UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

PROGRAMA DE PÓS GRADUAÇÃO EM CIÊNCIAS MÉDICAS: ENDOCRINOLOGIA

TESE DE DOUTORADO

EFEITOS NÃO ANTIHIPERGLICÊMICOS DA METFORMINA EM INDIVÍDUOS NÃO DIABÉTICOS

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Porto Alegre, abril de 2017

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Alguns jogadores de futebol, talvez por desconhecerem suas plenas potencialidades, ou simplesmente por se preocuparem mais com o corte de cabelo ou com tatuagens, muitas vezes não rendem tudo que podem. Felizmente, existem alguns bons técnicos que conseguem tirar o melhor do craque que perde o foco.

Reconheço que meus dois maiores defeitos são a teimosia e a procrastinação. Felizmente, minha "técnica", a professora Beatriz D. Schaan, soube lidar com isso para me ajudar a concluir este trabalho.

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Obrigado!

SUMÁRIO

ABREVIATURAS E SIGLAS

RESUMO, INTRODUÇÃO E CONCLUSÕES

ACC *American College of Cardiology*

AHA *American Heart Association*

AMPK enzima proteína quinase AMP-ativada

DM2 diabetes mellitus tipo 2

MAPA monitorização ambulatorial da pressão arterial

mGPD isoforma mitocondrial específica da glicerofosfato desidrogenase

NADH dinucleótido de nicotinamida e adenina

PAD pressão arterial diastólica

PAS pressão arterial sistólica

SOP síndrome dos ovários policísticos

T3L triiodotironina livre

T4L tiroxina livre

TRbeta receptor de hormônio tireoidiano nuclear específico

TRIV tempo de relaxamento isovolumétrico

TSH tireotrofina

ARTIGOS

ABPM *ambulatory blood pressure monitoring*

AC *abdominal circumference*

ACC *American College of Cardiology*

AHA *American Heart Association*

ANCOVA *analysis of covariance*

BMI *body mass index*

BP *blood pressure*

CNPq Conselho Nacional de Desenvolvimento Científico e Tecnológico

DBP *diastolic blood pressure*

DT *deceleration time of mitral E wave*

E/A relation *diastolic mitral flux*

E/e' relation *average between interventricular septum E/e' and basal lateral wall E/e'*

Echo *echocardiography*

FIPE-HCPA Fundo de Incentivo a Pesquisa e Eventos do Hospital de Clínicas de Porto Alegre

FT4 *free thyroxine*

HDL-c *high density lipoprotein cholesterol*

HOMA-IR *homeostatic model assessment for insulin resistance*

HR *heart rate*

ITT *intention to treat*

IVRT *isovolumetric relaxation time*

LDL-c *low density lipoprotein cholesterol*

LVSVI *left ventricular stroke volume index*

MPI *myocardial performance index*

NYHA *New York Heart Association*

PCOS *polycystic ovarian syndrome*

RCT *randomized clinical trial*

SBP *systolic blood pressure*

SH *subclinical hypothyroidism*

T3 *triiodothyronine*

TC *total cholesterol*

TG *triglycerides*

TSH *thyrotropin*

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Artigo 2 - Metformin effect on cardiovascular risk in non-diabetic users: a post-hoc analysis of two randomized controlled trials

RESUMO

A metformina é um medicamento classicamente usado como primeira linha no tratamento do diabetes mellitus tipo 2. Além de seus efeitos antihiperglicêmicos, existem evidências sugerindo que a metformina possa ter impacto positivo na redução do peso e da pressão arterial, na melhora do perfil lipídico, com consequente redução de marcadores de risco cardiovascular mesmo em indivíduos não diabéticos. Outro possível efeito da metformina observado a relativamente pouco tempo é a redução dos níveis de tireotrofina (TSH).

Na última década, diversos estudos têm sugerido que o uso de metformina em indivíduos com hipotireoidismo primário, tanto em tratamento com levotiroxina, quanto virgens de tratamento, possa reduzir os níveis de TSH. Esta redução não parece alterar os níveis de hormônios tireoidianos e tem sido observada em indivíduos com algum grau de resistência à insulina.

Com o objetivo de avaliar alguns destes efeitos em indivíduos não diabéticos, foram desenhados dois ensaios clínicos randomizados, duplo-cegos, placebo controlados, com até três meses de duração. O primeiro teve como objetivo principal avaliar o efeito da metformina sobre o TSH de indivíduos com hipotireoidismo subclínico. O outro estudo procurou avaliar o efeito da metformina sobre a pressão arterial de indivíduos hipertensos. São apresentados aqui os resultados do primeiro estudo e uma análise *post hoc* do grupo total de indivíduos dos dois estudos.

Para avaliar o efeito da metformina sobre o TSH de indivíduos com hipotireoidismo subclínico, 48 pacientes com este diagnóstico foram randomizados para receber metformina 850 mg duas vezes ao dia ou placebo por 3 meses. Não houve diferença estatisticamente significativa nos níveis de TSH entre os grupos, nem dentro dos grupos na comparação entre o período pré e pós-tratamento. No entanto, o tamanho da amostra se mostrou pequeno para afastar a hipótese de erro tipo beta.

Procurou-se avaliar também se o uso de métodos não invasivos de avaliação de risco cardiovascular (escores de risco de Framingham e ACC/AHA 2013) seria capaz de detectar redução de risco após o

uso da metformina nos 48 indivíduos do primeiro estudo somados aos 100 indivíduos do segundo. Não houve diferença estatisticamente significativa. Foi estimado um tamanho de amostra de 1960 indivíduos para mostrar uma redução de risco de 1%, com poder de 80% e erro alfa bilateral de 5% para o escore ACC/AHA 2013. No entanto, o significado desta possível pequena redução deve ser interpretado dentro do contexto clínico de diferentes faixas de risco.

Como a magnitude dos efeitos não antihiperglicêmicos da metformina em indivíduos não diabéticos parece ser modesta, estudos com amostras maiores e em diferentes populações são necessários para que se consiga identificar quem realmente pode se beneficiar do seu uso.

APRESENTAÇÃO

Este trabalho consiste na tese de doutorado “Efeitos não antihiperglicêmicos da metformina em indivíduos não diabéticos”, apresentada no Programa de Pós-Graduação em Ciências Médicas: Endocrinologia da Universidade Federal do Rio Grande do Sul em abril de 2017. O trabalho será apresentado em 3 partes, descritas a seguir:

1. Introdução

2. Desenvolvimento

2.a. Artigo 1

2.b. Artigo 2

3. Conclusões

INTRODUÇÃO

A metformina (dimetilbiguanida) é um fármaco usado no tratamento do diabetes mellitus tipo 2 (DM2), considerada primeira escolha no manejo desta doença (1). É o único representante da classe das biguanidas ainda no mercado. As biguanidas são formadas pela ligação de dois anéis guanidinas. A molécula de metformina é a menos lipofílica dentre elas, o que a tornou mais segura para o uso clínico. Possui biodisponibilidade oral de 50 a 60%, não se liga avidamente às proteínas e tem alto volume de distribuição, com acumulação máxima no intestino delgado. É excretada inalterada através da urina, o que pode levar a acúmulo e risco de acidose lática na presença de insuficiência renal (2).

O principal efeito da metformina é diminuir a liberação de glicose hepática através da inibição da gliconeogênese (3). Além disso, a metformina aumenta a utilização de glicose mediada pela insulina nos tecidos periféricos (como músculos e fígado), especialmente após as refeições, e apresenta efeito antilipolítico que reduz a concentração de ácidos graxos livres, desta forma reduzindo a disponibilidade de substrato para a gliconeogênese (3)(4). Como consequência do melhor controle glicêmico, os níveis séricos de insulina diminuem levemente (5).

O processo de supressão da gliconeogênese pela metformina acontece através da inibição de uma isoforma mitocondrial específica da glicerofosfato desidrogenase (mGPD), uma enzima responsável por converter o glicerofosfato em fosfato de dihidroxiacetona, desta forma, evitando com que o glicerol participe da via gliconeogênica. A inibição da mGPD também leva à acumulação de NADH citoplasmático e à diminuição da conversão de lactato em piruvato, desta forma limitando a contribuição do lactado na gliconeogênese hepática (6).

A metformina também ativa a enzima proteína quinase AMP-ativada (AMPK) nos hepatócitos, que parece ser o mecanismo pelo qual ocorre redução nos níveis dos lipídios séricos (7). A fosforilação inibitória dependente de AMPK das acetil-CoA carboxilases Acc1 e Acc2 suprimem a lipogênese e

diminuem a síntese celular de ácidos graxos no fígado e nos músculos (8). A regulação da AMPK é feita através da proteína LKB1 de Peutz-Jeghers (9). A LKB1 é supressora tumoral, e a ativação da AMPK através da LKB1 pode ter papel na inibição do crescimento celular (10).

Além da melhora em parâmetros do metabolismo glicêmico e lipídico já descritos, o uso da metformina também está associado à perda de peso, redução da obesidade centrípeta, redução do colesterol LDL, aumento do colesterol HDL e redução da insulinemia (4)(5).

Alguns estudos sugerem que a metformina possa ter efeito antihipertensivo independente dos seus efeitos metabólicos. Landin et al, avaliou 8 homens hipertensos, não obesos e não diabéticos, mostrando redução na pressão arterial sistólica (PAS) e diastólica (PAD) após 6 semanas de uso de metformina 850 mg duas vezes dia. Suspenso o tratamento por 2 meses, houve elevação da PAS e PAD a valores semelhantes aos basais. Apesar dos dados animadores, a principal crítica ao estudo é a ausência de um grupo controle (11). Ensaio clínico randomizado com 24 semanas de duração comparou o efeito da metformina 850 mg duas vezes dia vs. anticoncepcional oral (etinilestradiol + ciproterona) sobre a PA aferida por monitorização ambulatorial da pressão arterial (MAPA) em pacientes com síndrome dos ovários policísticos (SOP), mostrando que as 19 participantes que receberam metformina tiveram redução da PAS e PAD durante o dia, sem mudança à noite (12). Por fim, uma revisão sistemática com metanálise de 26 estudos, incluindo 4113 participantes demonstrou efeito hipotensor da metformina na PAS em indivíduos não diabéticos (- 1,98 mmHg; IC 95%: - 3,61 a - 0,35, p = 0,02) (13).

Também existem evidências de possível efeito benéfico da metformina sobre a função cardíaca diastólica. A variável de função diastólica mais estudada nesse contexto é o tempo de relaxamento isovolumétrico (TRIV), que é o intervalo de tempo compreendido entre o fim do período de ejeção através da válvula aórtica e o início da onda E, que é a primeira fase do enchimento ventricular. Quando há disfunção diastólico, há aumento do TRIV. Em pacientes com diabetes, o uso da metformina está associado redução do TRIV. Um estudo de coorte retrospectivo avaliou através de

ecocardiograma 242 pacientes com diabetes. Após ajuste em modelo de regressão linear, os pacientes que faziam uso de metformina tiveram um TRIV 9,9 ms menor quando comparados aos indivíduos que usavam insulina ou sulfonilureia (p=0,045) (14). Efeitos sobre a função ventricular esquerda também já foram observados em modelos animais, onde o uso da metformina teve efeito positivo no remodelamento miocárdico por mecanismo possivelmente associado a ativação da AMPK e da óxido nítrico sintetase endotelial (15).

Uma vez que o tratamento medicamentoso da hipertensão arterial sistêmica tem sua eficácia em reduzir desfechos duros é amplamente reconhecida (16), a melhor avaliação do efeito hipotensor da metformina pode ajudar a explicar seu impacto positivo na diminuição do risco cardiovascular amplamente relatado na literatura. Diferentes estudos demonstraram que o uso da metformina se associa com redução da mortalidade por causas cardiovasculares (17).

Assumindo sua eficácia como antihiperglicemiante, seu perfil de segurança e, além disso, assumindo a existência de efeitos benéficos não antihiperglicêmicos, outras aplicações têm sido propostas para metformina além do tratamento para o DM2, tais como: prevenção do DM2 em indivíduos com tolerância diminuída à glicose (18), no tratamento do diabetes mellitus gestacional (19), no tratamento da SOP (20), no manejo do ganho de peso associado ao uso de antipsicóticos atípicos (21) e no tratamento adjuvante do câncer (22).

Na última década, alguns estudos têm sugerido que a metformina possa influenciar a função tireoidiana de pacientes diabéticos, mais especificamente reduzindo os níveis de tireotrofina (TSH) sem efeito significativo sobre os hormônios tireoidianos (T4 e T3)(23)(24)(25). A redução do TSH aconteceu somente nos indivíduos com hipotireoidismo primário, recebendo ou não tratamento com levotiroxina (24)(25). No estudo de Isidro et al o valor do TSH foi reduzido de 3,11 ± 0,57 µUI/mL para 1,18 ± 0,36 µUI/mL após 3 meses de tratamento com metformina 1700 mg/dia, com um aumento não significativo no T4 e T3 livres (24). No estudo de Capelli et al, a redução do TSH nos grupos de pacientes diabéticos com hipotireoidismo em tratamento e com hipotireoidismo

subclínico sem tratamento foram de 2,37 ± 1,17 µUI/mL para 1,41 ± 1,21 µUI/mL e de 4,52 ± 0,37 µUI/mL para 2,93 ± 0,48 µUI/mL, após 12 meses de uso de metformina, respectivamente, novamente sem diferença nos valores de T4L basal vs. pós-tratamento (25). Rezzónico et al demonstrou redução de nódulos hiperplásicos em mulheres eutireoideas com resistência insulínica de 30% no grupo que recebeu apenas metformina e 55% no grupo que recebeu metformina + levotiroxina. Nos grupos de pacientes que não receberam tratamento ou que receberam apenas levotiroxina, não se evidenciou redução no tamanho dos nódulos. Neste estudo também não se observou alteração significativa nos valores de TSH, contudo as participantes receberam doses menores de metformina (1000 mg/dia) e eram eutireoideas (26). Taghavi et al comparou o efeito da metformina 1500 mg/dia vs. placebo sobre o TSH de 27 mulheres com síndrome dos ovários policísticos e hipotireoidismos subclínico. Após 6 meses de tratamento, o grupo metformina apresentou redução dos níveis de TSH vs. grupo placebo: 7,78 ± 1,74 µUI/mL para 6,14 ± 2,47 µUI/mL vs. 8,02 ± 2,21 µUI/mL para 8,82 ± 2,89 µUI/mL. Como já observado nos outros estudos, o efeito da metformina se restringiu ao TSH, uma vez que não houve diferença nos níveis de T4 livre (T4L) e T3L (27). Por fim, a revisão sistemática de Lupoli et al (28), que compilou dados de sete estudos, evidenciou redução nos níveis de TSH em indivíduos com hipotireoidismo evidente e subclínico induzida por metformina.

O mecanismo pelo qual a metformina possa induzir queda do TSH ainda está sendo estudado. É possível que haja sensibilização do TRbeta (receptor de hormônio tireoidiano nuclear específico) em nível central, aumentando sua sensibilidade aos hormônios tireoidianos, principalmente em pacientes com baixa reserva e fazendo com que os níveis de TSH caiam (29). A ativação da AMPK pela metformina em nível hipotálamo-hipofisário pode ajudar a explicar este efeito (30). Outros possíveis mecanismos seriam a modulação da atividade das deiodases, especialmente a deiodase tipo 2 em nível hipotálamo-hipofisário, modulação do tônus dopaminérgico sobre a secreção de

TSH (23) e mecanismo dependente da sensibilização à insulina (30). A literatura ainda carece de modelos experimentais que avaliassem essas hipóteses.

Uma condição, que acomete pacientes geralmente assintomáticos, definida por TSH elevado com níveis normais de T4 livre, onde o efeito da metformina poderia ser avaliado, é o hipotireoidismo subclínico (31)(32). Sua prevalência nos Estados Unidos foi estimada em 4,3% da população (33). Estudo transversal realizado no Brasil em 314 mulheres com mais de 40 anos que trabalhavam na Universidade de São Paulo mostrou prevalência de hipotireoidismo subclínico de 7,3% (34). Várias consequências do hipotireoidismo subclínico foram identificadas, embora seu tratamento ainda seja questionável, uma vez que o efeito deste sobre aquelas ter sido pouco estudado. A taxa de progressão anual para hipotireoidismo foi avaliada em 4,3% (35) e possível aumento dos níveis séricos de colesterol total foi observado (36). Além disso, há uma possível relação direta entre os níveis de TSH e os níveis pressóricos aferidos por MAPA de 24 horas ($r = 0,477$; $p = 0,004$), mas que não torna diferente a pressão arterial (PA) entre indivíduos com hipotireoidismo subclínico e eutireoideos (37). Outro estudo, no entanto, mostrou que a PAS e PAD foram maiores nos pacientes com hipotireoidismo subclínico, assim como o índice de massa corporal (38). Também foram observadas em pacientes com hipotireoidismo subclínico mudanças na função cardíaca similares, mas em intensidade menor, às que ocorrem em indivíduos com hipotireoidismo evidente. No hipotireoidismo subclínico ocorre piora na função ventricular esquerda evidenciada por disfunção diastólica, avaliada principalmente pelo TRIV através do ecocardiograma. O tratamento com levotiroxina melhora a função diastólica do ventrículo esquerdo, conforme dados de uma revisão sistemática (39).

Essas alterações cardiovasculares e metabólicas induzidas pelo hipotireoidismo subclínico, talvez possam explicar a maior morbimortalidade cardiovascular frequentemente demonstrada na literatura. Em uma metanálise de 7 estudos de coorte prospectivos contabilizando um total de 25.977 indivíduos e 2.020 com hipotireoidismo subclínico, os indivíduos com TSH > 10 µUI/mL

tiveram um aumento significativo [risco relativo de 1,89 (IC 95%: 1,28 – 2,80)] nos desfechos cardiovasculares combinados (infarto agudo do miocárdio não fatal, morte por doença arterial coronariana, hospitalização por angina ou revascularização) quando comparados com os indivíduos eutireoideos (40).

O tratamento com levotiroxina parece melhorar o perfil lipídico e a função ventricular esquerda (39). Contudo, apesar das evidências apontarem o hipotireoidismo subclínico como uma condição que agrega diversos fatores de risco cardiovascular, inclusive com aumento de mortalidade, a literatura ainda carece de estudos que demostrem que qualquer intervenção seja capaz de reduzir desfechos duros.

O papel da metformina na função tireoidiana, sobre a pressão arterial e sobre o risco cardiovascular de indivíduos não diabéticos foram até agora avaliados por poucos estudos, muitos destes com limitações metodológicas. Isso torna imprescindível a realização de ensaios clínicos randomizados para jogar luz sobre essas questões. Uma vez que os efeitos não antihiperglicêmicos da metformina sejam melhor caracterizados, será possível que esta droga de grande sucesso no tratamento do DM2 ganhe novas aplicações.

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ARTIGO 1

Metformin effect on TSH in subclinical hypothyroidism: randomized, double-blind, placebo-controlled clinical trial

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Abbreviated title: Metformin on TSH in subclinical hypothyroidism

Key terms: TSH, metformin, subclinical hypothyroidism, clinical trial

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Abstract:

1- Context: Non-randomized trials suggest that metformin may reduce TSH levels through unknown mechanisms.

2- Objective: To evaluate whether metformin can reduce TSH levels in subjects with subclinical hypothyroidism.

3- Design: randomized, double-blind, placebo controlled clinical trial with three months duration.

4- Setting: outpatients from a region in southern Brazil.

5- Patients: forty-eight individuals, between 18 and 65 years, with subclinical hypothyroidism.

6- Interventions: patients were randomized to the use of metformin 850 mg or placebo twice a day for three months.

7- Main outcome measures: primary outcome was the absolute decrease in TSH levels. Secondary outcomes were changes in the clinical and laboratory assessment, as well as in the diastolic function assessed by transthoracic echocardiography and in blood pressure assessed by ambulatory blood pressure monitoring.

8- Results: after three months, 93.75% of participants completed the follow up. The post treatment value of TSH in the metformin and placebo groups were 6.48 ± 3.11 and 7.02 ± 3.28 μIU/mL, respectively ($p = 0.57$). Patients that achieved status of euthyroidism in the metformin and placebo groups were 21.7 and 18.2 percent, respectively ($p = 0.76$). There was no significant reduction of TSH within the groups [delta for TSH of 0.63 ± 0.56 ($p = 0.28$) and 0.54 ± 0.60 μIU/mL ($p = 0.38$),

in metformin and placebo groups, respectively].There was a small increase in HDL cholesterol (62.8 ± 17.5 *vs.* 51.7 ± 15.3 mg/dL, p = 0.03) favoring the metformin group.

9- Conclusions: Metformin may not be superior to placebo in reducing TSH in subjects with subclinical hypothyroidism.

Précis:

This study evaluated if metformin would be able to reduce TSH levels in individuals with subclinical hypothyroidism. After follow-up, metformin was not superior to placebo.

Introduction:

Subclinical hypothyroidism (SH) is a condition defined by normal plasma levels of free thyroxine (FT4) in the presence of high plasma levels of thyrotropin (TSH) (1). According to the survey NHANES III, the prevalence of SH was 4.3% in a population of 16,533 individuals, after excluding those with overt thyroid disease (2). In Brazil, the prevalence of elevated TSH is between 6.1 and 19.1%, depending on the age or sex of the sample evaluated (3).

As shown in a meta-analysis of seven prospective cohort studies, compared with euthyroid subjects, individuals with TSH higher than 10 μIU/mL had a significant increased risk of developing combined cardiovascular events (nonfatal acute myocardial infarction, death from coronary artery disease, hospitalization for angina or revascularization; relative risk of 1.89; 95% CI: 1.28 to 2.80) (4). Slight changes in blood pressure, diastolic function and lipid profile could explain this increased risk. (5)(6). However, despite some evidence that SH is a condition that aggregates multiple cardiovascular risk factors, including increased cardiovascular mortality, there is no evidence that any intervention can reduce hard outcomes.

Some studies suggest that metformin may influence thyroid function of diabetic patients, specifically reducing TSH levels without significant effect on circulating levels of thyroid hormones [thyroxine (T4) and triiodothyronine (T3)] (7). The reduction in TSH levels only occurred in patients with primary hypothyroidism, regardless of whether they were being treated with levothyroxine (8)(9). Possible hypothesized mechanisms for this effect of metformin are: sensitization of thyroid hormone receptors, modulation of the activity of deiodinases, especially type II deiodinase at hypothalamic-pituitary level, and modulation of dopaminergic tone on TSH secretion (10). However, evidence that metformin may present such pleiotropic effects are supported by observational data or by uncontrolled studies with methodological limitations (7). Therefore, randomized controlled trials are necessary to confirm the effect of metformin in TSH reduction.

In this randomized, double-blind, placebo controlled study, we evaluated the effect of metformin on TSH of individuals with SH. In addition, the study participants were evaluated for other clinical and laboratory outcomes that could improve with this treatment, including echocardiographic variables of diastolic function and blood pressure assessed by ambulatory blood pressure monitoring (ABPM).

Material and methods:

The CONSORT Statement was used for reporting this trial.

Study design:

This is a randomized, parallel, double-blind, placebo controlled clinical trial with three months follow-up. The study was carried out in Santa Maria, a town in Southern Brazil.

Participants:

The sample consisted of 48 individuals aged 18-65 years, with SH, recruited through media announcements. The diagnosis of SH was confirmed in individuals with TSH higher than the upper limit of normal for the method in two steps, the first by any method and the second measured by chemiluminescence (Centaur XP, Erlangen, Germany, reference value: 0.27 - 4.2 μIU/mL). Free T4 assessed by chemiluminescence (Centaur XP, Erlangen, Germany) should be within normal limits (0.89 – 1.76 ng/dL). Patients with previous diagnosis of diabetes mellitus, use of levothyroxine, with contraindications to the use of metformin (related hypersensitivity to the drug, chronic kidney disease with calculated glomerular filtration rate ≤ 30 mL/min, abnormal liver function, heart failure NYHA III-IV despite optimal drug treatment, severe mental illness, any illness in terminal stage, malignancy not cured or in treatment, contrast studies in the last three months) were excluded, as well as those using drugs known to interfere with thyroid function (methimazole, propylthiouracil, lithium, iodine, amiodarone, aminoglutethimide, interferon, interleukin-2, sunitinib, corticoids, dopaminergic agonists, analogs of somatostatin, retinoids, carbamazepine/oxicarbamazepina, metyrapone, furosemide, phenytoin, probenecid and heparin).

Interventions:

The subjects received metformin or placebo, according to randomization, in same appearance capsules with 425 mg and in identical containers. They were instructed to take two capsules at lunch in the first three days and two more capsules at dinner beginning in the fourth day, totalizing a dose of 1700 mg per day. If there were adverse effects that precluded the taking of the medication, the participants were instructed to call the researcher. On these occasions, the dose was reduced to 425 mg per day with lunch, with an increase of 425 mg every 3 days until the highest tolerated dose.

Outcomes:

The prespecified primary outcome was the absolute decrease in TSH levels in patients with SH treated with metformin compared to placebo. Secondary exploratory outcomes were the proportion of patients achieving euthyroid state when taking metformin compared to placebo; and changes in clinical, laboratory and echocardiographic parameters of SH [disease progression, blood pressure modification measured in the office or by ABPM, body mass index (BMI), abdominal circumference and lipid profile].

Measurement of outcomes:

Patients with the first measure of high TSH were clinically evaluated and sent for biochemical evaluation after 12 hours of fasting. Blood collection was performed between 7:00 and 9:00 AM. If the diagnosis of SH was confirmed, they were included in the study and submitted to echocardiogram and ABPM. After the completion of these two exams, they were instructed to start using metformin/placebo and maintain contact with investigators if necessary. About four weeks after treatment initiation, participants received a call and were asked about adherence to treatment and adverse effects. In the last 10 days of the follow up, still in use of metformin/placebo, the patients were invited to return for further clinical evaluation, blood collection, echocardiogram and ABPM. Prespecified 45 days clinical evaluation and pill counting were not made because of logistical difficulties.

Clinical evaluation:

In clinical consultation, after a brief questionnaire, the patient was weighed on a digital scale with a sensitivity of 100 grams and measured in fixed length stadiometer graduated in millimeters. Abdominal circumference was measured with the patient standing, at the end of expiration, with an inextensible tape parallel to the ground, passing just above the iliac crests and below the umbilicus. Blood pressure was measured after 15 minutes of rest, with the patient seated, with the feet flat on

the floor, using an automated device (OMRON, model HEM 742, Kyoto, Japan) with appropriate size cuff for arm circumference. Patients with arm circumference ≥ 33 cm used a 32 cm x 42 cm cuff and with lower values, a 24 cm x 32 cm cuff. The average of two measures of blood pressure in the dominant arm was considered.

Biochemical assessment:

The following parameters were analyzed after 12 hours of fasting: TSH (chemiluminescence, Centaur XP, Erlangen, Germany, the limit of detection was 0.001 μIU/mL and the interassay coefficient of variation ranged from 4.01 to 4.19%), FT4 (chemiluminescence, Centaur XP, Erlangen, Germany, the lower limit of detection was 0.1 ng/dL, the interassay coefficient of variation ranged from 4.34 to 5.77% and cross reactivity did not exceed 0.02%), anti-TPO (chemiluminescence, Immulite 2000, Erlangen, Germany), glucose (enzyme hexokinase, Bioclin, Belo Horizonte, Brazil), insulin (chemiluminescence, Centaur XP, Erlangen, Germany), total cholesterol (enzymatic colorimetric, Advia 1800, Erlangen, Germany), HDL cholesterol (enzymatic colorimetric, Advia 1800, Erlangen, Germany), triglycerides (enzymatic colorimetric, Advia 1800, Erlangen, Germany). The LDL cholesterol was estimated using the Friedewald formula. Plasma glucose and insulin were used for the calculation of HOMA-IR by formula: HOMA-IR = glucose (mmol/L) X insulin (IU/mL) / 22.5 (11).

Transthoracic echocardiography:

Patients underwent two-dimensional transthoracic echocardiography with color Doppler with use of M mode for measurements of cardiac chambers and the calculation of ventricular function and use of pulsed, continuous, tissue and colorful Doppler for trans-valvular flow analysis. Philips HD7 equipment (Andover, MA, USA) with 2-4 MHz transducer and harmonic imaging was used. The following parameters were evaluated: isovolumetric relaxation time (IVRT), myocardial

performance index (MPI), left ventricular stroke volume index (LVSVI), E/A relation (diastolic mitral flux), E/e' relation (average between interventricular septum E/e' and basal lateral wall E/e'), deceleration time of mitral E wave (DT). The measurements were performed in 3 consecutive beats in patients in sinus rhythm.

Ambulatory blood pressure monitoring (ABPM):

Patients were submitted to ABPM using Spacelabs 90207 monitor (Hawthorne, CA, USA) programmed to measurements every 15 minutes between 7:00 and 22:00 and every 20 minutes from 23:00 to 6:00. Two cuff sizes according to arm circumference were used. Patients with arm circumference ≥ 33 cm used a 32 cm x 42 cm cuff and with lower values, a 24 cm x 32 cm cuff. A diary was delivered to notes of the periods of sleep and wakefulness. Exams with at least 80% of valid measures and with at least two valid measures every hour were considered for analysis. Mean systolic and diastolic blood pressure in sleep, wakefulness and total period of 24 hours were evaluated.

Sample:

Considering: 1. The reduction in TSH levels in individuals with diabetes mellitus from 4.52 ± 0.37 μIU/mL to 2.93 ± 0.48 μIU/mL ($p < 0.001$) after 12 months of metformin use (9), bilateral alfa error of 5%, power of 90%, difference detection 0.5 μIU/mL in TSH levels, loss of follow up of 20%, the study sample was calculated in 48 patients (24 in the placebo group and 24 in the metformin group). 2. Only 37.5% of patients with TSH above the upper limit of normal maintains high values in the second measure (12); the prevalence of SH among individuals with TSH above the upper limit of normal is 93% (2): 130 patients with elevated TSH should be screened to obtain 48 patients with SH to be randomized. The study was performed aiming to select 48 patients from 130 patients screened.

Randomization:

The randomization sequence was made with the help of software Random Allocation V2.0 in blocks of four participants by an external researcher. After the generation of the sequence, the bottles of the same appearance containing metformin or placebo were numbered sequentially from 1-48 by the member of the group responsible for the acquisition of medication/placebo (TSA). The bottles numbered sequentially were then made available for the researcher responsible for screening and clinical evaluation of participants (MDS). After the screening of the participants, confirming the case of SH, the bottle was delivered respecting pre-established numerical sequence.

Blinding:

In this context, the participants, the researcher doing the clinical evaluation and the team responsible for the collection and analysis of biochemical tests, realization of echocardiograms and ABPM were blinded to the procedures.

Statistical analysis:

The data were presented as mean and standard deviation (continuous variables with normal distribution) or median and confidence intervals (continuous variables with nonparametric distribution). Comparisons between groups of interest were made through Student t test for independent variables or non-parametric tests (U test), as appropriate. The relationship between categorical variables was evaluated by chi-square test. The intragroup TSH variation was evaluated through a T-test for paired variables. Analysis of covariance (ANCOVA) with inclusion of sex and BMI after intervention as covariates was also conducted. The level of significance was 5%. The analyses were performed with SPSS 18.0 (SPSS, Chicago, IL, USA). Analyses for clinical and

laboratory outcomes followed the intention to treat (ITT) principle. Echocardiographic and ABPM analyses were evaluated considering *per protocol*.

The study was approved by the Ethics committee of the responsible institution and is registered in the Brazilian Registry of Clinical Trials (www.ensaiosclinicos.gov.br) under the code RBR-6crdyx.

Results:

Between June 2013 and May 2015, 48 subjects with SH of the 112 individuals screened were randomized (Figure 1); 93.75% of participants completed the follow up of three months.

Clinical and laboratory characteristics of the participants are shown in Table 1. The groups were similar except that there are more women in the metformin group (91.7% *vs.* 62.5%, p = 0.02). The average TSH value at screening was 7.73 ± 2.35 μIU/mL, and the average TSH of randomized patients was 7.42 ± 2.16 μIU/mL.

After three months of treatment, there was no difference in the proportion of subjects who achieved euthyroid state, as well as the absolute fall of TSH (Table 2). There was no significant reduction of TSH within the groups [delta for TSH of 0.63 ± 0.56 (p = 0.28) and 0.54 ± 0.60 μIU/mL (p = 0.38), in metformin and placebo groups, respectively] (Figure 2). There was a slight increase in HDL cholesterol (+3.4%), statistically significant, favoring the metformin group (Table 2).

After adjustment, the p values for weight (p = 0.003), abdominal circumference (p < 0.001) HDL cholesterol (p = 0.001), insulin (p = 0.006) and HOMA-IR (p = 0.005) were smaller. These differences, especially in insulin and HOMA-IR, were considered a strong evidence of appropriate adherence to intervention. For TSH and other variables, the p value remained without significance, even after adjustment (Table 2).

Regarding the variables of ABPM and echocardiography, no differences were observed between groups after the three-month treatment period (*per protocol* analysis) (Table 3 and Table 4). However, despite not reaching statistical significance, the metformin group had consistently lower systolic and diastolic blood pressure measurements than the placebo group throughout the day and at night. Adverse effects, especially gastrointestinal (nausea, diarrhea and abdominal pain), were more frequent in the metformin group (Table 5).

Discussion:

This is the first randomized clinical trial to evaluate the effect of metformin on TSH of individuals with SH without diabetes mellitus. In our trial, the use of metformin was not superior to placebo in reducing TSH or inducing an euthyroid state.

A systematic review by Lupoli and colleagues (7) compiled data from seven studies that evaluated the effect of metformin on the TSH. In this review, only two studies included patients with SH (9)(13). The Capelli study was a cohort of TSH dosage before and after metformin in type 2 diabetic patients (9). The Morteza study was a non-randomized placebo controlled clinical trial in women with polycystic ovary syndrome (PCOS) (13). Since there can be spontaneous TSH decline in individuals with hypothyroidism (12), such designs are not the best to answer if metformin can really reduce TSH levels. Moreover, as these studies showed only positive results, publication bias is a plausible hypothesis. In our study, there was a decrease and even normalization of TSH in the metformin group and the placebo group in a similar magnitude.

In a randomized controlled trial performed by Karimifar (14) in patients with pre-diabetes, the analysis of the subgroup of participants with TSH between 2.6 and 5.5 μIU/ml showed an apparent greater decrease in TSH after three months of metformin use. This study did not present analysis between the groups.

Our study showed the most appropriate design to answer this kind of question. However, the statistical power to accept the negative result as true was only 14.5 percent. We believe that the loss of power occurred due to the characteristics (individuals with *vs.* without diabetes) and follow-up (3 *vs.* 12 months) different from the population used to calculate the sample. Thus, the power of the study does not allow accepting the negative result as definitive. Furthermore, despite the randomization, the groups were different in terms of number of women. There is a possibility that the effect of metformin on TSH levels is sex dependent (15). Men and women also have different body compositions, and this may influence insulin sensitivity, a possible mechanism by which metformin can cause TSH reduction, since previous studies have evaluated individuals with varying degrees of insulin resistance (PCOS, pre-diabetic and diabetic). The limited size of our sample limits the subgroup analysis. Moreover, such analyses were not part of the initial planning.

Although not statistically significant, the blood pressure lowering effect of metformin assessed by ABPM was notorious. This secondary analysis was included in the study to explore a possible hypotensive effect of metformin, since it is associated with reduction of cardiovascular risk in patients with diabetes mellitus (16)(17). This hypotensive effect deserves better research in populations of diabetic, insulin resistant and hypertensive patients, because, in associtión with the increase in HDL and reduced weight also checked in our study, cardiovascular benefits of metformin observed in diabetic patients would be expected.

Echocardiographic analysis of diastolic function was included to try to measure any tissue effect of the improvement of hypothyroidism after treatment (6). As in Capelli study that assessed the effect of metformin on electrocardiographic variables (18), we found no statistically significant changes of cardiac variables of diastolic function determined by metformin.

The short follow-up period is the main limitation of the study. However, careful with methodological rigor makes it an exception among the studies that assessed the effect of metformin on the TSH levels to date. As previously mentioned, the evaluation of conditions that may have

spontaneous remission as SH requires well-designed intervention studies, since improvement in some parameters may be due to the natural history of the disease itself. Also the results of an unicenter study may limit its external validity.

Finally, we await the TRUST trial data (19), which will bring more information if treatment of SH can prevent relevant clinical outcomes. Despite the inconclusive results regarding TSH, the use of metformin in subjects with SH can have a positive effect on parameters such as body weight, HDL and blood pressure, so further studies will clarify potential benefits of its use in reducing cardiovascular risk in this patient profile.

Conclusion:

The use of metformin in subjects with subclinical hypothyroidism is not superior to placebo in reducing the TSH.

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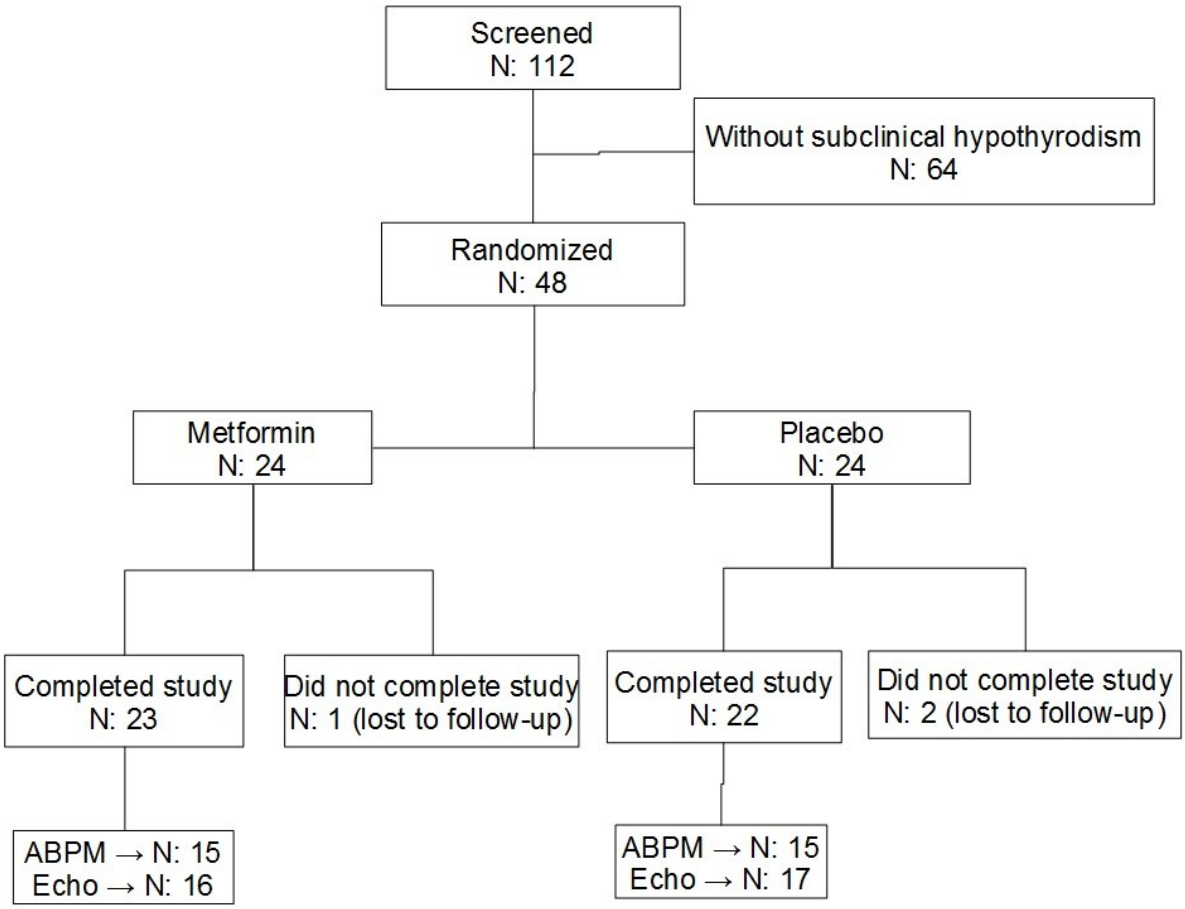
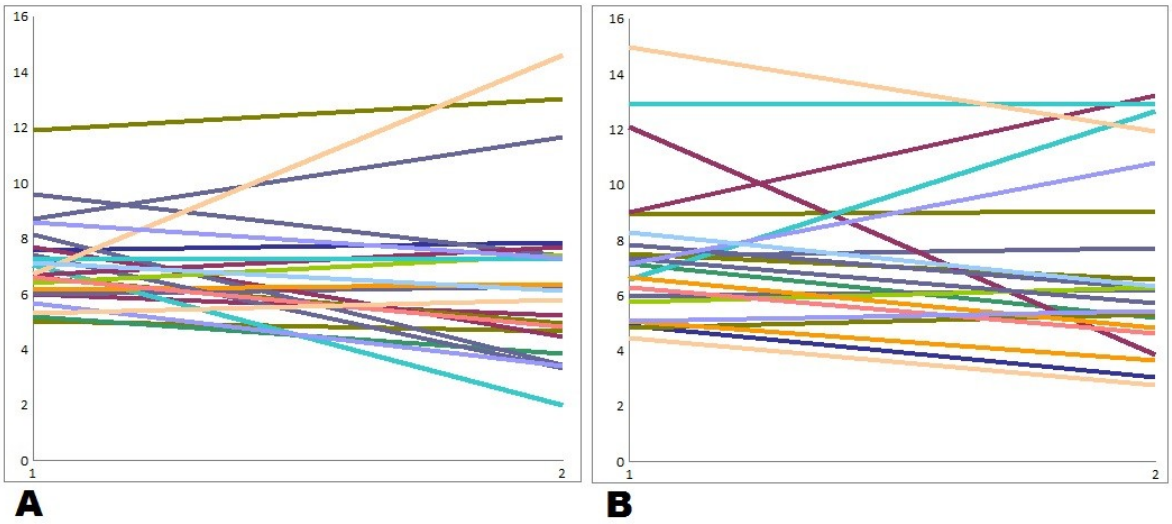
Figure 1. Study flowchart.



ABPM: ambulatory blood pressure monitoring, Echo: echocardiography.

Figure 2. Variation of TSH within groups.



A. TSH variation of subjects taking metformin. B. TSH variation of subjects taking placebo.

Table 1. Baseline characteristics of participants.

	Metformin (n = 24)	Placebo (n = 24)	p
Age (years)	42.7 (14.2)	42.9 (13.5)	0.95
Women n (%)	22 (91.7)	15 (62.5)	0.02
BMI (kg/m²)			
Men	28.2 (8.1)	29.7 (2.3)	
Women	26.9 (4.2)	27.5 (6.2)	
Total	26.9 (4.4)	28.3 (5.1)	0.35
AC (cm)			
Men	103.0 (14.1)	105.2 (6.8)	
Women	95.7 (9.1)	96.3 (15.7)	
Total	96.3 (9.4)	99.6 (13.6)	0.33
HR (bpm)	78.4 (11.8)	72.8 (12.6)	0.14
SBP (mmHg)	129.0 (15.3)	129.5 (15.4)	0.92
DBP (mmHg)	82.63 (7.9)	82.4 (8.7)	0.94
TSH screening (µIU/mL)	7.55 (2.17)	7.94 (2.59)	0.59
TSH (µIU/mL)	7.21 (1.61)	7.62 (2.62)	0.52
FT4 (ng/dL)	1.04 (0.16)	1.04 (0.12)	0.89
Anti-TPO$^{\epsilon}$ (IU/L)	99.7 (15.1 - 206)	130.0 (16.3 – 316.2)	0.69
Anti-TPO greater than 35 IU/L n (%)	13 (60.0)	12 (64.3)	0.81
Glucose (mg/dL)	90.6 (7.0)	93.3 (8.1)	0.24
Insulin (µIU/mL)	12.0 (7.2)	12.2 (6.0)	0.94
TC (mg/dL)	213.1 (33.3)	192.8 (36.7)	0.05
HDL-c (mg/dL)			

Men	41.0 (9.9)	42.4 (8.7)	
Women	62.4 (16.4)	59.5 (14.9)	
Total	60.7 (16.9)	53.1 (15.3)	0.11
LDL-c (mg/dL)	126.1 (28.5)	111.9 (38.3)	0.15
TG$^{\varepsilon}$ (mg/dL)	130.5 (99.0 – 164.5)	114.0 (97.7 – 169.2)	0.95
HOMA-IR	1.55 (0.93)	1.58 (0.77)	0.89

Variables are presented as mean and standard deviation or median and interquartile interval (ε). Statistical analysis: t test for independent variables, U test and chi-square test. BMI: body mass index, AC: abdominal circumference, HR: heart rate, SBP: systolic blood pressure, DBP: diastolic blood pressure, TC: total cholesterol, TG: triglycerides.

Table 2. Comparison between metformin and placebo groups 3 months after intervention.

	Metformin (n = 23)	Placebo (n = 22)	p
Euthyroid n (%)	5 (21.7)	4 (18.2)	0.76
BMI (kg/m²)			
Men	28.2 (7.9)	29.7 (2.2)	
Women	26.7 (3.8)	27.9 (6.1)	
Total	26.8 (4.1)	28.7 (4.9)	0.17
AC (cm)			
Men	100.5 (13.4)	105.0 (5.8)	
Women	95.5 (7.4)	97.5 (14.8)	
Total	95.9 (7.8)	100.6 (12.3)	0.13
HR (bpm)	81.5 (12.3)	77.4 (10.2)	0.22
SBP (mmHg)	127.5 (11.0)	129.2 (15.0)	0.66
DBP (mmHg)	83.5 (7.8)	82.8 (8.8)	0.79
TSH (µIU/mL)	6.48 (3.11)	7.02 (3.28)	0.57
FT4 (ng/dL)	1.08 (0.17)	1.07 (0.11)	0.83
Anti-TPO[ε] (IU/L)	98.4 (19.3 - 250.0)	143.5 (13.15 – 418.0)	0.38
Anti-TPO greater than 35 IU/L n (%)	11 (60.0)	13 (61.1)	0.95
Glucose (mg/dL)	89.3 (8.5)	93.0 (10.7)	0.20
Insulin (µIU/mL)	10.4 (6.1)	14.0 (7.6)	0.09
TC (mg/dL)	200.1 (29.1)	189.7 (31.1)	0.25
HDL-c (mg/dL)			
Men	40[#]	40.6 (6.3)	
Women	63.9 (17.2)	59.5 (14.9)	
Total	62.8 (17.5)	51.7 (15.3)	0.03

LDL-c (mg/dL)	113.3 (26.6)	108.2 (28.2)	0.54
TG$^{\varepsilon}$ (mg/dL)	109.0 (66.0 – 174.0)	136.0 (81.7 – 206.2)	0.10
HOMA-IR	1.32 (0.93)	1.80 (0.77)	0.08

The variables are presented as mean and standard deviation or median and interquartile interval (ε). Statistical analysis: t test for independent variables, U test and chi-square test. $p < 0.05$. BMI: body mass index, AC: abdominal circumference, HR: heart rate, SBP: systolic blood pressure, DBP: diastolic blood pressure, TC: total cholesterol, TG: triglycerides. (#) Data from only one individual.

Table 3. Evaluation of blood pressure measured by ABPM before and after intervention.

		Metformin (n=15)	Placebo (n=15)	Delta$^{\varepsilon}$	p
SBP 24h (mmHg)	Before	117.8 (15.2)	117.9 (9.7)		
	After	117.6 (12.8)	122.7 (15.5)	-5.1 (5.1)	0.33
DBP 24h (mmHg)	Before	72.2 (9.4)	70.4 (5.7)		
	After	71.6 (6.2)	73.7 (10.6)	-2.1 (3.1)	0.51
Daytime SBP (mmHg)	Before	121.0 (15.7)	120.4 (10.0)		
	After	119.3 (13.0)	124.3 (15.3)	-5.0 (5.1)	0.33
Daytime DBP (mmHg)	Before	75.0 (9.4)	72.6 (6.6)		
	After	73.4 (5.9)	75.7 (10.6)	-2.3 (3.1)	0.47
Night SBP (mmHg)	Before	106.7 (14.5)	107.5 (10.6)		
	After	112.1 (13.5)	116.3 (19.7)	-4.1 (6.2)	0.51
Night DBP (mmHg)	Before	61.5 (10.3)	61.4 (5.3)		
	After	64.6 (8.1)	66.1 (12.5)	-1.7 (3.8)	0.66

The variables are presented as mean and standard deviation. Statistical analysis: t test for independent variables. $p < 0,05$. (ε) Standard error of difference. ABPM: ambulatory blood pressure monitoring, SBP: systolic blood pressure, DBP: diastolic blood pressure. Delta is the difference between metformin and placebo groups after 3 months of intervention.

Table 4. Evaluation of diastolic function assessed by transthoracic echocardiography before and after the intervention.

		Metformin (n=16)	Placebo (n=17)	p
E/A	Before	1.45 (0.58)	1.55 (0.54)	
	After	1.45 (0.56)	1.37 (0.43)	0.65
E/e’	Before	6.63 (2.16)	6.22 (1.60)	
	After	6.71 (1.80)	6.57 (1.86)	0.82
IVRT (ms)	Before	94.79 (18.43)	98.63 (14.30)	
	After	97.77 (19.77)	104.56 (18.26)	0.31
DT (ms)	Before	171.24 (25.35)	191.36 (43.67)	
	After	181.96 (35.51)	174.21 (33.18)	0.52
MPI	Before	0.56 (0.18)	0.52 (0.19)	
	After	0.57 (0.22)	0.58 (0.19)	0.85
LVSVI (mL/m²)	Before	42.18 (5.33)	41.87 (8.64)	
	After	43.59 (5.04)	45.91 (10.26)	0.43

The variables are presented as mean and standard deviation. Statistical analysis: t test for independent variables. $p < 0,05$. E/A: E/A relation, E/e': E/e' relation, IVRT: isovolumetric relaxation time, DT: deceleration time, MPI: myocardial performance index , LVSVI: left ventricular stroke volume index. p refers to t test between metformin and placebo groups after 3 months of intervention.

Table 5. Adverse effects.

	Metformin (n=23)	Placebo (n=22)
Nausea	7 (30.4)	2 (9.1)
Diarrhea	8 (34.8)	0 (0.0)
Abdominal distension	4 (17.4)	5 (22.7)
Abdominal pain	4 (17.4)	1 (4.5)
Constipation	0 (0.0)	1 (4.5)
Flatulence	0 (0.0)	2 (9.1)
Headache	1 (4.3)	1 (4.5)
Dry mouth	2 (8.7)	0 (0.0)
Eructation/reflux	2 (8.7)	0 (0.0)
Muscle pain	1 (4.3)	0 (0.0)
Fatigue	2 (8.7)	0 (0.0)
Dizziness	3 (13.0)	1 (4.5)
Palpitations	0 (0.0)	1 (4.5)
Drownsiness	0 (0.0)	1 (4.5)
Sweating	1 (4.3)	0 (0.0)

Data are presented as n (%).

ARTIGO 2

Metformin effect on cardiovascular risk in non-diabetic users: a post-hoc analysis of two randomized controlled trials

Abbreviated title: Metformin and cardiovascular risk in non-diabetics

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Abstract

Background: The use of metformin in diabetic patients is associated with a reduction in cardiovascular events. Its global effect in metabolic profile may modify risk scores in non-diabetic populations.

Objectives: To evaluate the cardiovascular risk estimated through the Framingham and ACC/AHA 2013 risk scores in non-diabetic individuals.

Methods: One hundred subjects with a diagnosis of arterial hypertension and 48 subjects diagnosed with subclinical hypothyroidism were randomized to metformin 850 mg or placebo twice daily and were followed for two and three months, respectively, in two clinical trials. The cardiovascular risk in 10 years was calculated for each individual with the available clinical data.

Results: After intervention the Framingham score was 12.5% (5.3 – 21.6) vs. 10.6% (4.8 – 24.4), p=0.69 and ACC/AHA 2013 score was 6.1% (2.6 – 12.3) vs. 9.4% (3.0 – 15.7), p=0.45, for the metformin and placebo groups, respectively.

Conclusions: No cardiovascular risk reduction estimated by risk scores was detected with the use of metformin in subjects taking the drug during the evaluation period.

Background

Metformin use by individuals diagnosed with type 2 diabetes mellitus is associated with a reduction in cardiovascular events and mortality (1). Part of this risk reduction may be explained by modest non-antihyperglycaemic effects of metformin on weight reduction, on increasing HDL cholesterol, on insulin sensitivity and on lowering blood pressure (BP) (2)(3). However, studies aiming to evaluate the reduction of cardiovascular risk in non-diabetic individuals are scarce. We present here a post-hoc analysis of two randomized clinical trials describing whether a possible cardiovascular

risk reduction with metformin use in non-diabetic subjects could be detected by non-invasive methods such as the Framingham (4) and ACC/AHA 2013 (5) risk scores.

Methods

Data from 148 subjects from two randomized, parallel, double-blind placebo-controlled clinical trials with up to three months follow-up that aimed to assess non-antihyperglycemic effects of metformin in non-diabetic subjects were included. From the study that evaluated the effect of metformin on BP in hypertensive individuals (RCT1) (Clinical Trial Registration: NCT 02072382 at clinicaltrials.gov) came 100 subjects, 48 of whom were randomized to metformin and 52 randomized to placebo. From the study that evaluated the effect of metformin on TSH levels of individuals with subclinical hypothyroidism (RCT2) (Clinical Trial Registration: RBR-6crdyx at Brazilian Registry of Clinical Trials) came 48 subjects, 24 of whom were randomized to metformin and 24 randomized to placebo.

The sample consisted of individuals of both sexes, older than 18 years (age limit of 65 years for RCT2), non-diabetic, with no previous cardiovascular event and without contraindications to the use of metformin, from the South of Brazil. Patients with at least two BP measurements at different times greater than 140/90 mmHg or who were already taking medication for hypertension were considered hypertensive. Individuals with at least two TSH measurements above the upper limit of normality with free T4 within the reference range were considered as having subclinical hypothyroidism.

Subjects received metformin or placebo, according to randomization, in same appearance capsules with 425 mg and in identical containers. They were instructed to take two capsules at lunch in the first three days and two more capsules at dinner beginning in the fourth day, totalizing a dose of 1700 mg per day. If there were adverse effects that precluded the taking of the medication, the

participants were instructed to call the researcher. On these occasions, the dose was reduced to 425 mg per day with lunch, with an increase of 425 mg every three days until the highest tolerated dose.

In clinical consultation, after a brief questionnaire, the patient was weighed on a digital scale with a sensitivity of 100 grams and measured in fixed length stadiometer graduated in millimeters. The waist circumference was measured with the patient standing, at the end of expiration, with the inextensible tape parallel to the ground, passing just above the iliac crests and below the umbilicus. Blood pressure was measured after 15 minutes of rest, with the patient seated, with the feet flat on the floor, using an automated device (OMRON model HEM 742, Kyoto, Japan) with appropriate size cuff for arm circumference. Patients with arm circumference ≥ 33 cm used cuff 32 x 42 cm and with lower values, 24 x 32 cm. The average of two measures of BP in the dominant arm was considered.

The following parameters were analyzed after 12 hours of fasting for both trials: glucose (enzyme hexokinase Bioclin , Belo Horizonte, Brazil), total cholesterol (enzymatic colorimetric, Advia 1800, Erlangen, Germany), HDL cholesterol (enzymatic colorimetric, Advia 1800, Erlangen, Germany), and triglycerides (enzymatic colorimetric, Advia 1800, Erlangen, Germany).

The randomization sequence was made with the software V2.0 Random Allocation in blocks of four participants by an external researcher. After the generation of the sequence, the bottles of the same appearance containing metformin or placebo were numbered sequentially by an external researcher responsible for the acquisition of medication/placebo. The bottles numbered sequentially were then made available for the researcher responsible for screening and clinical evaluation of participants. After the screening of the participants, the bottle was delivered respecting preestablished numerical sequence.

The participants, the researcher who clinically evaluated in addition to the team responsible for the collection and analysis of biochemical tests were blind to the procedure.

After the conclusion of both trials, the Framingham (4) and ACC/AHA 2013 (5) risk scores were calculated for each subject before and after the intervention.

The data were presented as mean and standard deviation or median and confidence intervals, as appropriate. Comparisons between groups of interest were made through Student t test for independent variables or using non-parametric tests (U test), as appropriate. The relationship between categorical variables was evaluated by chi-square test. The level of significance was 5%. The analyses were performed with SPSS 18.0 (SPSS, Chicago) and followed the intention to treat (ITT) principle. Both studies were approved by the Ethics committee of the responsible institution and are registered (see text).

Results

Clinical and laboratory characteristics of the participants at baseline are shown in Table 1. The groups were similar in all aspects. The majority of the population studied was women, white and hypertensive. After the intervention, the cardiovascular risk assessed through the Framingham and ACC/AHA 2013 scores was not different between metformin and placebo groups (Table 2). Cardiovascular risk after the intervention was also not different within RCT1 [Framingham score 18.4% (9.4 – 25.3) vs. 15.9% (6.3 – 25.3), p=0.86 and ACC/AHA 2013 score 9.1% (3.9 – 13.9) vs. 11.3% (4.1 – 17.4), p=0.95, for the metformin and placebo groups, respectively] and RCT2 [Framingham score 4.5% (2.4 – 7.9) vs. 6.3% (1.8 – 10.6), p=0.43 and ACC/AHA 2013 score 2.0% (1.0 – 4.2) vs. 3.0% (2.3 – 8.3), p=0.13, for the metformin and placebo groups, respectively].

Discussion

Our analysis was not able to detect cardiovascular risk reduction assessed by the Framingham (4) and ACC/AHA 2013 (5) scores in metformin users, although evidence points to a positive effect of its use on BP and on the lipid profile (2)(3).

Despite statistically significant and clinically important reductions in cardiovascular disease morbidity and mortality, some optimally treated patients will experience one or more adverse outcomes. In multivariable analyses, the significant factors associated with residual risk were older age, increased body-mass index, male gender, hypertension, diabetes, baseline apolipoprotein B, and blood urea nitrogen (6). So, a reduction in cardiovascular risk assessed by the Framingham (4) and ACC/AHA 2013 (5) scores would be expected with metformin use, as both scores take into account total cholesterol, HDL cholesterol and systolic BP to estimate risk. However, our analysis was not able to detect this risk reduction.

The small sample size and short follow-up period were limitations of our study. Considering that metformin effects on lipid profile and BP are modest, we calculated that a sample size of 1960 individuals would be able to show a positive effect, considering a difference of 1% between the groups for the ACC/AHA 2013 score, 20% beta error and 5% bilateral alpha.

Another limitation was that the sample consisted of two different populations with different cardiovascular risks. This may have hampered adequate stratification of the patients with a greater chance of clinically benefiting from positive non-anti-hyperglycemic effects of metformin use.

Finally, perhaps the cardiovascular risk assessment through the Framingham and ACC/AHA 2013 scores is not able to detect pleiotropic effects of metformin (7), so studies that assess hard outcomes are also needed.

Conclusions

The use of the Framingham and ACC/AHA 2013 scores may not be able to detect cardiovascular risk reduction in non-diabetic subjects using metformin.

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Table 1. Baseline characteristics of participants.

	Metformin n=72	Placebo n=76	p
Age (years)	52.4 (13.3)	52.4 (12.9)	0.98
Male n (%)	23 (31.9)	33 (43.8)	0.14
Causasian n (%)	69 (95.8)	72 (94.5)	0.71
Smoker n (%)	4 (5.6)	7 (9.6)	0.67
Hypertensive n (%)	69 (95.8)	72 (94.5)	0.71
Weight (kg)	76.8 (13.9)	78.8 (16.8)	0.45
BMI (kg/m2)	28.5 (4.8)	28.6 (5.4)	0.90
AC (cm)	95.9 (10.2)	95.6 (13.9)	0.90
Glucose (mg/dL)	94.3 (12.4)	96.9 (16.4)	0.29
TC (mg/dL)	199.2 (33.2)	204.7 (43.5)	0.40
HDL-c (mg/dL)	49.2 (14.5)	48.2 (13.0)	0.66
TG (mg/dL)#	132.5 (99.2 – 168.2)	114.0 (93.0 – 174.0)	0.41
SBP (mmHg)	137.5 (17.6)	135.7 (14.9)	0.51
DBP (mmHg)	84.9 (8.6)	85.1 (9.7)	0.88
HR (bpm)	76.5 (13.5)	74.4 (11.9)	0.31
Framingham score (%)#	11.7 (6.3 – 24.8)	11.7 (6.3 – 21.6)	0.85
ACC/AHA 2013 score (%)#	6.5 (2.5 – 12.0)	7.6 (2.7 – 15.1)	0.90

The variables are presented as mean and standard deviation. The variables marked with (#) are presented as median and interquartile range. Statistical analysis: t test for independent variables, U test and chi-square test. BMI: body mass index, AC: abdominal circumference, HR: heart rate, SBP: systolic blood pressure, DBP: diastolic blood pressure, TC: total cholesterol, TG: triglycerides.

Table 2. Comparison between metformin and placebo groups after intervention.

	Metformin n=65	Placebo n=66	p
Weight (kg)	75.3 (13.4)	78.3 (16.4)	0.26
BMI (kg/m2)	27.9 (4.6)	28.0 (6.1)	0.88
AC (cm)	94.9 (9.5)	96.9 (16.5)	0.41
Glucose (mg/dL)	93.2 (12.6)	95.5 (14.7)	0.34
TC (mg/dL)	195.3 (32.8)	204.7 (43.3)	0.16
HDL-c (mg/dL)	49.5 (14.2)	48.4 (12.4)	0.63
TG (mg/dL)#	117.0 (87.0 – 170.5)	114.0 (84.5 – 189.0)	0.93
SBP (mmHg)	135.9 (16.2)	133.8 (15.4)	0.46
DBP (mmHg)	84.2 (9.5)	83.7 (9.6)	0.75
HR (bpm)	77.6 (13.4)	75.1 (12.8)	0.29
Framingham score (%)#	12.5 (5.3 – 21.6)	10.6 (4.8 – 24.4)	0.69
ACC/AHA 2013 (%)#	6.1 (2.6 – 12.3)	9.4 (3.0 – 15.7)	0.45

The variables are presented as mean and standard deviation. The variables marked with (#) are presented as median and interquartile range. Statistical analysis: t test for independent variables, U test and chi-square test. BMI: body mass index, AC: abdominal circunference, HR: heart rate, SBP: systolic blood pressure, DBP: diastolic blood pressure, TC: total cholesterol, TG: triglycerides. The trial evaluating the effect of metformin in hypertensive individuals was two months in duration. The trial evaluating the effect of metformin in subjects with subclinical hypothyroidism was three months in duration.

CONCLUSÕES

A metformina é um fármaco com grande tempo de uso dentro da prática clínica e com potencial de ganhar outras indicações além do diabetes. Em indivíduos com hipotireoidismo subclínico, o uso da metformina não foi capaz de promover redução significativa nos níveis de TSH, além de não promover melhora expressiva nos parâmetros clínicos e laboratoriais avaliados, com exceção de discreta elevação do colesterol HDL. Já na avaliação conjunta dos indivíduos com hipotireoidismo subclínico somados aos indivíduos com hipertensão arterial sistêmica, o uso da metformina não foi capaz de reduzir o risco cardiovascular medido através de escores de risco não invasivos.

A partir dos dados deste trabalho, pode-se inferir que os ditos efeitos não antihiperglicêmicos da metformina provavelmente são modestos, já que precisariam de tamanho de amostra maior para serem demonstrados. No entanto, dentro do contexto de busca por novas terapêuticas para redução do risco cardiovascular residual, o resgate de opções seguras e acessíveis é bem-vindo e deve ser priorizado. Mais estudos com diferentes populações de indivíduos não diabéticos e com diferentes perfis de risco precisam ser realizados. Assim, o perfil de paciente com maior probabilidade de apresentar benefício do uso de metformina poderá ser identificado, e o tamanho do benefício poderá ser medido de maneira mais acurada.