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Obesity

Dietary Supplementation with Omega-3 Fatty Acids and Oleate Enhances Exercise Training Effects in Patients with Metabolic Syndrome

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Objective: We studied the effects of exercise training alone or combined with dietary supplementation of omega-3 polyunsaturated fatty acids (Ω-3PUFA) and oleate on metabolic syndrome (MSyn) components and other markers of cardiometabolic health.

Methods: Thirty-six patients with MSyn underwent 24 weeks of high-intensity interval training. In a double-blind randomized design, half of the group ingested 500 mL/day of semi-skim milk (8 g of fat; placebo milk) whereas the other half ingested 500 mL/day of skim milk enriched with 275 mg of Ω-3PUFA and 7.5 g of oleate (Ω-3 + OLE).

Results: Ω-3 + OLE treatment elevated 30% plasma Ω-3PUFA but not significantly ($P = 0.286$). Improvements in VO_{2peak} (12.8%), mean blood pressure (−7.1%), waist circumference (−1.8%), body fat mass (−2.9%), and trunk fat mass (−3.3%) were similar between groups. However, insulin sensitivity (measured by intravenous glucose tolerance test), serum concentration of C-reactive protein, and high-density lipoprotein improved only in the Ω-3 + OLE group by 31.5%, 32.1%, and 10.3%, respectively (all $P < 0.05$). Fasting serum triacylglycerol, glucose, and plasma fibrinogen concentrations did not improve in either group after 24 weeks of intervention.

Conclusions: Diet supplementation with Ω-3PUFA and oleate enhanced cardiometabolic benefits of intense aerobic exercise training in patients with MSyn.

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Introduction

Metabolic syndrome (MSyn) is characterized by central obesity, dyslipidemia, hypertension, and hyperglycemia (1). Prevalence of MSyn is as high as ∼80% among adults who are inactive and have obesity (2). Insulin resistance is a major underlying factor in the development of MSyn (3). Therefore, interventions targeting insulin resistance may be effective strategies for treating MSyn. Obesity and high-fat diets have been highlighted as important risk factors in the pathogenesis of insulin resistance (4) and MSyn (5). In addition, the saturation state of dietary fat may also influence the development of insulin resistance (6), but this finding is not universal (7). Alternatively, mounting evidence has suggested that dietary supplementation with omega-3 polyunsaturated fatty acids (Ω-3PUFA) may improve insulin action (8-11). In animal models, dietary intake of Ω-3PUFA improved insulin sensitivity via upregulation of several genes and mediators involved in carbohydrate metabolism (9). In humans, Ω-3PUFA appears to readily incorporate into cell membranes resulting in increased cell fluidity (10). Ultimately, this may improve glucose transport through the enhanced ability of glucose transporter 4 to imbed in the cell membrane. Additionally, Ω-3PUFA supplementation has been shown to enhance resting fat oxidation and glycogen storage (11), potentially contributing to improve insulin sensitivity. However, it remains unclear whether these effects translate to measurable improvements in humans with insulin resistance.

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Author contributions: JFO, VFE, NH, and RMR were involved in the research design. RMR was the principal investigator of the exercise physiology laboratory at University of Castilla-La Mancha, and he was responsible for supervising the study activities. JFO wrote the initial draft and had primary responsibility for final content. FMP, VFE, and NH participated in the data collection and patient exercise training. JFO performed the statistical analysis. FJB and RCMD were responsible for the fatty acids measurements. RKN, JFH, and RMR were responsible for contributing to the interpretation of the results and for contributing to the manuscript. All authors contributed to the critical revisions of the article and read and approved the final version of the manuscript for submission.

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TABLE 1 Baseline anthropometric variables and metabolic syndrome components

	PLAC (n = 18)	Ω-3 + OLE (n = 18)	P
Age (years)	53 ± 2	54 ± 2	0.674
Weight (kg)	88.2 ± 3.3	93.9 ± 2.7	0.186
BMI (kg/m^2)	32.9 ± 0.8	33.8 ± 1.2	0.549
Waist-C (cm)	106.4 ± 1.8	107.5 ± 2.2	0.715
Fat mass (kg)	32.1 ± 1.5	34.4 ± 1.8	0.341
Trunk fat (kg)	18.5 ± 0.8	20.0 ± 1.1	0.256
TG (mmol/L)	1.38 ± 0.16	1.39 ± 0.21	0.966
HDL (mmol/L)	0.96 ± 0.05	0.93 ± 0.03	0.897
FG (mmol/L)	6.55 ± 0.33	6.55 ± 0.28	0.983
SBP (mm Hg)	130 ± 4	138 ± 4	0.198
DBP (mm Hg)	80 ± 2	84 ± 3	0.162
MSyn *Z*-score	2.0 ± 1.8	2.5 ± 2.4	0.469

Results as means ± SEM.
DBP, diastolic blood pressure; FG, fasting glucose; HDL, high-density lipoprotein; MSyn, metabolic syndrome; SBP, systolic blood pressure; TG, fasting triacylglycerol; waist-C, waist circumference.

Ω-3PUFA supplementation may also reduce cardiovascular disease risk by reducing circulating very-low-density lipoproteins (LDL) (12), triacylglycerols, and free fatty acids (FFA) (13). It also has additional anti-inflammatory and antithrombotic properties (10). Current recommended daily intake for Ω-3PUFA is 250 mg for the general population and higher for patients with cardiovascular disease (14). When this recommendation is combined with monounsatured fatty acid (MUFA) intake, additional improvements in cardiovascular disease risk such as reduced circulation of LDL, homocysteine, and vascular adhesion molecule 1 and increased high-density lipoprotein (HDL) have been reported (15,16). Data in animals and humans have indicated that oleate improves insulin sensitivity and prevents endoplasmic reticulum stress and inflammation through activation of AMP-activated protein kinase (17,18). Thus, Ω-3PUFA alone or in combination with MUFA (e.g., oleate) has been shown to be useful in the prevention and treatment of cardiovascular and metabolic disease risk factors.

In addition to dietary interventions, exercise also remains a cornerstone in the treatment of MSyn (19). More specifically, aerobic endurance exercise training can ameliorate many of the components of MSyn (20). Within endurance training modalities, studies using high-intensity interval training (HIIT) have reported better outcomes in reversing MSyn than studies using continuous moderate-intensity exercise (21). Because the proposed mechanisms by which HIIT may improve metabolic health share some of the putative actions of nutritional supplementation with "healthy fats," combining HIIT with daily supplementation of Ω-3PUFA and oleate may act additively to reduce MSyn factors. Thus, the main objective of this study was to determine the effects of 24-week HIIT with and without Ω-3PUFA plus oleate supplementation in a group of patients with MSyn.

Methods

Participants

Thirty-six patients participated in this investigation. All participants met the criteria for MSyn according to Alberti et al. (1) and were previously physically inactive (<120 min/week of moderate-vigorous self-reported physical activity). Table 1 shows participant baseline characteristics. Eighteen participants (50%) were under pharmacologicical treatment for at least one MSyn component. According to the last MSyn consensus (1), to be medicated for a factor counted as having that component. This study was conducted in accordance with a protocol approved by the Virgen de la Salud Hospital's Ethics Committee at Toledo, Spain.

Experimental design

Before participation, all volunteers underwent medical screening to confirm the inclusion criteria and to exclude individuals with untreated disease, which could interfere with the experiment or could be worsened by the exercise training. Subjects were randomly allocated into one of the two experimental groups after balancing for hypoglycemic agent treatment (four in each group), since insulin sensitivity was the main variable of this study. All participants completed two experimental trial days (Figure 1A) before ("Pre") and again after ("Post") 24 weeks of HIIT combined with ingestion of 500 mL/day of either (a) placebo milk (PLAC) or (b) milk with added Ω-3PUFA and oleate (Ω-3 + OLE). Participants were instructed to refrain from exercise for at least 72 h after their last exercise training session to "wash out" the acute exercise effects. Before beginning the exercise training program, participants reported to our laboratory in the morning to assess body composition and resting blood pressure. Participants then completed a graded exercise test until volitional exhaustion on a cycle ergometer to assess cardiorespiratory fitness. Seventy-two hours later, participants returned to the laboratory after an overnight fast that followed a standardized dinner (i.e., 714 kcal, 75% carbohydrate). We collected a baseline blood sample followed by a 50-min intravenous glucose tolerance test (IVGTT) to determine insulin sensitivity as previously described by Tura et al. (22) (i.e., CS_I).

Exercise training

Subjects underwent supervised HIIT at a frequency of three times per week for 24 weeks. Each training session was completed on a cycle ergometer and included a 10-min warm up at 70% of peak heart rate (i.e., HR_{peak}) followed by 4 × 4 min intervals at 90% HR_{peak} interspersed with 3-min active recovery at 70% HR_{peak} and a 5-min cooldown period for a total exercise duration of 43 min. Heart rate was measured with a telemetric heart rate monitor (Accurex coded, Polar, Finland). Participants included in post-training assessment attended at least 85% of all the prescribed exercise sessions and were instructed to maintain their normal dietary patterns for the duration of the study.

Ω-3 + OLE supplementation and placebo

Ω-3 + OLE were supplemented using commercial skim milk (Puleva, S.A., Spain) as vehicle containing 5.2% of carbohydrate, 3.1% of protein, and 2.3% of fat. Fat was composed of 0.5% saturated fatty acids, 1.5% MUFA, and 0.3% PUFA, being 0.055% eicosapentanoic + docosahexanoic acids (i.e., EPA + DHA). Daily the Ω-3 + OLE group received 7.5 g of MUFA and 1.5 g of PUFA including 275 mg of EPA + DHA. Placebo was semi-skim commercial milk (Puleva, S.A., Spain) containing 5.1% of carbohydrate, 3.9% of protein, and 1.6% of fat (8 g of total fat, 1% saturated fatty acid, 0.6% PUFA plus MUFA). Placebo and supplemented milk were packed in identical unlabeled white containers and had a

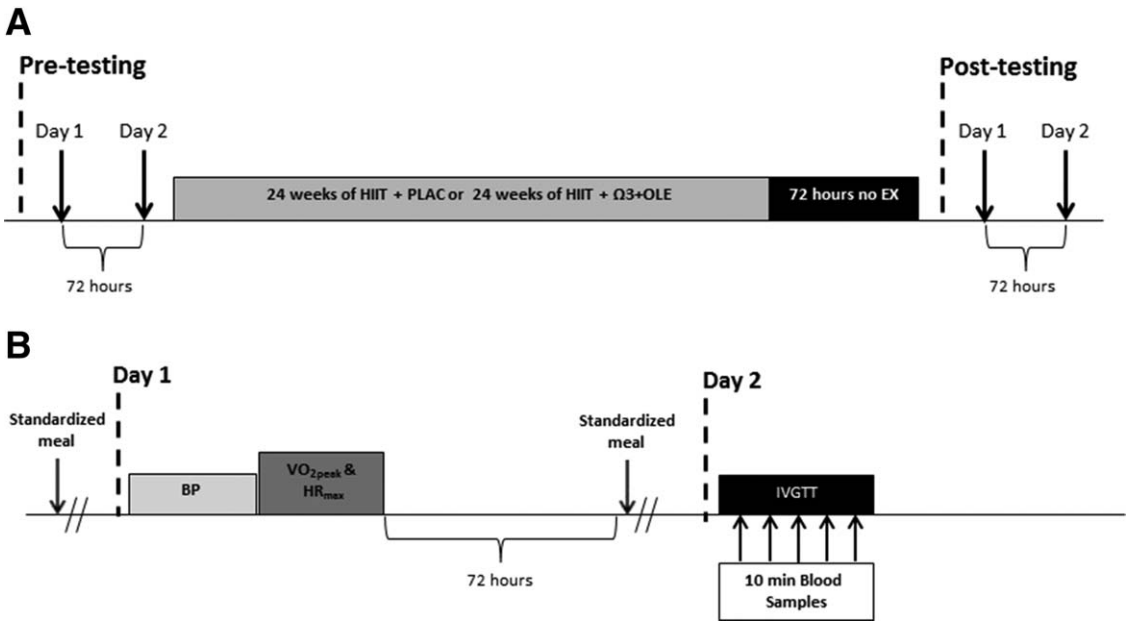

Figure 1 Timeline of experimental design. (**A**) General timeline of the study. (**B**) Timeline of the pre- and post-testing procedures.

similar flavor. Participants ingested 500 mL of the assigned milk daily during the 24 weeks of the study. Milk was used as a vehicle because previous research has shown that 8-week administration of Ω-3 + OLE enriched milk resulted in a significant increase in EPA and DHA plasma levels (16).

Anthropometric measurements

Anthropometric measurements were performed on day 1 of testing (Figure 1B). Body mass was determined using a ±0.05 kg sensitive scale (WildCat; Mettler-Toledo). Waist circumference was measured as proposed by Alberti et al. (23). Body composition was determined by dual energy X-ray absorptiometry (DXA, Hologic Series Discovery Wi QDR, Bedford).

Cardiorespiratory fitness

Cardiometabolic measurements were performed on day 1 of testing (Figure 1B). Resting blood pressure was assessed after 15 min of supine resting using an electronic monitor (Tango; Suntech Medical Instruments Inc.). Afterward, participants underwent a graded exercise test until volitional exhaustion on an electrically braked cycloergometer (Ergoselect 200, Ergoline, Germany) to assess peak oxygen uptake (VO_{2peak}), peak pulmonary ventilation ($V_{E\ peak}$), peak power output (PO_{peak}), peak respiratory exchange ratio (RER_{peak}), and heart rate (HR_{peak}). During exercise testing, integrated standard 12-lead ECG (Quark T12, Cosmed, Italy) and blood pressure were monitored during each stage to ensure that all subjects had a normal cardiovascular response to exercise. HR_{peak} during the test was recorded and used to prescribe training intensity.

Analytical procedures

Serum glucose, HDL, and triacylglycerols were analyzed using enzymatic methods. High-sensitive C-reactive protein (hsCRP), glycated hemoglobin, and fibrinogen were measured using immune-turbidimetry tests. All the above analyses were run in an automated chemistry analyzer (Mindray BS 400, Mindray Medical Instrumentation). Insulin concentration was measured using chemiluminescent microparticle immunoassay (Architect ci4100, Abbott Laboratories). The composition of plasma FFA was measured using gas chromatography-mass spectrometry (GC/MS; Agilent 5973 Networks, Mass Selective Detector; Agilent Technologies) after calibration with standards of known concentrations (i.e., WAKO Chemicals).

Insulin sensitivity

The IVGTT was performed according to the recommendations of the Islet Cell Antibody Registered User's Group (24). A 20-gauge 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). Afterward, every 10 min (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 each sample to ensure patency. We have recently shown that this IVGTT procedure has a day-to-day intraclass reproducibility for insulin sensitivity assessments of 0.955 (25). The 50-min IVGTT data allowed us to calculate insulin sensitivity index (CS_I) following Tura et al. (22).

MSyn *Z*-score

Calculations of MSyn *Z*-score (20) were performed using the criteria cut points of the consensus statement of the International Diabetes Federation (23). Standard deviations used for calculations were obtained from our database of patients with MSyn ($n = 169$).

TABLE 2 Anthropometric and cardiometabolic variables in both intervention groups

	PLAC		Ω-3 + OLE		*P*	
	Pre	Post	Pre	Post	Time	Time × group
Resting						
Weight (kg)	88.2 ± 3.3	86.9 ± 2.9	93.9 ± 2.7	91.0 ± 12.8	0.025	0.950
Body fat mass (kg)	32.1 ± 1.5	31.2 ± 1.2	34.4 ± 1.8	33.4 ± 2.0	0.013	0.950
Trunk fat mass (kg)	18.5 ± 0.8	17.9 ± 0.7	20.0 ± 1.1	19.3 ± 1.1	0.012	0.783
Lean mass (kg)	52.3 ± 1.9	52.1 ± 1.9	55.8 ± 1.5	55.5 ± 1.5	0.145	0.871
Exercise						
VO_{2peak} (L/min)	1.98 ± 0.15	2.20 ± 0.18	2.07 ± 0.16	2.37 ± 0.18	<0.001	0.442
$V_{E\ peak}$ (L/min)	82 ± 7	99 ± 10	88 ± 9	112 ± 9	<0.001	0.247
PO_{peak} (W)	161 ± 59	188 ± 22	166 ± 22	205 ± 24	<0.001	0.086
RER_{peak}	1.17 ± 0.02	1.14 ± 0.02	1.13 ± 0.02	1.12 ± 0.02	0.149	0.271

Results as means ± SEM.
PO_{peak}, peak power output; RER_{peak}, peak respiratory exchange ratio; $V_{E\ peak}$, peak pulmonary ventilation; VO_{2peak}, peak oxygen consumption.

Statistical analysis

Results are presented as means ± standard error of the mean (SEM). Shapiro–Wilks test revealed that data were normally distributed. Possible preintervention differences between groups were studied using *t*-test for unrelated measurements. Mixed-design ANOVA was run to analyze differences across time (repeated measures) and between experimental groups (PLAC vs. Ω-3 + OLE) in all reported variables. Vertical multiple comparisons among pairwise group means were performed with a Bonferroni correction for type I error when the time × group interaction was significant. SPSS, v22 (IBM Corporation) was used for statistical analysis with statistical significance set at $P \leq 0.05$.

Results

Changes in body weight, body composition, and cardiorespiratory fitness

Anthropometric measurements and cardiorespiratory fitness markers results are presented in Table 2. Body fat mass and trunk fat mass decreased significantly in both groups without differences between experimental groups (interaction $P > 0.05$). Lean mass did not change at the end of the intervention in any group ($P > 0.05$). VO_{2peak} improved in both groups similarly (interaction $P = 0.442$).

Plasma FFA composition

FFA species are expressed as percentage of the total pool of measured FFA in Table 3. Overall, we observed a 37 ± 14% reduction in FFA concentration after training in both groups, but this did not reach statistical significance ($P = 0.218$). Also, despite a 28% increase in plasma DHA + EPA concentration after training in the Ω-3 + OLE cohort (57 ± 16 vs. 73 ± 30 μmol/L) and a 49% reduction after training in the PLAC cohort (78 ± 23 vs. 40 ± 11 μmol/L), changes in plasma Ω-3PUFA concentration between groups were not statistically significant (interaction $P = 0.411$).

Changes in blood lipids, blood pressure, and assessment of MSyn

MSyn components before and after intervention are depicted in Table 4. Before the experimental intervention, four participants from each group were taking antihypertensive medication, five in PLAC, and six in Ω-3 + OLE received statins, two participants in each group were in fibrates treatment, and four participants in each group were taking metformin. At the end of the experiment, primary care physician discontinued statins in two volunteers from PLAC and in one participant from Ω-3 + OLE. From the MSyn components, waist circumference and blood pressure improved similarly in both groups (interaction $P > 0.05$). HDL improved only in the Ω-3 + OLE group

TABLE 3 Free fatty acids (FFA) as percentage of total FFA measured

	PLAC		Ω-3 + OLE		*P*	
	Pre	Post	Pre	Post	Time	Time × group
SFA (%)	29.0 ± 2.9	30.6 ± 4.6	31.4 ± 4.3	26.2 ± 3.8	0.553	0.266
MUFA (%)	28.4 ± 5.1	31.7 ± 5.6	31.8 ± 4.3	32.0 ± 4.8	0.594	0.648
PUFA (%)	42.6± 3.2	37.7 ± 4.2	36.8 ± 2.8	41.8 ± 4.7	0.989	0.167
Ω-3PUFA (%)	4.2 ± 0.8	4.4 ± 0.9	3.2 ± 0.4	4.9 ± 1.4	0.288	0.411

Results as means ± SEM.
SFA, saturated fatty acids; MUFA, monounsatured fatty acids; PUFA, polyunsatured fatty acids.

TABLE 4 Progression of metabolic syndrome components after intervention (PLAC vs. Ω-3 + OLE)

	PLAC		Ω-3 + OLE		*P*	
Variable	Pre	Post	Pre	Post	Time	Time × group
Waist-C (cm)	106.4 ± 1.8	104.5 ± 1.6	107.5 ± 2.2	105.6 ± 2.0	<0.001	0.925
TG (mmol/L)	1.38 ± 0.16	1.66 ± 0.26	1.39 ± 0.21	1.20 ± 0.10	0.717	0.083
HDL (mmol/L)	0.96 ± 0.05	0.98 ± 0.05	0.93 ± 0.03	1.04 ± 0.04[a,b]	0.025	0.040
FG (mmol/L)	6.55 ± 0.33	6.49 ± 0.39	6.55 ± 0.28	6.67 ± 0.44	0.875	0.506
SBP (mm Hg)	130 ± 4	122 ± 2	138 ± 4	129 ± 3	<0.001	0.612
DBP (mm Hg)	80 ± 2	74 ± 1	84 ± 3	77 ± 2	<0.001	0.424
MSyn *Z*-score	2.0 ± 1.8	1.6 ± 2.3	2.5 ± 2.4	1.1 ± 2.7	<0.001	0.071

Results as means ± SEM.
[a]Different from PRE.
[b]Different from PLAC at the same time point.
DBP, resting diastolic blood pressure; FG, fasting glucose concentrations in plasma; HDL, fasting high-density lipoprotein; MSyn, metabolic syndrome; SBP, resting systolic blood pressure; waist-C, waist circumference; TG, fasting triacylglycerols.

and serum triacylglycerols and glucose did not change in any group. Total cholesterol and atherogenic index [log(TG/HDL)] remained unchanged after intervention (Table 5). MSyn *Z*-scores improved 20% in PLAC and 56% in the Ω-3 + OLE group, but there were no statistical differences in the time × group interaction ($P = 0.071$; Table 4).

Insulin sensitivity, inflammatory stress, and prothrombotic response

The time course of glucose and insulin concentrations during IVGTT is presented in Figure 2. During the IVGTT, glucose disappearance rate did not change across time or between groups (Figure 2A, B). However, the area under the curve of insulin concentration was significantly lower after the intervention with Ω-3 + OLE (Figure 2C, D). Therefore, the calculated CS_I from 50-min IVGTT improved only in Ω-3 + OLE ($P = 0.008$; Figure 3A). This was accompanied by a reduction in circulating proinflammatory stress with a significant reduction in hsCRP in the Ω-3 + OLE group only, ($P = 0.028$; Figure 3B). Fasting insulin, glycated hemoglobin, homeostatic model assessment (HOMA2)-IR, and fibrinogen did not change in any group (Table 5).

Discussion

We recently reported that 16 weeks of HIIT improved a group of markers of cardiometabolic health such as VO_{2peak}, blood pressure, body fatness, hsCRP, and HDL (26). In this study, we used a non-pharmacological intervention (i.e., dietary supplementation) aiming to enhance the cardiometabolic health benefits of HIIT. We found that 24 weeks of HIIT with Ω-3 + OLE supplementation resulted in greater insulin sensitivity improvements (CS_I) and HDL (improving lipid profile), as well as reduced hsCRP (marker of inflammation stress), compared to HIIT and placebo. However, Ω-3 + OLE supplementation did not further augment the improvements in VO_{2max}, blood pressure, or body fatness beyond HIIT alone. The novel finding from this study is that dietary Ω-3 + OLE supplementation improved the benefits associated with a 24-week exercise training program in patients with MSyn. While some previous studies have demonstrated the benefits of HIIT (21,26) or Ω-3 + OLE supplementation (15,16) in patients with MSyn when administered separately, to our knowledge, this is the first study demonstrating the additive effects of HIIT in conjunction with dietary Ω-3 + OLE supplementation. Therefore, our findings provide evidence for a therapeutic approach to help improve cardiometabolic health in patients with MSyn based on lifestyle modifications (exercise and dietary supplementation).

TABLE 5 Additional clinical variables

	PLAC		Ω-3 + OLE		*P*	
Variable	Pre	Post	Pre	Post	Time	Time × group
TChol (mmol/L)	4.92 ± 0.23	4.74 ± 0.23	4.64 ± 0.18	4.66 ± 0.22	0.355	0.238
Atherogenic index	0.48 ± 0.09	0.52 ± 0.12	0.48 ± 0.07	0.41 ± 0.05	0.659	0.102
FI (μU/mL)	88.9 ± 8.3	94.5 ± 20.8	93.1 ± 9.0	95.8 ± 12.5	0.665	0.865
HOMA2-IR	1.74 ± 0.71	1.83 ± 1.69	1.84 ± 0.78	1.88 ± 1.07	0.673	0.959
HbA1c (%)	6.1 ± 0.2	6.0 ± 0.2	6.1 ± 0.2	6.1 ± 0.3	0.091	0.507
Fibrinogen (g/L)	2.87 ± 0.73	2.76 ± 0.55	3.03 ± 0.76	2.89 ± 0.55	0.231	0.861

Results as means ± SEM.
Atherogenic index, log [TG/HDL (mg/dL)]; FI, fasting insulin; HOMA2-IR, homeostatic model assessment; TChol, total cholesterol.

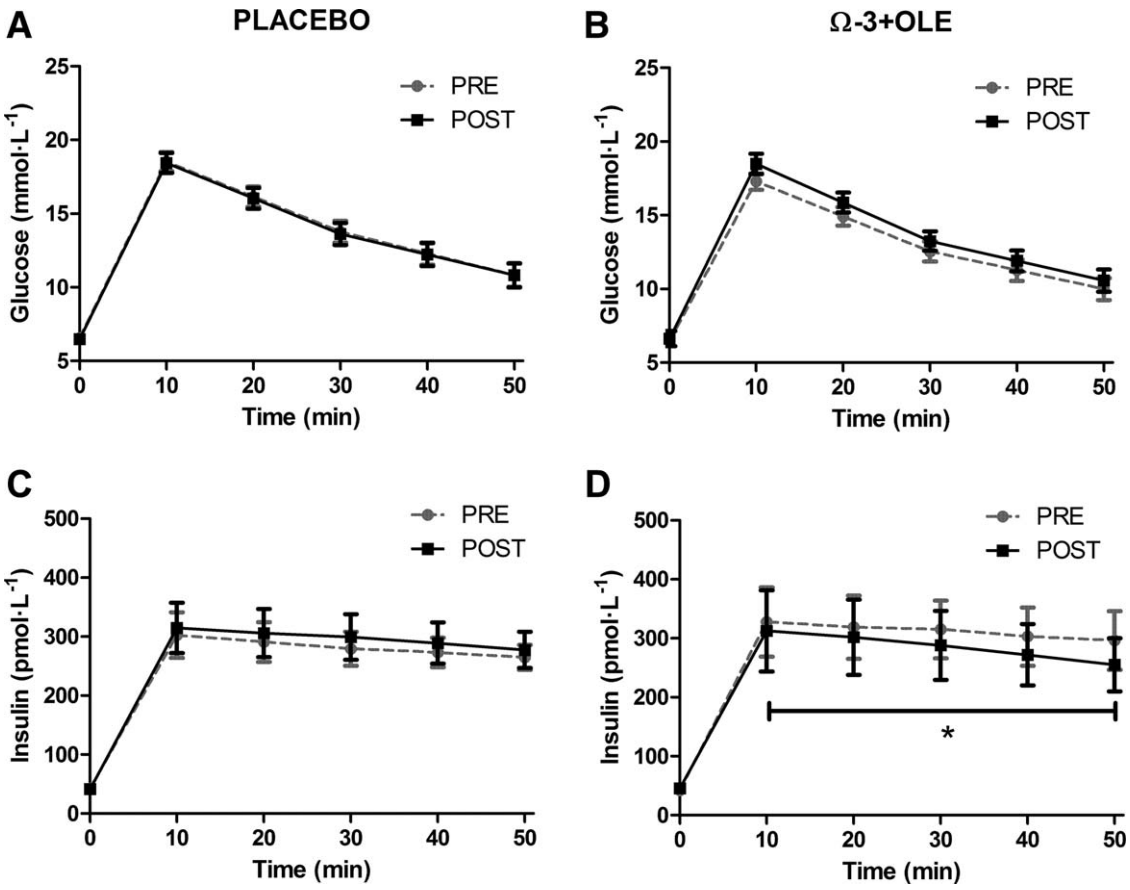

Figure 2 Time course of plasma glucose and insulin concentrations during IVGTT before and after intervention in (**A**,**C**) placebo (PLAC) and (**B**,**D**) supplemented (Ω-3 + OLE) groups. *The area under the curve of insulin concentration in the Ω-3 + OLE group is significantly lower in POST vs. PRE ($P < 0.05$).

Insulin resistance is a key factor underlying many of the symptoms of MSyn. On one hand, insulin sensitivity has been related to muscle phospholipid fatty acid composition. Although the mechanisms are not fully elucidated, it has been suggested that changes in membrane fluidity could influence insulin receptor function (27). In addition, research conducted in rodents showed that Ω-3PUFA supplementation improved insulin sensitivity by upregulating several genes implicated in insulin actions (9) as well as in the expression of glucose transporter 4 (9) on target cells. In addition, insulin sensitivity improvements may depend on exercise modality. We have recently reported that a bout of

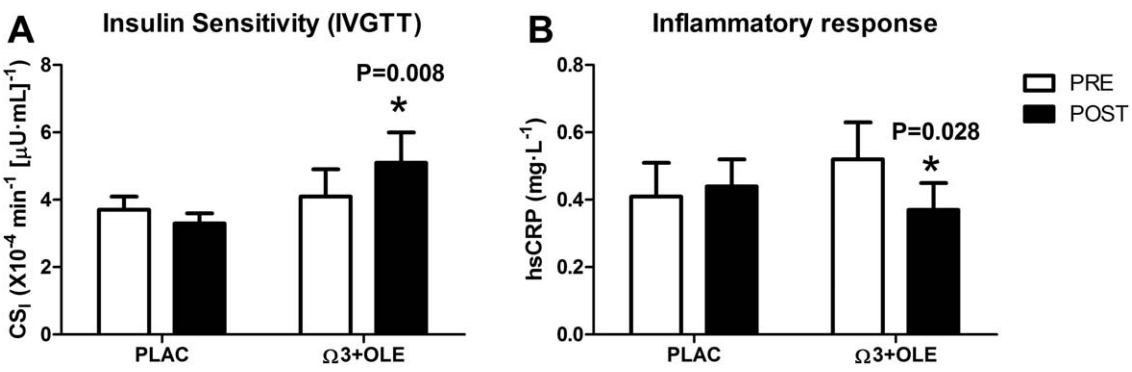

Figure 3 (**A**) Calculated insulin sensitivity (CS_I) from 50-min IVGTT and (**B**) inflammatory response assessed by high-sensitive C-reactive protein (hsCRP) before (PRE) and after (POST) a double-blind, 24-week intervention with placebo (PLAC) or supplement (Ω-3 + OLE). *Different from PRE ($P < 0.05$).

HIIT resulted in greater improvements in insulin sensitivity when compared with a more "conventional" steady-state endurance exercise bout (28). In fact, a single session of HIIT in patients with MSyn improved insulin sensitivity to a similar extent as pharmacological treatment with metformin (29), emphasizing the utility of this exercise modality in this population. Similarly, using the HOMA index, Tjonna et al. and our laboratory have reported insulin sensitivity improvement after 16 weeks of HIIT (21,26). In contrast, Stensvold et al. did not find differences in insulin sensitivity after 12 weeks of a similar intervention (30). The discrepancies between studies could be related to the high variability reported in the insulin sensitizing effects of exercise assessed by HOMA (31). For this reason, in this study, we implemented IVGTT, which has a greater reproducibility than HOMA and oral glucose tolerance tests (25). Another possible explanation for the discrepancy between our findings and others reporting no improvement in insulin resistance with HIIT may be the combination of dietary manipulation and HIIT. In this study, insulin sensitivity only improved after HIIT in the Ω-3 + OLE supplemented group. In addition, we have recently reported that a reduced-calorie diet was required to improve insulin sensitivity in patients with MSyn undergoing an HIIT exercise program (32). Therefore, longer-term insulin sensitizing effects of HIIT in patients with MSyn may require dietary manipulations (e.g., reduced calorie intake or Ω-3 + OLE supplementation) to induce beneficial clinical outcomes. Of note, insulin sensitivity improved despite some of our subjects taking medications that could interfere with insulin actions [i.e. metformin and statins (33,34)].

Systemic proinflammatory stress is associated with insulin resistance (35). Conversely, interventions resulting in reduced inflammatory stress including weight loss also showed parallel improvements in insulin sensitivity (36). Here, we found that despite similar weight loss after 24 weeks of HIIT in our PLAC and Ω-3 + OLE cohorts, systemic hsCRP concentrations were only reduced with Ω-3 + OLE. This suggests that increased Ω-3PUFA + OLE intake contributed to a reduction in proinflammatory stress. Similarly, Tartibian et al. found in postmenopausal women decreased levels of the proinflammatory cytokines interleukin-6, tumor necrosis factor-α, and prostaglandin E2 after a combined intervention based on continuous moderate-intensity exercise and Ω-3PUFA supplementation (37). When released into systemic circulation, these proinflammatory cytokines promote the secretion of hsCPR from the liver (marker of inflammation) and contribute to peripheral insulin resistance (38). Finucane et al. (18) recently observed a reduction in adipose tissue proinflammatory secretion following MUFA supplementation through a series of cell culture and rodent experiments. Consistent with our findings, they also observed a positive association between dietary MUFA intake and insulin sensitivity. Moreover, nitroalkenes are generated when unsaturated FFA react with nitric oxide derived species. Nitroalkenes have been linked with activation of anti-inflammatory signaling pathways, which could partly explain the effects of Ω-3 + OLE supplementation on insulin sensitivity (39). Unfortunately, we did not measure nitroalkene generation and cannot add more insight about this relevant topic. Collectively, evidence suggests that dietary Ω-3PUFA + OLE supplementation may relieve systemic proinflammatory stress and its inhibitory effect on the insulin-signaling cascade via reduced cytokine secretion from adipose tissue. Overall, Ω-3PUFA + OLE supplementation appears to be an effective intervention strategy for improving insulin sensitivity in clinical populations including patients with MSyn.

Combined use of MUFA and lower doses of Ω-3PUFA have been shown to improve cardiovascular risk factors (15,16). Visioli et al. found reductions in triacylglycerols and increases in HDL serum concentrations in participants who were healthy after 6 weeks of Ω-3PUFA + MUFA supplemented milk (15). In addition, Baro et al. showed a significant decrease in plasma concentration of homocysteine, LDL, and vascular cell adhesion molecule after 2 months of daily ingestion of skim milk supplemented with Ω-3PUFA + OLE (16). Thus, previous research and the present results indicate that the incorporation of Ω-3PUFA and MUFA into a functional food (skim milk) improve cardiovascular and metabolic risk factors. Data from our laboratory (32) showed that 16 weeks of HIIT improved cardiorespiratory fitness, blood pressure, HDL, and waist circumference while it was not effective on decreasing triacylglycerols or LDL and had a modest impact on insulin sensitivity. We currently hypothesized that the additive action of exercise and Ω-3PUFA + MUFA supplementation could improve those variables resilient to change with training alone.

In our study, participants in the Ω-3 + OLE group ingested 275 mg/day of EPA + DHA for 24 weeks, which increased plasma concentrations of DHA + EPA from 3.2 ± 0.4 to 4.9 ± 1.4% without reaching statistical difference. Baro et al. (16) provided healthy volunteers 330 mg/day of EPA + DHA for 8 weeks and found increases in plasma concentrations of DHA but not of EPA (1.6 ± 0.1% to 2.4 ± 0.1%). Although we did not find a statistically significant increase in plasma concentration of DHA and EPA, it is possible that the daily intake of 275 mg of Ω-3PUFA amounting to 46.2 g after 6 months of the experiment would have resulted in incorporation of these FFA into membrane phospholipids. Exercise by itself increases unsaturated membrane phospholipids (40), and perhaps the elevated blood supply of Ω-3PUFA with our supplementation may had led to greater incorporation into these membrane lipids. In turn, this incorporation may improve fluidity and transport across membranes (10). Our data are limited to FFA concentrations in blood and thus we can only speculate that our supplementation resulted in a change in membrane phospholipid composition.

Studies have shown that dietary supplementation of Ω-3PUFA (9,11-13,15) or oleate (17,18) improves blood lipid profile, insulin sensitivity, and inflammatory response. The combined supplementation of Ω-3PUFA and oleate has additional favorable effects on blood lipid profile and markers of atherosclerosis (16). On the other hand, exercise has been shown to improve MSyn components and cardiorespiratory fitness, the latter being a major determinant of cardiovascular mortality (20,21,26). In other studies, the combination of Ω-3PUFA supplementation and exercise training modulated immune response in postmenopausal women (37). Our research shows for the first time the combined effects of dietary supplementation with Ω-3PUFA plus oleate and exercise on insulin sensitivity and MSyn components. In this study, we observed an improvement in the inflammatory and insulin sensitivity responses mediated by the combination of dietary supplementation and exercise (Ω-3 + OLE). These benefits took place in addition to the improvements in body weight, body composition, and cardiorespiratory fitness provided by exercise alone (i.e., PLAC).

Conclusion

Dietary supplementation of Ω-3PUFA plus oleate (Ω-3 + OLE group) during 24 weeks of HIIT enhanced cardiometabolic health

benefits of HIIT in patients with MSyn. Metabolic (i.e., insulin sensitivity) and cardiovascular (HDL, CRP) risk factors were reduced only when HIIT was accompanied by Ω-3PUFA plus oleate supplementation. Importantly, our data suggest that the combination of lifestyle modifications (HIIT and Ω-3PUFA plus oleate supplementation) could have a greater impact on some of the factors composing MSyn than exercise alone.O

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