

## ORIGINAL ARTICLE

# Statins effect on insulin resistance after a meal and exercise in hypercholesterolemic pre-diabetic individuals

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**Aim:** To study if statins, a widely prescribed, inexpensive medication to prevent coronary artery diseases may cause insulin resistance (IR).

**Methods:** Fasted (HOMA-IR) and post-meal insulin resistance were assessed in 21 pre-diabetic hypercholesterolemic individuals treated with statins (STA trial). Measurements were compared to another trial conducted 96 h after statin withdrawal using placebo pills (PLAC trial). Trials were duplicated 16–18 h after a bout of moderate-intensity exercise (500 kcal of energy expenditure) to reduce IR and better appreciate statin effects (EXER+STA and EXER+PLAC trials).

**Results:** Statin withdrawal did not affect fasting (HOMA-IR;  $2.35 \pm 1.05$  vs.  $2.18 \pm 0.87$  for STA vs. PLAC trials;  $p = 0.150$ ) or post-meal insulin resistance (i.e., Matsuda-index, STA  $6.23 \pm 2.83$  vs. PLAC  $6.49 \pm 3.74$ ;  $p = 0.536$ ). A bout of aerobic exercise lowered post-meal IR ( $p = 0.043$ ), but statin withdrawal did not add to the exercise actions ( $p = 0.564$ ). Statin withdrawal increased post-meal plasma free glycerol concentrations ( $0.136 \pm 0.073$  vs.  $0.185 \pm 0.090$  mmol·L<sup>-1</sup> for STA vs. PLAC trials;  $p < 0.001$ ) but not plasma free fatty acids or fat oxidation ( $p = 0.981$ , and  $p = 0.621$ , respectively). Post-meal fat oxidation was higher in the exercise trials ( $p = 0.002$ ).

**Conclusions:** Withdrawal of statin medication does not affect fasting or post-meal insulin resistance in pre-diabetic hypercholesterolemic individuals. Furthermore, statin use does not interfere with the beneficial effects of exercise on lowering IR.

## KEYWORDS

aerobic exercise, Hydroxymethylglutaryl-CoA reductase inhibitor, hyperglycemia, metabolic syndrome X, pre-diabetes

## 1 | INTRODUCTION

Statin (3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitor) is the most prescribed medicine to treat

hypercholesterolemia and its accompanying cardiovascular risks. Statins are very effective in lowering cholesterol levels and are one of the safest drugs in clinical practice. Furthermore, statins can reduce postprandial

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hypertriglyceridemia<sup>1-3</sup> decreasing the risk of atherogenic plaque formation. Thus, in adults with a 10-year atherosclerotic cardiovascular disease risk (Framingham Heart Study<sup>4</sup>) of 20% or higher, statin therapy is indicated to reduce low-density cholesterol levels (i.e., LDL-c<sup>5</sup>). However, in the light of recent studies, some physicians are reluctant to prescribe statins to pre-diabetic, dyslipidemic patients despite reaching high atherosclerotic cardiovascular risk levels.<sup>6</sup>

In 2010, an influential meta-analysis by Sattar and co-workers gathering 13 randomized statin trials showed that 4 years of statin treatment was associated with a 9% increased risk for incident diabetes mostly in older participants.<sup>7</sup> Although in absolute terms this represented one case of diabetes per 255 patients taking statin therapy for 4 years, the study caught large scientific attention. Finally, the rapidly accumulating evidence suggesting that treatment with statins increased the risk of type 2 diabetes has led the U.S. Food and Drug Administration (FDA) to issue a warning on statin labels that remains since 2012.

Fewer studies report that statins lower the risk of diabetes<sup>8,9</sup> or at least, do not increase it when using pravastatin<sup>10</sup> or pitavastatin.<sup>11</sup> In the last few years, papers have been published, some supporting a link between statin use and diabetes<sup>12,13</sup> and others questioning that association.<sup>14</sup> All these studies have ignited a debate about the balance between the risks of statins on promoting diabetes, compared with the expected cardiovascular risk benefits from reducing LDL cholesterol.

The association between the use of statins and incident diabetes is puzzling. The health benefits of statin extend beyond their cholesterol-lowering properties reducing systemic inflammation, oxidative stress, and improving endothelial function<sup>15</sup> all of which would promote rather than impair carbohydrate metabolism. Furthermore, dyslipidemia (high levels of circulating fat) would favor tissue accumulation of non-esterified intermediates from lipid metabolism inhibit insulin signaling.<sup>16</sup> Therefore, statins, reducing dyslipidemia (LDL cholesterol and triglycerides), should prevent rather than compound insulin resistance.

In this study, we are taking an alternative experimental approach (i.e., drug withdrawal intervention) to help shed some light into the diabetogenic effects of statins. The aim of this study was to evaluate whether withdrawal of statin therapy reduces insulin resistance in hypercholesteremic pre-diabetic individuals. We hypothesized that if statins were causing insulin resistance, then statin withdrawal should improve it. Exercise, a well-known stimulus to reduce insulin resistance, is included in the experiment to assess if participants are responsive to improvements in glucose metabolism.

TABLE 1 Participants' characteristics

Variables	Mean ± SD
Age and anthropometry	
Age (years)	61 ± 7
BMI (kg·m <sup>-2</sup> )	30 ± 4
Body weight (kg)	85 ± 6
Body fat (%)	32 ± 6
Metabolic syndrome	
Waist circumference (cm)	105 ± 6
Fasting glucose (mmol·L <sup>-1</sup> )	6.11 ± 1.28
Triglycerides (mmol·L <sup>-1</sup> )	1.34 ± 0.67
HDL-c (mmol·L <sup>-1</sup> )	1.24 ± 0.34
Resting SBP (mmHg)	128 ± 10
Resting DBP (mmHg)	79 ± 7
Number of MetS factors	3 ± 1
Dyslipidemia	
Cholesterol (mmol·L <sup>-1</sup> )	4.38 ± 1.04
LDL-c (mmol·L <sup>-1</sup> )	2.95 ± 0.80
Years under statin treatment	5 ± 2
Cardiorespiratory fitness	
VO <sub>2MAX</sub> (ml·kg <sup>-1</sup> ·min <sup>-1</sup> )	31 ± 6
HR <sub>MAX</sub> (bt·min <sup>-1</sup> )	156 ± 3

Note: Values are means ± SD for 21 individuals with hypercholesterolemia and metabolic syndrome while taking their habitual dose of statin medication.

## 2 | METHODS

### 2.1 | Participants and preliminary testing

Twenty-one individuals with metabolic syndrome (one woman and 20 men) were included in this study. All of them were previously diagnosed with MetS based on the criteria of the International Diabetes Federation<sup>17</sup> as shown in Table 1. All subjects were medicated by their primary care physicians with statins for at least 3 years before the onset of the study. Statins were the only lipid-lowering therapy medication used by participants. The type and dose of the statin treatment are detailed in Table 2. In addition, eight subjects were on treatment with metformin to treat pre-diabetes and the remaining 13 were not medicated but had fasting blood glucose in the pre-diabetic range (>5.55 mmol·L<sup>-1</sup>). All participants signed a witnessed, informed consent of the protocol approved by the local Hospital's Ethics Committee following the latest declaration of Helsinki.

Drug	n	Dose (mg/day)	Bio-availability (%) <sup>19</sup>	Half-life (h) <sup>19</sup>	Solubility
Statins					
Atorvastatin	3	20–80	12	14	Lipophilic
Pitavastatin	2	2	80	11	Lipophilic
Pravastatin	1	20	18	2	Hydrophilic
Rosuvastatin	6	5–20	20	19	Hydrophilic
Simvastatin	7	10–20	5	2	Lipophilic
Ezetimibe/ Atorvastatin	1	10/80	35–60/12	22/14	Lipophilic
Ezetimibe/ Rosuvastatin	1	20/10	35–60/20	22/19	Hydrophilic
Antidiabetics					
Metformin	5	850–1000	55	5	Hydrophilic
Canagliflozin/ Metformin	1	50/850	65/55	12/5	Hydrophilic
Dapagliflozin/ Metformin	1	50/850	78/55	13/5	Hydrophilic
Vildagliptin/ Metformin	1	50/850	85/55	2/5	Hydrophilic

TABLE 2 Drug prescription and pharmacokinetics data

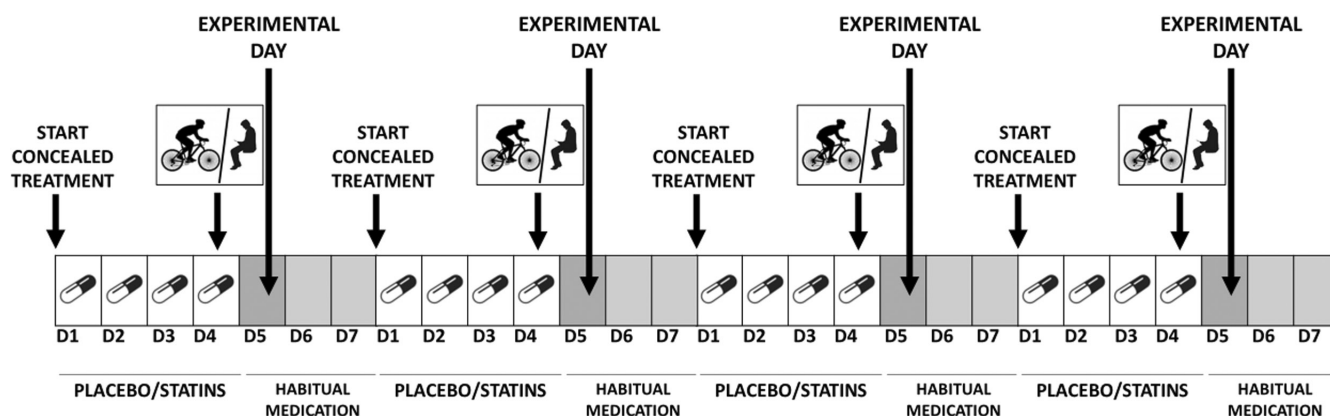


FIGURE 1 Experimental protocol

Subjects underwent a medical physical examination and completed a maximal cardiopulmonary graded exercise test (GXT) on an electronically braked cycle ergometer (Ergoselect 200, Ergoline, Germany) with ECG monitoring (Quark T12, Cosmed, Italy) to screen for myocardial diseases and determine their maximal oxygen consumption ( $VO_{2MAX}$ ). Maximal heart rate ( $HR_{MAX}$ ) was recorded to accordingly set exercise intensity during the exercise trials.

This study is part of a larger clinical trial ([ClinicalTrials.gov](https://clinicaltrials.gov) Identifier: NCT 04477590). In this study, we present data of a subset of individuals with metabolic syndrome whose main medication is statins. The statin dosage was prescribed by participant's primary care physicians, following the Spanish National Institute of Health guidelines for the management

of dyslipidemia.<sup>18</sup> Those guidelines require lifestyle advice progressing to pharmacological prescription with statins when a combination of two of the following fasting blood lipid level is reached; total cholesterol  $\geq 5.17$  mmol·L<sup>-1</sup>, LDL-c  $\geq 3.23$  mmol·L<sup>-1</sup>, and HDL-c  $\leq 1.04$ – $1.30$  mmol·L<sup>-1</sup> for men and women, respectively.

## 2.2 | Experimental design

The study followed a repeated-measures crossover, randomized control trial design. The experimental protocol is summarized in Figure 1. Subjects completed 4 trials in a random order sequence, generated with the macro feature of Excel (Microsoft Office), without repetition.

The team physician performed the randomization and concealed it to the rest of the team until data analysis completion. Upon study enrollment, the team physician masked individuals' prescribed statin medication in larger capsules. The same capsules were used for placebo but filled with dextrose. Prescription and placebo capsules were placed into plastic bottles identified with an alphanumeric code only known to the physician. These prescription bottles were provided to subjects as substitute of their habitual statin medication. In that way, we altered participants' drug intake between placebo and statins in a double-blinded fashion.

Placebo was taken for the 4 days (i.e., 96 h) preceding the trial (REST+PLAC and EXER+PLAC) because this time exceeds by fivefold the longer-lasting statin half-life of subjects (i.e., 19 h for rosuvastatin<sup>19</sup>; Table 2). During the first trial, subjects filled out a two-day activity and diet diary and were instructed to replicate those for the 48 h before every trial. Subjects underwent 4 trials to measure glucose tolerance after a mixed meal (i.e., MMTT) containing  $86 \pm 7$  gr of simple sugars under the following conditions, *a*) substituting their habitual statin medication by placebo (REST+PLAC trial), *b*) taking their habitual statin medicine (REST+STA trial), *c*) placebo combined with a bout of intense aerobic exercise (EXER+PLAC trial), and *d*) combining exercise and statin medicine (EXER+STA trial). Trials took place for 4 consecutive weeks and were scheduled on the same day of the week for each subject to reduce variability.

## 2.3 | Exercise trials

In the EXER+PLAC and EXER+STAT trials, exercise was performed the evening prior to the MMTT (~14 hour before) using continuous pedaling at a moderate intensity that elicited 60% percent of  $HR_{MAX}$ , until subjects reached 500 kcal of energy expenditure (60–75 min). Oxygen consumption was monitored every 15 min to determine when they accomplished that energy expenditure target. Subjects were provided with 500 ml of water after exercise to promote rehydration. The laboratory temperature remained at  $22 \pm 1$  °C.

## 2.4 | Experimental trials

Subjects arrived at the laboratory between 7 and 8 AM after 10–12 h overnight fast preceded by a standardized 411 kcal dinner (5 gr of fat, 12 gr of carbohydrate, 7 gr of protein per 100 g, 500 ml of water, and a medium-sized apple). Upon arrival, subjects provided a first urine sample to ensure hydration (i.e., urine specific gravity <1.020) and

following, subjects' body weight (Hawk, Mettler Toledo, USA) and body composition (i.e., bioimpedance analysis, Tanita BC-418-MA, Japan) were assessed. Subjects lie in a gurney while a catheter (20G, BD Insyte, Becton and Dickinson, Spain) was inserted in an antecubital vein and a 3-way stopcock attached (Luer-lock, CPK IV, Farmaban, Spain). Then, a blood sample was withdrawn (i.e., –90 min blood sample). This blood sample was used as a baseline for the calculations of fasted metabolite levels. After 65 min of lying in a quiet room, ( $22 \pm 1$  °C and  $25 \pm 6\%$  humidity) resting metabolic rate was assessed for 20 min, using indirect calorimetry (Quark b<sup>2</sup>, Cosmed, Italy). Fat oxidation was calculated according to Frayn's equations<sup>20</sup> with protein oxidation considered negligible.

## 2.5 | Mixed Meal tolerance test (MMTT) and resting metabolic rates

After resting metabolic rate assessment, subjects sat and ingested within 5 min a meal (i.e., MMTT) of  $248 \pm 27$  g (Granderroble Deserts, Spain) containing  $86 \pm 7$  gr of simple sugars. The meal amounted to  $11.6 \text{ kcal}\cdot\text{kg}^{-1}$  body weight for a group average of  $995 \pm 82$  kcal. During the 5 hours following test meal ingestion, blood samples were collected every 20 min, while the catheter was maintained patent by flushing with 5 cc of 0.9% saline (Grifols, Spain) after each blood collection. Each 60 minutes after meal ingestion, resting metabolic rate<sup>21</sup> and fat oxidation<sup>20</sup> were assessed using indirect calorimetry (Quark b2, Cosmed, Italy) for 20 min while subjects rested supine in a gurney.

## 2.6 | Blood analysis

5-cc blood samples were collected in tubes with a clot activator (Vacutainer®; USA) and serum obtained upon centrifugation. Glucose was analyzed using glucose oxidase peroxidase method with the intra-inter assay coefficient of variation (iCV; 0.9–1.2%) in an automated analyzer (Mindray BS 400 Medical Instrumentation, China). Insulin concentration was analyzed by chemiluminescence microparticle immunoassay (iCV; 2.0–2.8%; Architect System Insulin, Abbott Diagnostics Division, Germany). Fasting insulin resistance was estimated using the homeostasis model assessment of insulin resistance (HOMA-IR;<sup>22</sup>). Matsuda-index was used to calculate post-meal insulin resistance during the 5 h after oral ingestion of carbohydrate in the MMTT.<sup>23</sup>

Plasma FFA and free glycerol were analyzed using colorimetric commercial kits (Fujifilm Wako, USA, and Sigma, USA, respectively; iCV; <1.5%). Blood samples were collected every 20 min for 5 hours after meal ingestion and

blood metabolites were expressed as an average of those 15 blood sample determinations.

## 2.7 | Statistical analysis

Shapiro–Wilk test confirmed that the principal dependent variable, fasting glucose, was normally distributed. Calculations suggested that at least 18 number of paired comparisons would be required to achieve a difference between trials of  $0.44 \pm 0.61 \text{ mmol}\cdot\text{dl}^{-1}$  in glucose with a power of  $\beta = 0.80$  and a level of significance  $\alpha = 0.05$  (two-sided). Data comparing fasting parameters were analyzed using paired Student's *t*-test. A two-way ANOVA with repeated-measures (Exercise x Statin) was conducted for all dependent variables. Data collected in repeated time points (insulin, glucose, FFA, glycerol, and resting fat oxidation) were unified as a single value by conversion to area under the curve. Pearson's coefficient of correlation was conducted to test the association between insulin resistance (HOMA-IR, Matsuda-index) and fat metabolism indexes (fat oxidation, glycerol, and FFA concentration). Data are presented as mean  $\pm$  SD unless otherwise noted. SPSS version 21 (Chicago, IL) was used for statistical analysis with statistical significance set at  $p \leq 0.05$ .

## 3 | RESULTS

### 3.1 | Baseline characteristics and exercise results

Table 1 presents subjects' characteristics. All participants were overweight or obese and met at least three of the five MetS factors. All individuals were chronically medicated with statins to treat their dyslipidemia for an average of 5 year (Table 1). In addition, eight of them were treated with antihyperglycemic agents (i.e., metformin; Table 2) and the remaining 13, had fasting blood glucose levels in the range of pre-diabetes ( $\text{IFG} > 5.61 \pm 0.55 \text{ mmol}\cdot\text{L}^{-1}$ ;<sup>24</sup>). In all trials, subjects arrived at the laboratory well hydrated (urine specific gravity  $< 1.020$ ) with similar body weight ( $< 1\%$  variation) after ingestion of the standard dinner 10–12 h prior to the trials. No differences existed between the exercise trials in the percent of  $\text{HR}_{\text{MAX}}$  (i.e., PLAC  $64 \pm 8$  vs. STAT  $64 \pm 7\% \text{ HR}_{\text{MAX}}$ ;  $p = 0.621$ ) or the caloric expenditure (i.e., PLAC  $529 \pm 30$  vs. STAT  $544 \pm 32 \text{ kcal}$ ;  $p = 0.722$ ).

### 3.2 | Statin withdrawal effects on glycemic control

Withdrawal of statin treatment during 96 h did not increase glucose plasma fasting concentrations (i.e., STAT

$5.77 \pm 1.51$  vs. PLAC  $5.86 \pm 1.57 \text{ mmol}\cdot\text{L}^{-1}$ ;  $p = 0.185$ ). Moreover, postprandial glucose concentration (MMTT) was neither affected by statin withdrawal (Figure 2A). Insulin was not altered by statin withdrawal in the fasted state (STAT  $9 \pm 4$  vs. PLAC  $8 \pm 3 \mu\text{IU}\cdot\text{mL}^{-1}$ ;  $p = 0.121$ ) or during the MMTT (Figure 2B). Consequently, HOMA-IR did not show a response to statin withdrawal (Figure 2C), and the glycemic response to a meal (Matsuda-index) neither showed an effect of statins (Figure 2D).

### 3.3 | Exercise effect on glycemic control

Exercise resulted in a tendency to lower fasting plasma glucose (i.e., REST  $6.14 \pm 1.28$  vs. EXER  $5.48 \pm 1.70 \text{ mmol}\cdot\text{L}^{-1}$ ;  $p = 0.058$ ), but not in postprandial plasma glucose concentration ( $p = 0.300$ ). While basal plasma insulin levels were not affected by exercise (i.e., REST  $8.72 \pm 3.51$  vs. EXER  $8.30 \pm 2.83 \mu\text{IU}\cdot\text{mL}^{-1}$ ;  $p = 0.336$ ), insulin was reduced in the postprandial state by exercise, in the statin and placebo trials (Figure 2B). Exercise effect was not significant for HOMA-IR ( $p = 0.195$ ), but exercise significantly reduced Matsuda-index ( $p = 0.043$ , Figure 2D).

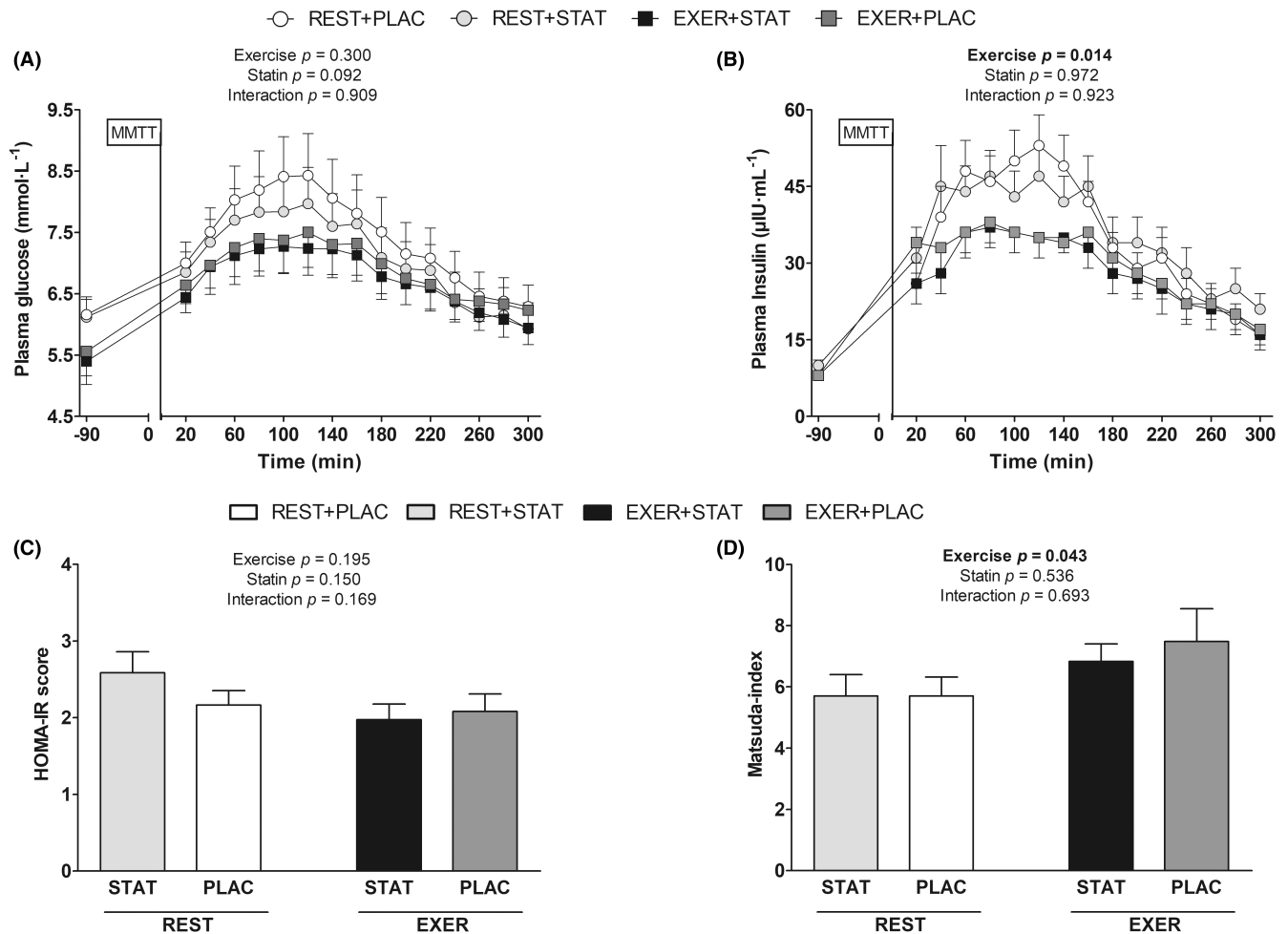
### 3.4 | The effects of statin withdrawal on fat metabolism

As designed, 96 h of withdrawal of statins resulted in 15% increase in total blood cholesterol levels compared to STAT trials (i.e., STAT trials  $4.19 \pm 1.4$  vs.  $4.94 \pm 1.22 \text{ mmol}\cdot\text{L}^{-1}$  PLAC trials;  $p < 0.001$ , Figure 3C). Furthermore, basal LDL-c levels were also significantly increased in the placebo trials (i.e., STAT trials  $2.56 \pm 0.83$  vs.  $3.20 \pm 0.80 \text{ mmol}\cdot\text{L}^{-1}$  PLAC trials;  $p < 0.001$ ). HDL-c levels were not affected by statin withdrawal or by the exercise remaining at  $1.20 \pm 0.23 \text{ mmol}\cdot\text{L}^{-1}$  in all trials. We found a significant reduction of postprandial plasma glycerol concentration in the trials with statins (Figure 2A). However, statins did not induce significant alterations in plasma FFA concentrations, nor in resting fat oxidation (Figure 3B and D).

### 3.5 | Exercise effects on fat metabolism

Previous exercise tended to elevate postprandial glycerol ( $p = 0.068$ ) and FFA ( $p = 0.118$ ) concentrations without reaching statistical significance (Figure 3A and B). However, during MMTT, exercise allowed fat oxidation to remain at pre-meal levels while it declined in the non-exercise trials (effect exercise,  $p = 0.002$ ; Figure 3D).





**FIGURE 2** Responses of, (A) plasma glucose concentration, (B) plasma insulin concentration, (C) insulin resistance index (HOMA-IR), and (D) Matsuda-index after 10–12 h fast and in response to a meal-test containing 86 gr of simple sugars in the four experimental trials. Data are presented as mean  $\pm$  SEM for 21 hypercholesterolemic pre-diabetic individuals with metabolic syndrome. Data collected during MMTT in repeated time points (plasma insulin and glucose) were unified as a single value by conversion to area under the curve

### 3.6 | Correlations

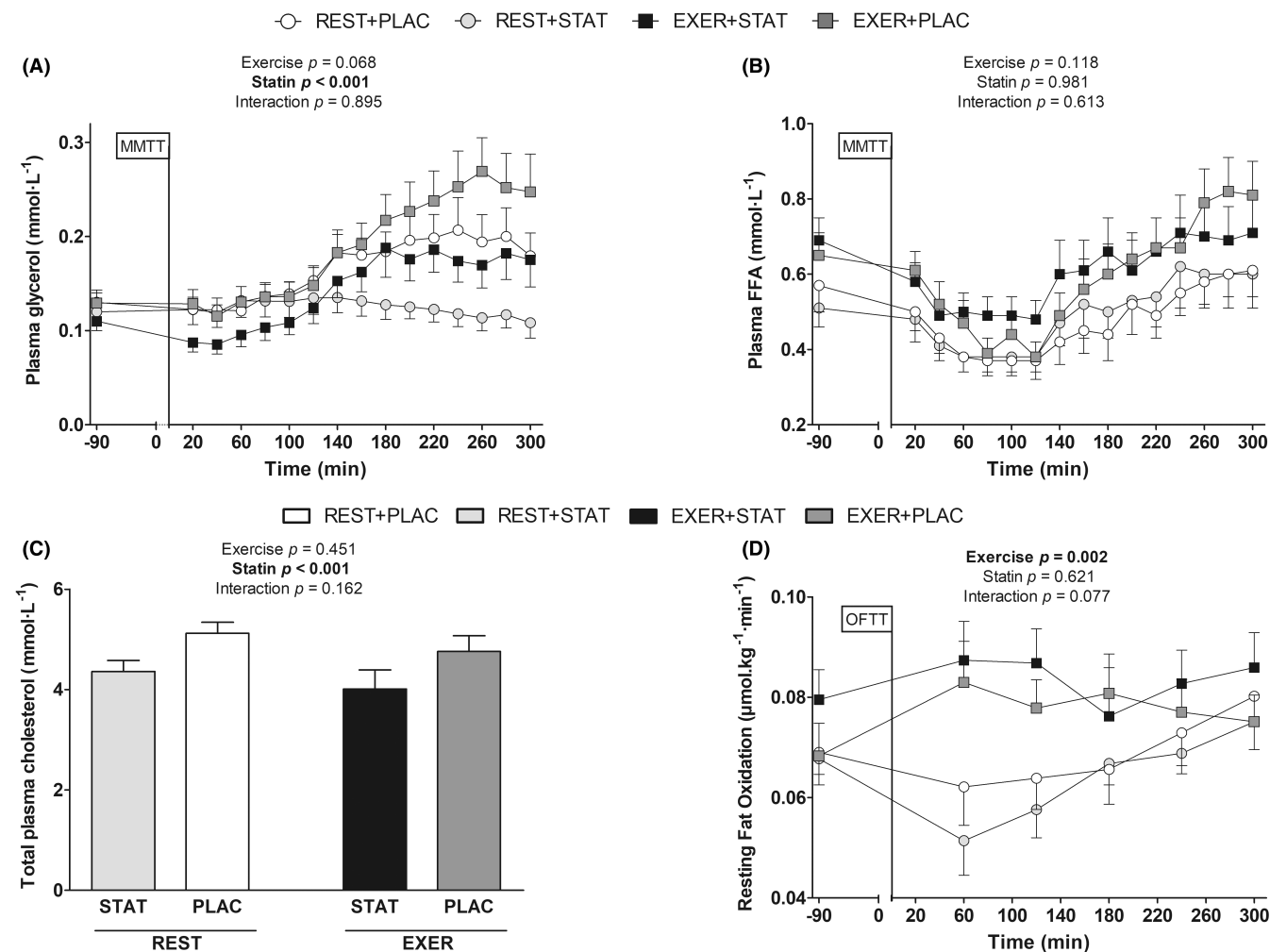
Pearson's coefficients of correlation were low and non-significant between insulin resistance (HOMA-IR, Matsuda-index) and lipid metabolism (i.e., fat oxidation, plasma glycerol, FFA). The doses of statin prescribed (% DDD<sup>25</sup>) were not associated with changes in plasma glucose ( $r = 0.131$ ;  $p = 0.593$ ) or insulin ( $r = -0.142$ ;  $p = 0.561$ ) upon 96 h of statin withdrawal.

## 4 | DISCUSSION

The current view that statin use increases the risk of type 2 diabetes has led the U.S. Food and Drug Administration (FDA) to issue a warning on statin labels. To confirm the diabetogenic effect, for 4 consecutive days, we withdrew statin medication of 21 hypercholesterolemic, insulin resistance/diabetic, individuals with metabolic syndrome

by masking their pills with a dextrose placebo. Our hypothesis was that if statins are contributing to elevate fasting or postprandial blood glucose or insulin levels, then, statin withdrawal should lower those values. To our surprise, statin withdrawal did not affect fasting insulin or glucose concentrations (i.e., neither HOMA-IR) and neither the glycemic responses to a meal containing 86 gr of simple sugars (Figure 2). Thus, our data suggest that in hypercholesterolemic individuals their chronic statin treatment does not worsen their pre-diabetes (i.e., insulin resistance).

Our experimental approach is open to two main criticisms. The first is, that perhaps, 96 h drug withdrawal is not enough time to release the effects of statins causing insulin resistance, resulting in the reported no change on glucose or insulin responses. However, 96 h withdrawal is 4 times longer than the half-life time of the more long-lasting statin type (i.e., rosuvastatin, Table 2). The sharp increase in plasma cholesterol concentration upon 96 h



**FIGURE 3** Response of, (A) plasma glycerol concentration, (B) plasma free fatty acid concentration, (C) total plasma cholesterol concentration, and (D) fat oxidation after 10–12h fast and in response to a meal-test containing 86 gr of simple sugars in the four experimental trials. Data are presented as mean  $\pm$  SEM for 21 hypercholesterolemic pre-diabetic individuals with metabolic syndrome. Data collected during MMTT in repeated time points (plasma glycerol, FFA and resting fat oxidation) were unified as a single value by conversion to area under the curve

withdrawal (Figure 3C) suggests successful cessation of statin metabolic effects. Furthermore, our experimental approach of drug withdrawal rather than drug provision, to assess a drug effect, is customary in the endocrinology and pharmacology fields and has been used in numerous experiments.<sup>26</sup>

The second criticism is that by studying a sample of individuals chronically treated with statin (>3 y) the diabetogenic effects of the drug had become permanent, and thus, irreversible. However, a bout of aerobic exercise, significantly lowered the insulin levels and improved whole-body IR in response to a meal (Figure 2D). Thus, our data suggest that chronic treatment with statins does not irreversibly interfere with insulin actions that still respond to a bout of prolonged moderate-intensity exercise. Since insulin action improved with exercise but not with statin withdrawal, it is logical to

assume that statins were not affecting insulin resistance and probably not compounding the insulin resistance state of the subjects.

Most of the literature defending a diabetogenic effect of statins use either retrospective cross-sectional designs or prospective randomized control trials. The first ones measure some index of diabetes (fasting hyperglycemia or HbA1C) in subjects that in retrospective, were or were not medicated with statins.<sup>27,28</sup> However, the matching of individuals using statins with others without treatment is challenging. Even when those groups are well-matched by age, body mass index, and sex, it is difficult to establish whether the worsening of glucose metabolism is directly due to statins or to the development of some previous metabolic disorder that leads to both hypercholesterolemia and diabetes.

The studies composing the prospective randomized control trials are well summarized in a meta-analysis by

Sattar and co-workers<sup>7</sup> revealing that statin treatment increases by 9% the risk of developing diabetes in a 4-year follow-up, mostly in older participants. While meta-analysis confers the higher weight of scientific evidence, the authors describe their findings to conclude that statin therapy is associated with a slightly increased risk of development of diabetes (i.e., one case of diabetes per 255 patients taking statin therapy for 4 years). It is possible that studies like ours using a crossover randomized design in 21 individuals and others using RTC with reduced number of subjects<sup>14</sup> are not able to detect such a low incidence of diabetes.

Since study participants were taking statins as prescribed by their primary care physicians for an average of 5 years (Table 1), subjects did not report statin-induced myalgia, a side-effect of statins typically manifested at the beginning of treatment. On the contrary, subjects were attended by different physicians which resulted in prescription of different types and dosages of statins among participants as presented in Table 2. Although simvastatin and atorvastatin have been deemed to have special diabetogenic effects<sup>29–31</sup>, insulin resistance did not improve when withdrawing those drugs on the 11 subjects taking these statins in our study (i.e., half of the sample). On the contrary, we did not find that pitavastatin and pravastatin (4 subjects) were beneficial for glucose control or at least innocuous as has been pointed out in some studies.<sup>10,11,14</sup> We also explored if the dose of statin was associated with the purposed negative effects on insulin resistance. Neither the type nor the dose of statin was correlated with changes in fasting insulin resistance or the glycemic response to a meal upon withdrawal.

It has been reported that a sustained reduction in plasma FFA turnover preventing intramuscular long-chain fatty Acyl-CoA accumulation improves insulin actions in type 2 diabetic patients.<sup>32,33</sup> This has led to the proposal, that inhibition of lipolysis may improve insulin resistance.<sup>34</sup> Based on experimental evidence with glycerol isotopic tracers, a statin's effect on serum triglycerides may be explained by an increase in triglyceride-rich lipoproteins turnover, possibly due to increased intravascular lipolysis.<sup>35</sup> Other authors suggest that statins induced an increase in lipolysis due to the inactivation of apolipoprotein CIII levels which was, in turn, inhibiting lipoprotein lipase.<sup>36</sup> In contrast to those studies, we detected lower glycerol concentration (marker of lipolysis) with statins after the meal.

Despite the possible inhibition of lipolysis with statins, resting fat oxidation was not affected fasting or postprandially (Figure 3D). Statin therapy is associated with reduced complex II-linked respiration,<sup>37</sup> which can affect the mitochondria oxidative capacity and could potentially lower fat oxidation.<sup>38</sup> In fact, we have recently reported that

metabolic syndrome individuals chronically medicated with statins had a 29% lower  $\beta$ -oxidation enzyme activity than non-medicated counterparts, reflected in lower peak fat oxidation during low-intensity exercise.<sup>39</sup> The reason by which we presently do not detect a reduction in resting fat oxidation may be because resting fat oxidation is around 10-fold lower than exercise fat oxidation, and thus, differences due to statins are impossible to detect at rest.

Our study is not free of limitations. We did not use a euglycemic hyperinsulinemic clamp, which is the gold standard procedure to study peripheral insulin resistance, but oral ingestion of glucose in food which affects both hepatic and peripheral insulin sensitivity. A limitation of the study is that eight out of the 21 subjects used antidiabetic medication (i.e., metformin; Table 2) that may reduce liver glucose output and increase insulin secretion.<sup>40</sup> Thus, in those subjects, metformin may shadow an improvement in glucose control upon statin withdrawal. However, that group of subjects did not differ from the rest in their HOMA-IR or Matsuda-index responses during the MMTT. Thus, as a whole group, the postprandial insulin response likely provided a valid surrogate of clamp-assessed whole-body IR. We provided subjects with a mixed meal containing  $86 \pm 7$  gr of simple sugars and treated data as with a standard OGTT (75 gr of glucose). Since we used a mixed meal and the fat could delay glucose incorporation into the blood,<sup>41</sup> we extended blood collection from the standard 2–3 h protocol to a 5 h protocol while collecting blood every 20 min. Finally, although the sample size might seem small ( $n = 21$ ), the crossover design reduced random variability and allows reaching statistical significance in many comparisons.

Some studies suggest that the risk of developing diabetes with statin therapy is larger in those individuals with initial impaired fasting glucose, elevated glycated hemoglobin, metabolic syndrome, or severe obesity.<sup>42,43</sup> Therefore, it is crucial to establish the effects of statins on this population at risk of developing diabetes. In this study, we chose hypercholesterolemic subjects with impaired glucose metabolism and chronic statin treatment and studied the effects of statin on insulin resistance and its associations with fat metabolism (i.e., lipolysis and fat oxidation). We found that manipulation of pharmacological statin treatment (withdrawal or continuation) in a double-blind fashion, does not affect insulin resistance. Our data suggest that the prediabetes that accompanies the metabolic syndrome is not induced by taking statins. Furthermore, statin prescription does not interfere with the exercise improvements in the glycemic response to a meal.

## AUTHOR CONTRIBUTIONS

Laura Alvarez-Jimenez: Formulated the research question, designed the study, collected data, analyzed the data,



revised the article. Ricardo Mora-Rodriguez: Formulated the research question, designed the study, collected data, wrote the article. Juan Fernando Ortega: Formulated the research question, designed the study, collected data, revised the article. Felix Morales-Palomo: Formulated the research question, designed the study, collected data, analyzed the data, revised the article. Alfonso Moreno-Cabañas: Formulated the research question, designed the study, collected data, revised the article.

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## CONFLICT OF INTEREST

The authors report no potential conflicts of interest. The team physician had direct clinical responsibility for patients.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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