Tese de doutorado

IMPACTO DO USO DE GLIBENCLAMIDA *VS.* VILDAGLIPTINA SOBRE A VARIABILIDADE DA GLICEMIA APÓS UMA SESSÃO DE EXERCÍCIO AERÓBICO EM PACIENTES COM DIABETES TIPO 2

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“On peut toujours plus que

ce que l’on croit pouvoir”

Joseph Kessel

SUMÁRIO

LISTA DE ABREVIATURAS

DM2: Diabetes Mellitus tipo 2

HbA1c: hemoglobina glicada

MAGE: amplitude media das oscilações glicêmicas

DPP-IV: Dipeptidil peptidase-IV

GLP-1: peptídeo -1 semelhante ao glucagon

HbA1c: hemoglobina glicada

MAGE: amplitude media das oscilações glicêmicas

RESUMO

Objetivos: Avaliar a variabilidade glicêmica, respostas metabólicas e cardiovasculares após uma sessão de exercício aeróbico em pacientes com diabetes em tratamento com vildagliptina ou glibenclamida. **Métodos:** Foi realizado ensaio clínico aberto e paralelo que incluiu 13 pacientes com diabetes tipo 2 tratados com vildagliptina (50mg bid) ou glibenclamida (5mg bid) por 12 semanas. Antes e após a intervenção, a variabilidade glicêmica (glicose média, variância da glicose, coeficiente de variação e média da amplitude das oscilações glicêmicas), respostas metabólicas (HbA1c, glicose, insulina e 8-iso prostaglandina F2α) e cardiovasculares (débito cardíaco, variabilidade da frequência cardíaca e componentes do controle autonômico) foram avaliadas no repouso, durante e após uma sessão de exercício aeróbico de 30 minutos. A variabilidade da pressão arterial foi aferida nas 24 horas após o exercício. **Resultados**: Doze semanas de tratamento resultou em redução da glicemia de 18% com vildagliptina e 35% com glibenclamida (p grupo=0.007). A HbA1c reduziu significativamente (1.24 % e 1.52%) nos grupos vildagliptina e glibenclamida, respectivamente. A variabilidade glicêmica não se alterou após o tratamento com glibenclamida ou vildagliptina (MAGE=55.8 ±5.3 mg/dL e 69.9 ± 13.3 mg/dL, respectivamente; p grupo=0.091; p tempo=0.234). Foi encontrada uma diminuição da glicose avaliada por monitoramento contínuo durante as 3 horas de recuperação do exercício, com AUC (de 6h) menor no grupo glibenclamida vs vildagliptina (p=0.04). A glibenclamida induziu maiores concentrações de insulina na recuperação do exercício. O grupo tratado com vildagliptina obteve 6.3mmHg de redução da pressão arterial sistólica, enquanto a glibenclamide reduziu 3.6mmHg, sem diferença entre os grupos. Os pacientes tratados com vildagliptina apresentaram menor variabilidade da pressão arterial sistólica (0.0445 ±0.05 mm/Hg), medida por *time rate*, comparados aos tratados com glibenclamida (0.601 ±0.12 mm/Hg), p=0.012. **Conclusão:** Este é o primeiro estudo conduzido em pacientes com DM2 que fornece dados sobre a influência da terapia medicamentosa padrão (metformina e glibenclamida) *vs* outra classe disponível (metformina e vildagliptina) em respostas a uma sessão de exercício aeróbico, um dos tratamentos recomendados para pacientes com DM2. Além de melhora no controle glicêmico e redução da pressão arterial sistólica obtidas por ambos tratamentos, foi observada menor variabilidade da pressão arterial nos

pacientes submetidos ao tratamento com vildagliptina. Não foi encontrada menor variabilidade da glicemia após o exercício nos pacientes tratados com vildagliptina comparados aos tratados com glibenclamida, refutando a hipótese de estudo.

Palavras-chave: hipoglicemiantes, variabilidade glicêmica, exercício aeróbico

1. INTRODUÇÃO

O Diabetes Mellitus tipo 2 (DM2) é caracterizado por hiperglicemia crônica, a qual determina, em longo prazo, inúmeras complicações, dentre elas, doenças cardiovasculares, retinopatia e nefropatia (1) . Bom controle glicêmico é a melhor alternativa para diminuir o aparecimento e progressão destas complicações (2).

Um dos indicadores mais confiáveis de controle do diabetes é a hemoglobina glicada (HbA1c), marcador biomolecular que reflete a média da glicemia dos últimos três meses. Em indivíduos saudáveis, cerca de 4% a 6% do total da hemoglobina apresenta-se glicada, enquanto que no paciente com diabetes e descontrole acentuado, esta porcentagem pode atingir valores de duas a três vezes acima do normal. As diretrizes recomendam um valor inferior a 7% para adultos com DM2 como indicador de bom controle glicêmico (3). Recentemente foi realizada uma revisão sistemática com metanálise de ensaios clínicos randomizados sobre controle glicêmico (por HbA1c) e desfechos cardiovasculares, totalizando 882666 pessoas com DM2. Nesta revisão, foi encontrada uma redução de 9% na incidência de infarto agudo do miocárdio apenas com valores de HbA1c abaixo de 7% (4).Entretanto, estudos de impacto como ACCORD (5, 6), ADVANCE (7) e VADT (8) demonstraram que o controle metabólico mais intensivo, chegando a HbA1c próxima de 6,5%, não determinou maiores reduções em eventos cardiovasculares em pacientes com DM2 e média de 8 a 11 anos de doença.

Apesar de ser consenso a utilização da glicemia de jejum e HbA1c para avaliação do controle glicêmico, na última década a variabilidade glicêmica, tanto em curto quanto em longo prazo, vem sendo estudada como um método alternativo (9), por potencialmente se associar ao desenvolvimento de complicações crônicas do diabetes (10). De forma prática, a variabilidade da glicose capilar pode ser avaliada pelo automonitoramento, método que consiste na colocação de uma gota de sangue capilar em uma fita que contém glicose oxidase acoplada a um glicosímetro. A avaliação da glicose pode ser feita em vários momentos no dia e serve para acompanhamento do estado glicêmico e fornece informações para adequação do tratamento (11). Em curto prazo a variabilidade glicêmica pode ser avaliada através da utilização do sistema de monitoramento contínuo da glicose (CGMS), o qual utiliza um sensor inserido no

tecido subcutâneo e mede a glicose intersticial, por meio da obtenção de 288 medidas a cada 24 horas, que podem ser realizadas em um período de até 5 dias (12). Este exame possibilita calcular a variação da glicose por meio de inúmeros índices. Estudos utilizam índices convencionais tais como *absolute means of daily differences* (MODD), *means of detrended fluctuation analysis (DFA), continuous overlapping net glycemic action* (CONGA), *glucose variance* (VAR), *glucose coefficient of variation* (CV%), *glucose standard deviation* (SD), *mean absolute glucose* (*MAG)* e *mean amplitude of glucose excursions* (MAGE) (13). Os cálculos integram médias, variações e oscilações entre momentos diários distintos, refletindo diferenças em períodos pré e pós-prandiais. O MAGE é considerado o meio mais utilizado para avaliar oscilações significativas na glicemia (14).

Neste contexto, o seguimento de 1441 pacientes com Diabetes Mellitus tipo 1 durante 9 anos mostrou que o desenvolvimento de complicações microvasculares do diabetes é maior naqueles com maior variabilidade de HbA1c (15), porém a análise de variabilidade glicêmica de curto prazo não foi relacionada com maior risco de complicações (16). Entretanto a literatura ainda é controversa, e não há consenso se a variabilidade glicêmica de curto prazo pode ser considerada como fator de risco independente em relação ao uso de HbA1c para complicações microvasculares em diabetes (17).

A variabilidade glicêmica pode estar associada ao desenvolvimento de distúrbios implicados na gênese de complicações do diabetes, dentre eles o estresse oxidativo (18) e a disfunção endotelial (19, 20). Um marcador urinário do estresse oxidativo é o 8-iso prostaglandina F2 alfa, que reflete a peroxidação lipídica. Em um estudo, foi demonstrado que a excreção urinária de 8-iso prostaglandina F2 alfa se correlacionava positivamente com a variabilidade glicêmica avaliada por MAGE ($r=0,86$ $p<0,001$) (21). Recentemente Tang et al (22) mostraram que elevação do MAGE se associou a risco cardiovascular alto, avaliado pelo *Framingham risk score.* Devido a sua possível relação entre variabilidade glicêmica e processos ligados a complicações do diabetes, é importante investigar as influências dos tratamentos clássicos e contemporâneos sobre as respostas e variações glicêmicas ao longo do dia de pacientes acometidos do distúrbio do metabolismo glicêmico.

Tratamento do DM2, conceitos clássicos e novas opções:

O controle glicêmico fisiológico é realizado primariamente por meio de hormônios, como insulina, glucagon e incretinas. A partir da descoberta da insulina, em 1920, o entendimento do diabetes ficou mais claro. Sabe-se que este hormônio possui efeito direto sobre o metabolismo, induzindo em segundos a captação da glicose pelas células musculares e adiposas, e mediando cascatas de sinalização que influenciam processos de regulação de atividade enzimática e de transcrição proteica em diversos tecidos. Tais processos em conjunto promovem redução da glicemia. Não obstante a função da insulina, o controle glicêmico depende também do glucagon, visto que ele induz glicogenólise hepática. A implicação bi-hormonal no DM2 não é unicamente utilizada atualmente, pois outros fatores e hormônios estão intimamente ligados ao processo de regulação da glicemia (23).

Nas últimas décadas, vasto interesse foi dado a outros dois hormônios, o polipeptídeo insulinotrópico glicose-dependente (GIP) e o peptídeo-1 semelhante ao glucagon (GLP-1), liberados pelas células endócrinas do epitélio do intestino e chamados incretinas (24). Estes hormônios são rapidamente degradados no plasma, sendo que o GLP-1 possui meia vida de 2 a 5 minutos e o GIP de 7 a 9 minutos. O GLP-1 possui inúmeros efeitos, tais como: estimular a secreção de insulina de modo glicose-dependente, inibir a secreção do glucagon, diminuir o apetite, retardar o esvaziamento gástrico e, em modelos experimentais, capacidade de preservar/aumentar a massa de células beta-pancreáticas. A alimentação com carboidratos pode aumentar até 6 vezes a liberação de GLP-1, outro estímulo citado na literatura é o exercício físico (25). A deficiência na secreção de insulina encontrada em pacientes com DM2 pode ser em parte, devido à redução de efeito do GLP-1 nestes pacientes (26, 27). Devido ao fato das incretinas possuírem efeitos benéficos na manutenção da homeostase da glicemia, os hormônios GIP e GLP1 são utilizados em alguns tratamentos do DM2.

O tratamento do DM2 é baseado em três pilares: dieta saudável, antidiabéticos orais ou insulina e exercício físico (3, 28, 29). Dentre os antidiabéticos orais, a metformina é a primeira opção, por ter se associado à redução de mortalidade e complicações macrovasculares (3, 4). Quando a metformina é utilizada unicamente e não se obtém bom controle glicêmico, é

recomendada a terapia combinada (30), havendo necessidade de acrescentar um segundo medicamento, o qual pode ser de qualquer uma das classes disponíveis. Diretrizes determinam os seguintes medicamentos como terapia sequencial: as sulfonilureias que aumentam a secreção de insulina; as tiazolidinedionas, também conhecidas como glitazonas (TZD) possuem como ação o aumento da sensibilidade à insulina; os inibidores do cotransportador sódio/glicose 2 (SGLT2), os quais inibem o SGLT2 no túbulo proximal renal; os agonistas de receptores de GLP-1 que apresentam a função de intensificar a ação do hormônio na corrente sanguínea, os inibidores da enzima dipeptidil peptidase-4 (DPP-4), que reduzem e retardam a degradação do GLP-1 natural e insulina. Estas classes possuem efeito de redução da HbA1c de aproximadamente 1% (31, 32) Dentre os inibidores da enzima DPPIV, que cliva o GLP-1, podemos citar a vildagliptina, sitagliptina e linagliptina (33). A glibenclamida é uma sulfonilueria muito utilizada como terapia sequencial para tratar o DM2 devido ao seu efeito potente em reduzir a glicemia. Dentre estas classes orais, a metformina e a glibenclamida foram adotadas como tratamento padrão pelo Ministério da Saúde no Brasil e fornecidas gratuitamente aos pacientes atendidos pelo Sistema único de Saúde (SUS).

Comparadas a inibidores de DPPIV as sulfonilureias mostraram melhor controle glicêmico em análises realizadas em uma revisão sistemática com metanálise que comparou eficácia e segurança destas duas terapias em seguimento entre 18 e 104 semanas. Pacientes tratados com sulfonilureias apresentaram mais episódios de hipoglicemias, ganho de peso e eventos adversos totais, já o tratamento com inibidores de DPPIV foi associado a menos eventos cardiovasculares (34).

Os antidiabéticos orais podem reduzir a variabilidade glicêmica de forma variável. Em um ensaio clínico a acarbose foi superior à glibenclamida na redução da variabilidade glicêmica, avaliada por diversos índices, inclusive por MAGE (35). Marfella et al (36) realizaram um estudo sobre a avaliação da eficácia sobre a glicemia de um tratamento de 3 meses com vildagliptina ou sitagliptina em pacientes com DM2 mal controlados com metformina. Os dois grupos apresentaram diminuições similares na HbA1c, glicemia de jejum e pós-prandial, enquanto o MAGE diminuiu significativamente somente nos sujeitos que receberam vildagliptina (34 ± 7 mg/dl, com 51% de redução) comparados ao grupo sitagliptina (59 ±16 mg/dl e redução de 14,5%). Este estudo mostrou um

aumento de GLP-1 e diminuição na concentração plasmática de glucagon, no período interprandial. Uma intervenção de 4 semanas de sitagliptina ou glimepirida foi suficiente para Kim et al encontrarem redução no MAGE no grupo que recebeu o inibidor de DPPIV e diminuição tanto na glicemia de jejum, como na HbA1c após o tratamento nos dois grupos (37).

Em relação ao terceiro pilar do tratamento do DM2, o exercício físico foi recentemente associado, além da redução da HbA1c já classicamente conhecida (28), a efeito em reduzir a variabilidade glicêmica (38).

O metabolismo glicêmico é ativado devido aos efeitos agudos do exercício, os quais são: 1) um aumento da captação da glicose pelo músculo equilibrado pela produção de glicose hepática, havendo uma maior dependência dos carboidratos como fonte energética na medida que a intensidade aumenta; 2) a captação de glicose pelo músculo esquelético estimulada pela insulina predomina em repouso e é prejudicada em pacientes com DM2, enquanto contrações musculares estimulam o transporte da glicose através da membrana por mecanismo adicional separado, sendo este não prejudicado pela resistência à insulina ou o DM2 e 3) atividades físicas podem resultar em melhoras agudas da ação da insulina sistêmica com duração de 2 a 72 horas (39)

Macdonald et al (40), alertados pelo fato de que a glicemia diminui durante o exercício na maioria (41, 42), mas não em todos indivíduos com DM2 (43), realizaram um estudo com 10 sujeitos, sendo 6 pacientes obesos com DM2 e 4 controles saudáveis. Os sujeitos foram avaliados com CGMS por 3 dias e realizaram uma sessão de exercício a 90% do limiar de lactato durante uma hora. Durante o exercício, a glicemia e os valores do CGMS reduziram nos pacientes com DM2 ($p< 0,001$), mas este resultado não foi encontrado nos saudáveis ($p = 0,085$). A redução significativa foi justificada pelo retardo da produção hepática de glicose, juntamente com a manutenção ou aumento da utilização da glicose induzidos pelo exercício. Este mesmo estudo discute que a acurácia do CGMS é influenciada pelo estado glicêmico, fornecendo dados fidedignos em estados hiperglicêmicos, condição comum em pacientes com DM2 tornando então, um método de avaliação útil para estes pacientes. A acurácia do CGMS antes e após modalidades distintas de exercício foi identificada pelo nosso grupo de pesquisa (38). A partir disso, os mesmos pesquisadores realizaram um ensaio clínico randomizado para avaliar duas sessões distintas de exercício em pacientes com

DM2. O estudo demonstrou mesma redução da variabilidade glicêmica, avaliada por métodos não convencionais, após sessões de exercício aeróbico (40 minutos entre 60 e 70% da FCmáx) ou de força em pacientes com DM2 em uso apenas de metformina (44). Na recuperação da sessão, a redução da glicose medida por CGMS teve duração de 3 horas. Este achado é contrário à duração do efeito de 72h citado na literatura (45).

A homeostase glicêmica em períodos pós-prandiais nas 24 horas após uma sessão de exercício aeróbico ou atividades de vida diária foi o desfecho em um estudo em pacientes com DM2 em uso de antidiabéticos orais submetidos a 45 minutos em um cicloergômetro a 50% da carga máxima, 3 atividades diárias de15 minutos cada ou a situação controle (sedentária). Somente o exercício aeróbico moderado reduziu tanto a prevalência de hiperglicemias em 24h (duração de ~4h *vs.* ~6h nas outras situações) como a média glicêmica. Esta intervenção também mostrou efeito atenuante sobre o aumento da insulina plasmática pós desjejum, resultados não encontrados na condição controle ou submetidos a atividades de vida diária (46).

Sharof et al (47) realizaram um estudo a fim de avaliar o impacto da combinação de uma sessão de exercício ao tratamento com metformina sobre a sensibilidade a insulina, atividade da AMPK e os substratos metabólicos em indivíduos insulino-resistentes, e mostraram que em resposta ao exercício, o grupo tratado com metformina não demonstrou aumento na sensibilidade a insulina, já o grupo placebo aumentou em 52% esta variável. Mulheres com DM2 tratadas por 4 meses com metformina ou glibenclamida e sujeitos controles sem uso de hipoglicemiantes foram avaliadas por Cunha et al (48). Após o tratamento e uma sessão de exercício físico de 45 minutos a 50% do VO_2pico não foi encontrada diminuição da glicemia em resposta ao exercício nos grupos avaliados. As concentrações de insulina diminuíram durante o exercício nos grupos que receberam glibenclamida e controle, sugerindo que a glibenclamida não interferiu na inibição fisiológica da secreção de insulina durante o exercício. A concentração de glucagon não foi alterada durante o exercício em todos os grupos.

A partir da importância dos pilares do tratamento do diabetes supracitados, um estudo recente avaliou os efeitos de um treinamento aeróbico (duas vezes por semana) e de força (uma vez semanal) sob tratamento de

liraglutida ou placebo por 16 semanas. Enquanto o grupo que fez o treinamento e recebeu a medicação diminuiu 2% a HbA1c ($p<0,001$), o grupo placebo diminuiu apenas 0,3%, sem significância estatística. Este comportamento também foi visto na glicemia de jejum. A análise da pressão arterial também foi realizada no mesmo estudo e os pesquisadores encontraram redução significativa na pressão arterial sistólica apenas no grupo tratado com liraglutida.

O efeito agudo de uma sessão de 35 minutos de exercício aeróbico sobre concentrações de GLP-1 em pessoas com DM2 foi estudada por Boulé et al em um ensaio clínico randomizado e cruzado (49). O trabalho não encontrou mudança no GLP-1 após a sessão de exercício aeróbico, enquanto que o aumento desta incretina foi encontrado apenas sob o tratamento de metformina de 28 dias, independente da realização do exercício. Recentemente a metformina não se mostrou atenuante aos benefícios do exercício crônico (50) e agudo (51) sobre o controle glicêmico, conforme hipótese levantada em estudos anteriores (47, 52, 53).

2. JUSTIFICATIVA E OBJETIVOS

Uma vez que tanto o exercício físico, como a classe de antidiabético oral que estiver sendo utilizada além da metformina podem interferir sobre a variabilidade glicêmica, é de interesse avaliar diferentes combinações destas intervenções sobre este desfecho. Os efeitos destas combinações podem decorrer de respostas agudas do exercício ou mesmo de alterações metabólicas específicas de cada antidiabético testado.

Este estudo pretende testar a hipótese que os pacientes tratados com vildagliptina associada a metformina possuem menor variabilidade glicêmica comparados aos tratados com glibenclamida e metformina (tratamento padrão) após uma sessão de exercício aeróio. Assim, este ensaio clínico pretende avaliar a variabilidade glicêmica após uma sessão de exercício aeróbio em pacientes com DM2 em tratamento com vildagliptina ou glibenclamida. Secundariamente, a proposta é avaliar respostas metabólicas e cardiovasculares ao exercício em pacientes com DM2 em tratamento com vildagliptina ou glibenclamida.

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4. ARTIGO 1

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STUDY PROTOCOL

Effects of vildagliptin as compared to glibenclamide on glucose variability after a submaximal exercise test in patients with type 2 diabetes – DIABEX VILDA

ClinicalTrials.gov Identifier: NCT01867502

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Abstract

Background**:** Cardiovascular disease, endothelial dysfunction and oxidative stress are common complications among patients with type 2 diabetes (T2DM). Besides the average blood glucose concentration, glycemic variability may be important factor for the development of chronic diabetes complications. Patients with T2DM are treated with different types of oral glucose-lowering drug. Exercise is considered a way to benefit the health of unhealthy and healthy individuals. This is confirmed by different scientific research studies in which the participants' health improved. Our general aim will be to evaluate glucose variability after the submaximal exercise test under the treatment with vildagliptin or glibenclamide, and the specific aims of this study are to evaluate the oxidative stress, endothelial function, metabolic and cardiovascular responses to exercise under the treatment with vildagliptin or glibenclamide. All these responses are important in patients with T2DM. Methods/Design: This study is a randomized, open label-PROBE design clinical trial (parallel group with a blinded end point). The sample estimated is 20 patients with T2DM. In addition to the patient's routine treatment (metformin), they will receive a second drug orally during 12 weeks: METV group - vildagliptin (50 mg bid) or METG group - glibenclamide (10 – 20mg q.d.). Before and after intervention, the evaluation of glycemic variability, endothelial function, oxidative stress, metabolic and cardiovascular response will be performed at rest, during and after a sub-maximal exercise test (30 minutes, with an intensity based on 10% under the heart rate at the second threshold). Discussion: Besides the drug action, exercise is also recommended in the treatment of glycemic control in patients with T2DM, especially for its beneficial effects on blood glucose and HbA1C. Few studies determine the effects of the association between exercise and oral glucose lowering drugs. The study will be conducted to report the metabolic and cardiovascular responses at rest, during and after sub-maximal exercise under two oral glucose-lowering drug (vildagliptin vs glibenclamide).

Keywords: Diabetes mellitus, type 2; Hypoglycemic Agents; Exercise

Background

Cardiovascular disease is the main cause of mortality among people with type 2 diabetes mellitus (T2DM) [1, 2]. Accelerated atherosclerosis in these patients is preceded by endothelial dysfunction, inflammatory burden and increased lipid peroxidation, all leading to enhanced macrophage foam cell formation [2]. Besides average blood glucose concentration, acute glycemic fluctuations from peaks to nadirs (glucose variability) may be involved in the development of diabetic complications [3], as they contribute to the generation of excessive protein glycation and oxidative stress [4]. High glucose variability was shown to be associated with endothelial dysfunction in patients with T2DM and optimal metabolic control [5]. Currently, the mean amplitude of glycemic excursion (MAGE) is one of the most used method for detecting significant swings in glycemia [6], but other tools may be useful to identify disturbances in glucose variability [7].

Some current treatments for T2DM have already been tested concerning their possible effects in reducing glucose variability besides reducing glycated hemoglobin (HbA1c) [8]. Exercise, one of the cornerstones for the treatment of hyperglycemia in T2DM, because of its beneficial effect on HbA1c [9] was recently shown to reduce glucose variability, besides its acute effects on reducing glucose levels [7]. Vildagliptin and sitagliptin were recently evaluated focusing on possible differences in daily glucose fluctuations in patients with T2DM inadequately controlled with metformin, showing that the first agent was more effective in flattening acute glucose fluctuations over a day [8]. Moreover, acarbose was superior, as compared to glibenclamide, in reducing MAGE. Therefore, aside from their absolute glucose lowering effect, it is evident that other effects could be different among different antidiabetic agents [10].

The present study will be conducted to test the hypothesis that vildagliptin associated with metformin may have more impact in improving glucose variability after a sub-maximal exercise test, as compared to glibenclamide. Our general aim will be to evaluate glucose variability after the submaximal exercise test under the treatment with vildagliptin or glibenclamide. The specific aims of this study are to evaluate the oxidative stress, endothelial function, metabolic and cardiovascular responses to exercise under the treatment with vildagliptin or glibenclamide.

Methods

Research design

This study is a randomized, open label-PROBE design clinical trial (parallel group with a blinded end point).

Outcomes:

The primary outcome will be glucose variability reduction, as evaluated by conventional and nonconventional methods. Secondary outcomes will include oxidative stress, endothelial function, metabolic and cardiovascular responses to exercise. Table 1 shows the measures to reach the outcomes.

Sample size calculation

According to data reported by Marfella and col [8], a total sample size of 20 patients (including 10% drop out) could allow detecting a difference between groups of 25.0 mg/dl on MAGE at week 12, assuming statistical power of 90%, and a significance level of 1% (two-sided two-sample t test).

Inclusion and exclusion criteria

The inclusion criteria will be: T2DM patients, with recent HbA1C between 7.5 and 10%, patients not involved in regular physical activities, older than 18 years and in use of metformin. The exclusion criteria will be: current smokers, in use of analgesic or anti-inflammatory drugs during the week of the study, body mass index (BMI) > 40 kg/m², proliferative diabetic retinopathy, ischemic heart disease, peripheral vascular disease, hepatic enzyme levels threefold higher than the reference values, lactose intolerance, glomerular filtration rate lower than 60ml/min, and blood pressure over 180/100mmHg at rest.

Eligibility assessment and follow-up visits

A search for eligibility and exclusion criteria will be performed in medical records, patient interviews and laboratory tests.

A physician will be responsible for the cardiovascular assessment, according to the American Heart Association guidelines [11]. The same physician will assess the presence of peripheral vascular disease using the ankle brachial index [12].

Two follow-up visits will be made after 4 and 8 weeks of treatment to measure blood pressure, heart rate, and body weight.

Randomization

The randomization sequence will be obtained through the R software version 2.12.1, with a block size of 5. Randomization to vildagliptin group (METV) or glibenclamide group (METG) will be performed by a researcher responsible only for this task, who will not participate in the recruitment, assessment or intervention phases of the study.

Data collection

Eligible patients will initially perform a maximum effort test. Forty-eight hours later, patients will begin the study protocol, as follows:

- Day 1: Begin a 24-hour urinary collection, perform vascular doppler ultrasound to evaluate endothelial function and then the glucose sensor will be inserted subcutaneously (begin continuous glucose monitoring system – CGMS evaluation);
- Day 2: End the 24-hour urinary collection, submit to the submaximal test (blood collection at baseline, 15 and 30 min of the session, and 60 min after recovery). On the same day, the patients will begin 24h ambulatory blood pressure monitoring (24h-ABPM).
- Day 3: Removal of the glucose sensor; end of the 24h ABPM, randomization.

This same protocol will be repeated at the end of the 12-week treatment with vildagliptin or glibenclamide.

The experimental sessions will occur at the Exercise Pathophysiology Research Laboratory, in Hospital de Clínicas de Porto Alegre (LaFiEx-HCPA), mantaining the ambient temperature between 20 and 22ºC.

Patients will be oriented to follow a habitual diet on the day before and go fasted to LaFiEx-HCPA on the test day. In addition, a day before, participants will be instructed to avoid intense activities and not to consume caffeinated beverages in order to exclude any residual effect before and after the effort test. During the drug treatment period (12 weeks) patients will not follow any kind of physical exercise programme. The flow diagram of the study design is shown in figure 1.

Study intervention

The METV group will receive 50mg of vildagliptin orally twice a day and the METG group will receive glibenclamide 5mg orally per day during the first week of the study, a dose that will be increased later to 10 mg a day (5mg twice a day). The dose may be adjusted, targeting HbA1c around 7.0% without frequent or severe hypoglycemias, reaching the maximum dose allowed (20 mg a day).

Patients will be instructed to measure capillary glycemia using a glucose monitor (Accu-Check Performa, Roche Diagnostics, Mannheim, Germany) twice a week and at any time if they present symptoms of hypoglycemia. These measured values will be shown in the follow-up visits or informed to the researchers by a phone call when two consecutive values lower than 70 mg/dl occur. This procedure will be explained at the beginning of the study. Medication adjustments will be guided by the researcher coordinator (Professor BDS).

At each follow-up visit and last visits (post-intervention) patients will be instructed to bring the medicine tablets with them to count the pills.

Maximal exercise and sub-maximal exercise tests:

A maximal exercise test will be performed to determine peak oxygen consumption (VO2peak) and ventilatory thresholds. The exercise capacity will be defined by a progressive maximal exercise test performed on a cycle ergometer, with increments of 20W/min. The test will have a 60 rpm intensity until exhaustion. Oxygen consumption (VO2) and carbon dioxide production (VCO2) will be determined by averaging the gas exchange by a computerized system (Oxycon Delta, VIASYS, Healthcare GmbH, Jaeger, Germany). The test will be finished when the individual is unable to maintain the 60 rpm speed. The VO2 peak is defined as the VO2 peak reached at the end of this exercise [13]. Heart rate will be continuously monitored by a 12-lead electrocardiogram (Nihon Kohden Corporation, Tokyo, Japan).

The ventilatory thresholds will be determined at the break point between the highest point of the CO2 production curve and VO2 (V-slope), or at the point where the curves of ventilatory equivalent for oxygen (VE/VO2) and end tidal oxygen (PETO2) reach their respective minimum values and begin to increase. The respiratory compensation point will be determined when the levels of ventilatory equivalent for carbon dioxide (VE/CO2) reach minimum values before they start to rise, and when end tidal carbon dioxide (PETCO2) reaches maximum values before these begin to decline [14].

The sub-maximal exercise tests will be done to simulate a typical aerobic exercise session on a cycle ergometer. The test will last 30 minutes, with an intensity based on 10% under the heart rate at the second threshold, which will be obtained in advance. Each patient will undergo two sub-maximal exercise tests, which will be performed before and after intervention. Before, at 15 minutes, at the end (at 30 minutes) and 60 minutes after each sub-maximal exercise test, 20 ml of blood will be collected and the variables described later will be measured, with the exception of HbA1c (measured just before exercise). The cardiovascular responses will be obtained as described afterwards.

Glucose variability evaluation

The continuous glucose monitoring system (CGMS) will be utilized for this evaluation. Subjects will be admitted to the laboratory in the morning, at approximately 8:00 a.m., 24 h before the exercise session, when the glucose sensor (GS) (Sof-SensorTM, Medtronic Mini-Med, Northridge, USA) will be inserted subcutaneously. The sensor is a glucose oxidase based platinum electrode that is inserted through a needle into the subcutaneous tissue of the anterior abdominal wall, using a spring-loaded device (Senserter, Medtronic, Northridge, USA).

Glucose oxidase catalyzes the oxidation of glucose in the interstitial fluid, which generates an electrical current. The current is carried by a cable to a pager-sized monitor that analyzes the data every 10 s and reports average values every 5 min, totalizing 288 readings per day. Glucose profiles will be collected the day before (day 1), the day of the test (day 2), and the day following (day 3) the submaximal exercise test. Sensor readings will be calibrated with a glucose monitor (Accu-Check Performa, Roche Diagnostics, Mannheim, Germany) using 4 finger-stick blood samples for each 24 h. Each sensor will be used continuously for up to 48h. All patients will be previously instructed about the operation of the monitor, which includes event registration for meals, and inserting capillary glucose values for calibration.

Glucose variability will be assessed from series of absolute values of glucose, obtained by the CGMS, sampled every 5 minutes. Each method will be used as reported in the literature and according to its limitations. Glucose variability will be evaluated using conventional analysis and other mathematical methods generally applied to biological series, here called non-conventional analysis of glucose variability. Conventional analysis of glucose variability will be

constructed from the statistical properties of the series, obtaining the following indices: MAGE [6], glucose variance (VAR), glucose coefficient of variation (CV%), and glucose standard deviation (SD), all normalized by the mean blood glucose in each period [15] [16]. These indices, except MAGE, will be calculated for every 6-h block of glucose values to obtain the measures according to the period of the day. The MAGE index will be calculated for the whole signal (24 h), and its calculation is based on the differences between consecutive points considering those which are higher than one standard deviation [16].

Non-conventional analysis of glucose variability will be 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 widely applied to heart rate and arterial pressure series [17]. Symbolic dynamics relies on the calculation of Shannon entropy of the distribution of patterns lasting three measures and the classification of frequent deterministic patterns lasting three measures and distributes deterministic patterns of the group into four categories according to the number and pattern: 1) no variation (0 V); 2) one variation (1 V); and 3) two like variations (2 LV); 4) two unlike variations (2 UV) [18]. This method was fully described and validated previously to glucose curves [7].

Metabolic evaluation

Peripheral venous blood will be collected into 10 mL vacutainer tubes to perform the blood tests; these samples will be stored at -20º. Glucose, HbA1c, insulin, glucagon and gucagon like peptide 1 (GLP-1) will be assayed.

Oxidative stress evaluation

The 24-hour urinary samples will be collected at visit 1 and 6 to evaluate creatinine and 8-iso prostaglandin F2α (8-iso PGF2 α), which is considered a well-recognized marker of oxidative stress [19]. In the current study, this isomer will be measured using an enzyme immunoassay method (Cell Biolabs, Inc San Diego, CA USA).

Cardiovascular evaluation

Cardiac output and heart rate will be measured before the sub-maximal tests (10min at rest), during the submaximal test and 60 minutes at recovery applying a noninvasive method. The data will recorded with Biopac MP150 system (Santa Barbara, CA, USA) using a general purpose amplifier module (DA100), impedance cardiography (NICO100C; Santa Barbara, CA, USA) and electrocardiogram (ECG100C, Santa Barbara, CA, USA), according to methodological guidelines provided by Sherwood et al. [20].

To assess the cardiovascular autonomic control, heart rate variability analysis will be applied. Pulse intervals (PI, tachograms) series will be obtained from the electrocardiogram records. Stationary segments (300 beats), coincident in tachogram, will be selected and spectral analysis will be performed using an autoregressive model, which estimates the center frequency and power of each relevant oscillatory component. The spectral bands for humans are defined as very low frequency (VLF), for 0.0-0.04 Hz, low frequency (LF), for 0.04-0.15 Hz and high frequency (HF) for 0.15-0.40 Hz intervals, defined according to previous references [17]. Tachogram spectra will be evaluated quantitatively and values of heart rate variability (HRV) will be obtained, as well as, its spectral components will be expressed in absolute (ms^2) and normalized units (NU). These NU will be obtained by calculating the power of LF and HF and correlating them to the total power without the very low frequency component [17].

Among the parameters obtained by spectral analysis, those distinguished for their physiological significance are the LF and HF components, which are mainly related to sympathetic and parasympathetic cardiac modulations, respectively. The relationship between them - the LF/HF index - or sympathetic-vagal balance [21] will be evaluated.

Patients will be submitted to a 24h-ABPM on a usual work day, using a monitor (Spacelabs 90207, Spacelabs, Redmond, WA), which will be programmed to automatically measure the blood pressure (BP) every 15 minutes during the day (06:00 to 22:00 h), and every 20 minutes during the night (22:00 to 6:00 h) [22, 23]. Blood pressure variability will be assessed from BP behavior in different windows of a 24-h period, daytime and nighttime periods. The cuff size will be adapted to the circumference of the arm of each patient according to the manufacturer’s recommendations.

Based on the results of 24h-ABPM, the mean 24-hour systolic BP (SBP) and diastolic BP (DBP) will be calculated for each patient, before and after treatment. Three different parameters of SBP variability will be calculated: 1) Time-rate index (rate of change in SBP over time in mm Hg/min, defined as the first derivative values of SBP by time); 2) Coefficient of variation of the 24h systolic BP (SD/mean pressure x 100%); and 3) Mean of standard deviation of 24h systolic BP. The time-rate index allows the calculation of the sum of angular coefficients and aims to measure how fast or how slow and in which direction SBP values change. The measure will be calculated using the following formula, where r is the rate of BP variability over time (considering the differences between BP measurements at each time interval) and N is the number of recordings[24]:

$$R = |\bar{r}| = \frac{\sum_{i=1}^{N-1} |ri|}{N-1}$$

Endothelial function evaluation

This analysis will be performed by a high-resolution ultrasound of the brachial artery (vascular doppler), which characterizes the flow mediated dilatation (FMD) that is expressed by changes in basal diameter in response to the increased flow and in response to nitroglycerin, that will be administered in a single dose (0.4 mg, sublingual spray). The equipment used will be an HD7XE (Philips, Bothell, WA, USA) with a high frequency transducer (3-12 MHz; L12-3, Philips, Bothell, WA, USA)[25]

Security assessment

Adverse events, including serious adverse events or pregnancies will be collected and included in the medical reports.

The reports containing serious adverse events or pregnancies will be forwarded to the laboratory of the respective manufacturer within 24 hrs, and to the health authorities.

To ensure patient safety, the individuals participating in the research will be monitored for the occurrence of all adverse events after beginning the specific protocol procedures until 4 weeks after the patient discontinues participation in this study.

Statistical analysis plan

Data will be analyzed using the SPSS software (Statistical Package for Social Sciences), version 18.0 for Windows. The description of values will be expressed as means ± SD. The statistical procedures used will be: Students t-test for independent samples (intergroups), paired t-test (intragroup), analysis of variance (ANOVA) for repeated measures to compare both groups at different times. A Pearson correlation will be performed between measures of glucose variability and 8-iso prostaglandin F2 alpha/Crn. The statistical power will be 90% and the accepted level of significance will be $p < 0.01$.

Discussion

Several antidiabetic agents are available for T2DM treatment. These treatments differ concerning drug action mechanism and long term outcomes. Besides these, nonpharmacological treatments are important, especially exercise as it is a major tool to achieve target blood glucose and HbA1c. Non-glucose beneficial effects can also be obtained. The many studies reporting the effects of the association between exercise and oral glucose lowering drugs usually focus on attaining better metabolic control. This study will be conducted to report the glucose variability and cardiovascular responses at rest, during and after sub-maximal exercise under two oral glucose-lowering drug (vildagliptin *vs.* glibenclamide).

Ethical and data protection issues

Participation will be voluntary and it will follow the ethical aspects of confidentiality and data protection. Procedures will be explained and information about the aim, design, potential risks and benefits associated and all relevant details of the research is given in the informed consent form. The data obtained by the study will be available to the participant and to whoever is authorized and may be used anonymously for academic scientific purposes.

The study was approved by the Ethics Committee of Hospital de Clínicas de Porto Alegre (Brazil), which is part of National Committee of Ethics in Research. Approval Number: 10662912.3.0000.5327

Trial status

Not yet recruiting. Enrolment will begin in April 2014. Each patient will have 10 visits to the hospital. Total data collection time will be 16 months.

Competing interests

This academic study is financially supported by Novartis®. The authors did not receive any reimbursement or financial benefits and declare that they have no competing interests. Novartis® provided vildagliptin and played no role in the design, methods, data management or analysis or in the decision to publish.

Authors' contributions

AF is involved in conception, design, drafting the protocol and she will coordinate the recruitment.

JPR made substantial contributions to the conception and design of the study. He provided the main encouragement to the idea of the study. Unfortunately, he passed away just before this protocol was sent to the Ethics Committee.

KRC is involved in drafting the protocol and will be responsible for data analysis procedure.

BDS is the principal investigator of the study, she has full access to all the data in the study and responsible for recruitment and trial coordination. She is involved in conception, design and drafting the protocol.

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Table

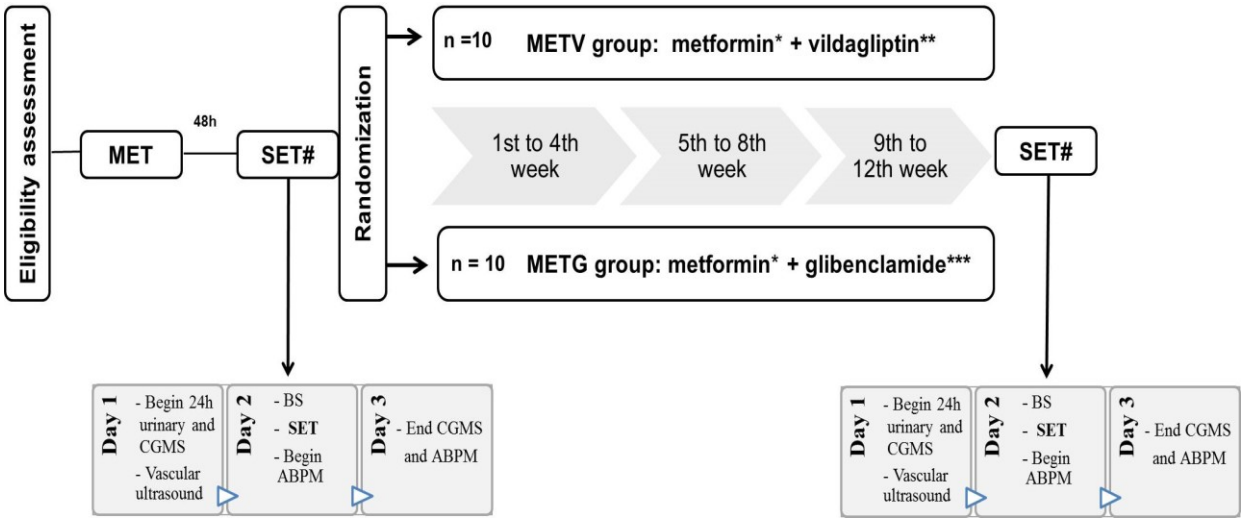
Table 1: Measures evaluated in order to reach the outcomes.

Moment	Data Collection	Measures	Outcomes
At rest	Continuous glucose monitoring system	Glucose values	Glucose variability
	Vascular doppler ultrasound	Flow mediated dilatation	Endothelial function
	Blood sample	Glucagon, glucose, HbA1c, Insulin and GLP-1	Metabolic responses
	Impedance cardiography and electrocardiogram	Cardiac output; Heart rate	Cardiovascular response
	24 hour urine	F2 isoprostane 8-iso prostaglandin F2α/ creatinine	Oxidative stress
During sub-maximal exercise tests (*0, 15', 30')	Continuous glucose monitoring system	Glucose values	Glucose variability
	Impedance cardiography and electrocardiogram	*Cardiac output; Heart rate	Cardiovascular response to exercise
	Blood sample	*Glucagon, glucose, Insulin and GLP-1	Metabolic responses
After sub-maximal exercise tests (continuous monitoring or *60' post exercise)	Continuous glucose monitoring system	Glucose values	Glucose variability
	Impedance cardiography and electrocardiogram	*Cardiac output; Heart rate	Cardiovascular response to exercise
	Ambulatory blood pressure monitoring	24 hour blood pressure variability	
	Blood sample	*Glucagon, glucose, HbA1c, Insulin and GLP-1	Metabolic responses

GLP-1: glucagon like peptide 1; HbA1c: glycated hemoglobin

Figure

Figure 1: Flow diagram of the study design.
MET: maximal exercise test. # The submaximal exercise test (SET) will occur in the second day of a three-day period of tests. METV: 12-week treatment with vildagliptin added to metformin; METG: 12-week treatment with glibenclamide added to metformin. *500 to 2000 mg/day according to tolerance; **50mg bid; ***10-20mg q.d.; CGMS: continuous glucose monitoring system; BS: blood sample; ABPM: ambulatory blood pressure monitoring.



5. ARTIGO 2

Este artigo será submetido a *Diabetologia*.

Keywords: Diabetes mellitus, hypoglycemic agents, exercise

Running Title: Oral antidiabetic agents and exercise in type 2 diabetes

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Conflicts of Interest:

This academic study was financially supported by Novartis®. The authors did not receive any reimbursement or financial benefits and declare that they have no competing interests. Novartis® provided vildagliptin and played no role in the design, methods, data management or analysis or in the decision to publish.

ABSTRACT

Glucose variability is a component of glycemic disorders and have received clinical importance because it might predict diabetic complications. Given an interaction between exercise and oral anti diabetic agents on glycemic control, the magnitude of this interaction may vary with different antidiabetics. Our general aim in this study was to evaluate glucose variability after an aerobic exercise session in patients receiving treatment with either vildagliptin or glibenclamide. The specific aims were to evaluate the metabolic and cardiovascular responses to exercise under treatment with vildagliptin or glibenclamide. A randomized open label clinical trial was performed. Patients with type 2 diabetes received a second drug orally added to metformin during 12 weeks: METV group - vildagliptin (50 mg bid) or METG group - glibenclamide (10 – 20mg q.d.). Before and after intervention, the glycemic variability, cardiovascular and metabolic response were performed at rest, during and after an aerobic exercise session. Both groups presented an improvement in metabolic control. This study showed reduction in 24h-ABPM SBP and daytime 24h-ABPM. Patients treated with vildagliptin and glibenclamide showed different responses in time rate of BP variation, nighttime time rate, SD 24h ABPM SBP variation in all periods, CV of the 24h and nighttime CV of the SBP. Beyond better metabolic control obtained from both treatments, blood pressure lowering was observed, and lower BP variability was observed in the group treated with vildagliptin.

LIST OF ABBREVIATIONS

8-iso PGF2 α: 8-iso prostaglandin F2α

ABPM: ambulatory blood pressure monitoring

BP: blood pressure

CGMS: continuous glucose monitoring system

CV: coefficient of variation

DBP: diastolic BP

DPP-IV: Dipeptidyl peptidase-IV

GLP-1: gucagon like peptide 1

HbA1c: glycated hemoglobin

MAGE: mean amplitude of glycemic excursion

METG: glibenclamide group

METV: vildagliptin group

SBP: systolic BP

SD: standard deviation

T2DM: patients with type 2 diabetes

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a disease characterized by chronic hyperglicemia and complications directly caused by this metabolic derangement. It is well-known that a good glycemic control is associated, in the long term, with lower incidence of chronic complications (1). Recently, beyond glycated hemoglobin (HbA1c) as a tool for monitoring glycemic control, glucose variability has been evaluated as a possible target for treatment (2). Glucose variability has received clinical importance because it might predict diabetic complications (3). Several indexes are used to estimate glucose variability, such as standard deviation (SD), mean absolute glucose (MAG), mean of daily differences (MODD), continuous overlapping net glycemic action (CONGA), and the mean amplitude of glucose excursions (MAGE), which is the most common measure of glucose spikes, swings, or excursions (4).

Some current treatments for T2DM have already been tested concerning their possible effects in reducing glucose variability as well as reducing HbA1c (5, 6). Considering that metformin, the first choice in the treatment of T2DM, cannot guarantee long term good glycemic control, other antidiabetics should be added as a second agent, such as dipeptidyl peptidase-IV (DPP-IV) inhibitors and sulfonylureas (7). Despite better glycemic control showed by sulfonylureas in studies included in a meta-analysis, they induce more hypoglycaemias, weight gain, and total adverse events as compared to DPP-IV inhibitors (8).

The role of antidiabetics on glucose variability is still controversial. Vildagliptin and sitagliptin are two antidiabetics that were evaluated in a study focusing on possible differences in daily glucose fluctuations in patients with T2DM inadequately controlled with metformin. In this study, vildagliptin was more effective in flattening acute glucose fluctuations over a day (5). Moreover, acarbose was superior to glibenclamide in reducing MAGE (6). Kim et al evaluated four weeks of sitagliptin *vs.* glimepiride and showed lower MAGE in the group that received sitagliptin. This study found the same results concerning glucose control in both groups (9).

In addition to the above, non-pharmacological treatments are very important, especially exercise (10). The acute effect of aerobic and combined exercise on changing glucose variability was firstly shown by our group in patients with T2DM (11). In other study, a single bout of a moderate exercise was comparable to a control session in insulin and non-insulin treated patients.

Exercise induced a comparable reduced in glycemic variability in both groups (12).

Given the interaction between exercise and oral antidiabetic agents on glycemic control, the magnitude of this interaction may vary with different antidiabetics. However, there is no information on the effects of the DPP-IV inhibitor vildagliptin *vs.* the sulphonylurea glibenclamide on glucose variability and other responses to exercise in T2DM patients. The aim of this study was to evaluate glucose variability, metabolic and cardiovascular responses after an aerobic exercise session in patients receiving treatment with either vildagliptin or glibenclamide. Our hypothesis was that vildagliptin associated with metformin would have more impact in improving glucose variability after an aerobic exercise session, as compareted to glibenclamide.

METHODS

The design of the DIABEX VILDA study has been described in detail elsewhere (13).

Research Design and Participants

This was an open label, PROBE (parallel group with a blinded end-point) design, randomized clinical trial.

The inclusion criteria were age older than 18 years, presence of T2DM, use of metformin, a recent HbA1c between 7.5% and 10%, and no involvement in regular physical activity. The exclusion criteria were: current smoking, body mass index (BMI) >40 kg/m^2, proliferative diabetic retinopathy, ischemic heart disease, peripheral vascular disease, cognitive decline or dementia, recent neurological events, severe depression, or current diagnosed cancer, lactose intolerance, hepatic enzyme levels three-fold higher than the reference values, glomerular filtration rate lower than 60 ml/min, blood pressure (BP) over 180/100 mmHg at rest, use of analgesic or antiinflammatory drugs during the week of the study, use of insulin and untreated thyroid dysfunction.

The study was previously approved by Scientific Committee and Research Ethical Comission of Hospital de Clínicas de Porto Alegre (Brazil), and clinicalTrials.gov identifier: NCT01867502, study release date: May-17-2013.

Pre-intervention

Eligible patients initially performed a maximum effort test on a cycle ergometer, with increments of 15 or 20 W/min, to determine peak oxygen consumption (VO_2peak) and peak heart rate. The test was carried out at 60 rpm until exhaustion. Oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were determined by averaging the gas exchange by a computerized system (Oxycon Delta; VIASYS Healthcare GmbH, Jaeger, Germany). The VO_2 peak and peak heart rate were defined as the values reached at the end of this exercise (14).

Intervention and data collection

In addition to metformin, patients received a second drug orally for 12 weeks: the METV group received vildagliptin (50 mg twice daily), and the METG received glibenclamide (10 mg once daily). After this treatment, evaluation of glycemic variability, metabolic and cardiovascular response was performed at rest, during and after an aerobic exercise session (30 minutes at 60 to 70% of the peak heart rate, as determined in maximal exercise test). Urinary oxidative stress was evaluated by 8-iso Prostaglandin F2α (8-isoPGF2α) excretion 24h before the exercise session by competitive enzyme-linked immunoassay, before and after treatment (Cell Biolabs, San Diego, CA, USA).

The study protocol was conducted during three days as follows:

- Day 1: Subjects were admitted to the laboratory in the morning at approximately 08.00 AM, 24 hours before the exercise session, when the glucose sensor (i-Pro2 digital recorder; Medtronic Mini-Med Inc., Northridge, CA,USA) was inserted subcutaneously to begin continuous glucose monitoring. Urinary collection was started.
- Day 2: End of the 24h urinary collection, carry out the exercise with blood collection 60 min before exercise (fasting), immediately before exercise (0 min), 15, and 30 minutes during the exercise session, and 90 minutes after beginning (60 minutes after recovery). Assessment of heart rate variability before and after exercise, and begin 24h ambulatory blood pressure monitoring (24h-ABPM).
- Day 3: Removal of glucose sensor; end of 24h-ABPM.

Glucose variability was assessed from a series of absolute values of glucose, obtained by the continuous gliucose monitoring system (CGMS), sampled every 5 minutes. Glucose variability was evaluated using conventional

analysis to obtain the following indices: MAGE, glucose variance (VAR), glucose coefficient of variation (CV%), and glucose SD. These indices, except MAGE, were calculated in a 6-hour timeframe of glucose values to obtain the measures according to the specific period of the day. The MAGE index was calculated for the whole signal (12.5 hours), and its calculation is based on the differences between consecutive points, considering those which are higher than 1SD (4).

In the second day of the protocol, blood samples were collected 60 min before exercise (fasting), immediately before exercise (0 min), 15, and 30 minutes during the exercise session, and 90 minutes after beginning (60 minutes after recovery), for measurement of plasma glucose by the glucose oxidase method (Sigma-Aldrich, St Louis, MO, USA), insulin by chemiluminescent enzyme immunoassay (Immulite 1000 Analyzer; Siemens Healthcare Diagnostics, Deerfield, IL, USA), glucagon-like-peptide-1 (GLP-1) by fluorescence enzyme immunoassay (EMD Millipore, Billerica, MA, USA) and glucagon by colorimetric enzyme immunoassay (R&D Systems – Bio-techne, Minneapolis, MN, USA).

After the exercise session, patients were submitted to a 24h-ABPM on a usual work day, using a monitor (model 90207; Spacelabs, Redmond, WA, USA) that was programmed to automatically measure BP every 15 minutes during the day (06.00 to 22.00 hours), and every 20 minutes during the night (22.00 to 6.00 hours) (15, 16). Blood pressure variability was assessed from BP behavior in different windows of a 24h period, covering both daytime and night-time periods. Based on the results of the 24h-ABPM, the mean 24h systolic BP (SBP) and diastolic BP (DBP) were calculated for each patient, before and after treatment. Three different parameters of SBP variability were calculated: 1) time–rate index (rate of change in SBP over time in mm Hg/min, defined as the first derivative values of SBP by time) (17); 2) coefficient of variation of the 24h SBP (SD/mean pressure $\times$ 100%); and 3) mean of the SD of the 24h SBP. The time–rate index allows the calculation of the sum of angular coefficients, and aims to measure how fast or how slow SBP values are and in which direction they change. We classified the subtypes of nocturnal decline in SBP as follows: dipper (≥ 10% nocturnal decline in SBP from the diurnal level), nondipper (< 10% nocturnal decline in SBP) and inverted dipper (< 0% nocturnal decline in SBP); for subtypes of nocturnal declines in DBP, the same cutoff values were used, but considering DBP (18).

Cardiac output (CO) and heart rate were measured before (10 minutes at rest), 2 and 60 minutes of recovery. The data were recorded with Biopac MP150 system (Santa Barbara, CA, USA) using a general purpose amplifier module (DA100), impedance cardiography (NICO100C) and electrocardiogram (ECG100C). Cardiovascular autonomic control was assessed by the following indices of heart rate variability: low frequency component (LF), high frequency component (HF) and LF/HF index. Heart rate variability was assessed by spectral analysis.

To ensure patient safety, the individuals participating in the research were monitored for the occurrence of adverse events after beginning the specific protocol procedures until 4 weeks after the patient discontinues participation in the study.

Randomization

The randomization sequence was generated by R software (v2.12.1 Vienna, Austria, 2011) with a block size of five and performed by a researcher responsible only for this task.

Data analyses

Data were analyzed using SPSS software (Statistical Package for Social Sciences; version 18.0 for Windows, SPSS Inc., Chicago, USA). The description of values was expressed as mean ± SE for parametric variables and median (P25-P75) for non-parametric variables. The effects of treatment (group; time in exercise; and group interaction) were estimated using a generalized estimating equation (GEE) followed by Bonferroni's post-hoc test ($p<0.05$).

RESULTS

Thirteen patients were included, seven in METV and six in METG. One patient from group METV did not finish the study. Figure 1 shows flow of participants through each stage.

Table 1 shows the patients' characteristics at baseline. Patients did not differ among groups: they were 46-65 years old, predominantly men, 83% were overweight/obese, and had T2DM for 6.5 years (1.4-14.2 years). Patients were sedentary, so this was reflected on reduced values of peak oxygen uptake in

most patients. No difference in body weight was observed at baseline between groups.

Both groups presented an improvement in metabolic control after the 12 weeks of treatment, as was observed by lower fasting plasma glucose levels (ΔMETV -31.83mg/dl with 95%CI -56.7, -6.97 and ΔMETG -60.17mg/dl with 95%CI -90.58, - 29.76), and higher fasting insulin (ΔMETV 1.27 with 95%CI -0.78, 3.32 and ΔMETG 5.9 with 95%CI 0.13, 11.67). After treatment, there was a similar decrease in HbA1c in both groups (HbA1c mean difference -1.24 % with 95%CI -1.70, -078%; p<0.001 in METV and mean difference -1.52 % with 95%CI -2.19, -0.85% in METG; p< 0.001).

In our patients, 8-isoPGF2α urinary excretion increased after 12 weeks of treatment in both groups (before treatment = 2275.9 ±185.9 pg/mg creatinine; METV = 3155.3 ±285.0 pg/mg creatinine; METG= 2736.0 ±394.4 pg/mg creatinine, without difference between them (p time=0.03; p group=0. 075).

Figure 2 (panel A) shows plasma glucose 60 min before exercise (fasting), immediately before exercise (0 min), 15, and 30 minutes during the exercise session, and 90 minutes after beginning (60 minutes after recovery) in METV and METG. Plasma glucose decreased in both groups in the end of exercise and after recovery (p<0.05). Panel B shows glucose values obtained from CGMS 60 min before exercise (fasting) and during the first 6 hours after exercise. Decreasing glucose was seen for 3h after the exercise session for both groups. Considering the incremental area under the curves (AUC – panel B, insets), it was lower in METG as compared to METV (p=0.04).

Table 2 shows plasma insulin, GLP-1 and glucagon 60 min before exercise (fasting), immediately before exercise (0 min), 15, and 30 minutes during the exercise session, and 90 minutes after beginning (60 minutes after recovery), after 12 weeks of vildagliptin or glibenclamide. Plasma insulin at fasting did not differ between groups after treatment, but after breakfast (0 min) insulin is higher in METG than in METV (p=0.006). Insulin level was smaller in METV at 30 min (exercise) and 90 min as compared to METG, with p=0.019 and 0.007, respectively. Plasma GLP-1 was higher in METV than in METG after treatment (p<0,05), in all measures.

Glucose variability, showed in a time frame of 6 hours and measured by conventional analysis, showed no change after treatment with vildagliptin or glibenclamide, in both groups (table 3). Before exercise, MAGE (showed in a time

frame of 12 hours) in METV was 65.8 ± 9.8 mg/dL and 64.4 ± 8.6 mg/dL in METG and after exercise METV had MAGE 55.8 ±5.3 mg/dL and METG 69.9 ± 13.3 mg/dL (p group=0.091; p time=0.234).

Cardiovascular autonomic control parameters did not change after 12 weeks of treatment between groups. Comparing data obtained in 2 and 60 minutes of recovery with data obtained before exercise, an aerobic exercise session did not change low and hight frequency components and heart rate variability. Cardiac output did not change after treatment neither in response to exercise (table 4).

Table 5 shows results of 24h-ABPM parameters after the exercise session, including blood pressure variability. After 12 weeks of treatment patients decreased 24h-ABPM SBP and daytime 24h-ABPM SBP (p=0.012 and p= 0.009, respectively) without difference between groups. Daytime mean BP in 24h showed a borderline statistic difference, trending to decrease in both groups (p=0.052). Time rate of SBP variation was lower in group METV compared to METG (METV= 0.0445 ±0.05 mm/Hg and METG= 0.601 ±0.12 mm/Hg, p=0.012). The same reduction was seen in nighttime time rate (p=0.001). Daytime time rate of BP increased after treatment (p time=0.01), with no significance between groups. In SD of SBP parameter, the groups were different after treatment, with lower values in METV group. CV of the 24h and daytime CV of SBP was lower in METV than METG. Nighttime CV of SBP variation was lower in METV compared to METG (p=0.018), with no difference in time measure (p=0.945).

In relation to adverse events, three patients in METG group reported dizziness and trembling, and one of them had capillary glucose of 57 mg/dL once. One patient in METV reported the following conditions: headache, nausea, loss of appetite, blurred vision, somnolence and abdominal discomfort; this patient did not finish the study. None of these events were considered to be severe.

DISCUSSION

Metformin and exercise are the most widely prescribed first-line therapies for T2DM. When good metabolic control is not reached with these therapies, other antidiabetic agents should be added (7) This study tested the effects of metformin plus vildagliptin *vs.* metformin plus glibenclamide on glucose variability before and after and acute exercise session. Although a better metabolic control was obtained after the 3-month treatment with both agents tested, no change in

glucose variability before or after exercise was observed. Vildagliptin determined lower BP variability after exercise, as shown by changes in time rate of SBP, nighttime time rate of SBP, SD of SBP, CV of SBP.

Although previous studies showed that glucose variability can be modulated by interventions such as exercise (19) or antidiabetics agents (5, 6), when evaluating different classes of antidiabetics and the response to exercise we did not observe this effect. In relation to antidiabetic drugs, Kim et al described a lower MAGE after 4 weeks of a DPP-IV inhibitor treatment (sitagliptin), but not when glimepiride was used (9). However, these authors did not evaluate the response to exercise, and did not compare the groups after treatment. The other report that sitagliptin could reduce glucose variability was described in patients who were evaluated by day-to-day glucose variability, not CGMS, thus, precluding comparability with our results (20). Consistent with our results, a combination of basal insulin and a GLP-1 receptor agonist dulaglutide for 26 weeks of treatment did not change MAGE (21). Aditionally, the effect of 12 weeks of treatment with gemigliptin *vs.* sitagliptin or glimepiride as initial combination therapy with metformin on glucose variability in drug-naïve patients with T2DM showed a significant decrease in MAGE and SD of glucose at week 12 compared with baseline in all groups, and MAGE was significantly lower in both gemigliptin and sitagliptin groups compared with that in the glimepiride group. Interestingly, SD of blood glucose was significantly decreased in the gemigliptin group compared with the sitagliptin, suggesting that different DPP-IV inhibitors could decrease glucose variability in a specific way (22). Since our treatment was with other DPP-IV inhibitor and other sulfonylurea, these different results could be explained. In accordance with our results, participants with T2DM on oral therapy including an insulinotropic agent, metformin or glitazones, plus DPP-IV inhibitors or sulfonylureas showed no difference in CV (23).

Evaluating the effect of an acute aerobic exercise session in reducing glucose (CGMS), we found a similar response as showed by our own previous report (11), i.e., a short-lived effect (until 3h of recovery). A single bout of moderate-intensity exercise substantially reduced glycemia and glucose variability, measured by CONGA throughout the subsequent day in insulin- and non–insulin treated T2DM (12). Aditionally, patients with T2DM who were taking metformin for at least 3 months without any other glucose-lowering medication did not improve glycemic response by adding a bout of exercise (24).

After treatment, higher insulin levels were observed in the present study during exercise and recovery in METG. This is in accordance with the mechanism of action of glibenclamide in comparison with vildagliptin. Massi et al (25) showed that an aerobic exercise session determined a reduced blood glucose and no change in insulinemia measured by AUC (1-3h) after exercise in patients with T2DM treated with glibenclamide when compared to no exercise state, explained by a blunting of the physiological response to exercise (suppression of endogenous insulin secretion) with glibenclamide. Although our evaluation was different from this study, because it was only one hour after exercise, we found similar response in patients receiving glibenclamidde, i.e. no change in plasma insulin after exercise, while patients receiving vildagliptin showed a plasma insulin decrease one hour after the aerobic exercise session, which is certainly more benefic for patients, as risk of hypoglycemia would be reduced.

As expected, GLP-1 levels were higher after treatment with vildagliptin than with glibenclamide, in accordance with the mechanism of action of these drugs. However, GLP-1 levels did not rise with exercise and were similar between groups after exercise. In according with these results, GLP-1 did not change comparing different points after exercise, in response to high-intensity intermittent exercise, but when compared area under the curve with rest it is increased (26). In normal weight or obese individuals, 60 min of moderate-intensity aerobic exercise performed the night before a mixed meal does not alter the postprandial responses of GLP-1 (27). In females, exercise increased GLP-1 concentrations, but not in males (28, 29). It is possible that we found no difference because most of our individuals were men, and all with T2DM, and these patients have an impaired secretion of GLP-1 (30), possibly even in response to exercise.

Urinary levels of 8-isoPGF2α rose after 12 weeks of treatment with vildagliptin or glibenclamide uncovering higher oxidative stress induced by the treatments. This increase after treatment is in accordance with a previous work, which showed a significant increase in urinary excretion rates of 8-iso PGF2 in patients treated with glibenclamide for 12 weeks (31). The 8-isoPGF2α is a well-recognized marker of oxidative stress (32). Patients with T2DM have sustained hyperglycemia, the cause of enhanced lipid peroxidation and increased oxidative stress (33). The increase of this marker could be related to liver metabolism of glibenclamide and vildagliptin, since they are metabolized by cytochrome monooxygenases P450 enzymes, that may trigger oxidative stress (34). Glucose

fluctuations were shown to have a more specific triggering effect on oxidative stress than chronic sustained hyperglycemia (35). Previous reports showed that urinary excretion rates of 8-iso PGF2 did not correlate with fasting plasma insulin, HbA1c and fasting plasma glucose, but was positively correlated with MAGE (36), and other variability parameters (3). However, although hyperglycemia is definitively related to high levels of 8-isoPGF2α not all studies could show relation between glucose variability and urinary 8-iso-PGF2α excretion (37, 38). In our work, we observed lower fasting plasma glucose levels and HbA1c after treatment, and higher fasting insulin, but glucose variability did not change by treatment.

Lower BP levels during treatment with vildagliptin or glibenclamide was already shown and suggested to be determined by improving adherence of patients to all treatments, both pharmacological and nonpharmacological (39, 40). In the RECORD study patients presented a 1.4 mmHg decrease in 24h-ABPM SBP after 6 months of treatment with metformin plus sulfonylurea (41). In a recent trial, patients with T2DM presented a reduction in 24h-ABPM SBP after a 12-week treatment with DPP-IV inhibitors (sitagliptin *vs.* vildagliptin). This study showed a mean -4.41 (2.03) mmHg 24h-ABPM reduction (42). Additionally, it is possible that GLP1 provides a favorable vascular effect because of its vasodilatory properties acting instead through an NO/cGMP-dependent mechanism (43). Since sulfonylureas act through the blocking of calcium-dependent potassium channels in several vascular beds, and arterial tone is influenced by the activation of these channels, this BP lowering mechanism by glibenclamide could not be discarded (44).

There are few trials comparing antidiabetic effects on BP, and they are controversial. A recent systematic review and network meta-analysis make a ranking of the impact of antidiabetic drugs. Among treatments, sitagliptin, that has a similar action as vildagliptin in lowering blood glucose, was in the 10th place while GLP1 receptor agonists occupied the first place on surface under the cumulative ranking curve of treatment hierarchy. In the ranking, sulfonylureas appear farther than others in probability of being the best treatment on lowering SBP and DBP, among the 14 types of agents and doses evaluated (45). We did not find differences between groups concerning SBP decrease, probably because of the small sample size and data obtained after one session of moderate exercise. Patients treated with vildagliptin presented a decrease of 6.3

mmHg in SBP while glibenclamide was associated with a 3.6 mmHg reduction in SBP after 12 weeks of treatment and after an acute exercise session. Thus, both treatments had the same efficacy concerning SBP changes. The values reached may be important, because even modest lowering of BP has been proved to be clinically meaningful for patients with T2DM (1, 46, 47). The results of the ADVANCE trial showed a 18% decrease in the risk of cardiovascular death with an approximately 5.6 mmHg reduction of SBP (46).

Although the SD of BP has limitation in evaluating BP variability, this index together with the time rate provided a full satisfactory quantification of BP variation over 24h and might represent a useful tool to assess the validity of therapeutic measures at controlling BP variability (48). Patients included in METV showed a smaller SBP variability as evaluated by time rate of 24h and nighttime ($p=0.012$ and 0.001 respectively), so vildagliptin added to metformin seems to have a beneficial effect on BP variability in our study. The same reduction was seen on CV of the 24h and daytime of SBP ($p=0.028$ and 0.027 respectively).

The major limitation of our study is the small sample size. Although our sample size was small we still were able to detect the meaningful effect of glibenclamide and vildagliptin on systolic BP decrease, which is an important clinical outcome for patients with T2DM. Concerning cardiovascular responses to treatment and exercise, our study present limited analyzes for the absence of a non-diabetic control group. Likewise, the comparative response with a control group would have helped provide a better understanding of some responses.

CONCLUSIONS

This is the first study conducted in patients with T2DM to provide data on the influence of a standard drug therapy (metformin plus glibenclamide) *vs.* other option availabile (metformin plus vildagliptin) on the response to an aerobic exercise session, which is also recommended to achieve a good glycemic control for patients with T2DM. Beyond better metabolic control obtained from both treatments, glucose variability did not change in response to exercise, however BP lowering was observed, such as lower BP variability in the group treated with vildagliptin.

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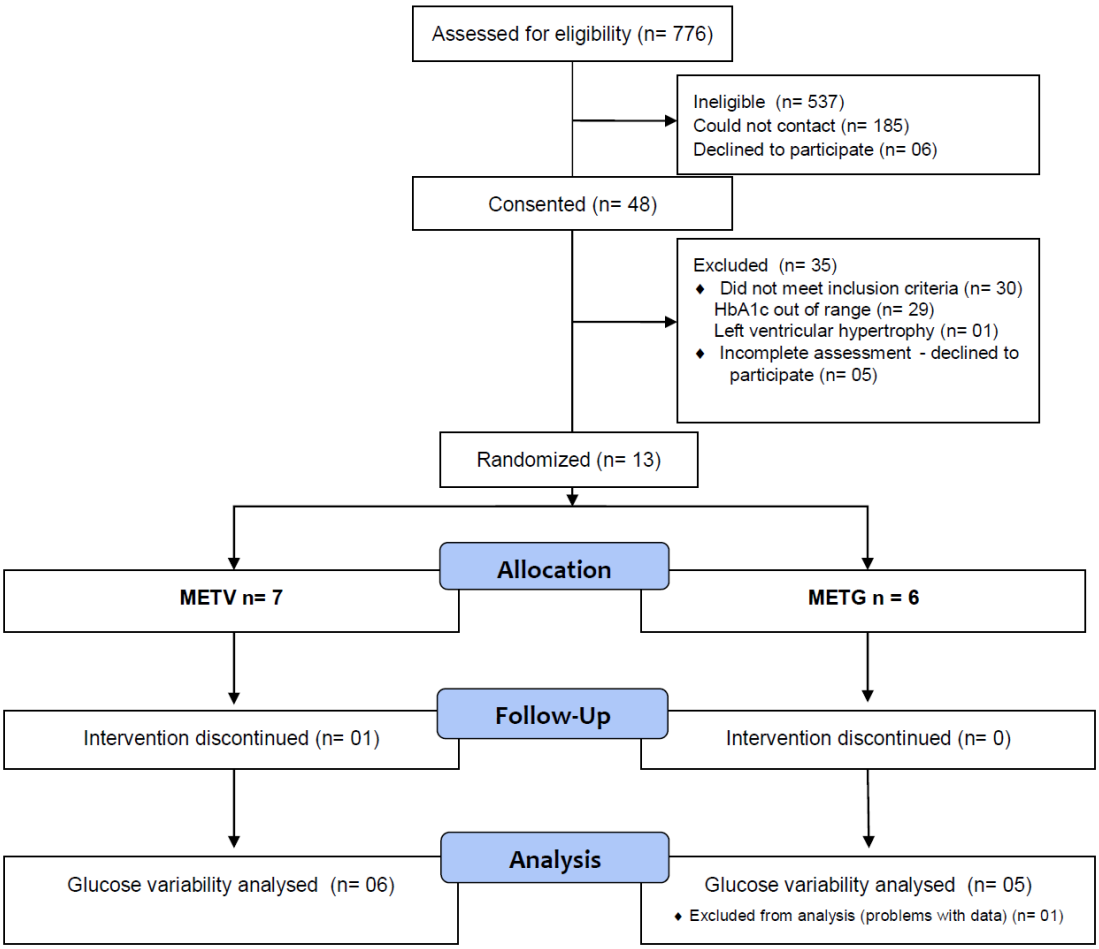

Figure 1: Flow diagram. HbA1c: glycated hemoglobin; METV: metformin + vildagliptin; METG: metformin + glibenclamide.

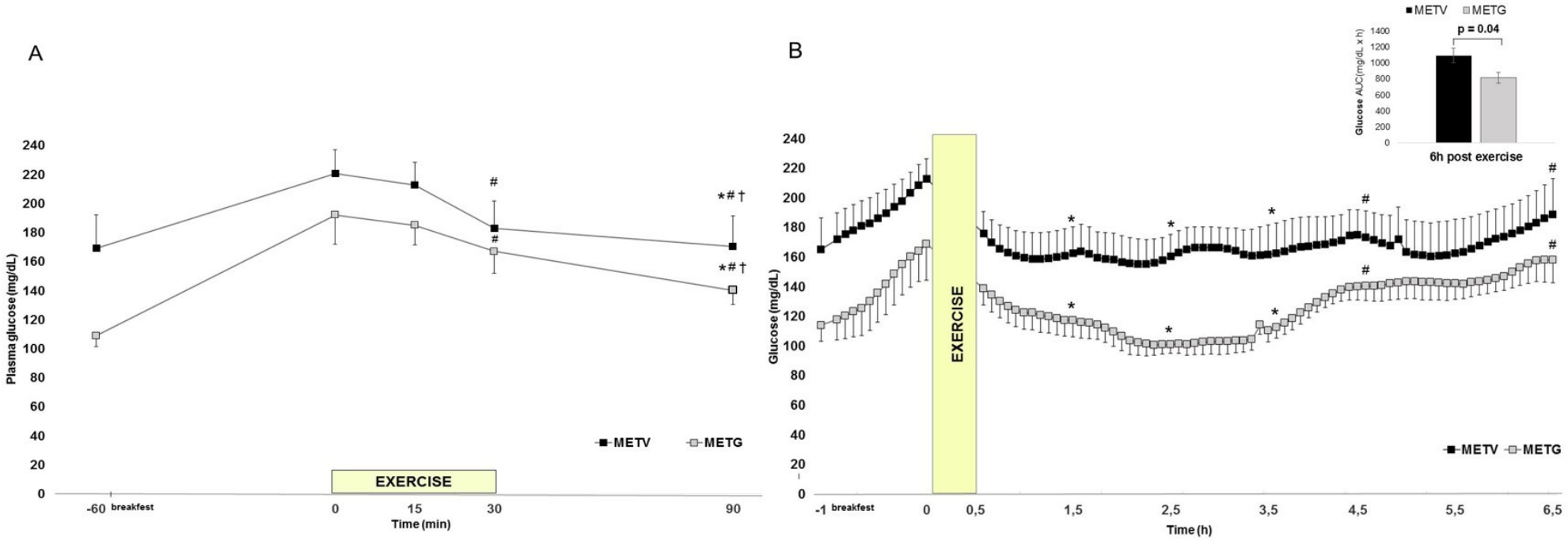

Figure 2: Plasma glucose at baseline (fasting , -60'), before (0'), during (15'), and after exercise (30' and 90' after beginning and 60' of recovery) (**panel A**). Glucose (continuous glucose monitoring, sampled every 5 minutes) before and after exercise (**panel B**). Data were obtained after 12 weeks of treatment with vildagliptin (METV, 50mg b.i.d n= 6) or glibenclamide (METG, 5mg b.i.d. n= 5 in patients with type 2 diabetes treated with metformin. Incremental area under the curves (AUC) are insets (panel B). Data are presented as means and SE. For panel A: *p<0.05 *vs.* 0; # p<0.05 *vs.* 15min; † p<0.05 *vs.* 30min (adjustment for baseline glucose). For panel B: *p<0.05 *vs.* 0; # p<0.05 *vs.* 2.5h after exercise (adjustment for baseline glucose). Groups (METV *vs.* METG) were not different, except at the baseline (-60).

Table 1: Characteristics of patients before treatment with vildagliptin or glibenclamide

Clinical	**METV (n=6)**	**METG (n=6)**	**p**
Age (years)	55.3 ± 2.7	56.8 ± 2.2	0.67
Male (n)	5	3	0.65
Diabetes duration (years)	6.5 (1.7-14.2)	6.5 (1.4-11.0)	0.68
BMI (Kg/m²)	28.9 ± 1.9	29.0 ± 1.5	0.97
SBP (mm/Hg)	134.5 ± 5.6	140.5 ± 3.8	0.40
DBP (mm/Hg)	85.3 ± 2.3	82.0 ±3.3	0.44
VO2peak (ml/Kg/min)	26.7 ± 7.2	24.5 ± 6.7	0.45
HbA1c (%)	9.1 ± 0.3	8.3 ± 0.4	0.12
Current therapy			
ACEi (n)	-	1	
□-blockers (n)	-	3	
ARB (n)	2	2	
Calcium-channel blockers (n)	1	3	
Mean daily dose of metformin (mg)	1478 ± 597	1700 ± 888	

HbA1c: glycated hemoglobin; VO2peak: peak oxygen uptake per kilogram of body weight; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure. ACEi: angiotensin converting enzyme inhibitor; ARB: angiotensin II receptor blocker. Data are expressed as mean ± SE, except duration of diabetes, which is expressed as median (P25-P75) and current therapy which is showed by number of patients.

Table 2: Hormonal levels after treatment with vildagliptin and glibenclamide in the day of the exercise evaluation

Insulin (µU/mL)	**METV**	**METG**
Fasting	10.3 (6.7-13.7)^{Aa}	15.8 (6.8-28.8)^{Aa}
0'	22.0 (4.7-28.2)^{Aab}	40.9 (26.7-69.3)^{Bb}
15'	25.2 (18.6-35.5)^{Ab}	44.8 (22.7-79.5)^{Aab}
30'	22.8 (17.6-29.0)^{Abc}	43.3 (29.2-77.4)^{Bb}
90'	13.7 (5.8-27.3) ^{Aac}	36.4 (20.2-47.3)^{Bb}
GLP-1 (pM/L)		
Fasting	14.88 ± 5.42^{A}	7.71 ± 1.56^{B}
0'	15.93 ± 7.06^{A}	8.22 ±1.15^{B}
15'	14.39 ± 3.13^{A}	9.29 ± 2.44^{B}
30'	13.43 ± 2.05^{A}	7.76 ± 0.99^{B}
90'	12.99 ± 5.11^{A}	8.57 ± 1.63^{B}

Hormonal levels at fasting (-60'), before (0'), during (15'), after exercise (30') and 90' after beginning (60' of recovery) after 12 weeks of treatment with vildagliptin (METV, 50mg b.i.d n=6) or glibenclamide (METG, 5mg b.i.d. n=6) in patients with type 2 diabetes inadequately controlled with metformin therapy. GLP-1: glucagon like peptide-1. Data are expressed as median and P25-P75 or as mean and SE. Different lowercase letter mean $p<0.05$ intragroup. Different capital letter mean $p<0.05$ intergroup.

Table 3: Glucose variability after 12-wk treatment with vildagliptin or glibenclamide in the patients studied

	METV			METG				
	before exercise	after exercise		before exercise	after exercise		P group	P time
	-6h - 0h	0h - 6h	6h - 12h	-6h - 0h	0h - 6h	6h - 12h		
Glucose (mg/dL)	194.4 ±30.7	174.4 ± 18.5	192.5 ± 30.0	167.1 ± 13.5	148.1 ± 21.6	152.2 ± 27.1	**0.275**	**0.092**
CV (%)	11.4 ± 2.4	11.6 ±3.1	10.2 ± 2.5	15.9 ±3.0	16.0 ±1.1	15.1 ±2.2	**0.086**	**0.921**
Glucose SD (mg/dL)	15.9 (15.2-21.5)	24.8 (9.2-27.1)	10.6 (8.2-25.4)	19.0 (11.6-27.6)	20.1 (18.7-32.4)	20.6 (9.8-29.9)	**0.228**	**0.709**
Glucose variance (mg^2/dL^2)	252.1 (231.2-493.3)	617.5 (98.9-736.0)	111.6 (69.2-706.1)	361.5 (152.4-788.0)	405.3 (349.1-1091.8)	508.5 (97.1-892.4)	**0.227**	**0.698**
Variance in normalized units (mg/dL)	1.9 (1.4-4.5)	2.7 (0.5-5.0)	1.2 (0.9-4.4)	3.6 (2.5-8.4)	3.1 (2.8-5.7)	3.6 (2.4-5.0)	**0.156**	**0.172**

Glucose variability evaluated after 12 weeks of treatment with vildagliptin (METV, 50mg b.i.d) or glibenclamide (METG, 5mg b.i.d.) in patients with type 2 diabetes inadequately controlled with metformin therapy. SD: standard deviation; CV%: coefficient of variation. Data are expressed as mean and SE in mean absolute glucose and CV%. SD, glucose variance and variance in normalized units data are expressed as median (p25-p75). n is 5 for METV and 4 for METG.

Table 4: Cardiovascular parameters after 12-wk treatment with vildagliptin or glibenclamide in the day of the exercise evaluation

	METV			METG				
	pre-exercise	**2' post exercise**	**60' post exercise**	**pre-exercise**	**2' post exercise**	**60' post exercise**	**p group**	**p time**
Cardiac output (L/min/m²)	6.7 (3.3-7.1)	6.6 (3.3-7.0)	6.6 (3.3-7.1)	6.6 (4.9-6.9)	6.6 (3.3-7.0)	3.0 (3.3-6.9)	0.54	0.73
HRV (ms²)	417.3 (332.5-817.6)	325.5 (165.1-426.7)	563.4 (66.0-920.2)	222.9 (139.9-302.1)	293.3 (128.3-546.3)	467.4 (162.9-550.9)	0.99	0.07
LF peak (Hz)	0.07 (0.06-0.09)	0.07 (0.06-0.09)	0.08 (0.05-0.08)	0.06 (0.05-0.07)	0.08 (0.06-0.09)	0.7 (0.06-0.10)	0.19	0.92
LF band (ms²)	132.7 (35.3-133.6)	61.1 (43.2-256.0)	80.0 (27.7-185.0)	189.5 (122.8-243.6)	57.6 (30.0-100.9)	80.3 (24.0-250.3)	0.89	0.13
LF band (nu)	49.5 (33.7-75.9)	61.5 (34.4 (78.5)	34.2 (23.4-64.1)	52.6 (32.7-82.4)	51.4 (30.5-67.1)	58.6 (30.3-79.7)	0.84	0.58
HF peak (Hz)	0.30 (0.30-0.34)	0.30 (0.28-0.30)	0.25 (0.23-0.29)	0.31 (0.29-0.31)	0.29 (0.25-0.30)	0.30 (0.22-0.30)	0.95	0.09
HF band (m/s²)	123.4 (23.8-125.5)	40.5 (31.5-141.4)	234.9 (8.9-511.4)	97.6 (26.4-545.0)	42.0 (8.1-216.4)	45.0 (5.4-203.3)	0.40	0.23
HF band (nu)	46.3 (15.9-52.0)	30.3 (19.8-58.5)	62.6 (19.1-73.3)	43.8 (14.5-63.6)	37.5 (23.0-65.2)	18.7 (10.1-67.7)	0.61	0.81
LF/HF index	1.1 (0.7-6.7)	1.8 (1.0-5.4)	0.55 (0.3-3.4)	1.2 (0.5-6.7)	1.4 (0.5-3.1)	3.8 (0.4-7.3)	0.54	0.73

Cardiovascular response to exercise after 12 weeks of treatment with vildagliptin (METV, 50mg b.i.d) or glibenclamide (METG, 5mg b.i.d.) in patients with type 2 diabetes inadequately controlled with metformin therapy. HRV: heart rate variability; LF: low frequency. HF: high frequency. Data are expressed as median (95%CI). After treatment n was 5 for both groups.

Table 5: Ambulatory blood pressure monitoring (ABPM) parameters before and after 12-wk treatment with vildagliptin or glibenclamide in the patients studied

24h-ABPM	**Before treatment**	**METV**	**METG**	**p group**	**p time**
24h-ABPM SBP (mmHg)	130.9 ±3.9	120.0 ±4.2	126.0 ±4.5	0.363	0.012
Daytime 24h-ABPM SBP (mmHg)	133.6 ±3.8	121.3 ±3.5	129.5 ±5.6	0.225	0.009
Nightime 24h-ABPM SBP (mmHg)	124.6 ±4.9	115.1 ±7.4	118.9 ±2.6	0.730	0.109
24h-ABPM DBP (mmHg)	78.4 ±3.4	73.6 ±1.1	73.0 ±3.6	0.814	0.272
Daytime 24h-ABPM DBP (mmHg)	81.0 ±3.4	75.3 ±1.0	75.9 ±4.1	0.895	0.179
Nightime 24h-ABPM DBP (mmHg)	72.5 ±3.8	68.0 ±2.9	66.8 ±2.9	0.702	0.388
24h-ABPM MBP (mmHg)	93.4 ±1.8	88.4 ±2.4	92.0 ±1.8	0.473	0.073
Daytime 24h-ABPM MBP (mmHg)	96.3 ±1.7	90.2 ±1.6	95.1 ±4.5	0.323	0.052
Nightime 24h-ABPM MBP (mmHg)	86.8 ±2.7	84.4 ±4.7	85.2 ±2.3	0.959	0.371
SBP variability indexes					
Time rate of SBP variation (mmHg/min)	0.468 ±0.02	0.445 ±0.05	0.601 ±0.12	0.012	0.272
Daytime time rate of SBP variation (mmHg/min)	0.456 ±0.04	0.476 ±0.03	0.528 ±0.08	0.447	0.010
Nightime time rate of SBP variation (mmHg/min)	0.438 ±0.03	0.417 ±0.05	0.523 ±0.05	0.001	0.602
SD 24h-ABPM SBP (mmHg)	12.5 ±0.9	10.9 ±0.8	15.0 ±1.0	0.010	0.816
Daytime SD 24h-ABPM SBP (mmHg)	11.6 ±0.9	10.4 ±0.7	13.7 ±1.4	0.015	0.638
Nightime SD 24h-ABPM SBP (mmHg)	10.6 ±0.7	8.6 ±0.4	13.2 ±1.0	<0.001	0.804
SD 24h-ABPM DBP (mmHg)	9.8 ±0.7	8.7 ±1.1	10.9 ±0.3	0.247	0.958
Daytime SD 24h-ABPM DBP (mmHg)	8.4 ±0.7	8.3 ±0.9	9.6 ±0.9	0.413	0.563
Nightime SD 24h-ABPM DBP (mmHg)	8.1 ±0.5	7.3 ±0.8	9.8 ±1.0	0.263	0.333
CV of the 24h SBP	9.7 ±2.5	9.1 ±0.9	11.9 ±0.8	0.028	0.471
Daytime CV 24h-ABPM SBP (mmHg)	8.8 ±0.6	8.6 ±0.7	10.7 ±1.3	0.027	0.370

Nightime CV 24h-ABPM SBP (mmHg)	9.3 ±0.8	7.6 ±0.7	11.2 ±1.1	0.018	0.945
CV of the 24h DBP	12.7 ±3.5	11.9 ±1.6	15.1 ±1.1	0.213	0.710
Daytime CV 24h-ABPM DBP (mmHg)	10.8 ±0.9	11.1 ±1.3	13.0 ±1.9	0.373	0.402
Nightime CV 24h-ABPM DBP (mmHg)	11.7 ±0.6	11.0 ±1.5	14.8 ±1.8	0.076	0.004
SBP dipping (%)	7.2 ±2.1	6.4 ±4.0	8.8 ±2.9	0.585	0.859
DBP dipping (%)	11.1 ±2.4	9.7 ±3.5	11.7 ±2.7	0.759	0.837

Ambulatory blood pressure monitoring parameters before and after 12 weeks of treatment with vildagliptin (METV, 50mg b.i.d) or glibenclamide (METG, 5mg b.i.d.) in patients with type 2 diabetes inadequately controlled with metformin therapy. 24h-ABPM: ambulatory blood pressure monitoring.; SBP: systolic blood pressure; DBP: diastolic blood pressure; SD: standard deviation; CV: Coefficient of variation. Data are expressed as mean and SE. Number of patients before treatment is 12 (both groups considered together); no differences between groups were found at this time. For most variables n is 5 for both groups. Dipping: nighttime SBP or DBP fall >10%.

6. CONCLUSÕES

Este é o primeiro estudo conduzido em pacientes com DM2 que fornece dados sobre a influência da terapia medicamentosa padrão (metformina e glibenclamida) *vs* outra classe disponível (metformina e vildagliptina) em respostas a uma sessão de exercício aeróbico, um dos pilares recomendados para alcançar um bom controle glicêmico em pacientes com DM2. Além de melhora no controle glicêmico e redução da pressão arterial sistólica obtidas por ambos tratamentos, foi observada menor variabilidade da pressão arterial nos pacientes submetidos ao tratamento com vildagliptina. A hipótese do estudo não se confirmou, visto que a variabilidade glicêmica não foi diferente entre os tratamentos utilizados.