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 ± 0.7 × 10⁻⁴ min⁻¹ (μU mL)⁻¹; \( P = 0.953 \)] but was 43 % higher during MET than during 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 ± 0.1 % 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 insulinsensitizing 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.,
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 to 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 ± SEM

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SEM</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>52.9 ± 2.3</td>
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<tr>
<td>BMI (kg m^-2^)</td>
<td>35.6 ± 1.9</td>
</tr>
<tr>
<td>VO_{2peak} (mL kg^-1^ min^-1)</td>
<td>22.1 ± 1.9</td>
</tr>
<tr>
<td>Waist perimeter (cm)</td>
<td>106.8 ± 3.3</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol L^-1^)</td>
<td>7.9 ± 0.69</td>
</tr>
<tr>
<td>Fasting blood insulin (pmol L^-1^)</td>
<td>88.9 ± 11.1</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>4.5 ± 0.7</td>
</tr>
<tr>
<td>HbA1c (% mmol mol^-1^)</td>
<td>6.7 ± 0.2 (50 ± 1.5)</td>
</tr>
<tr>
<td>Metformin daily dose (mg)</td>
<td>1,307 ± 220</td>
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</tbody>
</table>

#### 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, O_2consumption was measured by indirect calorimetry (Quark B^2^, 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_SG) 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 Optimun, 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 % \( \text{HR}_{\text{peak}} \) measured during the preliminary test, followed by 4 × 4 min intervals at 90 % of \( \text{HR}_{\text{peak}} \) interspersed with 3-min active recovery at 70 % \( \text{HR}_{\text{peak}} \) to finish with a 5-min cooldown period at 70 % \( \text{HR}_{\text{peak}} \) 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 (\( \text{VO}_2 \)) and carbon dioxide production (\( \text{VCO}_2 \)) were measured using indirect calorimetry (Quark B\( ^2 \), 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 Plasticpak, 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 (\( \text{C}_\text{SI} \)) was obtained according to Tura et al. [15].

Blood samples analysis

Blood samples were mixed in tubes with 3 K-EDTA (Vacuette\textsuperscript®*, 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 \(-80^\circ\text{C}\). 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–Wilks 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 ± 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, respectively).
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 %HRpeak), whereas the workload that elicited the 90 % HRpeak 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; \( P = 0.326 \)). \( \text{VO}_2 \) was higher during MET + EX than during EX (1.46 ± 0.17 and 1.38 ± 0.18 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\textsubscript{SI}) and glucose disappearance rate from the 50-min IVGTT data are shown in Fig. 2a. C\textsubscript{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 ± 0.75 × 10\(^{-4}\) min\(^{-1}\) (\( \mu \text{U} \text{mL}^{-1} \)); 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 ± 0.1 % 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 ± 7.3 mg dL\(^{-1}\), for MET + EX, MET, and EX).
Correlations

\[ \text{VO}_2, \text{ energy expenditure, carbohydrate oxidation rate, and workload at 90}\% \text{ HR}_{\text{peak}} \text{ correlated significantly with } K_g \]

\[ (r = 0.543, r = 0.524, r = 0.560, \text{ and } r = 502; P < 0.05, \text{ respectively}), \text{ but not with } C_{\text{SI}} \]

\[ (r = 0.147, r = 0.131, r = 0.155, \text{ and } r = 0.077; P > 0.05, \text{ respectively}). \]

Discussion

Metformin and exercise are considered compatible interventions for insulin-resistance treatment [2]. However, recent data suggests that metformin blunts the insulin-sensitizing effects of exercise [12, 13]. We measured insulin sensitivity and 2-h postprandial blood glucose concentration after three meals in a group of insulin-resistant participants treated with metformin (>6 months) enrolled in an exercise training program. Withdrawing metformin ingestion 24 h when half-life in plasma is 4–9 h [22] and refraining from exercise 72 h before trials [14] allowed us to study the isolated and combined effects of both interventions in these participants. We observed that metformin alone (MET; withdrawing exercise) and exercise alone (EX; withdrawing metformin) had similar effects on insulin sensitivity. However, when combined (i.e., MET + EX) glucose disappearance rate significantly increased in comparison with the EX alone or to the MET trial alone (Fig. 2b). In addition, in MET + EX, we found a 43 % increase in the insulin sensitivity index (C_{\text{SI}}) over the other trials that, however, did not reach statistical significance \((P = 0.081; \text{Fig. 2a}). \text{ The results of the statistical analysis prevent us from concluding about any summative effects of MET + EX on insulin sensitivity. However, we can reasonably argue that metformin does not undermine the insulin-sensitizing effect of exercise in our particular experimental situation. Thus, the novel finding of this study is that the addition of exercise on top of chronic metformin treatment far from blunting the exercise insulin-sensitizing response [12, 13] tends to enhance it.}

The effect of a bout of exercise on subjects taking metformin has been previously investigated. Sharoff et al. [12] recruited two matched groups of insulin resistant. One group received a daily dose of 2,000 mg of metformin 2–3 weeks before a hyperinsulinemic euglycemic clamp (HEC). Four hours before the HEC, participants performed a bout of 40 min of exercise at 65 % \text{VO}_{2\text{peak}}. The other group of subjects performed the bout of exercise without the influence of metformin (control group). They observed improved insulin sensitivity in the placebo but not in the metformin group suggesting that the short-term metformin treatment attenuated the insulin-sensitizing effects of 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 Sharoff 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 metformin or a placebo treatment in type 2 diabetes patients. They found post-exercise reduction in blood glucose in the placebo condition but not in the metformin condition. However, 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 metformin 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.

References


