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Omega-3 PUFAs improved endothelial function and arterial stiffness with a parallel antiinflammatory effect in adults with metabolic syndrome☆

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ABSTRACT

Objectives: Metabolic syndrome (MetS) is associated with adverse cardiovascular events, and impaired vascular function. In this study we evaluated the effects of omega-3 polyunsaturated fatty acids (PUFAs) supplementation on vascular function, inflammatory and fibrinolytic process in subjects with MetS.
Methods: We studied the effect of a 12 weeks oral treatment with 2 g/day of omega-3 PUFAs in 29 (15 male) subjects (mean age 44 ± 12 years) with MetS on three occasions (day0: baseline, day 28 and day 84). The study was carried out on two separate arms (PUFAs and placebo), according to a randomized, placebo-controlled, double-blind, cross-over design. The diagnosis of MetS was based on the guidelines of Adult Treatment Panel III definition. Endothelial function was evaluated by flow-mediated dilation (FMD) of the brachial artery. Carotid-femoral pulse wave velocity (PWV) was measured as an index of aortic stiffness. Serum levels of interleukin-6(IL-6) and plasminogen activator inhibitor-1(PAI-1) were measured by ELISA.
Results: Treatment with PUFAs resulted in a significant improvement from day 0 to 28 and 84 in FMD and PWV ($p < 0.001$ for all). Nevertheless, treatment with placebo resulted in no significant changes in FMD ($p = 0.63$) and PWV ($p = 0.17$). Moreover, PUFAs treatment, compared to placebo, decreased IL-6 levels ($p = 0.03$) and increased PAI-1 levels ($p = 0.03$). Finally, treatment with PUFAs resulted in a significant decrease in fasting triglyceride levels from day 0 to 28 and 84 ($p < 0.001$) and in serum total cholesterol levels ($p < 0.001$).
Conclusions: In subjects with MetS, treatment with omega-3 PUFAs improved endothelial function and arterial stiffness with a parallel antiinflammatory effect.

1. Introduction

The metabolic syndrome (MetS), a concurrence of impaired glucose and insulin metabolism, overweight and abdominal fat distribution, dyslipidemia, and hypertension, has gain awareness and interest recently, as it is directly correlated with the development and progression of atherosclerotic cardiovascular disease and with type 2 diabetes mellitus and identifies people at higher risk of cardiovascular disease than the general population [1,2]. Importantly, the close relationship between MetS, endothelial dysfunction and impaired arterial wall properties is linked to cardiovascular risk, coronary artery disease and mortality [3–7].

The use of marine omega-3 polyunsaturated fatty acids (omega-3 PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as presented either in oil fish or in concentrated pharmaceutical preparations has demonstrate substantial cardiovascular benefits [8,9]. Current guidelines suggest omega-3 PUFAs consumption in secondary prevention of myocardial infarction and mainly in hypertriglyceridemia. We have also shown previously that supplementation of healthy smokers with 2 g daily of omega-3 PUFAs can ameliorate smoking induce impairment of arterial

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function [10]. The effects of omega-3 PUFAs on vascular properties and inflammatory process remain unknown. Therefore in the present study we examined the impact of omega-3 PUFAs supplementation on the endothelial function, arterial wall properties, inflammatory and fibrinolytic process in subjects with MetS.

2. Research design and methods

2.1. Study population

Twenty-nine patients with MetS, mean aged ± SD (44 ± 12) years old, 14 females–15 males, were included in this double-blind, placebo controlled, cross-over trial. Individual characteristics of the participants are presented in Table 1. The diagnosis of metabolic syndrome was based on the guidelines of American Heart Association and the National Heart, Lung, and Blood Institute, which update the NCEP ATP III (US National Cholesterol Education Program Adult Treatment Panel III) definition in 2004 [11].

We select subjects with MetS and no evidence of cardiovascular disease, acute inflammatory or chronic disease. Diabetes mellitus was determined by fasting plasma glucose tests and was analyzed in accordance with the American Diabetes Association diagnostic criteria (fasting blood glucose levels >125 mg/dl or use of special medication, indicated the presence of diabetes) [12]. Five subjects (2 men) with diabetes mellitus were included in the study. From the analysis we excluded individuals taking regular cardiovascular medications, statins, antioxidant vitamin supplementation, anti-inflammatory or steroid substances during the past 2 months, or oral contraceptives (female participants). We also excluded women under hormone replacement therapy and premenopausal female subjects with irregular menstrual cycle. As in healthy young women endothelial function and arterial wall properties vary during menstrual cycles [13], measurements in each female participants, were performed at the same phase of the menstrual cycle (late luteal phase). The participants refrained from caffeine, alcohol, smoking and any food for 12 h before each study. Their body mass index (BMI) was between 20 and 35 kg/m^2 and their clinical examination and electrocardiogram were normal. All measurements were performed at the same place in the morning (between 8.00 and 10.00 a.m.). Participants were instructed to avoid changes in diet and physical activity habits during the study period to exclude any impact of these parameters in studied parameters. Moreover, daily physical activity and daily dietary logs of all food and beverages consumed during the study were reviewed to assure compliance.

Table 1
Characteristics of the study participants.

Age (years)	44.31 ± 12.23 (26–70)
Gender (males/females)	15/14
Metabolic syndrome components	
Three components (*n*)	10
Four components (*n*)	12
Five components (*n*)	7
Body mass index (kg/m^2)	28.29 ± 3.24 (21.85–34.86)
Peripheral systolic pressure (mmHg)	132.9 ± 22.8 (110–180)
Peripheral diastolic pressure (mmHg)	86.0 ± 5.5 (68–100)
Peripheral pulse pressure (mmHg)	46.8 ± 24.4 (30–112)
Diabetes mellitus (*n*)	5
Heart rate (bpm)	75.0 ± 12.2 (56.3–89.7)
AI75 (%)	5.64 ± 7.62 (−3 to 16)
PWV (m/s)	7.62 ± 1.59 (5.23–13.0)
FMD (%)	3.67 ± 3.57 (−0.5 to 13.2)
EID (%)	15.12 ± 4.56 (8.23–22.12)
Glucose (mg/dl)	102.6 ± 15.8 (72–144)
Total cholesterol (mg/dl)	192.8 ± 32.2 (144–299)
LDL (mg/dl)	107.2 ± 16.1 (80–162)
High density lipoprotein (mg/dl)	39.9 ± 6.7 (24–61)
Triglycerides (mg/dl)	180.1 ± 22.8 (152–257)
log IL-6 (pg/ml)	0.25 ± 0.37 (−0.39 to 1.49)
log PAI-1 (ng/ml)	2.31 ± 0.45 (1.06–3.23)

Values are presented as mean value ± SD. In parentheses we provide the range of values for categorical variables.
n: number of participants; bpm: beats per minute; AI75: augmentation index corrected for a steady heart rate of 75 beats/min; FMD: flow-mediated dilation; EID: endothelial independent dilation; PWV: pulse wave velocity; log IL-6: based 10 logarithm of interleukin 6; log PAI-1: based 10 logarithm of plasminogen activator inhibitor-1.

2.2. Study design

At baseline, the participants rested in a quiet dark room for 30 min, under controlled temperature 22–24 °C. Endothelial function as well as the elastic properties of arterial tree were evaluated in all patients at baseline. Endothelium-independent dilation (EID) was estimated at the end of each study. Blood samples were obtained at baseline. After the baseline visit, the patients were randomly allocated into 2 groups to receive an oral treatment with omega-3 PUFAs (dose of 2 g, 46% EPA–38% DHA) or placebo, for a period of 12 weeks. Both the active drug preparation and placebo were prepared as identical formulations (capsules) and were administrated once daily. Follow-up was performed at the end of fourth week and at the end of twelfth week. After a 4 weeks wash-out period, the same patients were called back for the second part of the study. At that time, the same measurements were performed, but those patients who received placebo in the first part of the study now received omega-3 PUFAs, vice versa, in a double-blind cross-over fashion (Fig. 1).

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee and each subject gave written informed consent.

2.3. Evaluation of endothelial function

Endothelial function was evaluated by estimating the flow mediated dilation in the brachial artery, as previously described [14]. Briefly, after 10 min rest, the right brachial artery was scanned in longitudinal section, 5 cm above the antecubital fossa using a Vivid e ultrasound system (General Electric, Milwaukee, Wisconsin, USA) equipped with a 5.0–13.0 MHz (harmonics) linear array ultrasound transducer. A pneumatic cuff placed distal to the ultrasound probe was then inflated to suprasystolic pressure on the forearm for 5 min to induce reactive hyperemia. After the release of ischemia cuff, brachial artery diameter was measured manually with electronic calipers (as the average derived from multiple diameter measurements along a segment of the vessel) at the boundaries of the media–adventitia interfaces, every 15 s for 2 min, and FMD was defined as the % change of vessel diameter from rest to the diameter 60 s after cuff release [14]. After 10 min rest, a further arterial diameter measurement was made between 2 and 5 min after a single sublingual spray of glyceryl trinitrate (400 μg). The same examiner throughout the study conducted examinations. The same observer who was blinded to the image sequence assignment measured images. Endothelium-independent dilation (EID) was defined as the %change of vessel diameter from rest to the maximum diameter post-nitrate administration. The repeatability of the technique in our institution for determining FMD was determined according to the Bland–Altman method. The repeatability coefficient, which was calculated as defined by the British Standard Institution, that is, according to the formula: repeatability coefficient $= 2 \times \sqrt{(\Sigma di^2/N)}$ (where N is the sample size and di the difference between the two measurements in a pair), was 5.0%.

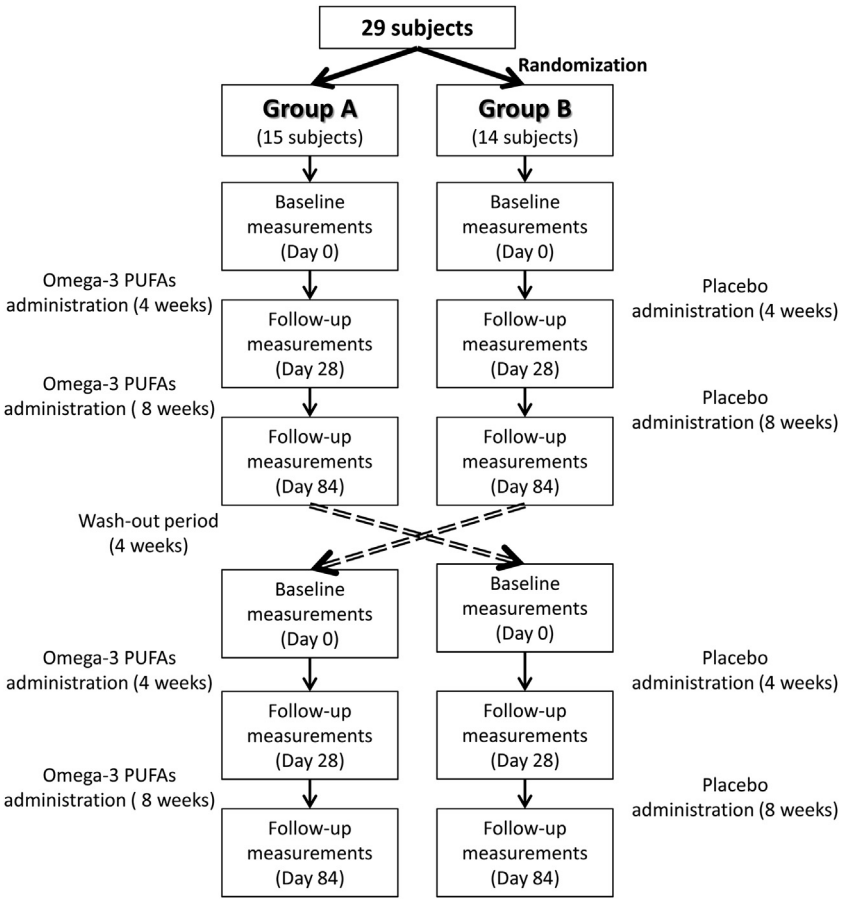

Fig. 1. Diagram showing the design of the study.

2.4. Evaluation of aortic elastic properties

Arterial stiffness was evaluated in all patients with pulse wave velocity (PWV) measurements. Carotid-femoral pulse wave velocity (PWV), which is considered to be an index of aortic stiffness was calculated from measurements of pulse transit time and the distance traveled between 2 recording sites (PWV = distance in meters divided by transit time in seconds) by using a well validated noninvasive device (SphygmoCor; AtCor Medical) as previously described [15]. Two different pulse waves were obtained at 2 sites (at the base of the neck for the common carotid and over the right femoral artery) with the transducer. Distance was defined as the distance from the suprasternal notch to femoral artery minus the distance from the carotid artery to the suprasternal notch.

2.5. Measurement of wave reflection indexes

Augmentation index (AIx) of the central (aortic) pressure waveform and aortic pressures was calculated, as a composite index of wave reflections and arterial stiffness, using a validated, commercially available system (SphygmoCor®, AtCor Medical, Sydney, Australia), which employs the principle of applanation tonometry as previously described [16]. In brief, from radial artery recordings, the central arterial blood pressure was derived with the use of a generalized transfer function which is an accurate estimate of the central arterial pressure waveform. Waveforms of radial pressure were calibrated according to sphygmomanometric systolic blood pressure and diastolic blood pressure measured in the brachial artery. Because augmentation index is influenced by changes in heart rate, it was corrected accordingly (corrected for a steady heart rate of 75 beats/min—AI75).

2.6. Biochemical measurements

A fasting venous blood sample was taken at each visit by venipuncture between 8.00 and 10.00 a.m. Venous blood samples were centrifuged at 3000 rpm and serum was collected and stored at −80 °C until assayed. Serum levels of interleukin-6 (IL-6) were measured as a well-established inflammatory marker by commercially available ELISA kits. Moreover, serum levels of plasminogen activator inhibitor-1 (PAI-1) were measured as a biomarker of fibrinolytic status. Biochemical measurements including lipids and glucose levels were measured by using colorimetric enzymatic method in a Technicon automatic analyzer (RA-1000, Dade-Behring Marburg GmbH).

2.7. Statistical analysis

All variables were tested for normal distribution of the data. Normally distributed data were expressed as means ± s.d. Values of IL-6 and PAI-1 were skewed and they were log-transformed. The effect of omega-3 PUFAs and placebo treatment on arterial wall properties and the impact of the two interventions were evaluated with 2-way repeated-measures ANOVA. In cases in which ANOVA yielded a significant interaction, appropriate post-hoc tests were done by comparing the changes between different examined days. Exact values of $p < 0.05$ were considered statistically significant. The sample size was decided based on a power analysis which

revealed that with a sample size of 28 subjects, a type I error of 0.05 and a moderate effect size of 0.25, our study, with 3 measurements and 2 groups, has a power of more than 80% to reveal differences between the examined parameters. Data analysis was performed with SPSS software, (version 18.0; SPSS, Chicago, Il).

Power analysis with a type I error of 0.05 and a moderate effect size of 0.25 revealed that with 3 measurements and 2 groups the sample of our study.

3. Results

The mean age of the twenty-nine participants (15 male) was 44 ± 12 years. At baseline the mean systolic blood pressure was 133 ± 23 mmHg, the mean diastolic blood pressure was 86 ± 6 mmHg and the heart rate was 75 ± 12 beats per minute. From the study population, five subjects had diabetes mellitus and ten, twelve and seven subjects had three, four and five metabolic syndrome components respectively. At baseline, the mean values of AI75, PWV, FMD and EID were 5.64 ± 7.62%, 7.62 ± 1.59 m/s, 3.67 ± 3.57%, 15.12 ± 4.56% respectively as it is shown in Table 1.

3.1. Effects of omega-3 PUFAs supplementation on FMD and arterial wall properties

Omega-3 PUFAs treatment resulted in a linear increase in FMD values ($p < 0.001$) while, there was no such improvement in FMD values at the placebo arm ($p = 0.63$), as it is shown in Table 2 and in Fig. 2 panel A. Importantly, omega-3 PUFAs treatment, compared to placebo, improved significantly over time ($p < 0.001$) FMD values (Fig. 2 panel A).

As concerning PWV, omega-3 PUFAs treatment resulted in a linear decrease in PWV values ($p < 0.001$) while, placebo administration has no impact in PWV values ($p = 0.17$) as it is shown in Table 2 and in Fig. 2 panel B. Importantly, omega 3 PUFAs treatment compared to placebo, improve significantly over time ($p = 0.006$) PWV values (Fig. 2 panel B).

As, concerning AI75 values, although treatment with omega-3 PUFAs resulted in a linear decrease, this improvement did not reach statistical significance ($p = 0.15$). In the placebo arm we did not observed any improvement ($p = 0.32$) as it is shown in Table 2 and in Fig. 2 panel C.

3.2. Effects of omega-3 PUFAs supplementation on inflammatory and fibrinolytic status

Although, omega-3 PUFAs improved significantly IL-6 levels ($p < 0.001$) and increased PAI-1 levels ($p < 0.001$), placebo administration has no impact on IL-6 levels ($p = 0.77$) and PAI-1 levels ($p = 0.71$) (Table 2). Importantly, omega-3 PUFAs treatment, compared to placebo, improved significantly over time ($p = 0.03$) IL-6 values (Fig. 3 panel A) and increased PAI-1 values ($p = 0.03$) (Fig. 3 panel B).

3.3. Effects of omega-3 PUFAs supplementation on body mass index, glucose and lipids levels

Treatment with omega-3 PUFAs had no significant impact in BMI (28.29 ± 3.24 kg/m^2 vs. 27.95 ± 3.18 kg/m^2 vs. 28.01 ± 3.32 kg/m^2, $p = 0.14$) for day 0, 28 and 84 respectively. Similarly placebo treatment had no impact on BMI (28.22 ± 3.32 kg/m^2 vs. 28.41 ± 3.15 kg/m^2 vs. 28.32 ± 3.15 kg/m^2, $p = 0.09$) for day 0, 28 and 84 respectively. Though, omega-3 PUFAs improved significantly total cholesterol ($p < 0.001$), triglycerides ($p < 0.001$), low density lipoprotein (LDL) cholesterol ($p < 0.001$) and glucose levels ($p = 0.02$) (Table 2). Placebo administration has no impact on total cholesterol ($p = 0.15$), triglycerides ($p < 0.65$), LDL cholesterol ($p < 0.61$) and glucose levels ($p = 0.55$). Neither treatment with omega-3 PUFAs ($p = 0.56$) nor placebo ($p = 0.28$) had an impact in high density lipoprotein (HDL) cholesterol (Table 2).

To further examine if the improvement in total cholesterol, triglycerides, LDL cholesterol and glucose levels observed through time, with omega-3 PUFAs treatment, is significantly different to

Table 2
Effects of treatment on vascular function and serum biomarkers.

	Day 0	Day 28	Day 84	*p*-Values
Omega 3 fatty acids				
FMD (%)	3.67 ± 3.57	5.13 ± 4.51	7.72 ± 4.17*	<0.001
EID (%)	15.12 ± 4.56	14.88 ± 5.02	15.89 ± 5.9	0.56
PWV (m/s)	7.62 ± 1.59	7.40 ± 1.84	7.22 ± 1.54*	<0.001
AI75 (%)	14.83 ± 17.88	14.64 ± 18.06	13.32 ± 17.58	0.15
Total cholesterol (mg/dl)	193 ± 32	187 ± 28*	183 ± 29*	<0.001
Triglycerides (mg/dl)	180 ± 22	175 ± 21*	166 ± 17*	<0.001
High density lipoprotein cholesterol (mg/dl)	40 ± 7	40 ± 6	40 ± 7	0.56
Low density lipoprotein cholesterol (mg/dl)	107 ± 16	104 ± 16*	100 ± 15*	<0.001
Glucose (mg/dl)	102 ± 15	101 ± 13	100 ± 13*	0.02
log Interleukin-6 (pg/ml)	0.26 ± 0.34	0.19 ± 0.36	−0.01 ± 0.36*	<0.001
log Plasminogen activator inhibitor-1 (ng/ml)	2.27 ± 0.46	2.34 ± 0.42	2.56 ± 0.38*	<0.001
Placebo				
FMD (%)	3.63 ± 2.92	3.80 ± 2.62	3.52 ± 2.21	0.63
EID (%)	15.67 ± 6.01	16.20 ± 4.32	15.08 ± 5.01	0.45
PWV (m/s)	7.58 ± 1.77	7.58 ± 1.76	7.54 ± 1.72	0.17
AI75 (%)	15.00 ± 17.63	15.55 ± 18.02	15.33 ± 17.48	0.32
Total cholesterol (mg/dl)	193 ± 32	193 ± 32	192 ± 32	0.15
Triglycerides (mg/dl)	181 ± 23	180 ± 22	182 ± 22	0.65
High density lipoprotein cholesterol (mg/dl)	39 ± 7	39 ± 7	39 ± 6	0.28
Low density lipoprotein cholesterol (mg/dl)	107 ± 16	107 ± 16	107 ± 16	0.61
Glucose (mg/dl)	102 ± 14	102 ± 14	103 ± 16	0.55
log Interleukin-6 (pg/ml)	0.29 ± 0.30	0.27 ± 0.37	0.26 ± 0.44	0.77
log Plasminogen activator inhibitor-1 (ng/ml)	2.36 ± 0.42	2.29 ± 0.39	2.38 ± 0.35	0.71

Variables are presented as mean ± SD. Interleukin-6 and plasminogen activator inhibitor-1 are transformed to based 10 logarithm.
p-Values are based on repeated measures ANOVA and expresses linear trend.
*$p < 0.05$ compared to day 0 after Bonferroni correction.
FMD: flow mediated dilation; EID: endothelium independent dilation; PWV: pulse wave velocity; AI75: augmentation index corrected for a steady heart rate of 75 beats/min.

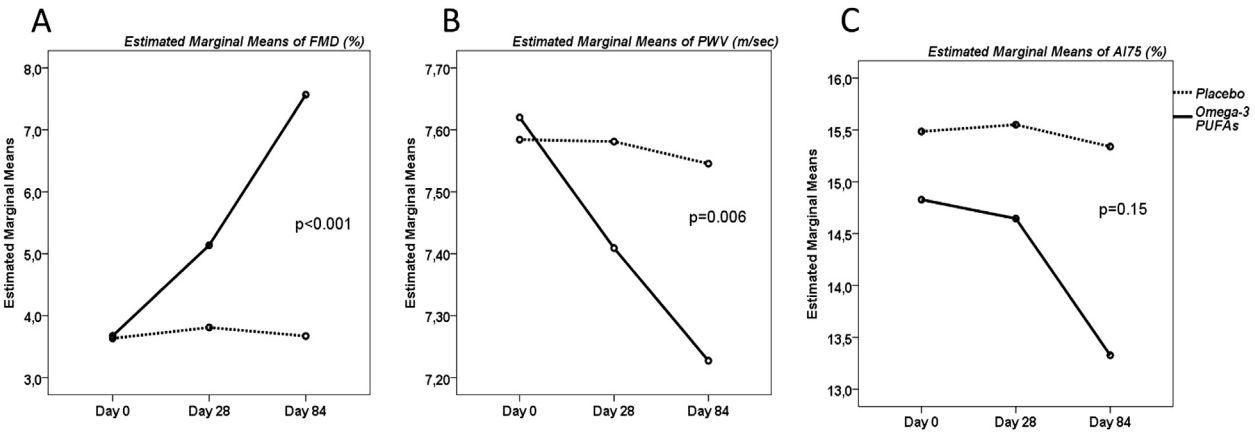

Fig. 2. Charts representing the mean values of FMD (panel A), PWV (panel B) and AI75 (panel C), in the different examined days (day 0, 28 and 84) in the omega-3 PUFAs treatment group and in the placebo group. *p*-Values are referred to the different impact of omega-3 PUFAs and placebo administration in FMD, PWV and AI75. FMD: Flow mediated dilation; PWV: Pulse wave velocity; AI75: Augmentation index adjusted for a stable heart rate of 75 beats per minute.

changes observed with placebo administration we performed repeated measures ANOVA and we examined for the presence of interaction between time and treatment. Accordingly, we found that treatment with omega-3 PUFAs compared to placebo significantly improved levels of total cholesterol ($p < 0.001$), triglycerides ($p < 0.001$) and LDL cholesterol ($p < 0.001$) but not glucose levels ($p = 0.29$) through time (Table 2).

4. Discussion

In the present study we found that a daily administration of omega-3 PUFAs (2 g dose, 46% EPA–38% DHA acid) resulted in an improvement in endothelial function, arterial stiffness, inflammatory and fibrinolytic status in MetS patients. Moreover, omega-3 PUFAs treatment improved the metabolic profile of these subjects by reducing total cholesterol, LDL-cholesterol and triglycerides levels. These findings introduce the hypothesis that favorable effects of omega-3 PUFAs on endothelial function and arterial stiffness in adults with MetS are partially mediated through modification of the metabolic profile and inflammatory process.

4.1. Metabolic syndrome, omega-3 PUFAs and arterial function

Studies have shown favorable effects of dietary modifications including low carbohydrate diets, low fat diets, diets rich in fibers, Mediterranean diets and diets rich in phytochemicals such as flavonoids and phenolic acids in MetS patients. Moreover, cardiometabolic risk can also be modified by an increase in the abundance of omega-3 PUFAs in the diet in MetS patients [17]. In recent years, growing evidence links the intake of omega-3 PUFAs with an improvement in endothelial function [18]. In the present study we documented a favorable effect of omega-3 PUFAs supplementation in endothelial function and central aortic stiffness of MetS subjects with a parallel improvement in metabolic and inflammatory components.

The mechanisms by which omega-3 PUFAs might influence endothelial function are likely to be multiple and complex. It is known that omega-3 PUFAs act via incorporation into cellular phospholipids partly at the expense of arachidonic acid. This incorporation results in a concomitant reduction of $n-6$ PUFAs suggesting that a specific ratio of $n-3$ to $n-6$ fatty acids is important in improving endothelial function. The mechanistic basis for the improved endothelium-triggered relaxation with omega-3 PUFAs includes the suppression of thromboxane A2 or cyclic endoperoxides, a reduced production of cytokines, the augmented endothelial synthesis of nitric oxide, an improvement of vascular smooth muscle cell sensitivity to nitric oxide, and a reduced expression of endothelial adhesion molecules [19,20]. We have also previously documented that a dose of 2 g (46% EPA–38% DHA acid) daily can substantially attenuate inflammation [10].

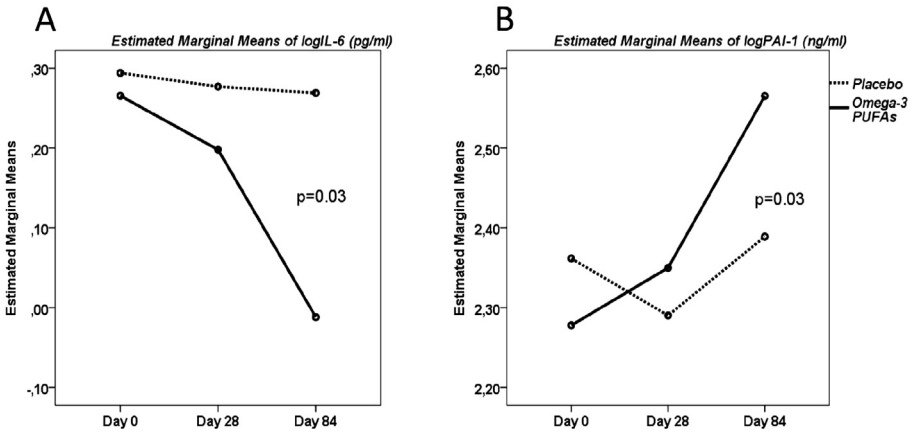

Fig. 3. Charts representing the mean values of log IL-6 (panel A) and log PAI-1 (panel B), in the different examined days (day 0, 28 and 84) in the omega-3 PUFAs treatment group and in the placebo group. *p*-Values are referred to the different impact of omega-3 PUFAs and placebo administration in log IL-6 and log PAI-1. log IL-6: Based 10 logarithm of interleukin 6; log PAI-1: Based 10 logarithm of plasminogen activator inhibitor-1.

Arterial stiffness is another common feature of MetS [4]. Several studies have documented favorable effects of omega-3 PUFAs administration on arterial stiffness in healthy subjects, in smokers, in subjects with dyslipidemia and hypertension [10,21,22]. In this study we documented a favorable effect of omega-3 PUFAs administration in the central aortic stiffness in subjects with MetS and a cluster of risk factors that adversely affect atherosclerosis and arterial compliance.

Several mechanisms have been proposed for the beneficial effects of omega-3 PUFAs in arterial stiffness and atherosclerosis progression. Indeed it was documented that incorporation of omega-3 PUFAs into advanced atherosclerotic plaques has a plaque stabilizing effect reducing number of macrophages in the plaque and stabilizing plaque morphology [23]. It has also proposed that omega-3 PUFAs reduce production of the vasoconstrictor thromboxane A2, improved vascular reactivity and compliance, and has a favorable effect on autonomic nerve function [24]. Moreover, an antagonistic effect of omega-3 PUFAs on angiotensin II receptors and inhibition of the rennin secretion and of angiotensin converting enzyme activity may further enhance arterial compliance [25].

4.2. Metabolic syndrome, omega-3 PUFAs and inflammation

Metabolic syndrome and its components, dyslipidemia, glucose intolerance, diabetes mellitus, obesity, arterial hypertension are characterized by chronic inflammation and an imbalance between proinflammatory and anti-inflammatory cytokines [26–28]. Omega-3 PUFAs compete with arachidonic acid as substrates for the formation of pro-inflammatory mediators, such as leukotrienes, prostaglandins, and cytokines. In addition to competitive inhibition of the $n-6$ fatty acid pathway, $n-3$ PUFAs may also inhibit production of inflammatory and fibrotic mediators including C reactive protein, interleukins, tumor necrosis factor alpha, matrix metalloproteinases 2 and 9, and tissue inhibitors of metalloproteinase [29–31].

In our cohort of subjects with MetS we documented a reduction in IL-6 levels which importantly was accompanied by an improvement in endothelial function and arterial stiffness. These results provide further inside into how omega-3 PUFAs administration can alter arterial wall properties and improve cardiovascular outcome of subjects with MetS.

4.3. Metabolic factors and omega-3 PUFAs

In our cohort of MetS patients we documented that treatment with 2 g daily of omega-3 PUFAs resulted in a decrease in triglycerides levels of the patients. Furthermore, we also documented favorable effects of omega-3 PUFAs treatment in total and LDL cholesterol levels.

Previous studies have shown that a consistent effect of omega-3 PUFAs treatment is a lowering of plasma triglyceride concentrations [32,33]. Current guidelines suggest omega-3 PUFAs consumption in hypertriglyceridemia (2–4 g/day). Importantly, we documented that the reduction in triglyceride levels was accompanied by an improvement in parameters of arterial function.

Concerning the effects of omega-3 PUFAs in glucose homeostasis and insulin resistance, the results are inconsistent. Most of the studies in human suggest that none of the nutritionally important $n-3$ fatty acids improve insulin sensitivity. This is probably due to the inverse association of insulin resistance and Δ^5 desaturase activity that could in turn reduce bioavailability of $n-3$ PUFA [30,34]. In contrast animal studies provide evidence that $n-3$ PUFAs reduce insulin resistance and improve glucose tolerance [30]. In line with previous studies in our cohort of MetS subjects fasting glucose levels were not affected by omega-3 PUFAs treatment.

4.4. Metabolic syndrome, fibrinolytic status and omega-3 PUFAs

In large epidemiologic studies impaired fibrinolytic status as it can be expressed by elevated plasma PAI-1 levels have invariably been demonstrated in subjects with MetS and this elevation may contribute to a thrombotic tendency [35]. Intake of omega-3 PUFAs has been associated with increased plasma PAI-1 activity in healthy individuals or in patients undergoing coronary bypass surgery [10,36]. In the present study we confirm that doses of 2 g/day of omega-3 PUFAs for 3 months increase PAI-1 serum levels in MetS subjects.

4.5. Limitations

The different impact of omega-3 PUFAs administration on PWV and on reflected waves should be attributed to multiple mechanisms of action of omega-3 PUFAs in vascular properties and this is in accordance with previous studies in diabetic subjects [37]. Moreover, multifactorial mechanisms, that determine reflected waves, are not directly associated with changes in endothelial function, nitric oxide production and arterial stiffness. Furthermore we cannot exclude the possibility that a different treatment period would have a significant impact in AI75.

In addition, we have no evidence on the impact of omega-3 PUFAs treatment on other inflammatory markers, such as C reactive protein. Finally, this study does not provide evidence concerning the long term impact of omega-3 PUFAs treatment on cardiovascular health.

5. Conclusions

Treatment with omega-3 PUFAs may favorably affect endothelial function and the elastic properties of the arterial tree in MetS subjects, with a parallel antiinflammatory effect. The effect of omega-3 PUFAs on vascular endothelium and endothelial activation provides a novel mechanism by which omega-3 PUFAs affects vascular compliance, which requires further investigation.

Conflict of interest

There are no conflicts of interest.

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