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Effects of chronic metformin treatment on training adaptations in men and women with hyperglycemia: A prospective study

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Abstract

Objective: This study aimed to determine whether chronic metformin use interferes with the improvements in insulin resistance (IR) and cardiorespiratory fitness with aerobic training in people with hyperglycemia and metabolic syndrome (MetS).

Methods: A total of 63 middle-aged (53 [7] years) individuals with MetS and obesity (BMI = 32.8 [4.5] kg/m^2) completed 16 weeks of supervised high-intensity interval training (3 d/wk, 43 min/session). Participants were either taking metformin (EXER+MET; $n = 29$) or were free of any pharmacological treatment for their MetS factors (EXER; $n = 34$). Groups were similar in their initial cardiorespiratory fitness (maximal oxygen uptake [VO_{2MAX}]), age, percentage of women, BMI, and MetS factors (z score). The effects of exercise training on IR (homeostatic model assessment of insulin resistance [HOMA-IR]), MetS z score, VO_{2MAX}, maximal fat oxidation during exercise, and maximal aerobic power output were measured.

Results: Fasting insulin and HOMA-IR decreased similarly in both groups with training (EXER+MET: −4.3% and −10.6%; EXER: −5.3% and −14.5%; p value for time = 0.005). However, metformin use reduced VO_{2MAX} improvements by half (i.e., EXER+MET: 12.7%; EXER: 25.3%; p value for time × group = 0.012). Maximal fat oxidation during exercise increased similarly in both groups (EXER+MET: 20.7%; EXER: 25.3%; p value for time = 0.040). VO_{2MAX} gains were not associated with HOMA-IR reductions (EXER+MET: $r = −0.098$; $p = 0.580$; EXER: $r = −0.255$; $p = 0.182$).

Conclusions: Metformin use was associated with attenuated VO_{2MAX} improvements but did not affect fasting IR reductions with aerobic training in individuals with hyperglycemia and high cardiovascular risk (i.e., MetS).

INTRODUCTION

Metabolic syndrome (MetS) is characterized by central obesity, dyslipidemia, hypertension, and hyperglycemia [1], which increase cardiovascular disease risk and all-cause mortality [2]. Some authors have supported insulin resistance (IR) as the triggering factor for MetS [3,4]. IR by itself is a predictor of disease, disability, and all-cause mortality [5]. On the other hand, longitudinal studies have shown that moderate and high levels of cardiorespiratory fitness ([CRF] assessed by maximal oxygen uptake [VO_{2MAX}]) confer protection

See Commentary, pg. 1141.

against developing type 2 diabetes mellitus and its related hyperglycemia (6,7). It has been shown that aerobic exercise training that improves CRF also reduces IR (8). However, the concomitant loss of body weight with exercise training may be partially responsible for the IR improvements (6,9). Body weight loss, and specifically visceral abdominal fat loss, reduces cardiovascular risk (10); therefore diet, in conjunction with aerobic exercise, is recommended to regain glycemic control in people with IR or hyperglycemia (11).

Metformin (i.e., 1, 1-dimethyl-biguanide) is the most-prescribed oral medicine in the world to reduce hyperglycemia. The primary target tissue of metformin is the liver, but it also influences metabolic processes in skeletal muscle, adipose tissue, the intestines, the brain, and the cardiovascular system (12). Although the main metabolic effect of metformin may be a reduction in hepatic glucose production by inhibiting gluconeogenesis (13), other authors have suggested that metformin also increases skeletal muscle glucose uptake (14). Metformin has been found to reduce mitochondrial respiration by inhibiting complex I of the electron transport chain (ETC) (15), which could result in energy unbalance. Through this mechanism, but also independently of the ETC, metformin activates AMP-activated protein kinase (AMPK) (16), which phosphorylates and inhibits the mechanistic target of rapamycin complex 1 (mTORC1). AMPK and mTORC1 are major cellular regulators of lipid and glucose metabolism (17); therefore, inhibition of mTORC1 could mediate the stimulation of carbohydrate metabolism with metformin.

Metformin inhibition of ETC complex I could limit the development of mitochondrial respiration with exercise training. It has been reported that metformin treatment blunts the exercise training improvements in VO_{2MAX} in young adults (18), older adults (19), and adults with prediabetes (20). Furthermore, Konopka et al. (19) found that metformin completely abolishes the exercise-induced improvements in mitochondrial respiration (i.e., complex I-linked respiration). Given the strong association between low CRF and IR (6), it is possible that training that increases VO_{2MAX} could alleviate IR. On the other hand, metformin treatment could limit training increases in VO_{2MAX} and, subsequently, improvements in IR with training (18-21). Conversely, if metformin actions on improving glucose metabolism are based on limiting mitochondrial function, the potentiation of mitochondrial development with exercise training may blunt metformin actions on glucose metabolism.

To our knowledge, there is only one study addressing the effects of metformin on training adaptations and MetS risk factors. In that study, 32 adults with glucose intolerance were separated into four groups (22), with two groups taking metformin and two groups completing exercise training for 12 weeks using continuous aerobic and resistance training. In this study, the groups taking metformin experienced initial body weight loss (4 kg), something that is typical when metformin prescription is first started. That body weight loss may have influenced the comparison between the groups that exercise trained with or without metformin. In this study, we propose to study the interactions between exercise training (specifically, high-intensity interval training [HIIT]) and metformin in a sample of individuals chronically medicated with metformin. In this way, we

Study Importance

What is already known?

► Exercise and metformin are first-line therapeutic options for the treatment of prediabetes and type 2 diabetes.
► Nevertheless, there is evidence of interfering effects of metformin on the insulin-sensitizing and cardiorespiratory fitness improvements induced by exercise training.

What does this study add?

► Chronic metformin treatment was associated with blunted gains in maximal oxygen uptake (VO_{2MAX}) with intense aerobic exercise training in people with metabolic syndrome and hyperglycemia.
► However, the associated blunted VO_{2MAX} development did not prevent exercise-induced improvements in fasting insulin resistance.

How might these results change the focus of clinical practice?

► Although fitness improvements may be blunted by the chronic use of metformin in people with metabolic syndrome, this does not hinder the clinical benefits of exercise training on reducing fasting insulin resistance; therefore, clinical practice should include exercise advice.

will avoid the confusing effect of metformin initially lowering body weight. Our hypothesis is that chronic metformin treatment would restrain the VO_{2MAX} increases induced by 16 weeks of HIIT and, in parallel, the expected reductions in IR in people with hyperglycemia and MetS.

METHODS

Participants

A total of 63 middle-aged (53 [7] years) volunteers (31 women and 32 men with overweight and obesity [BMI = 32.8 (4.5) kg/m^2] and MetS) completed the study. MetS was defined as the presence of three of the following five risk factors: elevated waist circumference (≥94 cm for men and ≥80 cm for women); elevated blood pressure (≥130 mm Hg for systolic and/or ≥85 mm Hg for diastolic); elevated fasting blood glucose (≥100 mg/dL); elevated triglycerides (TG; ≥150 mg/dL); and reduced high-density lipoprotein cholesterol (HDL-c; ≤40 mg/L for men and ≤50 mg/dL for women) (1). Participants were previously sedentary, as assessed by a 7-day International Physical Activity Questionnaire (IPAQ) (23), with less than 120 min/

wk of moderate-intensity activity (24). Exclusion criteria included the following: untreated cardiovascular disease (including severe hypertension, defined as systolic blood pressure ≥ 180 mm Hg and/or diastolic blood pressure ≥ 120 mm Hg); renal disease; or any disease associated with exercise intolerance. All individuals provided written, witnessed, informed consent in accordance with a protocol approved by the local Virgen de la Salud Hospital's Ethics Committee of Toledo (reference #170) and according to the Declaration of Helsinki.

Experimental design

This was a prospective intervention (exercise training) parallel group study (treated or untreated with metformin). The exercise training intervention took place from early October 2019 to February 2020. Investigators were blinded to group allocation during data collection and analysis. Volunteers were recruited and screened, and they completed the treatment and testing in the order presented in Figure 1, in compliance with Consolidated Standards of Reporting Trials (CONSORT). A total of 34 individuals with MetS under chronic metformin treatment (>6 months of treatment, 1,279 [489] mg/d) composed the metformin group (EXER+MET). Another 29 individuals with MetS who were not taking metformin, or any other pharmacological treatment, composed the control group (EXER).

At baseline and after 4 months of HIIT, participants attended the laboratory in the morning (7 AM to 9 AM) after 8 to 10 hours of overnight fasting and 48 hours after the last training bout. Participants were instructed to maintain their usual medication (i.e., EXER+MET group), physical activity, and dietary nutrition patterns during the duration of the study. Participants were not requested to record their diet because we were concerned that this might impact individuals' normal feeding pattern through a Hawthorne effect (25), potentially confounding the effects of exercise training on body composition assessment.

Exercise intervention

Participants completed 16 weeks of a supervised HIIT cycling program. HIIT sessions were composed of four 4-minute intervals at 90% of maximum heart rate (HR_{MAX}) interspersed with 3-minute active recovery at 70% HR_{MAX} plus warm-up and cooldown periods (26) for a total of 43 minutes. During all training sessions, HR was continuously displayed on a large screen (Seego Realtrack Systems, Almeria, Spain), and participants self-adjusted the workload to reach their individually prescribed percentage of HR_{MAX}. Monthly, during a regular training session, HR_{MAX} was reevaluated, and training workloads were adjusted accordingly to maintain training stimulus (26). The training monitoring program (Seego Realtrack Systems) recorded the time that individuals were in the target HR zone (i.e., 70%-90% HR_{MAX}) in each exercise session.

Clinical investigation

Before and after 16 weeks of training, participants arrived at the laboratory in the morning, well hydrated and after an overnight fast. Nude body weight (Hawk; Mettler Toledo, Columbus, Ohio), height (stadiometer; Seca 217; Seca GmbH, Hamburg, Germany), and waist circumference were assessed by the same researcher. Body composition was analyzed by bioelectrical impedance analysis (Tanita BC-418; Tanita Corp., Tokyo, Japan), and sectional analysis of right-leg fat-free mass (FFM) was used as an indirect measure of muscle gains with cycling HIIT. After 20 minutes of supine rest on a gurney, brachial resting blood pressure was measured after 15 minutes of supine rest using an electrocardiography (ECG) gated electro-sphygmomanometer (Tango; SunTech Medical Inc., Morrisville, North Carolina) as the average of three measurements. Afterward, 5 mL of blood was drawn from an antecubital vein for the determination of glucose, insulin, and lipids (i.e., TG and HDL-c). Last, metabolic and CRF assessments were carried out. After training, all testing was repeated 48 hours after the last training session to measure the chronic effects rather than the acute effects of the last bout of training.

Metabolic fitness and CRF

Maximal fat oxidation rate (FO_{MAX}) was assessed using a submaximal graded exercise test (GXT) on a calibrated cycle ergometer with breath-by-breath indirect calorimetry (Quark b2; COSMED srl, Rome, Italy) monitoring. Initial power output was set at 10 W for women and 30 W for men and was increased 10 or 15 W every 4 minutes for women and men, respectively (27). The last minute of each stage was averaged to calculate nonprotein respiratory quotient and fat oxidation rate (28). The FO_{MAX} test terminated when respiratory exchange ratio surpassed 1.0.

After resting and hydration, VO_{2MAX} was assessed using GXT, starting at 30 W for women and 50 W for men, with increases of 15 or 20 W for women and men, respectively, until volitional exhaustion. After a cooldown and 15 minutes of passive rest, a short (1-3 minutes) verification test was performed at 110% of the maximum workload (PO_{MAX}) reached during the GXT (29). During tests, a 12-lead standard ECG was continuously monitored (Quark T12, COSMED). The highest values of VO_2 and HR during GXT or verification test were determined as VO_{2MAX} and HR_{MAX} (29).

Blood analysis

Plasma glucose was analyzed using the glucose oxidase peroxidase method, with intra-interassay coefficient of variation (iCV) of 0.9% to 1.2%. HDL-c was analyzed using the accelerator selective detergent method (iCV = 1.7%-2.9%). TG was analyzed with the glycerol-3-phosphate oxidize method (iCV = 0.8%-1.7%). All the analyses were run in an automated Mindray BS 400 Chemistry
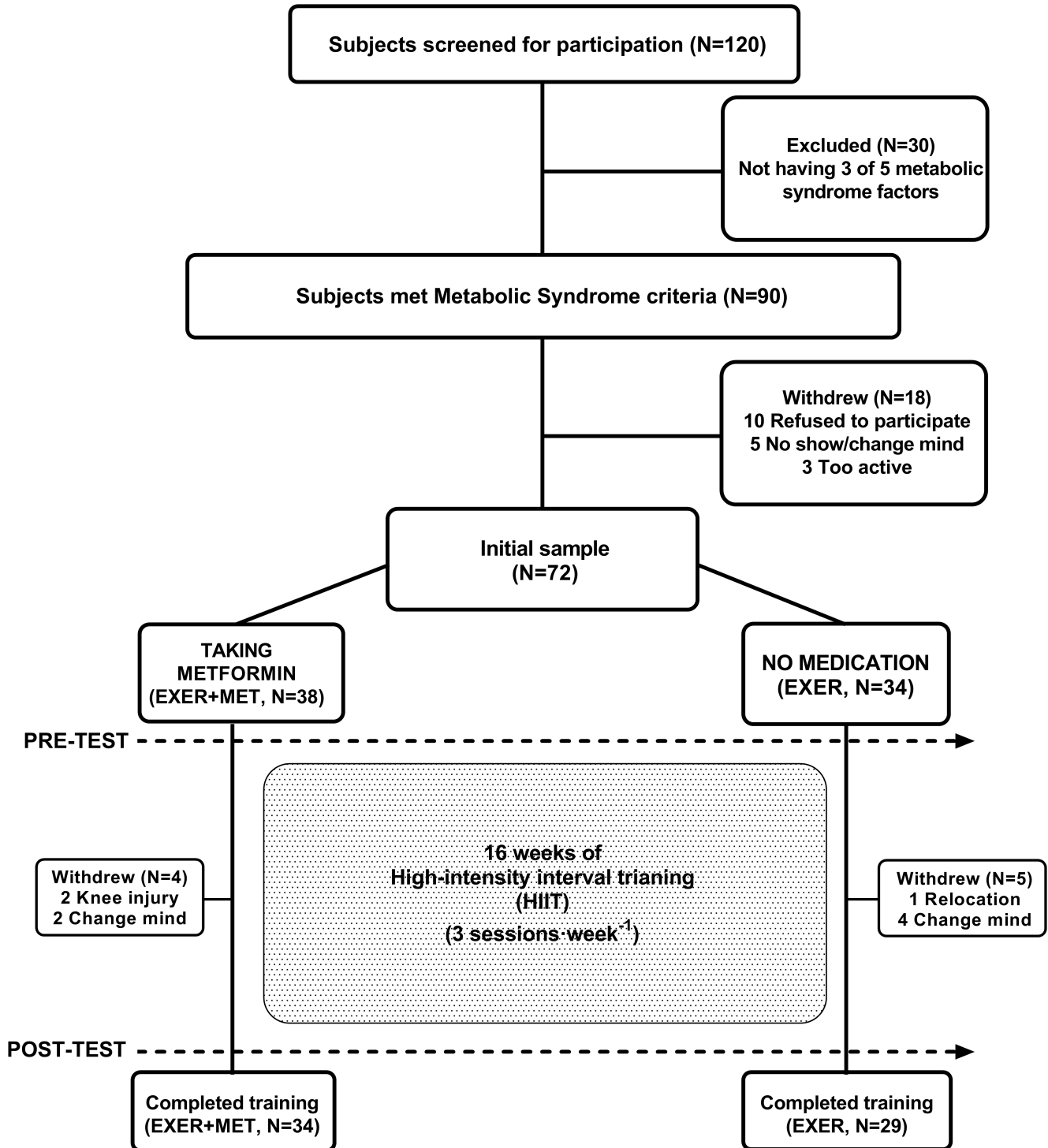
FIGURE 1 CONSORT (Consolidated Standards of Reporting Trials) schematic representation of the study procedures

Analyzer (Mindray Medical Instrumentation Ltd., Shenzhen, China). Insulin concentration was measured in duplicate using chemoluminescent microparticle immunoassay (iCV = 2.0%-2.8%) in an automated analyzer (Architect ci4100; Abbott Laboratories, Abbott Park, Illinois). IR was calculated using the homeostatic model assessment of IR (HOMA-IR) (30), following the proposed criteria of metabolic dysfunction-associated fatty liver disease (HOMA-IR ≥ 2.5) (31).

MetS *z* score

Sex-specific MetS *z* score provided information on the continuous evolution of MetS risk factors with the treatments. The sum of the *z* score for each MetS component was divided by five to compile the MetS risk score with units of standard deviation (SD) (32). The equations used to calculate MetS *z* score were the following:

$$\text{Men's MetS}\,z\,\text{score} = ([40 - \text{HDL} - c]/\text{SD})$$
$$+ ([\text{TG} - 150]/\text{SD}) + ([\text{glucose} - 100]/\text{SD})$$
$$+ ([\text{waist circumference} - 94]/\text{SD})$$
$$+ ([\text{mean arterial pressure} - 100]/\text{SD}) \tag{1}$$

$$\text{Women's MetS}\,z\,\text{Score} = ([50 - \text{HDL} - c]/\text{SD})$$
$$+ ([\text{TG} - 150]/\text{SD}) + ([\text{glucose} - 100]/\text{SD})$$
$$- ([\text{waist circumference} - 80]/\text{SD})$$
$$- ([\text{mean arterial pressure} - 100]/\text{SD}) \tag{2}$$

Statistical analysis

Per-protocol analysis was used, and only individuals who completed the protocol were included in the statistical analysis. A Kolmogorov–Smirnov test revealed that all variables showed a normal distribution except for TG, HDL-c, HOMA-IR, glucose, and insulin. Those variables were log-transformed for statistical analysis. Data are reported as mean (SD). Data in figures are mean (SEM) for easier display. Additionally, 95% CI was calculated. At baseline, variables were compared between groups with unpaired Student t test. Mixed-design ANOVA with baseline HOMA-IR as a covariate was used to detect differences within and between groups before and after training. Mixed-design ANOVA was used to detect differences on IR variables within and between groups before and after training. When a time by group interaction existed, a Bonferroni post hoc test was performed to identify the time point at which groups significantly differed. A McNemar test was used to evaluate the prevalence of MetS factors. Pearson coefficients of correlation (r) were conducted to test the association among measured variables. All statistical procedures were run on SPSS version 24 (IBM Corp., Armonk, New York) with statistical significance set at $p \leq 0.05$.

RESULTS

Baseline participant characteristics

Participants were White individuals living in Southern Europe, with similar age between groups (EXER+MET: 54 [6] years and EXER: 51 [8] years; $p = 0.189$). At baseline, EXER+MET and EXER groups were similar in body weight (86.8 [13.6] kg and 90.1 [15.4] kg, respectively; $p = 0.392$), number of MetS factors (3.9 [0.9] and 3.6 [0.6], respectively; $p = 0.106$), percentage of women in each group (50% and 48%, respectively; $p = 0.891$), and CRF (VO$_{2MAX}$: 22.2 [5.3] mL/min/kg and 21.1 [4.3] mL/min/kg, respectively; $p = 0.351$). Data were analyzed without sex differentiation because all women were postmenopausal and were not taking hormonal replacement, and their responses did not differ from men's responses. All participants in the EXER+MET group maintained their dose of metformin treatment during the 4-month intervention (i.e., average of 1,279 [489] mg/d). A similar number of individuals, with characteristics similar to their respective groups (number and MetS factors z score), withdrew from the experiment in each

group (Figure 1). Therefore, the statistical analysis was performed per protocol. The training monitoring program (Seego Realtrack Systems) revealed similar fulfillment of the training target HR and similar numbers of minutes trained between groups ($p = 0.731$ and $p = 0.752$, respectively). As expected, groups differed in their basal glucose concentration and HOMA-IR, which were higher in the EXER+MET group. In contrast, mean arterial pressure (MAP) and systolic and diastolic blood pressure were initially higher in the EXER group than in the EXER+MET group (102.4 [10.0] mm Hg and 95.3 [11.0] mm Hg, 126 [16] mm HG and 135 [12] mm Hg, and 80 [11] mm Hg and 86 [10] mm Hg, respectively; $p = 0.009$, $p = 0.016$, and $p = 0.018$; Table 1).

Body weight and composition

Evolution of body weight and composition following training is depicted in Table 1. After 16 weeks of HIIT, no time or time per group effect, using baseline HOMA-IR as covariate, was found in body weight (p value for time $= 0.907$, p value for time × group $= 0.191$), BMI (p value for time $= 0.833$, p value for time × group $= 0.163$), fat mass (p value for time $= 0.203$, p value for time × group $= 0.606$), FFM (p value for time $= 0.093$, p value for time × group $= 0.129$), and right-leg FFM (p value for time $= 0.368$, p value for time × group $= 0.771$).

MetS components

Evolution of MetS components after 16 weeks of training is depicted in Table 1. After 16 weeks of training, MAP was reduced in both groups (EXER+MET: −3.9, 95% CI: −6.5 to −1.3 mm Hg, $p = 0.003$; EXER: −8.5, 95% CI: −11.3 to −5.7 mm Hg, $p < 0.001$). In addition, systolic blood pressure was reduced in both groups (EXER+MET: −4.8, 95% CI: −8.3 to −1.4 mm Hg, $p = 0.007$; EXER: −10.8, 95% CI: −14.6 to −7.1 mm Hg, $p < 0.001$). However, the MAP- and systolic blood pressure-lowering effects of training were larger in the EXER group ($p = 0.021$ and $p = 0.027$, respectively). No time or time per group effect was found in waist circumference, TG, HDL-c, MetS z score, or MetS factors. A McNemar test revealed that MAP was reduced after training only in the EXER group ($p = 0.002$).

IR

Evolution of fasting glucose, insulin, and IR (i.e., HOMA-IR) after 16 weeks of training is depicted in Figure 2. Plasma glucose concentration and HOMA-IR were higher in the EXER+MET group before training ($p < 0.001$ and $p = 0.003$, respectively), whereas plasma insulin was similar among groups ($p = 0.534$). After 16 weeks of HIIT, fasting insulin and HOMA-IR decreased similarly (p value for time $= 0.005$) in the EXER+MET (-1.01, 95% CI: -2.45 to 0.43 µIU/mL and -0.61, 95% CI: -1.23 to 0.02, respectively) and EXER groups (-1.82, 95% CI: -3.38 to -0.26 µIU/mL and -0.47, 95% CI: -1.14 to 0.21, respectively). However, plasma glucose

TABLE 1 Changes in body composition and MetS factors with a 16-week HIIT program

	EXER+MET		EXER		Baseline	Time	Time × group
	Baseline	16 weeks	Baseline	16 weeks	p value	p value	p value
Age (y)	54 ± 6		51 ± 8		0.189		
Women (%)	50		48		0.891		
Body weight (kg)	86.8 ± 13.6	85.5 ± 12.6	90.1 ± 15.4	88.3 ± 14.6	0.364	0.907	0.191
BMI (kg/m^2)	32.5 ± 5.2	32.0 ± 5.2	33.1 ± 3.5	32.5 ± 3.4	0.585	0.833	0.163
Fat mass (kg)	31.9 ± 7.9	31.0 ± 7.8	33.9 ± 7.0	33.3 ± 7.0	0.286	0.203	0.606
FFM (kg)	54.9 ± 9.9	54.6 ± 9.7	56.2 ± 11.3	55.1 ± 11.3	0.635	0.368	0.771
Right-leg FFM (kg)	10.2 ± 3.5	10.1 ± 3.3	9.8 ± 3.0	9.6 ± 2.8	0.655	0.204	0.626
Waist circumference (cm)	106.5 ± 9.8	103.9 ± 9.8	106.3 ± 10.7	103.9 ± 10.0	0.926	0.372	0.717
(MetS prevalence)	(100%)	(100%)	(100%)	(97%)			
Glucose (mg/dL)	152.3 ± 47.3	145.3 ± 43.2	104.4 ± 10.1	103.2 ± 17.3	<0.001	0.344	0.452
(MetS prevalence)	(100%)	(100%)	(72%)	(59%)			
Triglycerides (mg/dL)	142.2 ± 85.5	138.3 ± 75.5	148.8 ± 83.6	138.7 ± 74.7	0.758	0.356	0.685
(MetS prevalence)	(30%)	(27%)	(41%)	(34%)			
HDL-c (mg/dL)	43.7 ± 11.1	44.2 ± 10.4	44.0 ± 12.8	43.9 ± 10.5	0.925	0.988	0.992
(MetS prevalence)	(64%)	(55%)	(69%)	(62%)			
Mean arterial pressure (mm Hg)	95.3 ± 11.0[a]	91.3 ± 10.7[b]	102.4 ± 10.0	93.8 ± 8.9[b]	0.009	0.005	0.021
(MetS prevalence)	(45%)	(30%)	(76%)	(41%)[b]			
Systolic blood pressure (mm Hg)	126 ± 16[a]	121 ± 14[b]	135 ± 12	124 ± 13[b]	0.016	0.019	0.027
Diastolic blood pressure (mm Hg)	80 ± 11[a]	76 ± 10	86 ± 10	78 ± 8	0.018	0.013	0.092
MetS z score	0.62 ± 0.65	0.43 ± 0.74	0.44 ± 0.43	0.19 ± 0.47	0.218	0.673	0.273
MetS factors	3.9 ± 0.9	3.7 ± 0.9	3.6 ± 0.6	3.1 ± 1.1	0.106	0.156	0.264

Note: Data are presented as mean ± SD for 63 patients with MetS divided into the EXER+MET and EXER groups. Baseline HOMA-IR was used as a covariate in the statistical analysis.

Abbreviations: EXER, exercise; EXER+MET, exercise + metformin; FFM, fat-free mass; HDL-c, high-density lipoprotein cholesterol; HIIT, high-intensity interval training; HOMA-IR, homeostatic model assessment of insulin resistance; MetS, metabolic syndrome.

[a]Significant difference from EXER group at that time point ($p < 0.05$).

[b]Significant change from baseline within each group.

concentration was not affected by training; therefore, no time or time per group effect was found.

Metabolic fitness and CRF

CRF (i.e., VO_{2MAX}) after 16 weeks of training is shown in Figure 3. Before training, VO_{2MAX} (EXER+MET: 1.94 [0.59] L/min, 22.2 [5.3] mL/kg/min, 35.0 [6.9] mL/kg FFM/min; EXER: 1.91 [0.55] L/min, 21.1 [4.3] mL/kg/min, 34.0 [5.8] mL/kg FFM/min), FO_{MAX} (EXER+MET: 0.22 [0.07] g/min; EXER: 0.23 [0.07] g/min), PO_{MAX} (EXER+MET: 153 [57] W; EXER: 155 [47] W), and HR_{MAX} (EXER+MET : 153 [17] beats/min; EXER: 156 [15] beats/min) were similar between groups ($p > 0.05$). After 16 weeks of training, absolute VO_{2MAX} improved in both groups (EXER+MET: 0.23, 95% CI: 0.13- 0.33 L/min, $p < 0.001$; EXER: 0.43, 95% CI: 0.32-0.54 L/min, $p < 0.001$). Moreover, relative VO_{2MAX} improved in both groups (EXER+MET: 2.97, 95% CI: 1.76-4.18 mL/kg/min, $p < 0.001$ and 4.44, 95% CI: 2.73-6.15 mL/kg FFM/min, $p < 0.001$; EXER: 5.17, 95% CI: 3.85-6.48 mL/kg/min, $p < 0.001$ and 8.19,

95% CI: 6.32-10.05 mL/kg FFM/min, $p < 0.001$). There was a time per group interaction, with higher VO_{2MAX} improvements in the EXER group when VO_{2MAX} was expressed in absolute terms, relative to body weight or FFM terms ($p = 0.012$, $p = 0.021$, and $p = 0.006$, respectively). Likewise, PO_{MAX} improved further in the EXER group (interaction $p = 0.05$; EXER+MET: 29, 95% CI: 20-38 W, $p < 0.001$; EXER: 42, 95% CI: 32-52 W, $p < 0.001$). In contrast, FO_{MAX} increased similarly (p value for time = 0.040, p value for time × group = 0.698) in both groups (EXER+MET: 0.05, 95% CI: 0.01-0.08 g/min; EXER: 0.06, 95% CI: 0.02-0.09 g/min). Last, HR_{MAX} had a similar response in both group after training (p value for time = 0.006, p value for time × group = 0.335).

Predictors of improved IR

Reductions in body weight were significantly correlated with reductions in fasting insulin and HOMA-IR in the EXER+MET ($r = 0.618$ and 0.740; $p < 0.001$) and EXER ($r = 0.508$ and 0.551; $p < 0.05$) groups. Reductions in body weight were also significantly correlated with reductions in
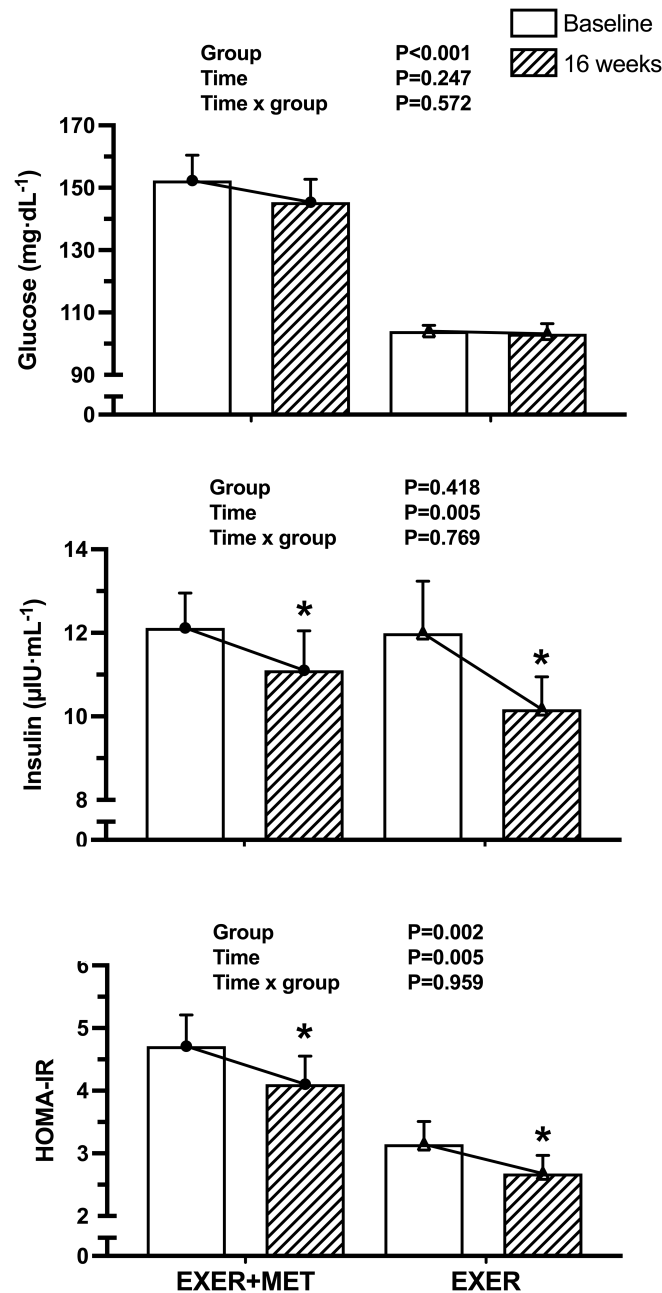
FIGURE 2 Effect of 16 weeks of high-intensity interval training on fasting hyperglycemia, insulin concentrations in plasma, and fasting insulin resistance, calculated with HOMA-IR, in the EXER+MET ($n = 34$) and EXER ($n = 29$) groups. Data are presented as mean ± SEM. *Significant change from baseline within each group. EXER, exercise group; EXER+MET, exercise + metformin group; HOMA-IR, homeostatic model assessment of insulin resistance

glucose in the EXER+MET group ($r = 0.580$; $p < 0.001$), but not in the EXER group ($r = 0.245$; $p = 0.201$). Moreover, metformin dose, as an indicator of diabetes severity, was not correlated with improvements (i.e., post minus pre) in glucose ($r = 0.195$; $p = 0.373$), insulin ($r = 0.190$; $p = 0.384$), and HOMA-IR ($r = 0.239$; $p = 0.272$) following training. Metformin dose was not associated with improvements in VO_{2MAX} ($r = -0.360$; $p = 0.092$), FO_{MAX} ($r = -0.299$; $p = 0.165$), and PO_{MAX} ($r = -0.409$; $p = 0.053$) in the EXER+MET group. Metformin dose was not associated with FO_{MAX} at baseline ($r = -0.137$; $p = 0.533$). VO_{2MAX}

improvements with training, in the EXER+MET or EXER groups, were not correlated with reductions in fasting glucose ($r = -0.032$; $p = 0.858$ and $r = -0.358$, $p = 0.056$), insulin levels ($r = -0.134$; $p = 0.449$ and $r = -0.162$; $p = 0.402$), or HOMA-IR ($r = -0.098$; $p = 0.580$ and $r = -0.255$; $p = 0.182$). Finally, we did not find an association between FO_{MAX} and HOMA-IR at baseline (EXER+MET: $r = -0.073$, $p = 0.683$; EXER: $r = 0.230$, $p = 0.230$) or between FO_{MAX} and HOMA-IR reductions after 16 weeks of training (EXER+MET: $r = -0.001$, $p = 0.995$; EXER: $r = -0.153$, $p = 0.429$) in any of the groups.
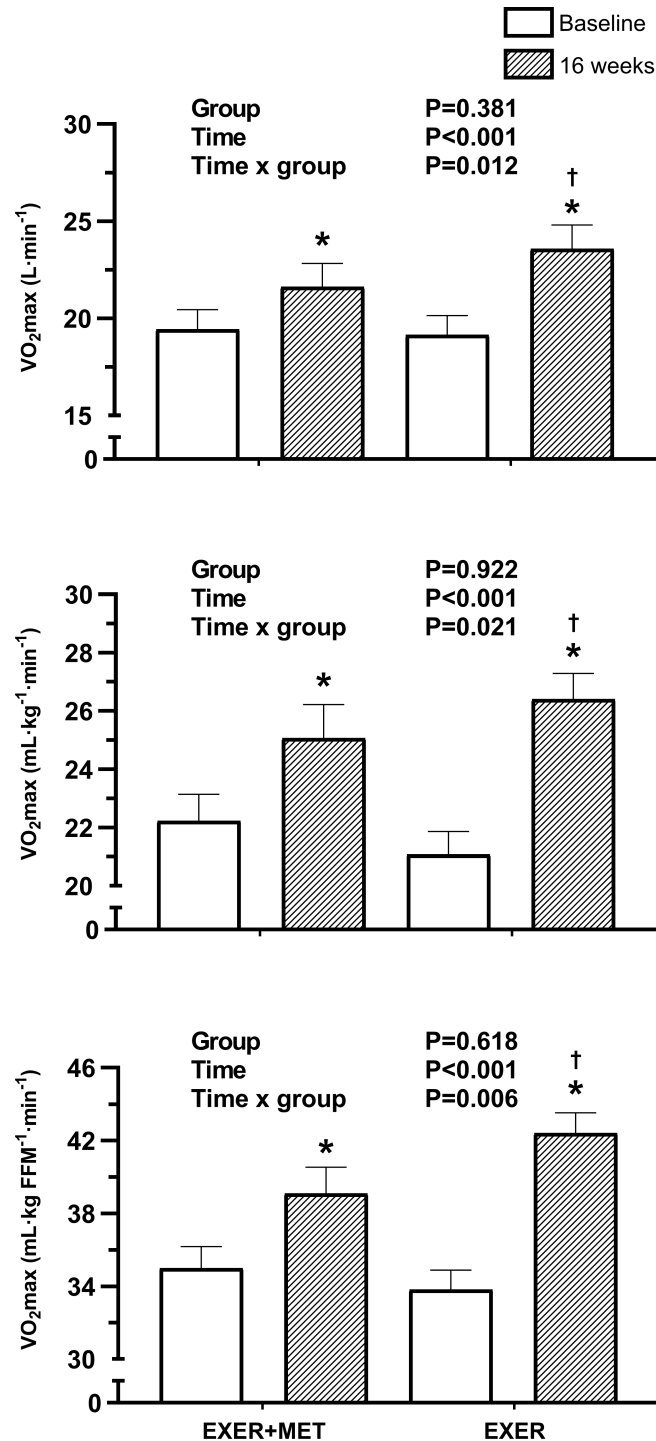
FIGURE 3 Absolute and relative (i.e., divided by body weight and FFM) VO_{2MAX} after 16 weeks of high-intensity interval training in the EXER+MET ($n = 34$) and EXER ($n = 29$) groups. Data are presented using baseline homeostatic model assessment of insulin resistance as a covariate in the statistical analysis. *Significant change from baseline within each group. †Significant difference in the VO_{2MAX} improvement between groups ($p < 0.05$). EXER, exercise group; EXER+MET, exercise + metformin group; FFM, fat-free mass; VO_{2MAX}, maximal oxygen uptake

DISCUSSION

We found that metformin treatment does not blunt the beneficial effects of 16 weeks of HIIT on reducing fasting IR (HOMA-IR) and fasting hyperinsulinemia (Figure 2). This finding contrasts with our hypothesis and the current literature, which has suggested that

metformin prevents the improvements in glucose uptake after 8 to 12 weeks of aerobic training in people with prediabetes (19-21,33). We recruited people with MetS already treated with metformin and compared them with another group of people with MetS whose fasting hyperglycemia (104.4 [10.1] mg/dL; Table 1) had not yet progressed to require metformin prescription. Both groups were similar

as far as cardiovascular disease risk (i.e., MetS z score; Table 1), but their type 2 diabetes stage was different. The prevailing view of metformin interfering with training adaptations could discourage metformin users from joining an exercise program. Our data importantly balance that view, suggesting that metformin does not interfere with training reductions in fasting IR (i.e., HOMA-IR) when prescription has been ongoing for more than 6 months.

Malin et al. (21) found that insulin sensitivity (tested 28 hours after training using euglycemic-hyperinsulinemic clamp) increased after 12 weeks of aerobic-resistance training (EXER group), and that adding metformin to training (EXER+MET group) tended to blunt this training effect. This happened despite a larger body weight loss in the EXER+MET group than in the EXER group (i.e., 4 kg vs. 0.5 kg). Although the information that prevailed from that paper was that metformin blunts the full effect of exercise training in improving insulin's glucose disposal, other relevant measurements of glucose metabolism did not differ between groups. For instance, fasting insulin decreased similarly in both groups (−1.7 [0.7] µIU/mL and −2.9 [1.5] µIU/mL for the EXER+MET and EXER groups, respectively) (22), similar to the present study (−1.0 [3.1] µIU/mL and −1.8 [5.2] µIU/mL; Table 1). Furthermore, HOMA-IR showed similar reductions in both groups (−24% and −12% for the EXER+MET and EXER groups, respectively (21), similar to the present study (−1.0 [3.1] µIU/mL and −1.8 [5.2] µIU/mL; Figure 2). Therefore, the blunting effect of metformin on reducing IR was evident only when insulin was elevated at 187 µIU/mL during the hyperinsulinemic clamp technique (21). Those insulin concentrations were observed only after ingestion of a high glucose load (75-g oral glucose tolerance test) in people with prediabetes in the phase of insulin overproduction (34). Therefore, metformin intake may not negatively affect exercise improvements in glucose transport at lower, more typical insulin concentrations and definitely not at fasting insulin concentrations (Figure 2).

We observed that chronic metformin treatment interfered with the VO_{2MAX} and PO_{MAX} gains of exercise training, as previously described (Figure 3) (19,20). We hypothesized that the lower improvement in VO_{2MAX} would also restrict exercise reductions in fasting IR in the EXER+MET group. However, this was not the case, and the gains in VO_{2MAX} and the reductions in IR with training were not associated (Figure 4). Longitudinal studies have shown that aerobic exercise training that improves VO_{2MAX} also reduces IR (8,35,36). Nevertheless, the underlying mechanism supporting this positive association is not well defined. Our data suggest that CRF and glucose metabolism improve simultaneously with exercise training, without a clear relationship between them (Figure 4).

In our training intervention study, only body weight loss correlated with the reductions in HOMA-IR in both groups of individuals. It was shown that, when the energy expended during training was reimbursed by increasing food intake to prevent body weight loss, glucose uptake during a clamp was not improved with training (37). Other investigators showed that exercise training reduced IR independently of body weight losses by improving cellular signaling mechanisms (i.e., insulin receptor and glucose transporter type 4 [GLUT4]) (35). We have recently found using an intravenous glucose load test that a minimal weight loss (i.e., >2%) is required for exercise training to reduce IR in people with MetS (9). In this study, we confirm a link between body weight losses and the reductions in IR.

Low CRF is strongly associated with elevated cardiovascular disease risk and mortality (38). Aerobic training is the cornerstone of lifestyle changes to promote CRF improvements in people with MetS (39). However, studies have suggested that metformin blunts training improvements in middle-aged individuals with prediabetes (21,40), older individuals (19), and even healthy, active individuals (18). However, no information is available for individuals with very low CRF (i.e., MetS; ~22 mL O_2/kg/min) under chronic metformin treatment (>6 months) like those studied here. Our findings also corroborate that, in people with MetS, chronic metformin treatment blunts the improvement in CRF by 50% after 16 weeks of HIIT, a type of training shown to promote gains in CRF (41).

Metformin use during training (EXER+MET) did not affect the gains in FO_{MAX} after 16 weeks of exercise training (i.e., 20%: EXER+MET group; 25%: EXER). FO_{MAX} is used as a surrogate of mitochondrial function improvement with exercise training (42). Therefore, our results suggest that the reduced increase in VO_{2MAX} in EXER+MET might not be related to impaired mitochondrial development. Konopka et al. (19) showed that metformin intake during training altered intrinsic mitochondrial function independently of influencing mitochondrial biogenesis or abundance. Thus, metformin, although influencing mitochondria function, may not alter training-induced mitochondria biogenesis. Moreover, Malin and Braun showed that metformin did not inhibit training increases in fat oxidation and the concomitant reductions in carbohydrate use (20). Our data also suggest that metformin does not inhibit the metabolic training adaptations to increase exercise fat oxidation (i.e., FO_{MAX}), which suggests normal mitochondria β oxidation development.

After 16 weeks of HIIT, the EXER+MET group lowered MAP by 4 mm Hg whereas the EXER group lowered it by 8 mm Hg ($p = 0.02$ for the interaction; Table 1). The higher initial blood pressure in the EXER group could have contributed to the larger blood pressure-lowering effect of the same training program in this group (43). However, this comparative resistance to lower blood pressure with training in the EXER+MET group coincides with a previous report from Malin and Braun's group (22). They suggest that metformin alters the effects of training on reducing vascular inflammation and improving endothelial function. Exercise combined with metformin was shown to lower AMPK activation in skeletal muscle (33), which is important for nitric oxide production and, therefore, for vasodilation. Ultimately, the limitation in vasodilation could affect blood flow to the contracting muscle and oxygen delivery, explaining the lower VO_{2MAX} development with EXER+MET.

Walton et al. (44) reported that metformin may inhibit muscle hypertrophy via inhibition of mTORC1, leading to decreased muscle protein synthesis or increased autophagy. In their study, metformin attenuated the gains in one-repetition-maximum knee extension (placebo group: 23.1% [18.9%]; metformin group: 15.3% [18.5%]; $p = 0.055$). In contrast, Boule et al. (40) observed that training while medicated with metformin did not affect strength gains following
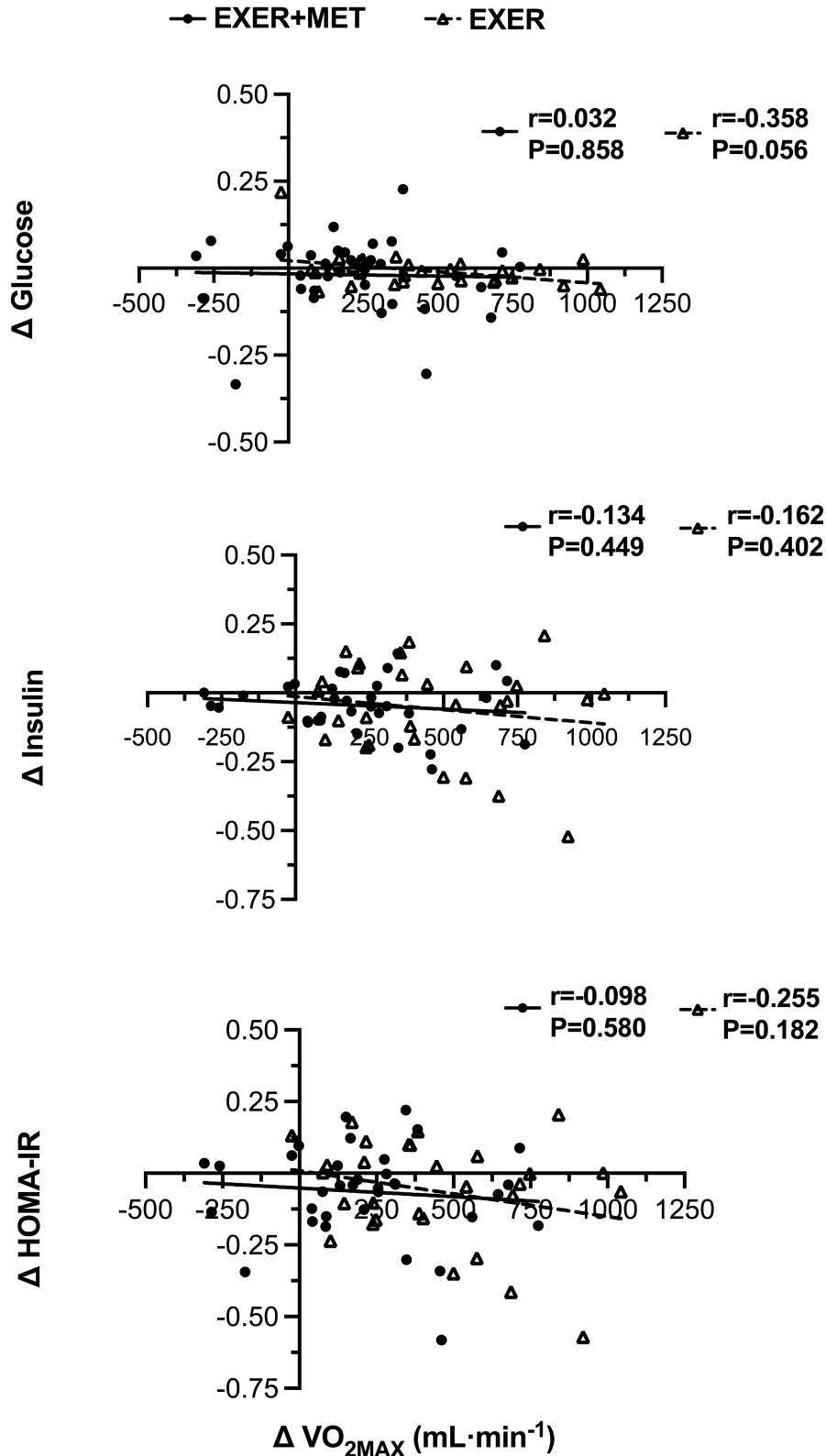
FIGURE 4 Pearson correlation between gains in cardiorespiratory fitness after 16 weeks of high-intensity interval training (ΔVO_{2MAX}) and changes in fasting glucose, insulin, and HOMA-IR in EXER+MET ($n = 34$) and EXER ($n = 29$) groups. EXER, exercise group; EXER+MET, exercise + metformin group; HOMA-IR, homeostatic model assessment of insulin resistance; VO_{2MAX}, maximal oxygen uptake

aerobic training in middle-aged patients with type 2 diabetes. Here we showed that metformin blunted the gains in cycling peak aerobic power (PO_{MAX}, watts) following 16 weeks of a HIIT program.

The reduced development in cycling leg power output with training in the EXER+MET group may be the main reason for the lower increase in VO_{2MAX}. It is unclear whether that effect was mediated by

metformin restraining leg power because our indirect measure of leg FFM did not reveal changes after 16 weeks of HIIT (Table 1).

Study limitations include our indirect method to assess body composition and our lack of direct measure of mitochondrial respiration and function. Another potential limitation is that only per-protocol statistical analysis was used. On the other hand, we used an available index of IR (HOMA-IR) in a moderately large sample of individuals with hyperglycemia, among other cardiovascular risks (i.e., MetS). Of note, HOMA-IR reflects liver glucose regulation, assuming a feedback loop between the liver and pancrea' β-cells, and it is less informative of IR in peripheral tissues. The strengths of the study are that we carefully measured whole-body maximal fat oxidation during exercise (27) and used a verification test to confirm the attainment of VO_{2MAX} (29). Moreover, to our knowledge, our study is unique in that the EXER+MET pharmacological dose (i.e., 1,279 [489] mg/d) was much less than the dose that other investigators have used (i.e., 2,000 mg/d), which may lead to hormesis effects (45,46). Furthermore, by recruiting individuals who were chronically medicated, we avoided the confounding effects of metformin on body weight loss at the onset of prescription.

As shown by other authors, we found that chronic metformin treatment (1,279 [489] mg/d for at least 6 months) is associated with blunted gains in VO_{2MAX} with intense aerobic exercise training in people with MetS and hyperglycemia. The blunted VO_{2MAX} development did not prevent other health benefits of training. Specifically, metformin did not interfere with the exercise-induced reductions in IR, at least at fasting insulin concentrations. Our study disputes that metformin interferes with training improvements in glucose metabolism (at least in resting IR), which could encourage metformin users to engage in exercise training.O

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

AUTHOR CONTRIBUTIONS

Alfonso Moreno-Cabañas and Ricardo Mora-Rodriguez contributed to the conceptualization of the study, designed and refined the methods, collected the data, performed the statistical analyses pertaining, and developed and revised the manuscript; Felix Morales-Palomo, Laura Alvarez-Jimenez, and Juan Fernando Ortega contributed to the conceptualization of the study and collection of data, performed statistical analyses, and revised the manuscript. The authors have read and approved the final version of the manuscript and agreed with authorship order.

CLINICAL TRIAL REGISTRATION

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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