Dietary supplementation with omega 3 fatty acids and oleate enhances exercise training effects in patients with metabolic syndrome

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Running title: Diet and exercise in abdominally obese people

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Word count: 3499 words.

Disclosure. The authors report no conflicts of interest.

Bullet-point answers

What is already known about this subject?

- Dietary intervention and exercise are cornerstones in the treatment of metabolic syndrome.

- Intense aerobic exercise training improves insulin sensitivity in obese people.

- Separately, exercise and dietary supplementation with omega-3 polyunsaturated fatty acids (Ω-3PUFA), both improve insulin sensitivity and reduce cardiovascular risk.

What does your study add?

- Twenty-four weeks of intense aerobic exercise training with Ω-3PUFA and oleate supplementation resulted in improved insulin sensitivity, C-reactive protein and HDL.

- Life-style modifications combining exercise and PUFA supplementation help improve cardio-metabolic health in patients with metabolic syndrome.

Abstract

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 cardio-metabolic health. **Methods:** Thirty-six MSyn patients underwent 24-wks of high intensity interval training. Using a double-blind randomized design, half of the group ingested 500 mL·day^{-1} of semi-skimmed milk (8 g of fat; PLAC), whereas the other half ingested 500 mL·day^{-1} of skimmed 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, however not significantly (P=0.286). Improvements in VO$_2$peak (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 IVGTT), serum concentration of C-reactive protein and HDL 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-wks of intervention. **Conclusions:** Diet supplementation with Ω-3PUFA and oleate enhanced cardio-metabolic benefits of intense aerobic exercise training in patients with metabolic syndrome.

Introduction

Metabolic syndrome (MSyn) is characterized by central obesity, dyslipidemia, hypertension

and hyperglycemia [1]. Prevalence of MSyn is as high as ~80% among obese, inactive adults [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]. Additionally, 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 suggests 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 (GLUT 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.

Ω-3PUFA supplementation may also reduce cardiovascular disease risk though by reducing

circulating very-low density lipoproteins [12], triacylglycerols, and free fatty acids (FFA) [13]. It also

has additional anti-inflammatory and anti-thrombotic properties [10]. Current recommended daily

intake for Ω-3PUFA is 250mg for general population and higher for patients with cardiovascular

disease [14]. When this recommendation are combined with monounsatured fatty acids (MUFA)

intake, additional improvements in cardiovascular disease risk such as reduced circulation of LDL,

homocysteine, vascular adhesion molecule 1, and increased HDL have been reported [15, 16]. Data

in animals and humans indicates that oleate improves insulin sensitivity, prevents endoplasmic

reticulum stress, and inflammation through activation of AMP-activated protein kinase (AMPK) [17,

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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 as 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, high-intensity interval training

(HIIT) has reported better outcomes in reversing MSyn than continuous moderate-intensity exercise

[21]. Because the proposed mechanisms by which HIIT may improve metabolic health shares 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 a 24-week HIIT with and without Ω-3PUFA

plus oleate supplementation in a group of patients with metabolic syndrome.

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 per week of moderate-vigorous self-reported physical activity). Table 1 shows participant's baseline characteristics. Eighteen participants (50%) were under pharmacologic treatment for at least one MSyn component. According to the last MSyn consensus [1], to be medicated for a factor accounted as having that component. This study was conducted in accordance with a protocol approved by the local Hospital's Ethics Committee.

Experimental design

Before participation, all volunteers underwent medical screening to confirm the inclusion criteria and to exclude individuals with untreated disease, which could interfere 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 (4 in each group), since insulin sensitivity was the main variable of this study. All participants completed two experimental trials days (Figure 1A) before ("Pre") and again after ("Post") 24 weeks of HIIT combined with ingestion of 500 mL·day^{-1} 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 in 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 (i.e., IVGTT) to determine insulin sensitivity as previously described by Tura et al. [22] (i.e., CS_I).

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Exercise training

Subjects underwent supervised high intensity interval training (HIIT) at a frequency of 3 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 their peak heart rate (i.e., HR_{peak}) followed by 4 x 4-min intervals at 90% HR_{peak} interspersed with 3-min active recovery at 70% HR_{peak} and a 5-min cool-down 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% fat. Fat was composed of 0.5% saturated fatty acids (SFA), 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-skimmed 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% SFA, 0.6% PUFA plus MUFA). Placebo and supplemented milk were packed in identical unlabeled white containers and had a similar flavor. Participants ingested 500 mL of the assigned milk daily during the 24 weeks of the study. Milk was used as a vehicle since previous research has shown that 8 weeks 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 in day 1 of testing (Figure 1B). Body mass was determined using a ± 0.05 kg sensitive scale (WildCat; Mettler-Toledo, USA). 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, USA).

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Cardiorespiratory fitness

Cardio-metabolic measurements were performed in day 1 of testing (Figure 1B). Resting

blood pressure was assessed after 15 minutes of supine resting using an electronic monitor (Tango;

Suntech Medical Instruments Inc., USA). Following, participants underwent a graded exercise test

until volitional exhaustion in an electrically braked cyclo-ergometer (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 (HbA1c), 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, USA). Insulin concentration was

measured using chemiluminescent micro particle immunoassay (Architect ci4100, Abbott

Laboratories, USA). The composition of plasma FFA was measured using gas chromatography-mass

spectrometry (GC/MS; Agilent 5973 Networks, Mass Selective Detector; Agilent Technologies, USA)

after calibration with standards of known concentrations (i.e., WAKO Chemicals, USA).

Insulin sensitivity

The intravenous glucose tolerance test (IVGTT) was performed according the

recommendations of the Islet Cell Antibody Registered User's Group (ICARUS) [24]. A 20 G

intravenous catheter (BD Insyte, Becton–Dickinson, Spain) was inserted in an antecubital vein and a

Luer-lock three-way stopcock attached (Vitroway, Vitromed Healthcare, India). Immediately after

delivering the glucose load, the stopcock, catheter, and vein were rapidly flushed with 10 ml of

saline solution (Salina 0.9 %, Grifols, Spain). Following, every ten minutes (i.e., 10, 20, 30, 40, and 50-min), a 5-mL blood sample was obtained and the catheter flushed with 3 mL 0.9 % saline after 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 (C_{SI}) following Tura et al., [22].

Metabolic syndrome *Z*-score

Calculations of MSyn *Z*-score [20] was 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 MSyn patients (n =169).

Statistical analysis

Results are presented as mean ± standard error of the mean (SEM). Shapiro–Wilk test revealed that data were normally distributed. Possible pre-intervention differences between groups were studied using t-test for non-related 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, U.S.A.) was used for statistical analysis with statistical significance set at P≤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-3 PUFA concentration between groups were not statistically significant (interaction P=0.411).

Changes in blood lipids, blood pressure and assessment of Metabolic Syndrome

MSyn components before and after intervention are depicted in Table 4. Before the experimental intervention, 4 participants from each group were taking antihypertensive medication, 5 in PLAC and 6 in Ω-3+OLE received statins, 2 participants in each group were in fibrates treatment, and 4 participants in each group were taking metformin. At the end of the experiment, primary care physician discontinued statins in 2 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 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

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the Ω-3+OLE group but there were no statistical differences in the time x group interaction (P=0.071; Table 4).

Insulin sensitivity, inflammatory stress and pro-thrombotic response

Time course of glucose and insulin concentrations during IVGTT are presented in Figure 2. During the IVGTT, glucose disappearance rate did not change across time or between groups (Figure 2 A-B). However, the area under the curve of insulin concentration was significantly lower after the intervention with Ω-3+OLE (Figure 2 C-D). Therefore, the calculated insulin sensitivity index (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 pro-inflammatory stress with a significant reduction in hsCRP in the Ω-3+OLE group only, (P=0.028; Figure 3B). Fasting insulin, HbA1C, 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 cardio-metabolic health such as VO$_{2peak}$, blood pressure, body fatness, hsCRP, and HDL [26]. Presently, we used a non-pharmacological intervention (i.e., dietary supplementation) aiming to enhance the cardio-metabolic health benefits of HIIT. We found that 24 weeks of HIIT with Ω-3+OLE supplementation resulted in greater insulin sensitivity improvements (C$_{SI}$), HDL (improving lipid profile), as well as reduced hsCRP (marker of inflammation stress), than 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 the present study is that dietary Ω-3+OLE supplementation, improved the benefits associated with a 24 weeks exercise training program in metabolic syndrome (MSyn) patients. While some previous studies have demonstrated benefits of HIIT [21, 26] or Ω-3+OLE supplementation [15, 16] in MSyn patients 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 cardio-metabolic health in patients with MSyn based on life-style modifications (exercise and dietary supplementation).

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 has shown that Ω-3PUFA supplementation improves insulin sensitivity by upregulating several genes implicated in insulin actions [9] as well as in the expression of GLUT4 transporters [9] on target cells. Besides, insulin sensitivity improvements may depend on exercise modality. We have recently reported that a bout of HIIT resulted in greater improvements in insulin sensitivity when compared with more "conventional" steady-state endurance exercise bout [28]. In fact, a single session of HIIT in MSyn patients, improved insulin sensitivity to a similar extent as pharmacological treatment with

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metformin [29], emphasizing the utility of this exercise modality in this population. Similarly, using

the homeostatic model assessment (HOMA), Tjonna and co-workers and our laboratory have

reported insulin sensitivity improvement after 16 weeks of HIIT [21, 26]. In contrast, Stensvold and

co-workers 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 the present study we

implemented intravenous glucose tolerance test, 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 due to the

combination of dietary manipulation and HIIT. Presently, 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 MetSyn patients undergoing an HIIT exercise

program [32]. Therefore, longer-term insulin sensitizing effects of HIIT in MSyn patients may require

dietary manipulations (e.g., reduced calorie intake or Ω-3+OLE supplementation) to induced

beneficial clinical outcomes. Of note, insulin sensitivity improved despite some of our subjects were

taking medications that could interfere with insulin actions (i.e. metformin and statins; [33, 34]).

Systemic pro-inflammatory stress is associated with insulin resistance [35]. Conversely

interventions resulting in reduced inflammatory stress including weight loss also show 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 and oleate intake contributed to a reduction in

pro-inflammatory stress. Similarly, Tartibian et al., found in post-menopausal women decreased

levels of the pro-inflammatory cytokines IL-6, TNF-a and PGE2 after a combined intervention based

on continuous moderate-intensity exercise and Ω-3PUFA supplementation [37]. When released into

systemic circulation, these pro-inflammatory cytokines promote the secretion of hsCPR from the

liver (marker of chronic inflammation) and contribute to peripheral insulin resistance [38]. Finucane

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et al., [18] recently observed a reduction in adipose tissue pro-inflammatory 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 nitroalkenes generation and cannot add more insight about this relevant topic. Collectively, evidence suggests that dietary Ω-3PUFA and oleate supplementation may relieve systemic pro-inflammatory stress and its inhibitory effect on the insulin-signaling cascade via reduced cytokine secretion from adipose tissue. Overall, Ω-3PUFA and oleate supplementation appears to be an effective intervention strategy for improving insulin sensitivity in clinical populations including MSyn patients.

Combined use of MUFA and lower doses of Ω-3PUFA improve cardiovascular risk factors [15, 16]. Visioli et al, found reductions in triacylglycerols and increases in HDL serum concentrations in healthy participants after 6 weeks of Ω-3PUFA + MUFA supplemented milk [15]. In addition, Baró et al., showed a significant decrease in plasma concentration of homocysteine, LDL, and vascular cell adhesion molecule, after 2 months of daily ingestion of skimmed milk supplemented with Ω-3PUFA and oleate [16]. Thus, previous research and the present results indicate that the incorporation of Ω-3PUFA and MUFA into a functional food (skimmed milk) improve cardiovascular and metabolic risk factors. Since data from our laboratory [32], showed that 16 weeks of high intensity interval training improved cardiorespiratory fitness, blood pressure, HDL and waist circumference, but it was not effective on decreasing triacylglycerols, LDL and have a modest impact on insulin sensitivity, we hypothesized that the additive action of exercise and Ω-3PUFA + MUFA supplementation, could improve those variables resilient to change after the same training alone.

In our study, participants of Ω-3+OLE group ingested 275 mg·day^{-1} of the EPA+DHA for 24 weeks, which increased plasma concentrations of DHA+EPA from 3.2±0.4 to 4.9±1.4% without

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reaching statistical difference. Baró et al. [16], provided healthy volunteers 330 mg·day^{-1} 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%; respectively). 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 is limited to FFA concentrations in

blood and thus we can only speculate that our supplementation resulted in a change in membrane

phospholipid composition.

Studies show that dietary supplementation of Ω-3PUFA [9, 11-13, 15] or oleate [17, 18]

improve 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 improves MSyn components and

cardiorespiratory fitness, being the latter, a major determinant of cardiovascular mortality [20, 21,

26]. In addition, the combination of Ω-3PUFA supplementation and exercise training modulates

immune response in post-menopausal 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. Presently, 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

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Dietary supplementation of Ω-3 polyunsaturated fatty acids plus oleate (Ω-3+OLE group) during 24 weeks of high intensity interval training (HIIT) enhanced cardio-metabolic health benefits of HIIT in patients with metabolic syndrome. Metabolic (i.e., insulin sensitivity) and cardiovascular (HDL, C-reactive protein) risk factors were reduced only when HIIT was accompanied by Ω-3PUFA plus oleate supplementation. Importantly, our data suggests that the combination of lifestyle modifications (HIIT and Ω-3PUFA plus oleate supplementation) could have a greater impact on some of the factors composing metabolic syndrome than exercise alone.

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Accepted Article

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Figure captions

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

Figure 2. Time course of plasma glucose and insulin concentrations during IVGTT before and after intervention in placebo (PLAC; A, C) and B. supplemented (Ω-3+OLE; B, D) 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).

Figure 3. A) Calculated insulin sensitivity (CS$_I$) from 50-min intravenous glucose tolerance test and B) Inflammatory response assessed by high sensitive C-reactive protein (hs CRP), before (PRE) and after (POST) a double blind, 24 weeks intervention with placebo (PLAC) or Ω-3 polyunsaturated fatty acids + oleate (Ω-3+OLE). * Different from PRE (P<0.05).

Table 1. Baseline anthropometric variables and metabolic syndrome components. Waist-C, waist

circumference; TG, fasting triacylglycerol; FG, fasting glucose; SBP, systolic blood pressure; DBP, diastolic

blood pressure; MSyn, metabolic syndrome.

Variable	PLAC (n=18)	Ω-3+OLE (n=18)	P value
Age (years)	53 ± 2	54 ± 2	0.674
Weight (kg)	88.2 ± 3.3	93.9 ± 2.7	0.186
BMI ($kg \cdot m^{-2}$)	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 ($mmol \cdot L^{-1}$)	1.38 ± 0.16	1.39 ± 0.21	0.966
HDL ($mmol \cdot L^{-1}$)	0.96 ± 0.05	0.93 ± 0.03	0.897
FG ($mmol \cdot L^{-1}$)	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.

Table 2. Anthropometric and cardio-metabolic variables in both intervention groups. Peak oxygen

consumption (VO_{2peak}).

Variable	PLAC		Ω-3+OLE		P value	
	Pre	Post	Pre	Post	Time	Time x 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} ($L \cdot min^{-1}$)	1.98 ± 0.15	2.20 ± 0.18	2.07 ± 0.16	2.37 ± 0.18	<0.001	0.442
$V_{E\ peak}$ ($L \cdot min^{-1}$)	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.

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

	PLAC		Ω-3+OLE		P value	
	Pre	**Post**	**Pre**	**Post**	**Time**	**Time x 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.

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Table 4. Progression of metabolic syndrome components after intervention (PLAC vs. Ω-3+OLE). Waist-C, waist circumference; TG , fasting triacylglycerols; HDL, fasting high density lipoprotein; FG, fasting glucose concentrations in plasma; SBP, resting systolic blood pressure; DBP, resting diastolic blood pressure; MSyn, metabolic syndrome. * Different from PLAC at the same time point. † Different from PRE.

Variable	PLAC		Ω-3+OLE		P value	
	Pre	Post	Pre	Post	Time	Time x 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})	1.38 ± 0.16	1.66 ± 0.26	1.39 ± 0.21	1.20 ± 0.10	0.717	0.083
HDL (mmol·L^{-1})	0.96 ± 0.05	0.98 ± 0.05	0.93 ± 0.03	1.04 ± 0.04 † *	0.025	0.040
FG (mmol·L^{-1})	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.

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Table 5. Additional clinical variables. TChol, total cholesterol; Atherogenic index, log [TG/HDL (mg/dl)]; FI, fasting insulin.

Variable	PLAC		Ω-3+OLE		P value	
	Pre	Post	Pre	Post	Time	Time x Group
TChol (mmol·L^{-1})	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^{-1})	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^{-1})	2.87 ± 0.73	2.76 ± 0.55	3.03 ± 0.76	2.89 ± 0.55	0.231	0.861

Results as means ± SEM.

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A.

Pre-testing   Post-testing

Day 1   Day 2   Day 1   Day 2

24 weeks of HIIT + PLAC or 24 weeks of HIIT + Ω3+OLE   72 hours no EX

72 hours   72 hours

B.

Day 1   **Day 2**

Standardized   Standardized
meal   meal

BP   VO$_{2peak}$ &
HR$_{max}$ IVGTT

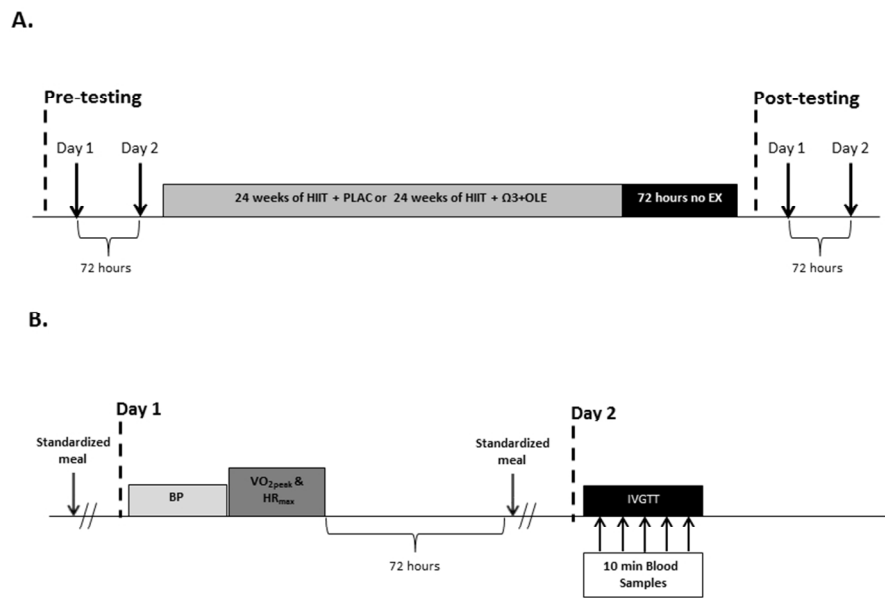72 hours   10 min Blood
Samples

Figure1 Timeline of experimental design. A) General timeline of the study. B) Timeline of the pre- and post testing procedures.
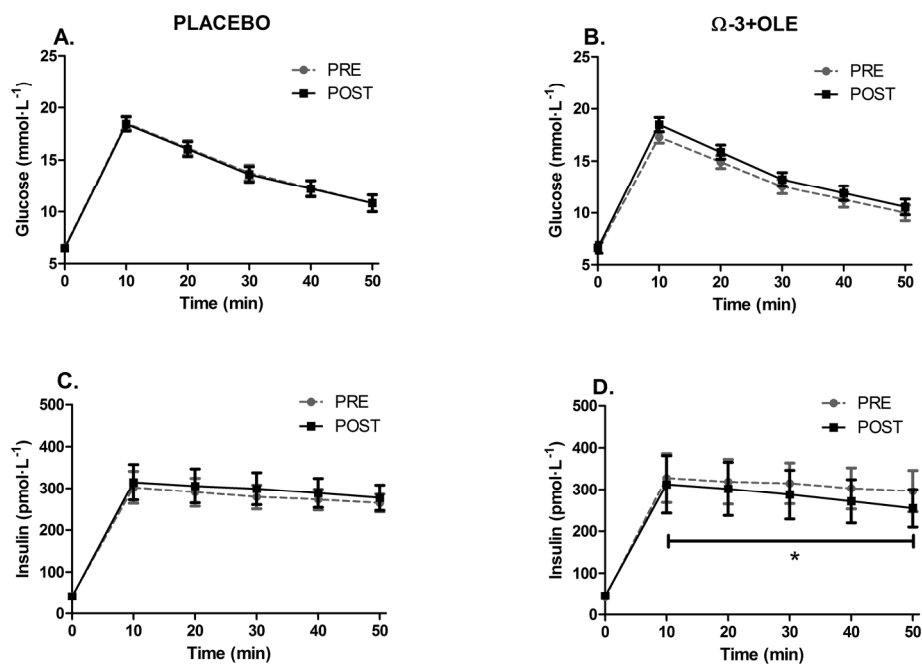254x190mm (96 x 96 DPI)

Figure 2. Time course of plasma glucose and insulin concentrations during IVGTT before and after intervention in placebo (PLAC; A, C) and B. supplemented (Ω-3+OLE; B, D) 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).
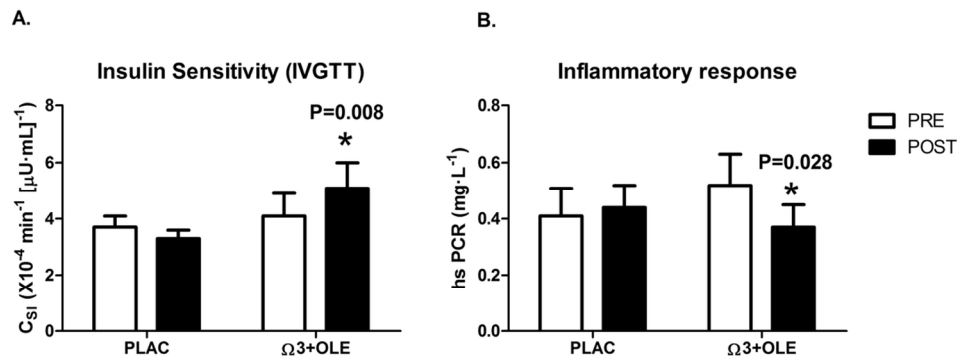177x125mm (300 x 300 DPI)

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110x43mm (300 x 300 DPI)