (-5.17, -3.63) 4.25
Kadoglou et al,[68] 2012 23 24 -5.00 (-10.67, 0.67) 2.87
Plotnikoff et al[69] 2010 27 21 -2.40 (-7.21, 2.41) 3.16
Sigal et al,[62] 2007 64 22 -1.00 (-10.02, 8.02) 1.90
All resistance training -4.44 (-6.76, -2.11) 26.92
I² = 55.7%; P for heterogeneity = .016
Combined training
Balducci et al,[35] 2010 288 275 -4.00 (-5.99, -2.01) 4.04
Balducci et al[44] 2010 22 11 -2.60 (-4.60, -0.60) 4.04
Dobrosielski et al[70] 2012 51 63 0.30 (-3.16, 3.76) 3.62
Gram et al[47] 2010 24 12 -1.00 (-12.03, 10.03) 1.49
Jorge et al[48] 2011 12 4 7.91 (-5.17, 20.99) 1.18
Kadoglou et al[54] 2013 22 7 -13.50 (-15.03, -11.97) 4.14
Okada et al,[71] 2010 21 17 -2.30 (-10.63, 6.03) 2.07
Sigal et al,[62] 2007 64 22 2.00 (-7.00, 11.00) 1.90
All combined training -2.45 (-7.04, 2.13) 22.47
I² = 94.1%; P for heterogeneity <.001
Overall -4.22 (-5.89, -2.56) 100.00
I² = 86.7%; P for heterogeneity <.001
-24
0
24
Systolic Blood Pressure Changes, mmHg (95% CI)

Figure 3.


a.
No. of patients
Source
Intervention
Control
SBP
Weighted Mean
Difference, % (95% CI)
Weight, %
Agurs-Collins et al,[72] 2007 30 23 -5.90 (-13.73, 1.93) 2.75
Pi-Sunyer et al,[17] 2007 2496 2463 -4.00 (-4.76, -3.24) 9.11
Allen et al,[73] 2008 21 25 -10.00 (-16.96, -3.04) 3.23
Andrews et al,[74] 2011 240 93 2.00 (-0.33, 4.33) 7.69
Araiza et al,[61] 2006 15 15 -12.10 (-21.98, -2.22) 1.94
Christian et al,[82] 2008 141 132 2.11 (-2.78, 7.00) 4.83
De Greef et al,[83] 2010 18 20 -2.00 (-12.29, 8.29) 1.82
Diedrich et al,[84] 2010 14 16 -5.62 (-16.36, 5.12) 1.70
Ferrer-Garcia et al,[85] 2011 44 40 -6.28 (-11.32, -1.24) 4.68
Goldhaber-Fiebert et al,[75] 2003 33 28 -1.00 (-10.83, 8.83) 1.96
Hare et al,[76] 2011 94 92 -4.50 (-9.33, 0.33) 4.88
Kirk et al,[86] 2003 32 31 -10.81 (-13.77, -7.85) 6.95
Krousel-Wood et al,[87] 2008 37 39 2.50 (-2.43, 7.43) 4.79
Piette et al,[88] 2011 145 146 -5.60 (-8.59, -2.61) 6.92
Plotnikoff et al,[89] 2011 45 43 -1.60 (-2.40, -0.80) 9.09
Schultz et al,[77] 2011 97 88 -1.00 (-4.32, 2.32) 6.52
Sun et al,[78] 2008 97 49 -5.80 (-10.31, -1.29) 5.20
Toobert et al,[79] 2003 137 108 0.33 (-2.22, 2.88) 7.44
Tudor-Locke et al,[90] 2004 24 23 -3.20 (-9.88, 3.48) 3.40
Wing et al,[80] 1988 13 15 4.00 (-6.82, 14.82) 1.68
Wisse et al,[91] 2010 32 29 2.00 (-4.65, 8.65) 3.43
Overall -2.97 (-4.52, -1.43) 100.00
I² = 79.5%; P for heterogeneity <.001
-22
0
22
Systolic Blood Pressure Changes, mmHg (95% CI)
b.
No. of patients
Source
Intervention
Control
DBP
Weighted Mean
Difference, % (95% CI)
Weight, %
Agurs-Collins et al,[72] 2007 30 23 -3.95 (-7.72, -0.18) 1.84
Pi-Sunyer et al,[17] 2007 2496 2463 -1.20 (-1.59, -0.81) 24.19
Allen et al,[73] 2008 21 25 -1.00 (-5.16, 3.16) 1.53
Andrews et al,[74] 2011 240 93 1.00 (-0.60, 2.60) 7.84
Araiza et al,[61] 2006 15 15 -0.70 (-6.22, 4.82) 0.89
Christian et al,[82] 2008 141 132 -0.06 (-7.32, 7.20) 0.52
De Greef et al,[83] 2010 18 20 4.10 (-3.90, 12.10) 0.43
Diedrich et al,[84] 2010 14 16 -6.66 (-11.70, -1.62) 1.06
Ferrer-Garcia et al,[85] 2011 44 40 -3.23 (-6.39, -0.07) 2.56
Goldhaber-Fiebert et al,[75] 2003 33 28 -4.00 (-8.27, 0.27) 1.46
Hare et al,[76] 2011 94 92 -1.80 (-5.00, 1.40) 2.50
Kirk et al,[86] 2003 32 31 -2.63 (-4.98, -0.28) 4.30
Krousel-Wood et al,[87] 2008 37 39 -0.20 (-3.08, 2.68) 3.02
Piette et al,[88] 2011 145 146 -2.00 (-3.71, -0.29) 7.12
Plotnikoff et al,[89] 2011 45 43 -1.20 (-1.80, -0.60) 20.47
Schultz et al,[77] 2011 97 88 -2.90 (-4.75, -1.05) 6.32
Sun et al,[78] 2008 97 49 -2.96 (-6.83, 0.91) 1.76
Toobert et al,[79] 2003 137 108 -1.34 (-2.93, 0.25) 7.97
Tudor-Locke et al,[90] 2004 24 23 0.40 (-3.29, 4.09) 1.92
Wing et al,[80] 1988 13 15 2.00 (-4.36, 8.36) 0.68
Wisse et al,[91] 2010 32 29 -2.00 (-6.02, 2.02) 1.63
Overall -1.41 (-1.94, -0.88) 100.00
I² = 27%; P for heterogeneity =.124
-22
0
22
Diastolic Blood Pressure Changes, mmHg (95% CI)

Association Between Physical Activity Advice Only or Structured Exercise Training With Blood Pressure Levels in Patients With Type 2 Diabetes: A Systematic Review and Meta-Analysis

Electronic Supplementary Material table headings, figure legends and reference list heading

Electronic Supplementary Material Table S1. Literature search strategy used for the PubMed database

#1” Search "Diabetes Mellitus, Type 2"[Mesh] OR Ketosis-Resistant diabetes [title/abstract] OR Ketosis Resistant diabetes [title/abstract] OR Maturity-Onset diabetes [title/abstract] OR Maturity Onset diabetes [title/abstract] OR Non Insulin Dependent diabetes [title/abstract] OR Non-Insulin-Dependent diabetes [title/abstract] OR Type 2 Diabetes [title/abstract] OR stable Diabetes [title/abstract] OR Diabetes Mellitus Type II [title/abstract] OR Maturity-Onset Diabetes Mellitus [title/abstract] OR Maturity Onset Diabetes Mellitus [title/abstract] OR MODY [title/abstract] OR NIDDM [title/abstract] OR Adult-Onset Diabetes Mellitus [title/abstract] OR Diabetes Mellitus Noninsulin Dependent [title/abstract].
#2 “Search "randomized controlled trial[pt] OR controlled clinical trial[pt] OR randomized controlled trials[mh] OR random allocation[mh] OR double-blind method[mh] OR single-blind method[mh] OR clinical trial[pt] OR clinical trials[mh] OR ("clinical trial"[tw]) OR ((singl*[tw] OR doubl*[tw] OR trebl*[tw] OR tripl*[tw]) AND (mask*[tw] OR blind*[tw])) OR ("latin square"[tw]) OR placebos[mh] OR placebo*[tw] OR random*[tw] OR research design[mh:noexp] OR follow-up studies[mh] OR prospective studies[mh] OR cross-over studies[mh] OR control*[tw] OR prospectiv*[tw] OR volunteer*[tw]) NOT (animal[mh] NOT human[mh])
#3 “ Search Exercises [title/abstract] OR Physical Exercise OR Physical Exercise [title/abstract] OR Physical Exercises [title/abstract] OR Isometric Exercises [title/abstract] OR Isometric Exercise [title/abstract] OR Warm Up Exercise [title/abstract] OR Aerobic Exercises [title/abstract] OR Aerobic Exercise [title/abstract] OR Exercise Therapies [title/abstract] OR Pilates Training [title/abstract] OR Strength Training [title/abstract] OR Strengthening Programs [title/abstract] OR Weight Lifting Exercise Program [title/abstract] OR Weight Bearing Strengthening Program [title/abstract] OR Weight Bearing Exercise Program [title/abstract] OR Exercise Isometric [title/abstract] OR Exercise Aerobic [title/abstract] OR Aerobic Exercises [title/abstract] OR Aerobic Exercise [title/abstract] OR Pilates Exercise [title/abstract] OR Training Resistance [title/abstract] OR Strength Training [title/abstract] OR Weight Lifting [title/abstract] OR Strengthening Program [title/abstract] OR Weight Bearing [title/abstract] OR (("Exercise Therapy"[Mesh] OR "Exercise Movement Techniques"[Mesh] OR "Resistance Training"[Mesh] OR "Muscle Stretching Exercises"[Mesh])) OR "Exercise"[Mesh].
#4 #1 AND #2 AND #3

Electronic Supplementary Material Table S2. *Risk of bias of studies included in the meta-analysis: structured exercise training*

	Adequate sequence generation	**Allocation concealment**	**Blinding of outcome****	**Description of losses and exclusions**	**Intention to treat analysis**
Arora et al, 2009[1]	Unclear	Unclear	No	Yes	No
Balducci et al, 2010[2]	Yes	Unclear	No	Yes	No
Balducci et al, 2010[3]	Yes	Unclear	Yes	Yes	yes
Belli et al, 2011[4]	Yes	Unclear	No	Yes	No
Bjorgaas et al, 2005[5]	Yes	Yes	No	Yes	No
Castaneda et al, 2002[6]	Unclear	Unclear	Yes	Yes	No
Dobrosielski et al, 2012[7]	Unclear	Unclear	No	Yes	No
Dunstan et al, 1998[8]	Yes	Unclear	No	Yes	No
Dunstan et al, 2002[9]	Unclear	Unclear	No	Yes	No
Gram et al, 2010[10]	Yes	Yes	Yes	Yes	Yes
Hameed et al, 2012[11]	Yes	Unclear	No	Yes	Yes
Jorge et al, 2011[12]	Unclear	Unclear	No	Yes	Unclear
Kadoglou et al, 2007[13]	Unclear	Unclear	No	Yes	No
Kadoglou et al, 2007[14]	Unclear	Unclear	No	Yes	No
Kadoglou et al, 2010[15]	Yes	Yes	No	Yes	No
Kadoglou et al, 2012[16]	Yes	Yes	Unclear	Yes	No
Kadoglou et al, 2012[17]	Yes	Unclear	No	Yes	No
Kadoglou et al, 2013[18]	Yes	Unclear	No	Yes	No
Kadoglou et al,2010[19]	Unclear	Unclear	No	Yes	No
Kurban et al, 2011[20]	Unclear	Unclear	No	Yes	Yes
Leehey et al, 2009[21]	Yes	Unclear	No	Yes	No
Madden et al, 2012[22]	Yes	Yes	Yes	Yes	Yes
Middlebrooke et al, 2006[23]	Yes	Unclear	No	Yes	No
Monteiro et al, 2010[24]	Unclear	Unclear	Unclear	Yes	Yes
	Adequate sequence generation	**Allocation concealment**	**Blinding of outcome**	**Description of losses and exclusions**	**Intention to treat analysis**
Negri et al, 2010[25]	Yes	Unclear	No	Yes	Yes
Okada et al, 2010[26]	Yes	Unclear	No	Yes	Yes
Plotnikoff et al, 2010[27]	Yes	Yes	Unclear	Yes	Yes
Shenoy et al, 2010[28]	Yes	Unclear	No	Yes	Yes
Sigal et al, 2007[29]	Yes	Yes	Yes	Yes	Yes
Yavari et al, 2010[30]	Unclear	Unclear	No	Yes	No

* Unclear: Used when allocation concealment was not unclear to use yes or no. **Blinding of outcome: Blinded = When blood pressure was assessed by automated device; Not blinded: When blood pressure was assessed by manual device.

Electronic Supplementary Material Table S3. *Risk of bias of studies included in the meta-analysis: physical activity advice only*

	Adequate sequence generation	Allocation concealment	Blinding of outcome**	Description of losses and exclusions	Intention to treat analysis
Agurs-Collins et al, 1997[31]	Yes	Unclear	Unclear	Yes	No
Allen et al, 2008[32]	Yes	Unclear	No	Yes	No
Andrews et al, 2011[33]	Yes	Yes	No	Yes	Yes
Araiza et al, 2006[34]	Unclear	Unclear	Yes	Yes	Yes
Christian et al, 2008[35]	Yes	Yes	No	Yes	Yes
De Greef et al, 2010[36]	Yes	Yes	No	Yes	Yes
Diedrich et al, 2010[37]	Unclear	Unclear	Unclear	Yes	No
Ferrer-Garcia et al, 2011[38]	Unclear	Unclear	No	Yes	Unclear
Goldhaber-Fiebert et al, 2003[39]	Yes	Unclear	Yes	Yes	No
Hare et al, 2011[40]	Yes	Unclear	Yes	Yes	Yes
Kirk et al, 2003[42]	Yes	Yes	Yes	Yes	No
Krousel-Wood et al, 2008[41]	Yes	Unclear	Unclear	Yes	Yes
Piette et al, 2011[42]	Yes	Yes	Unclear	Yes	Yes
Pi-Sunyer et al, 2007[43]	Yes	Yes	Unclear	Yes	No
Plotnikoff et al, 2011[44]	Yes	Unclear	Unclear	Yes	Yes
Schultz et al, 2011[45]	Unclear	Unclear	No	Yes	No
Sun et al, 2008[46]	Unclear	Unclear	No	Yes	Unclear
Toobert et al, 2003[47]	Unclear	Unclear	Unclear	Yes	Unclear
Tudor-Locke et al, 2004[48]	Unclear	Unclear	Unclear	Yes	No
Wing et al, 1988[49]	Unclear	Unclear	Unclear	Yes	No
Wisse et al, 2010[50]	Unclear	Yes	Yes	Yes	No

* Unclear: Used when allocation concealment was not unclear to use yes or no. . **Blinding of outcome: Blinded = When blood pressure was assessed by automated device; Not blinded: When blood pressure was assessed by manual device.

Electronic Supplementary Material Table S4. Absolute changes in systolic and diastolic blood pressure in individual studies of structured exercise training and physical activity advice *vs.* no intervention stratified according to the presence or absence of hypertension at baseline in patients with type 2 diabetes.

	Hypertensive patients		**Normotensive patients**		
	N	**Change (CI 95%)**	**N**	**Change (CI 95%)**	***P***
Structured Exercise Training					
Aerobic					
Systolic	221	-5.84 (-8.84; -2.84)	240	-3.10 (-5.92; -0.28)	.001
Diastolic	221	-1.35 (-2.88; 0.18)	240	-2.82 (-4.44; -1.21)	.001
Resistance					
Systolic	93	-7.21 (-12.32; -2.10)	148	-2.78 (-5.64; 0.08)	.001
Diastolic	93	-3.71 (-4.24; -3.17)	148	-1.80 (-3.34; -0.25)	.001
Combined					
Systolic	440	-2.90 (-7.70; 1.90)	64	2.00 (-5.21; 9.21)	.001
Diastolic	440	-1.71 (-4.04; 0.62)	64	1.00 (-2.94; 4.94)	.001
Physical activity advice					
Systolic	3158	-2.33 (-4.01; -0.64)	647	-2.91 (-6.04; 0.22)	.001
Diastolic	3158	-1.32 (-2.00; -0.65)	647	-1.47 (-2.28; -0.65)	.001

Student t test between weighted mean differences from hypertensive and normotensive groups, using pooled sample sizes, averaged mean and standard deviation for each group. Hypertension, studies including more than 70% of hypertensive patients and baseline BP >140/90 mmHg

Electronic Supplementary Material Table S5. Sensitivity analyzes by volume and intensity in absolute changes in systolic blood pressure in studies of structured exercise training in patients with type 2 diabetes

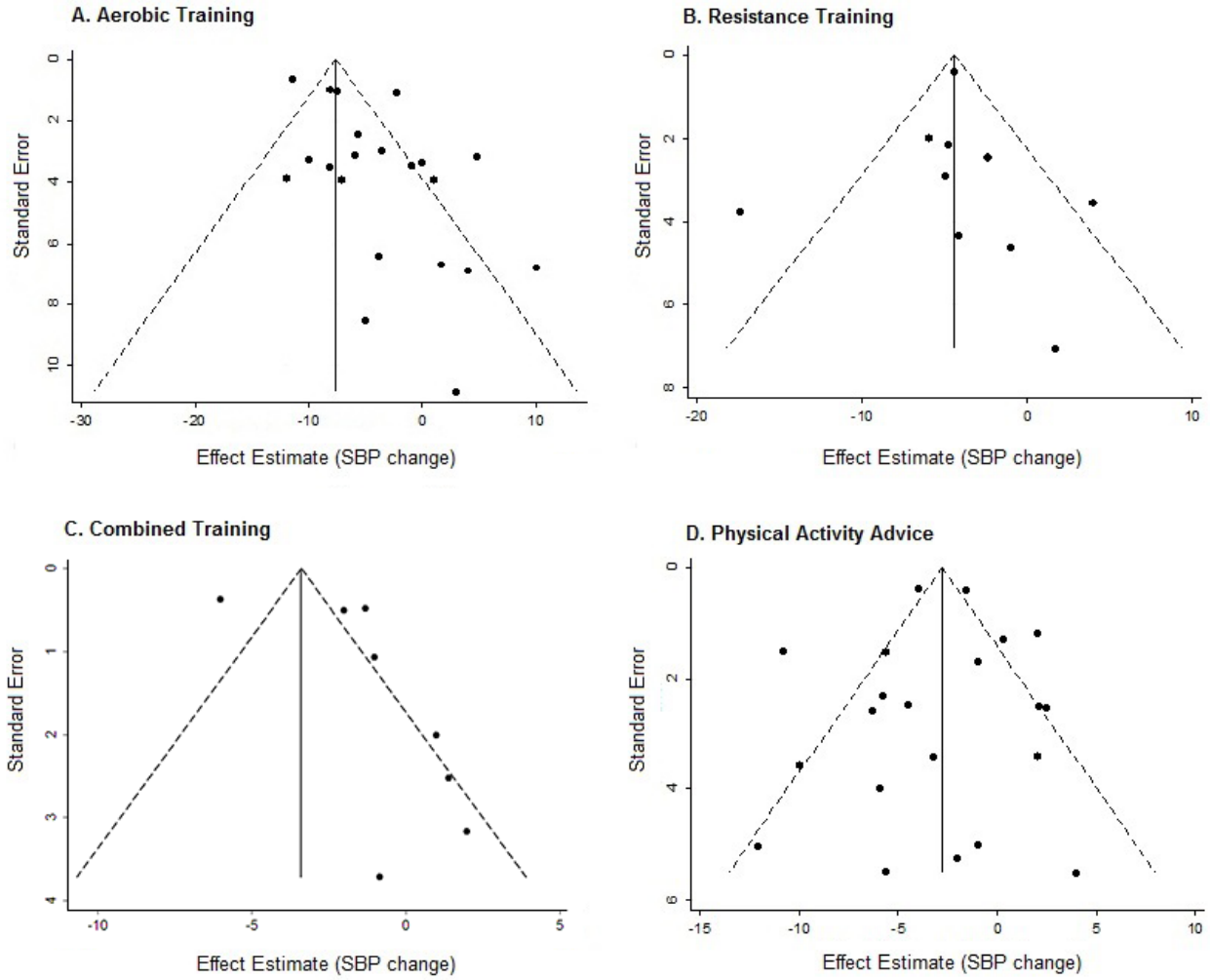
Subgroup	**N of studies (patients)**	**Change (95% CI)**	***p***	***I²***	***P***
Structured Exercise Training – Intensity					
Aerobic					
Low intensity (≤70% HRmax)	8 (289)	-1.99 (-8.59; 4.62)	0.555	85.5%	<.001
High intensity (>70% HRmax)	8 (333)	-5.47 (-7.94; -3.00)	<.001	67.9%	.003
Resistance					
Low intensity (≤70% 1-RM)	5 (209)	-5.21 (-9.29; 1.16)	.012	77.4%	.001
High intensity (>70% 1-RM)	5 (194)	-3.99 (-6.66; -1.32)	.003	0%	.644
Combined					
Low intensity (HRmax or 1-RM <70%)	3 (229)	-4.14 (-15.47; 7.19)	.447	96.6%	<.001
High intensity (HRmax & 1-RM ≥70%)	2 (596)	-3.30 (-4.71; -1.89)	.001	0%	.331
Structured Exercise Training – Volume					
Aerobic					
Low weekly volume (≤ 150min/wk)	10 (357)	-2.31 (-3.97; -0.64)	.007	0%	.438
High weekly volume (> 150min/wk)	11 (459)	-7.05 (-9.58; -4.53)	<.001	76.1%	<.001
Resistance					
Low volume (≤ 60 sets/wk)	4 (167)	-6.92 (-11.70; -2.13)	.005	74.4%	.008
High volume (> 60 sets/wk)	5 (215)	-3.14 (-5.95; -0.34)	.028	34.9%	.188
Combined					
Low volume (≤ 150min/wk or ≤60sets/wk)	3 (135)	-0.22 (-5.38; 4.95)	.934	39,1%	.194
High volume (>150min/wk & >60sets/wk)	2 (143)	-6.69 (-20.21; 6.83)	.332	98%	<.001

Electronic Supplementary Material Table S6. Structured exercise training and physical activity advice only in patients with type 2 diabetes: univariate and multivariate meta-regression models

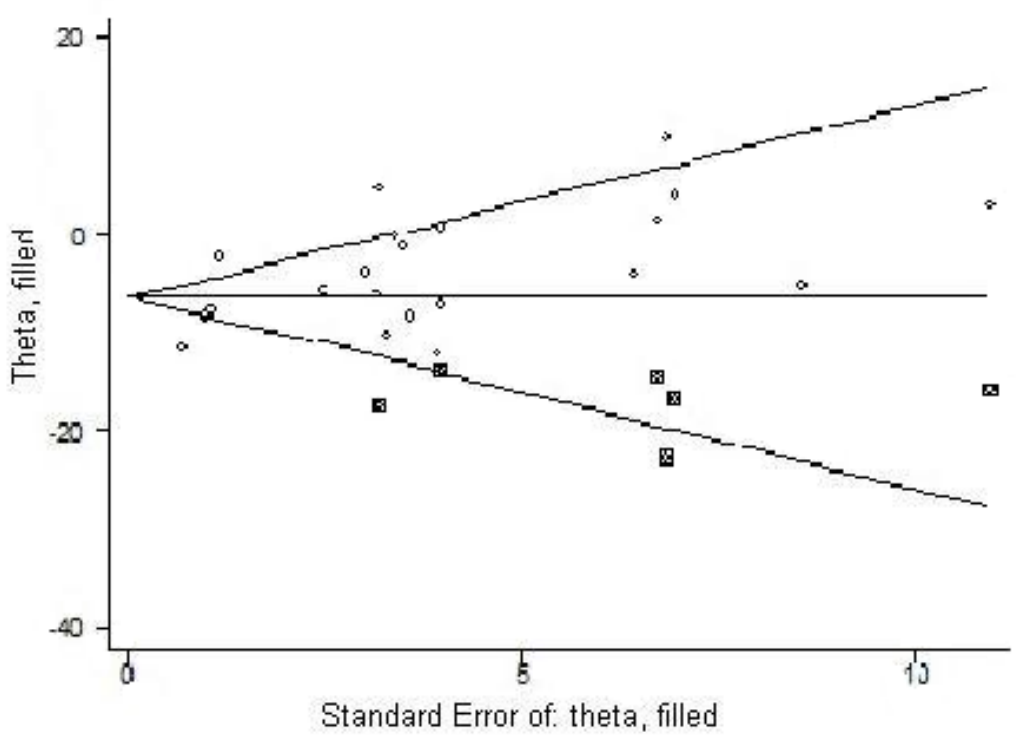
tructured exercise training				
Covariates	**Coefficient**	**95%CI**	***P***	**Adjusted R^2**
Supervised aerobic training, n=21				
Baseline SBP (mmHg), n=21	-0.05	-0.48; 0.37	0.802	NS
BMI change (kg/m²), n=17	-7.57	-15.1; -0.06	0.049	79.01%
Frequency (sessions/wk), n=21	-2.29	-5.39; 0,82	0.140	NS
Weekly volume (min/wk), n=21	-0.06	-0.09; -0.02	0.003	58.31%
Intensity (% $HR_{máx}$), n=16	-0.12	-0.46; 0.21	0.436	NS
Multivariate model: BMI change + Weekly volume, n=17			<.001	91.25%
Supervised resistance training, n=10				
Baseline SBP (mmHg), n=10	-0.29	-0.76; 0.17	0.187	NS
BMI change (kg/m²), n=7	-2.97	-13.58; 7.65	0.504	NS
Frequency (sessions/wk), n=10	0.72	-6.86; 8.30	0.832	NS
Weekly volume (sets/wk), n=9	0.10	-0.08; 0.28	0.248	NS
Weekly volume (repetitions/wk), n=9	0.001	-0.001; 0.02	0.088	NS
Intensity (% 1-RM)	-0.18	-0.67; 0.32	0.432	NS
Multivariate model: Baseline SBP + Weekly volume (repetitions/wk), n=9			0.089	NS
Supervised combined training, n=8				
Baseline SBP (mmHg), n=8	-0.22	-0.99; 0.55	0.508	NS
BMI change (kg/m²), n=8	-4.61	19.87; 10.64	0.487	NS
Frequency (sessions/wk), n=8	-2.64	-9.07; 3.79	0.354	NS
Aerobic volume (min/wk), n=8	-0.05	-0.15; 0.04	0.204	NS
Aerobic intensity (% $HR_{máx}$), n=5	0.12	-1.15; 1.40	0.775	NS
Resistance volume (sets/wk), n=5	0.13	-0.44; 0.19	0.289	NS
Resistance volume (repetitions/wk), n=3	-0.02	-0.43; 0.39	0.596	NS
Resistance intensity (% 1-RM), n=4	-0.03	-1.53; 1.47	0.938	NS
Multivariate model: Baseline SBP + Aerobic volume, n=7			.001	100%
Physical activity advice, n=21				
Baseline SBP (mmHg), n=21	-0.35	-0.64; -0.05	0.024	34.07%
BMI change (kg/m²), n=15	-0.19	-3.26; 2.89	0.897	NS
Encouragement by phone, n=21	-3.10	-6.89; -0.70	0.104	NS
Dietary recommendation, n=21	0.98	-3.12; 5.09	0.623	NS
Recommended frequency (sessions/wk), n=11	-1.01	-5.38; 3.36	0.613	NS
Recommended duration (min/session), n=9	0.17	-0.13; 0.49	0.222	NS
Multivariate model: Baseline SBP + Encouragement by phone + Recommended duration, n=9			0.007	97.42%

Studies in "low" or "high" categories were determined by the median of each variable. SBP, systolic blood pressure; BMI, body mass index; NS, *P* value is not significant.

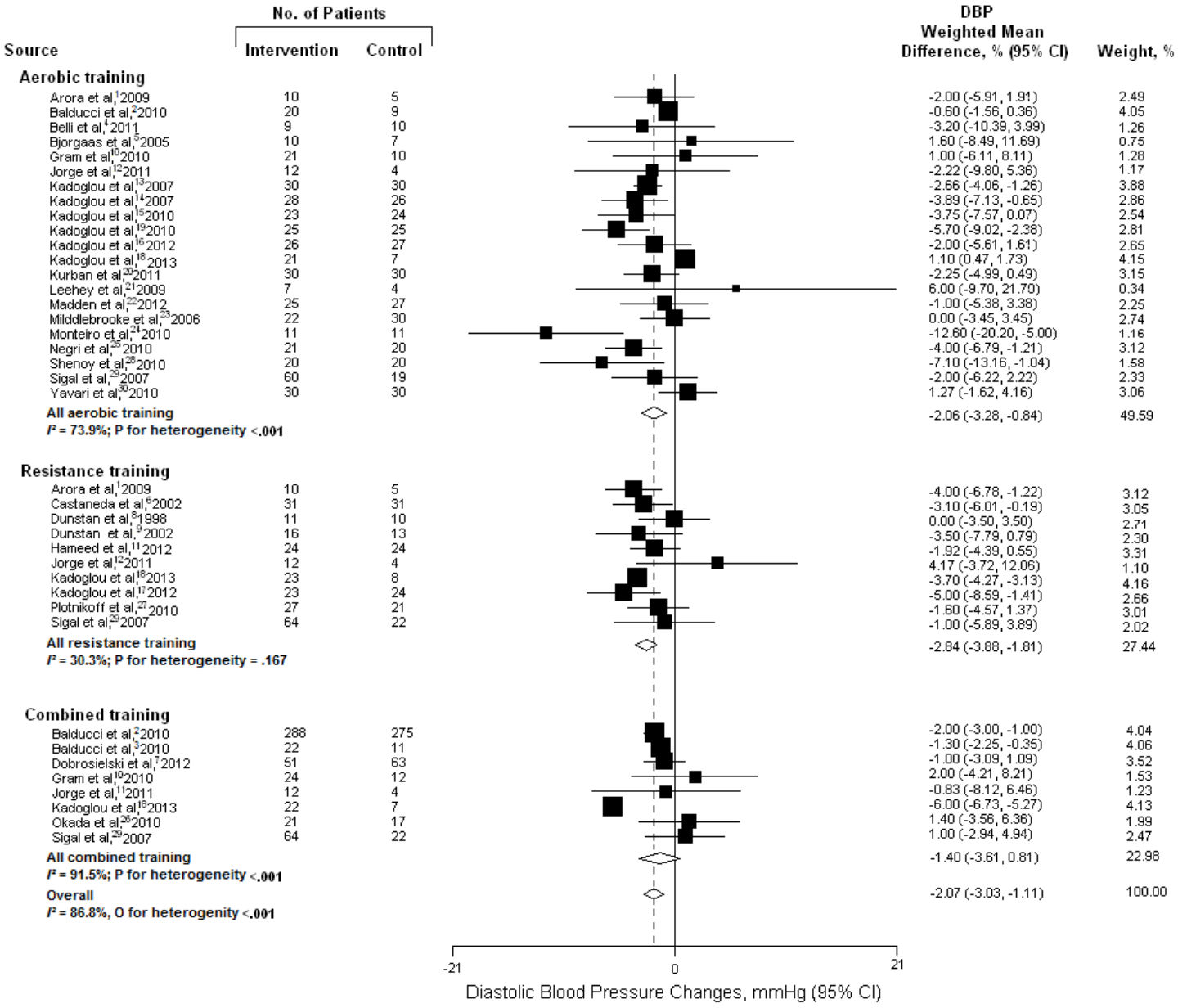
Electronic Supplementary Material Figure S1. Contour-enhanced funnel plot of each trial observation(s)



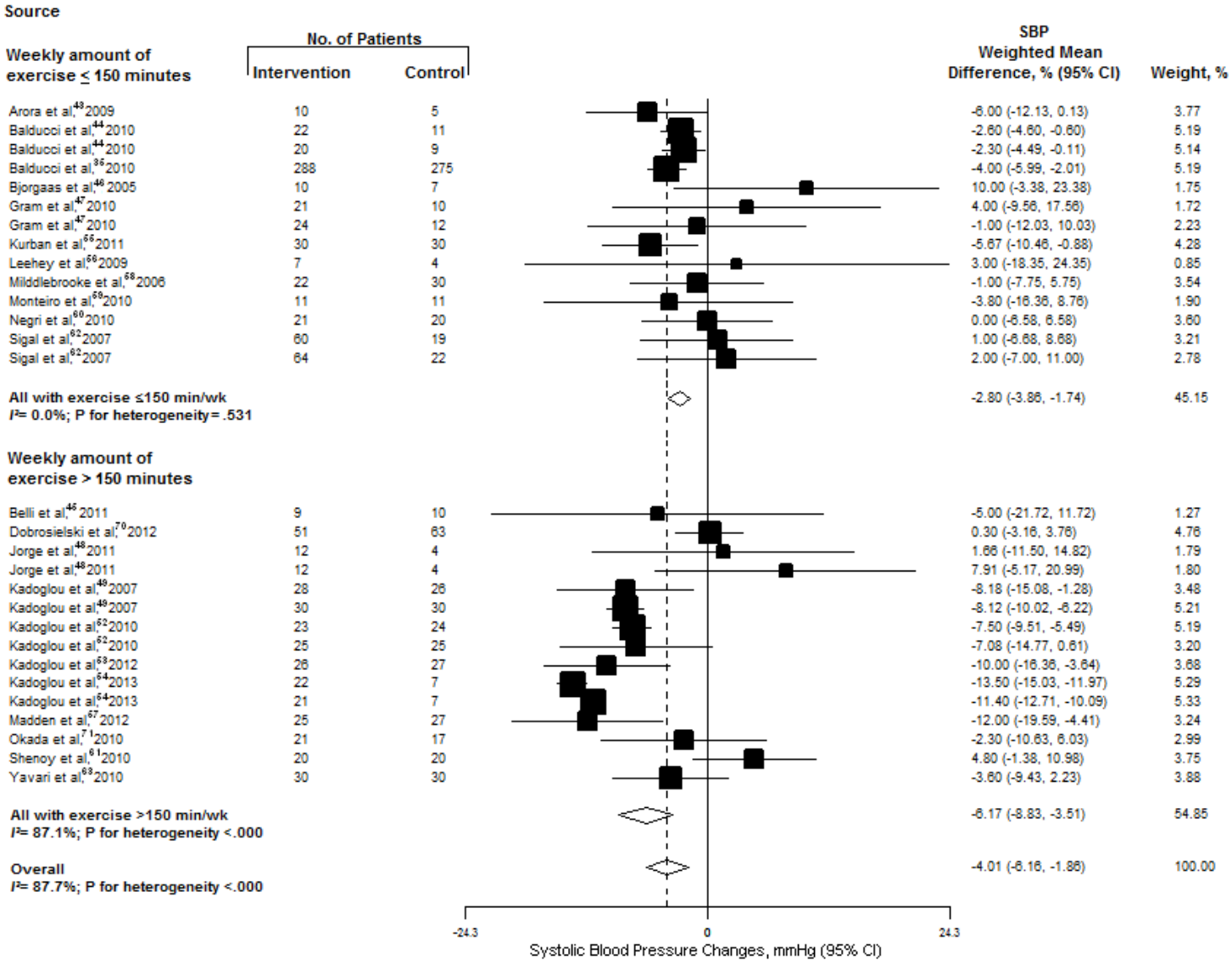
Electronic Supplementary Material Figure S2. Contour-enhanced trim-and-fill computation of each trial observation(s) in aerobic structured exercise training in patients with type 2 diabetes



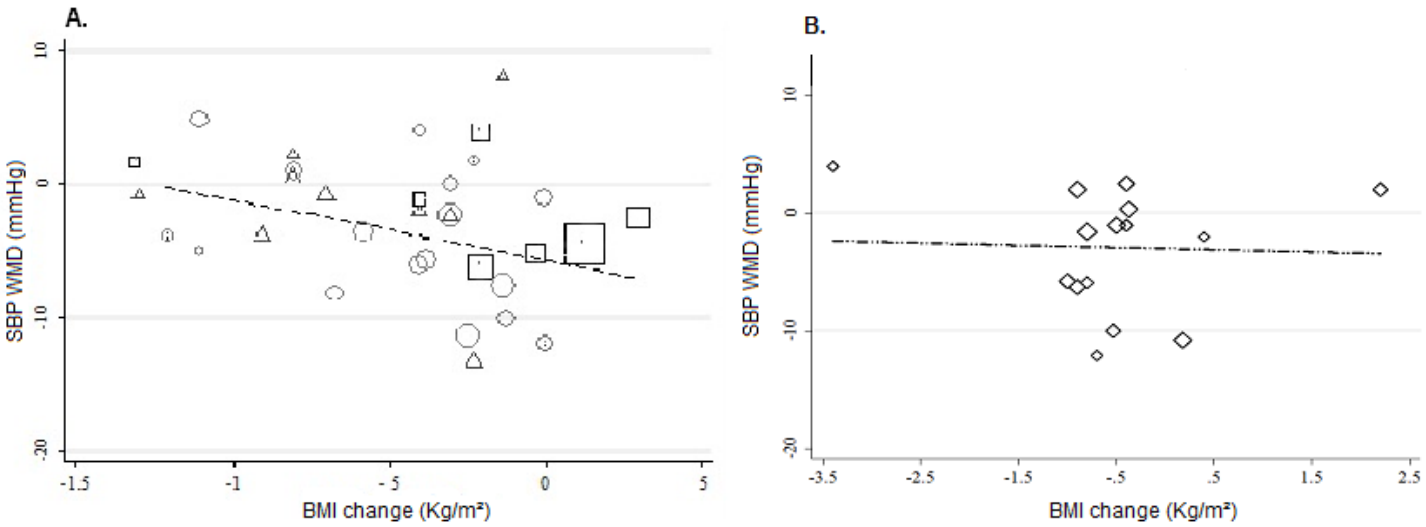
Electronic Supplementary Material Figure S3. Absolute changes in diastolic blood pressure of individual studies of structured exercise training *vs.* no intervention in patients with type 2 diabetes



Electronic Supplementary Material Figure S4. Absolute changes in systolic blood pressure in individual studies of structured exercise training (aerobic and combined) *vs.* no intervention, stratified by weekly amount of exercise

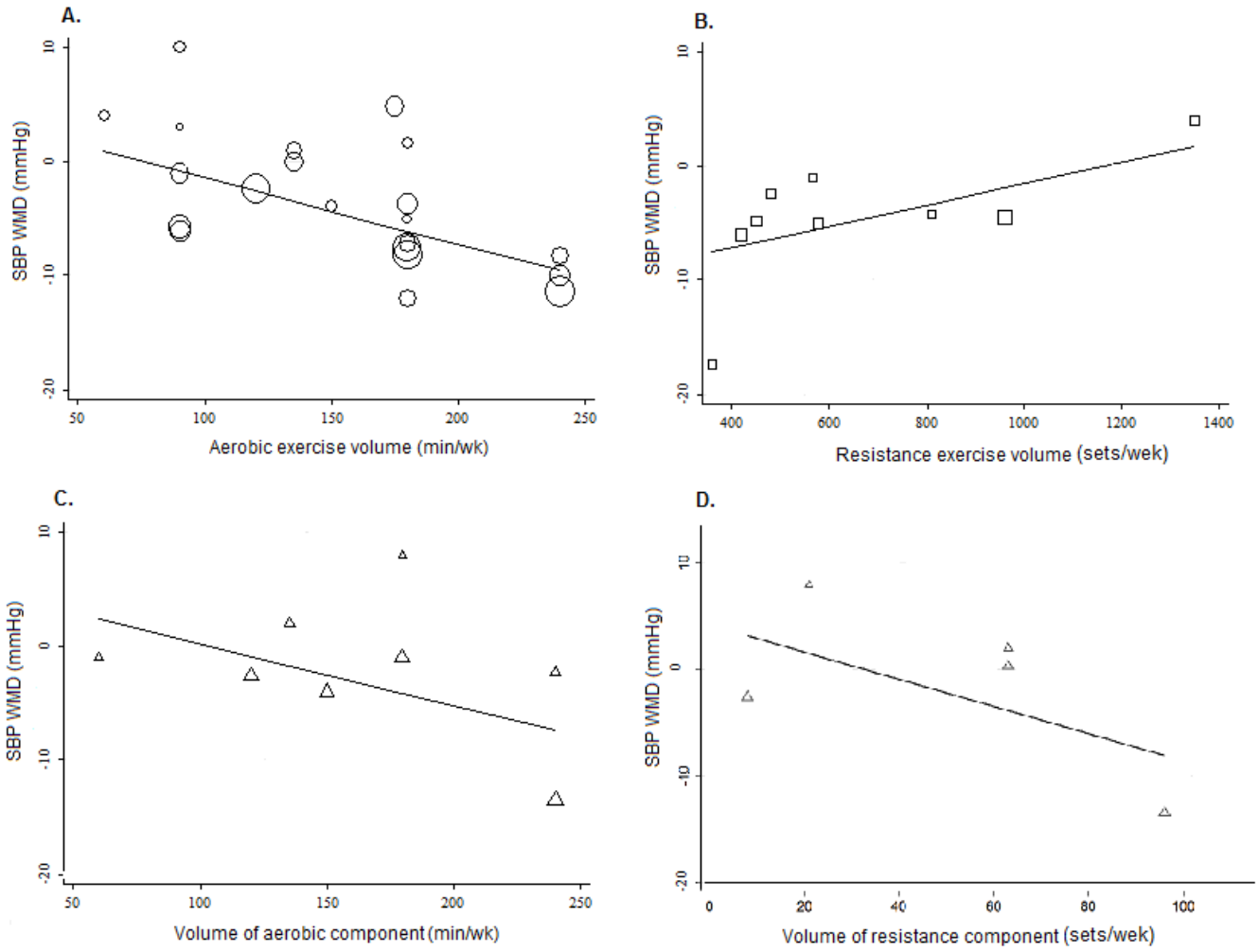


Electronic Supplementary Material Figure S5. Relationship between changes in body mass index and changes in systolic blood pressure



Each observation represents the WMD (***weighted mean difference)*** of systolic blood pressure between the different interventions of exercise training and control groups. The size of the symbols is proportional to the inverse variance of each study in the pooled analysis. Meta-regression line is show for all type of intervention (Panel A, structured exercise). Circles, aerobic training; squares, resistance training; and triangles, combined training. Slope for weighted regression, y= -4.38x -5.43. Weighted correlation: r = -0.35, *P* = .047. Panel B: dashed-dot line and diamonds, physical activity advice. Slope for weighted regression, y = -0.33x -2.91. Weighted correlation: r = -0.06, *P* = .831

Electronic Supplementary Material Figure S6. Relationship between exercise volume and changes in systolic blood pressure (SBP) in studies of aerobic training (A), resistance training (B), aerobic component (C) and resistance component (D) in combined training.



Exercise volume is expressed as minutes of aerobic exercise per week for the aerobic component or number of repetitions per week for de resistance component. Each observation represents the WMD (***weighted mean difference)*** of systolic blood pressure between the structured exercise training types and controls. The size of de circles, squares and triangles is proportional to the inverse variance of each study in the pooled analysis. Slopes for the weighted regressions and weighted correlations were, respectively: y= -0.59x + 4.86 and r= -0.59, *P*= .005 for the volume of aerobic training (A); y= 0.01x -11.36 and r= 0.63, *P*= .071 for the volume of resistance training (B); y= -0.05x + 5.69 and r= -0.51, *P*= .197 for the aerobic component in the combined exercise (C); y= -0.12x + 3.03 and r= -0.62, *P*= .268 for the resistance component in the combined exercise (D).

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