Metformin does not attenuate the acute insulin-sensitizing effect of a single bout of exercise in individuals with insulin resistance

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Abstract Combining metformin and exercise is recommended for the treatment of insulin resistance. However, it has been suggested that metformin blunts the insulin-sensitizing effects of exercise. We evaluated in a group of insulin-resistant patients the interactions between exercise and their daily dose of metformin. Ten insulin-resistant patients underwent insulin sensitivity assessment using intravenous glucose tolerance test after an overnight fast in the following conditions: (1) after taking their habitual morning dose of metformin (MET), (2) after 45 min of high intensity interval exercise having withheld 24 h from metformin (EX), and (3) after their habitual metformin dose plus an identical exercise bout (MET + EX). During the exercise trials (EX and MET + EX), energy expenditure and substrate oxidation were assessed by indirect calorimetry. In addition, 12-h postprandial blood glucose was measured in all three trials. Insulin sensitivity was similar between MET and EX [4.0 ± 0.8 and $4.1 \pm 0.7 \times 10^{-4} \text{ min}^{-1} (\mu\text{U mL})^{-1}$; $P = 0.953$] but was 43 % higher than both MET and EX after MET + EX (NS; $P = 0.081$). Blood glucose disappearance rate was higher after MET + EX than after MET or EX trials (1.7 ± 0.2 ,

1.0 ± 0.1 , and $1.2 \pm 0.1 \text{ % min}^{-1}$, respectively; $P = 0.020$). There was no difference in postprandial blood glucose concentration after the three meals that followed the trials ($P = 0.446$). Exercise energy expenditure was 9 % higher during MET + EX than during EX ($P = 0.015$) partly due to increased carbohydrate oxidation. Our data suggest that habitual metformin treatment in insulin-resistant patients does not blunt the acute insulin-sensitizing effects of a single bout of exercise that on the contrary, tends to enhance it.

Introduction

Insulin resistance is a metabolic abnormality commonly found in obese patients linked to metabolic syndrome and type 2 diabetes [1]. Metformin is a widespread prescribed medication indicated to treat insulin resistance. In patients with type 2 diabetes, oral intake of metformin is indicated as the first line of treatment in combination with lifestyle changes (mainly diet and exercise) to regain glycemic control [2]. In sort, metformin (dimethylbiguanide) is an efficacious antidiabetic agent increasing insulin sensitivity by 10–30 % [3–5]. The precise mechanism of action of metformin is not completely understood. However, it has been suggested that it works through activation of 5-AMP-activated protein kinase (AMPK) [6]. Alternatively, metformin may help to regain glycemic control by inhibition of hepatic endogenous glucose production [7] and controlling the inflammatory response.

A single bout of exercise enhances insulin-stimulated glucose uptake in both, healthy individuals [8] and those who are insulin resistant [9, 10]. Exercise improves insulin sensitivity mainly by triggering glucose transporter (i.e.,

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GLUT4) translocation to the membrane through mechanisms either related or unrelated to insulin signaling [11]. Although exercise and metformin could both target AMPK, it is not well established, if its combination has redundant, additive, or counteracting actions on insulin sensitivity improvements. Sharoff and coworkers [12] recently found that metformin treatment alone or in combination with a bout of exercise did not increase whole-body insulin sensitivity or AMPK activity. This study suggests that metformin treatment renders exercise less effective for improving insulin resistance. Nevertheless, metformin in combination with exercise is recommended in the current guidelines for prevention and treatment of type 2 diabetes [2], and thus, more studies are required that confirm or deny the possible interference effect of metformin on exercise.

While exercise and metformin are two important therapies for insulin resistance, most of the available literature proposes that metformin intake blunts the insulin-sensitizing effects of exercise [12, 13]. In these studies, the participants initiated metformin treatment with the experiment, and metformin consumption was maintained only for 3–4 weeks before testing. In an attempt to compensate reduced treatment time, in these studies, metformin was ingested at a high dose (i.e., 2,000 mg day⁻¹). We think that it is important to study whether metformin inhibits the insulin-sensitizing effects of exercise in patients undergoing chronic metformin treatment (>6 months) at the doses habitually prescribed for this population. Therefore, the aim of this study was to determine the separated and combined effects of exercise and metformin on insulin sensitivity in a group of insulin-resistant participants chronically medicated with metformin.

Materials and methods

Participants

Ten obese, insulin-resistant participants (8 type 2 diabetes patients and two impaired fasting glucose patients according to the current standards [2]), under metformin treatment for at least 6 months before the experimental trials, who were enrolled in a high intensity interval exercise program participated in the study (six women and four men; characteristics on Table 1). Subjects were recruited by verbal requesting during the group habitual exercise sessions. After detailed information about the benefits and risks involved with their participation in the study all gave their written informed consent to participate in the investigation which was approved by the local Hospital's Ethics Committee and conformed to the latest revision of the Declaration of Helsinki.

Table 1 Participant's characteristics; values are mean $\pm$ SEM

Age (years)	52.9 $\pm$ 2.3
BMI (kg m ⁻²)	35.6 $\pm$ 1.9
VO _{2peak} (mL kg ⁻¹ min ⁻¹)	22.1 $\pm$ 1.9
Waist perimeter (cm)	106.8 $\pm$ 3.3
Fasting blood glucose (mmol L ⁻¹)	7.9 $\pm$ 0.69
Fasting blood insulin (pmol L ⁻¹)	88.9 $\pm$ 11.1
HOMA-IR	4.5 $\pm$ 0.7
HbA1c (%) (mmol mol ⁻¹)	6.7 $\pm$ 0.2 (50 $\pm$ 1.5)
Metformin daily dose (mg)	1,307 $\pm$ 220

Preliminary testing

All volunteers underwent medical screening to exclude individuals with symptoms or signs of cardiorespiratory disease. Prior to the start of the experiment, participants underwent a cycling graded exercise test until volitional exhaustion in an electrically braked cycle ergometer (Ergoselect 200, Ergoline, Germany). Integrated standard 12-lead ECG (Quark T12, Cosmed, Italy) and blood pressure were monitored in every stage to ensure that all subjects had a normal cardiovascular response to exercise. During the maximal test, O₂ consumption was measured by indirect calorimetry (Quark B², Cosmed, Italy), and peak oxygen consumption (VO_{2peak}) and peak heart rate (HR_{peak}) were assessed.

Experimental design

All participants underwent three experimental trials in random counterbalanced order with a separation of at least 1 week between them. Trial started in the morning after an overnight fast that followed a standardized dinner (i.e., 714 kcal, 75 % from carbohydrate, 6 % from fat, and 19 % from protein). Participants were asked to refrain from exercise for at least 72 h prior to each trial since this has been shown to be enough time for remittance of the acute insulin sensitivity effects of a bout of high intensity interval exercise [14]. Participants ingested 500 mL of bottled water upon awakening to promote euhydration. A 50-min-long intravenous glucose tolerance test (i.e., IVGTT) was performed to measure peripheral insulin sensitivity as proposed by Tura et al. [15] (i.e., C_{SI}) in three experimental conditions; (1) 1 h after the usual morning dose of metformin (MET), (2) 1 h after the usual morning dose of metformin plus one bout of high intensity interval exercise (MET + EX), (3) after one bout of high intensity interval exercise with 24-h withdrawal from metformin (EX). After the trials, subjects were provided with portable glucose monitors (Free Style Optimum, Abbott, UK) and trained to obtain blood glucose measures 2 h after a standardized breakfast, lunch, and dinner. For the trial's day, breakfast,

lunch, and dinner were provided to the subjects in individualized bags (1,893 kcal, 65 % carbohydrates, 28 % fat, and 17 % protein). Each participant filled out a meal log to aid in the replication of their diet during the 24 h before the trials.

Experimental trials

Upon participant's arrival to the laboratory, nude body weight (Hawk, Mettler Toledo, USA) was measured. Then, the intravenous catheterization was performed and participants rested 20 min in supine position before extraction of baseline blood samples which was followed by the IVGTT. For the trials MET + EX and EX, exercise was performed before IVGTT and consisted on 10-min warm-up period at a workload that elicited 70 % HR_{peak} measured during the preliminary test, followed by 4×4 min intervals at 90 % of HR_{peak} interspersed with 3-min active recovery at 70 % HR_{peak} to finish with a 5-min cooldown period at 70 % HR_{peak} for a total of 43 min of exercise [16]. After exercise, subjects rested supine for 30 min to allow blood volume to return to pre-exercise values. Then, the baseline blood sample was collected and the IVGTT was performed.

Exercise measures

During both exercise trials (MET + EX and EX), breath-by-breath oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were measured using indirect calorimetry (Quark B², Cosmed, Italy), starting at the end of the warm-up, and following during the first and the third high intensity interval bout and their correspondent active recovery period. Out of these measurements, substrate oxidation and energy expenditure were calculated according to Jeukendrup and Wallis [17], and Brower [18], respectively. Heart rate was recorded at 5-s intervals during exercise using a heart rate monitor (RS400, Polar, Finland).

Insulin sensitivity

The intravenous glucose tolerance test (IVGTT) was performed with a glucose load of 0.5 g kg^{-1} body mass with a maximal dose of 35 g of glucose for subjects surpassing 70 kg of body weight. We used a 30 % glucose solution (Glucosada 30 %, Grifols, Spain) manually infused at an even pace during 3 min using two 60-mL syringes (BD Plastipak, Spain). These procedures comply with the recommendations of the Islet Cell Antibody Registered User's Group (ICARUS) [19]. A 20G intravenous catheter (BD Insyte, Becton–Dickinson, Spain) was inserted in an antecubital vein and a Luer-lock three-way stopcock attached (Vitroway, Vitromed Healthcare, India). Immediately after delivering the glucose load, the stopcock, catheter, and

vein were rapidly flushed with 10 ml of saline solution (Salina 0.9 %, Grifols, Spain). Following, every ten minutes (i.e., 10, 20, 30, 40, and 50-min), a 5-mL blood sample was obtained and the catheter flushed with 3 mL 0.9 % saline after every sample to ensure patency. We have recently shown that this IVGTT procedure has a day-to-day intraclass reproducibility of the insulin sensitivity assessments of 0.955 and a coefficient of variation of 13.4 % [20]. Using the 50-min IVGTT data, calculated insulin sensitivity index (C_{SI}) was obtained according with Tura et al. [15].

Blood samples analysis

Blood samples were mixed in tubes with 3 K-EDTA (Vacuette®, Greiner Bio-One GmbH, Austria) and centrifuged at 4,000 rpm during 10 min at 4 °C (MPW-350R, Med. Instruments, Poland) to obtain plasma that was stored at −80 °C. Glucose was measured with enzymatic glucose oxidase assay (Enzymatic Glucose Reagent, Thermoscientific, USA) using a multichannel spectrometer plate reader (Versamax, Molecular Devices, USA). Insulin concentration was analyzed by chemoluminescence (Architect System Insulin, Abbott Diagnostics Division, Germany).

Statistics

Shapiro–Wilk test revealed that data were normally distributed. Repeated measures ANOVA was used to identify differences between the three experimental trials (MET + EX, MET, and EX). After a significant F test (Geisser–Greenhouse correction for the assumption of sphericity), differences between means were identified using Tukey's HSD post hoc procedure. Only in the two exercise trials (MET + EX and EX), the exercise responses (energy expenditure, substrate oxidation and heart rate) were compared using Student's *T* test. Significance value was set at $P < 0.05$. Results were reported as mean $\pm$ SEM. All the tests were performed with SPSS for windows (Version 18, SPSS Inc., USA).

Results

Body weight and diet

Subjects' characteristics are shown in Table 1. Fasting blood glucose, glycosilated hemoglobin (HbA1c), and calculated insulin resistance (HOMA-IR) [21] confirmed the deregulated glycemic control in these subjects. Body weight did not differ between trials (90.7 ± 4.3 , 90.4 ± 4.3 , and 90.1 ± 4.4 kg for MET + EX, MET, and EX,

Fig. 1 Energy expenditure during exercise at the same workload in the metformin plus exercise (MET + EX) and exercise (EX) trials including substrate contribution. Carbohydrates oxidation (CH_{ox}) in white, and fat oxidation (FAT_{ox}) in black. Values are mean $\pm$ SEM. Asterisk different from MET + EX

Fig. 2 **a** Insulin sensitivity index from the intravenous glucose tolerance test (C_{SI}; Tura). **b** Rate of blood glucose disappearance during the IVGTT in the three experimental trials; metformin plus exercise (MET + EX), metformin alone (MET), and exercise alone (EX). Values are mean $\pm$ SEM. Asterisk different from MET + EX

Fig. 3 Mean blood glucose concentration measured after breakfast, lunch, and dinner during the 12 h that followed the experimental trials; metformin plus exercise (MET + EX), metformin alone (MET), and exercise alone (EX). Values are mean $\pm$ SEM

respectively; $P = 0.241$). Dietary records showed that participants adhered strictly to the indications and diet provided by the research team (data not shown).

Exercise workload, energy expenditure, and substrate oxidation

During both exercise trials the workload was 62 ± 10 watts during warm-up and the active recovery periods (70 %HR_{peak}), whereas the workload that elicited the 90 % HR_{peak} was 117 ± 18 watts in average. Heart rate was similar during both exercise trials (126 ± 5 and 124 ± 5 bpm for MET + EX and EX, respectively; $P = 0.326$). VO₂ was higher during MET + EX than during EX (1.46 ± 0.17 and $1.38 \pm 0.18 \text{ L min}^{-1}$ for MET + EX and EX, respectively; $P = 0.027$). However, respiratory exchange ratio was not different between trials (0.98 ± 0.02 and 0.97 ± 0.01 for MET + EX and EX, respectively; $P = 0.137$). Energy expenditure and carbohydrate oxidation were higher during MET + EX than during EX alone ($P = 0.015$ and $P = 0.010$, respectively; Fig. 1). On the other hand, fat oxidation was very low and not different between trials ($P = 0.410$).

Insulin sensitivity

Calculated insulin sensitivity index (C_{SI}) and glucose disappearance rate from the 50-min IVGTT data are shown in Fig. 2a. C_{SI} differences among trials did not reach statistical significance ($P = 0.081$) despite being 43 % higher in MET + EX than in both MET or EX trials, respectively (5.78 ± 1.47 , 4.04 ± 0.77 , and $4.07 \pm 0.75 \times 10^{-4} \text{ min}^{-1} (\mu\text{U mL})^{-1}$; Fig. 2a). Glucose disappearance rate was higher in MET + EX than in both MET and EX (1.7 ± 0.2 , 1.0 ± 0.1 , and $1.2 \pm 0.1 \text{ \% min}^{-1}$ for MET + EX, MET, and EX, respectively; $P = 0.020$; Fig. 2b).

Ambulatory blood glucose control

Capillary blood samples, obtained 2 h after breakfast, lunch, and dinner, were averaged, and those means were not statistically different between trials ($P = 0.452$; Fig. 3). However, in MET + EX, the blood glucose concentration mean was 6.0 and 5.7 % lower than during the MET and EX trials, respectively (119 ± 7.4 , 126.6 ± 6.7 , and $126.2 \pm 7.3 \text{ mg dL}^{-1}$, for MET + EX, MET, and EX).

284 Correlations

285 VO_2 , energy expenditure, carbohydrate oxidation rate, and
 286 workload at 90 % HR_{peak} correlated significantly with K_G
 287 ($r = 0.543$, $r = 0.524$, $r = 0.560$, and $r = 0.502$; $P < 0.05$,
 288 respectively), but not with C_{SI} ($r = 0.147$, $r = 0.131$,
 289 $r = 0.155$, and $r = 0.077$; $P > 0.05$, respectively).

290 Discussion

291 Metformin and exercise are considered compatible inter-
 292 ventions for insulin-resistance treatment [2]. However,
 293 recent data suggests that metformin blunts the insulin-
 294 sensitizing effects of exercise [12, 13]. We measured
 295 insulin sensitivity and 2-h postprandial blood glucose
 296 concentration after three meals in a group of insulin-
 297 resistant participants treated with metformin (≥ 6 months)
 298 enrolled in an exercise training program. Withdrawing
 299 metformin ingestion 24 h when half-life in plasma is 4–9 h
 300 [22] and refraining from exercise 72 h before trials [14]
 301 allowed us to study the isolated and combined effects of
 302 both interventions in these participants. We observed that
 303 metformin alone (MET; withdrawing exercise) and exer-
 304 cise alone (EX; withdrawing metformin) had similar
 305 effects on insulin sensitivity. However, when combined
 306 (i.e., MET + EX) glucose disappearance rate significantly
 307 increased in comparison with the EX alone or to the MET
 308 trial alone (Fig. 2b). In addition, in MET + EX, we found
 309 a 43 % increase in the insulin sensitivity index (C_{SI}) over
 310 the other trials that, however, did not reach statistical sig-
 311 nificance ($P = 0.081$; Fig. 2a). The results of the statistical
 312 analysis prevent us from concluding about any summative
 313 effects of MET + EX on insulin sensitivity. However, we
 314 can reasonably argue that metformin does not undermine
 315 the insulin-sensitizing effect of exercise in our particular
 316 experimental situation. Thus, the novel finding of this study
 317 is that the addition of exercise on top of chronic metformin
 318 treatment far from blunting the exercise insulin-sensitizing
 319 response [12, 13] tends to enhance it.

320 The effect of a bout of exercise on subjects taking
 321 metformin has been previously investigated. Sharoff et al.
 322 [12] recruited two matched groups of insulin resistant. One
 323 group received a daily dose of 2,000 mg of metformin
 324 2–3 weeks before a hyperinsulinemic euglycemic clamp
 325 (HEC). Four hours before the HEC, participants performed
 326 a bout of 40 min of exercise at 65 % $\text{VO}_{2\text{peak}}$. The other
 327 group of subjects performed the bout of exercise without
 328 the influence of metformin (control group). They observed
 329 improved insulin sensitivity in the placebo but not in the
 330 metformin group suggesting that the short-term metformin
 331 treatment attenuated the insulin-sensitizing effects of
 332 exercise. However, the group treated with metformin

started with baseline insulin sensitivity 50 % higher than
 the placebo group. This high baseline insulin sensitivity in
 the metformin group may have limited the chance for
 metformin or exercise increase an already high insulin
 sensitivity level. Magkos et al. [23] proposes that
 improvements of insulin sensitivity depend on the baseline
 insulin sensitivity. Thus, it is possible that the differences
 in the response after exercise between groups could be
 related to the different baseline insulin sensitivity.

Coinciding with Sharrof et al., Boulé et al. [13] also
 suggest that the improved glycemic response after exercise
 is impaired by metformin. They tested the glycemic
 response to incremental exercise after 28 days of metfor-
 min or a placebo treatment in type 2 diabetes patients. They
 found post-exercise reduction in blood glucose in the pla-
 cebo condition but not in the metformin condition. How-
 ever, no measure of insulin sensitivity was reported. In the
 same study, the authors reported an increased fat oxidation
 during the metformin plus exercise condition. By contrast,
 we found a 12 % increase in carbohydrate oxidation during
 the high intensity interval exercise bout ($P = 0.010$).
 Boulé et al. [13] exercise was preceded by breakfast and 20
 consecutive maximal leg extensions contractions. It is
 possible that breakfast and previous resistance exercise
 could have altered substrate metabolism, explaining the
 difference between our results.

In the studies that argue for a reductive effect of met-
 formin on exercise actions [12, 13], the dose of metformin
 provided was 53 % higher than the dose that our subjects
 chronically ingest (2,000 mg day⁻¹ in their studies vs.
 1,300 mg day⁻¹ in our subjects). It is counterintuitive to
 think that a higher dose could have produce lower insulin-
 sensitizing effect. However, some drugs when provided at
 the upper dosage range produce the opposite effect than
 intended (i.e., hormesis effect, stimulatory effect at low
 doses but inhibitory at high doses). Thus, it is possible that
 the differences in dosage may have played some role in the
 different findings between our and previous studies. The
 relevance of our findings in comparison with the available
 literature in this area arises from the fact that we tested
 insulin sensitivity with a reliable and valid method in a
 real-world scenario, where insulin-resistant subjects
 undergo metformin treatment by physician prescription. To
 avoid the potential confounding effect of between group
 differences, we chose a crossover design using the same
 group of participants in standardized conditions (diet and
 previous exercise) in our three experimental trials. With
 our design, no blunting effect of metformin was observed.

In an attempt to follow the glycemic control effect of
 our treatments, participants self-monitored their blood
 glucose concentration 2 h after a standardized breakfast,
 lunch, and dinner in each experimental condition
 (MET + EX, MET, and EX). Our objective was to test

whether the hypothesized blunting of metformin on the exercise improvements in insulin sensitivity [12, 13] occurred during the 12 h that followed our treatments. Our data did not show higher blood glucose concentration after any of the three meals in the MET + EX trial (Fig. 3). Conversely, the combination of metformin and exercise was associated with a 5.7 % reduction in 2-h postprandial blood glucose values that, however, did not reach statistical significance ($P = 0.446$). Thus, we do not observe that pharmacological treatment with metformin blunts neither the insulin-sensitizing effect of exercise, nor the postprandial blood glucose regulation when monitored during the 12 h that followed exercise.

In summary, our data suggest that chronic metformin treatment in insulin-resistant patients does not blunt the acute insulin-sensitizing effects of a single exercise bout. On the contrary, metformin treatment improves the rate of glucose disappearance (i.e., K_G , Fig. 2b) after exercise. Furthermore, metformin on top of exercise does not worsen the blood glucose response to a meal in comparison with exercise or metformin alone. Thus, the combined treatment of metformin and exercise seems to offer synergistic insulin-sensitizing effects in insulin-resistant patients undergoing metformin treatment. Our findings strongly imply that the clinical result of combining metformin and exercise treatment is positive in insulin-resistant patients.

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Conflict of interest None.

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