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DECREASE OF ATHEROSCLEROSIS MARKERS AND OXIDATIVE STRESS AFTER 1 YEAR ADMINISTRATION OF OMEGA-3 FATTY ACIDS SUPPLEMENTS IN PATIENTS WITH METABOLIC SYNDROME

Andreea Dragomir [1,✉], ***Emila Rusu*** [1], ***Mihaela Posea*** [1,2], ***Gabriela Radulian*** [1,2]

[1] University of Medicine and Pharmacy "Carol Davila", Bucharest
[2] National Institute of Diabetes, Nutrition and Metabolic Diseases "N. C. Paulescu", Bucharest

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Abstract

Background and Aims: *To assess the impact of 1 year administration of omega-3 fatty acids supplements on oxidative stress parameters and atherosclerosis progression.* ***Material and Methods:*** *A total of 284 patients with metabolic syndrome, aged 61±6.7 years, without clinical evidence of atherosclerosis were allocated to 2 groups, matched by sex and age: group A (140 patients) – diet according to ESC/EASD recommendations; group B (144 patients) – the same diet + capsules of fish oil (1g eicosapentanoic acid, 1g docosahexanoic acid, 0,1g α-tocopherol acetate). Body fat (BF) was measured by bioimpedance analysis. For oxidative stress evaluation we used the FormOx systems monitor on a blood drop and for progression of atherosclerosis the intima-media thickness (IMT) at common carotid artery. Patients were evaluated at baseline, after 6 months and 1 year.* ***Results:*** *IMT significantly decreased in group B vs group A at 1 year ($p<0.0001$) and was correlated with %BF ($p<0.001$), waist-to-hip ratio (WHR) ($p=0.002$), leptin ($p<0.001$), adiponectin ($p<0.05$), leptin/adiponectin ratio ($p<0.001$) and oxidative stress ($p<0.001$).* ***Conclusions:*** *One year administrations of omega-3 PUFA enriched diet reduces cardiovascular risk of metabolic syndrome patients, resulting in a significant decrease of oxidative stress and atherosclerosis progression.*

key words: *metabolic syndrome, atherosclerosis, oxidative stress, adipocytokines.*

Background and Aims

The Metabolic Syndrome (MetS) is defined as an association of the most dangerous risk factors for fatal heart diseases: type 2 diabetes mellitus (T2DM) or prediabetes (impared fasting glucose – IFG or Impared glucose tolerance – IGT), atherogenic dylipidaemia (high levels of triglycerides and/or decreased levels of HDL-cholesterol), and arterial hypertension.

It is estimated that almost a quarter of the general adult population has MEtS. The risk for development of myocardial infarction or stroke is three times higher among this population than in subjects without MetS, while the risk of cardiovascular death is two times higher. Also, the risk of type 2 diabetes development among this category of people is estimated to be five times higher than in general population [1].

✉ Str. Luncsoara Nr. 4-6, Bl. 63, Sc. B. Ap. 99, sector 2, Bucharest; Tel: 0724.114.869
corresponding author e-mail: andreeadiana.dragomir@gmail.com

According to International Diabetes Federation (IDF), in 2013, 5.1 million of deaths were caused by diabetes. T2DM has become the main cause of mortality among adults, especially because of the associated cardiovascular diseases that claim over 50% of these deaths [1].

Even before diabetes onset, the abnormal levels of blood glucose and lipids increase the risk of cardiovascular disease (CVD); the more components of MetS are associated, the higher is the risk.

According to the International Diabetes Federation (IDF), the following criteria must be met for the diagnosis of MetS [2]:

Central obesity (defined as waist circumference ≥ 94 cm in males and ≥ 80 cm in females) plus any two of the following: 1) Triglycerides (TG) levels ≥150 mg/dl or associated treatment for this condition; 2) HDL-cholesterol levels < 40 mg/dl in males and < 50 mg/dl in females or associated treatment for this condition; 3) Systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg or associated treatment for this condition; and 4) Fasting plasma glucose ≥ 100 mg/dl or previous diagnosis of T2DM.

For research studies, IDF recommends also the assessment of a number of other parameters linked to metabolic syndrome that might shed some light into the subject: distribution of body fat, evaluation of oxidative stress and endothelial dysfunction, adipose tissue biomarkers (leptin, adiponectin) and inflammatory cytokines (TNF-α, IL-6), insulin resistance (HOMA-IR) etc [2].

The study presented in this paper was part of a research grant that started in 2006 and this is why we used the IDF criteria from 2005.

Omega-3 polyunsaturated fatty acids (ω-3 PUFA) are mainly represented by linolenic acid, eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA). The most important dietary sources of ω-3 PUFA are marine seafood (like salmon or tuna), but also some fresh water fish and vegetable oils like soybean and canola.

The major effects of ω-3 PUFA are: decreased risk for cardiovascular mortality, myocardial infarction and ischemic heart disease; antiatherogenic effects and improvement of endothelial function, effects on lipid profile (reduction of triglycerides and increase of HDL-cholesterol levels), lower incidence of arrhythmias, metabolic syndrome and diabetes complications [3-6].

The relationship between fatty acids, atherosclerosis and other inflammatory diseases has been suggested by epidemiological, clinical, and in-/ex-vivo studies. Increased intake of saturated fatty acids is positively associated with development of atherosclerosis and inflammation [7]. In contrast, omega-3 fatty acids, such as EPA and DHA have shown protective effects against CVD.

Hypertriglyceridemia was proposed to be an independent risk factor for CVD [8,9], being associated with increases in both the size and number of circulating TG-rich lipoprotein particles [10]. This may contribute directly to CVD risk by increasing the delivery of atherogenic TG-rich particles to endothelial cells, thus promoting atherosclerosis progression [11]. Also, hypertriglyceridemia is often associated with other CVD risk factors, such as low levels of HDL-C, an increased concentration of small, dense LDL particles, obesity, physical inactivity, insulin resistance, glucose intolerance, prothrombotic and inflammatory states, and cigarette smoking [12,13].

American Heart Association (AHA) recommendations for the intake of omega-3 fatty acids are [14]:

- for individuals without documented CVD - an average intake of 500 mg/day EPA + DHA, consisting of fish, preferably oily fish;

- for secondary prevention in patients with established CVD - intake of 1 g/day EPA + DHA, in the form of oily fish or fish oil capsules;

- for individuals with hypertriglyceridemia, the recommendation is 2–4 g/day under the supervision of a physician.

In 2007, the European and American cardiology societies added to their recommendations 1 g/day EPA + DHA for secondary prevention of cardiovascular disease, postmyocardial infarction, and prevention of sudden cardiac death [15].

In a meta-analysis consisting of 18 trials, fish oil supplementation in patients withT2DM determined a significant decrease of TG and no effect on fasting glucose or HbA1c [16].

The objective of our study was to evaluate the differences between the effect of a standard diet recommended to metabolic syndrome patients and the same diet supplemented with ω-3 PUFA on the progression of atherosclerosis and oxidative stress.

Material and Methods

A total of 284 patients with metabolic syndrome, (defined according to the 2005 IDF criteria), without clinical evidence of atherosclerosis were included into the study. They were allocated to 2 groups, matched by sex, age and weight: group A (140 patients) – diet according to ESC/EASD recommendations and group B (144 patients) – the same diet + one capsule of fish oil (1g eicosapentanoic acid, 1g docosahexanoic acid, 0,1g α-tocopherol acetate).

The nutritional recommendations according to the ESC/EASD guideline [17] were the following: Total Fat intake < 30-35% of dietary energy; Saturated fatty acids < 10% dietary energy; Trans fatty acids < 2% dietary energy; Fiber intake > 30 g/day; and Salt < 6 g/day.

We measured the following anthropometric and clinical parameters: height, weight, waist and hip circumference, blood pressure. We calculated the body mass index (BMI) and the waist-to-hip ratio (WHR).

The laboratory measurements included: total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides; Fasting plasma glucose (FPG) and insulinemia (I); HbA1c; TGO, TGP, GGT; urea, creatinin, uric acid; leptin and adiponectin.

HOMA-IR was calculated accoding to the formula: I (mU/L) x FPG (mg/dL) / 405. We also determined the leptin to adiponectin ratio, because in the recent studies this ratio was correlated with atherosclerosis progression and was considered even a better marker than leptin and adiponectin.

Body fat mass (BFM) and body fat percent (%BF) were measured by bioimpedance analysis, using a BioSpace 3.0 InBody Analyser. The analyser performed whole body and segmental measurements, with three frequency ranges and eight touch electrodes.

Oxidative stress was assessed using FormOx systems monitor on a blood drop using the FORT (Free Oxygen Radicals Test) method. This is a colorimetric test that uses an amine derived employed as chromogen in order to express the oxidative status of the sample. The producers of this instrument (Callegary Spa, Catellani Group, Parma, Italy) defined a unit of measure for this test, called FORT units. One FORT unit corresponds to 7.6 mmol/l of H_2O_2 (equivalent to 0.26 mg/l) [18].

For progression of atherosclerosis, the intima-media thickness (IMT) at the level of the common carotid artery (CAA) was measured.

Patients were evaluated for all the above mentioned parameters at baseline, after 6 months and 1 year of ω-3 PUFA administration.

Statistical analysis

For statistical analysis and interpretation of results we used Microsoft Excel and Statistica var 4.3. All the numeric variables were expressed as mean value ± standard deviation. For comparison of mean values at baseline, 6 months and 12 months we used Student t-test. Value of $p<0.05$ was considered statistically significant.

Results

Some of the baseline parameters of the two study groups are shown in Table 1.

Table 1. Baseline characteristics of the study groups.

Parameters	Group A – Diet only	Group B – Diet + PUFA-omega 3
Age (years)	64 ± 8.4	63 ± 7.3
Total Cholesterol (mg/dl)	224 ± 18.6	226 ± 17.5
HDL – Cholesterol (mg/dl)	43 ± 10	44 ± 12
LDL – Cholesterol (mg/dl)	148 ± 19	147 ± 18
Triglycerides (mg/dl)	155 ± 68	158 ± 62
Fasting Plasma Glucose (mg/dl)	124 ± 22	121 ± 24
BMI (kg/m^2)	33.4 ± 9.2	32.8 ± 8.9
Leptin	20 ± 5.4	19 ± 6.2
Adiponectina	8.9 ± 2.32	9.2 ± 2.64
FORT units	346 ± 86	342 ± 73

After one year intervention a significant difference between groups was observed in terms of anthropometric parameters: in group B, BMI dropped from 32.8 ± 8.9 kg/m2 at baseline to 27.7 ± 5.4 kg/m^2, while in group A, the reduction was from 33.4 ± 9.2 kg/m2 to 30.14 ± 6.3 kg/m^2.

The most clinically significant results were obtained for body fat assessed using bioimpedance method. Thus, after one year, body fat mass (BFM) in group B was 24.82 ± 3.4 kg versus 28.67 ± 5.5 kg in group A. Body fat percent (%BF) dropped to 25.92 ± 1.6% in the group that received PUFA-omega 3, compared to 29.64 ± 4.8% in the group that followed diet only recommendations.

All the differences between groups were statistically significant, following the trend that appeared after 6 months of administration, as shown in Table 2.

Table 2. The evolution of anthropometric parameters at 6 months and 1 year.

Parameters 6 months	Group A – Diet only 6 months	Group B – Diet + PUFA 6 months	P value 6 months
BMI (kg/m2)	31.12 ± 7.8	29.1 ± 5.3	P < 0.5
BF (%)	30.48 ± 6.3	27.48 ± 4.8	P < 0.001
BFM (Kg)	29.42 ± 6.8	26.78 ± 4.2	P < 0.001
Parameters 1 year	Group A – Diet only 1 year	Group B – Diet + PUFA 1 year	P value 1 year
BMI (kg/m2)	30.14 ± 6.3	27.7 ± 5.4	P < 0.002
BF (%)	29.64 ± 4.8	25.92 ± 1.6	P = 0.016
BFM (kg)	28.67 ± 5.5	24.82 ± 3.4	P < 0.001

In terms of metabolic parameters and oxidative stress, after 6 months, the most significant results were obtained for LDL-cholesterol and HDL-cholesterol; although all the results were statistically significant, with a p value < 0.05 as shown in Figure 1.

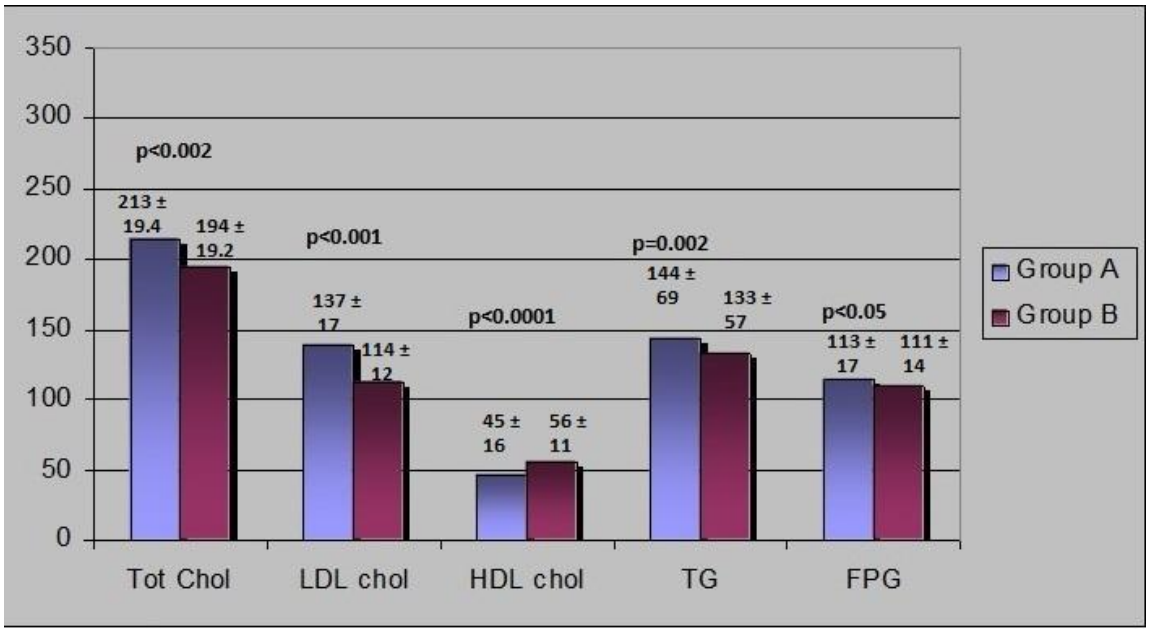

Figure 1. *Modification of metabolic parameters after 6 months.*

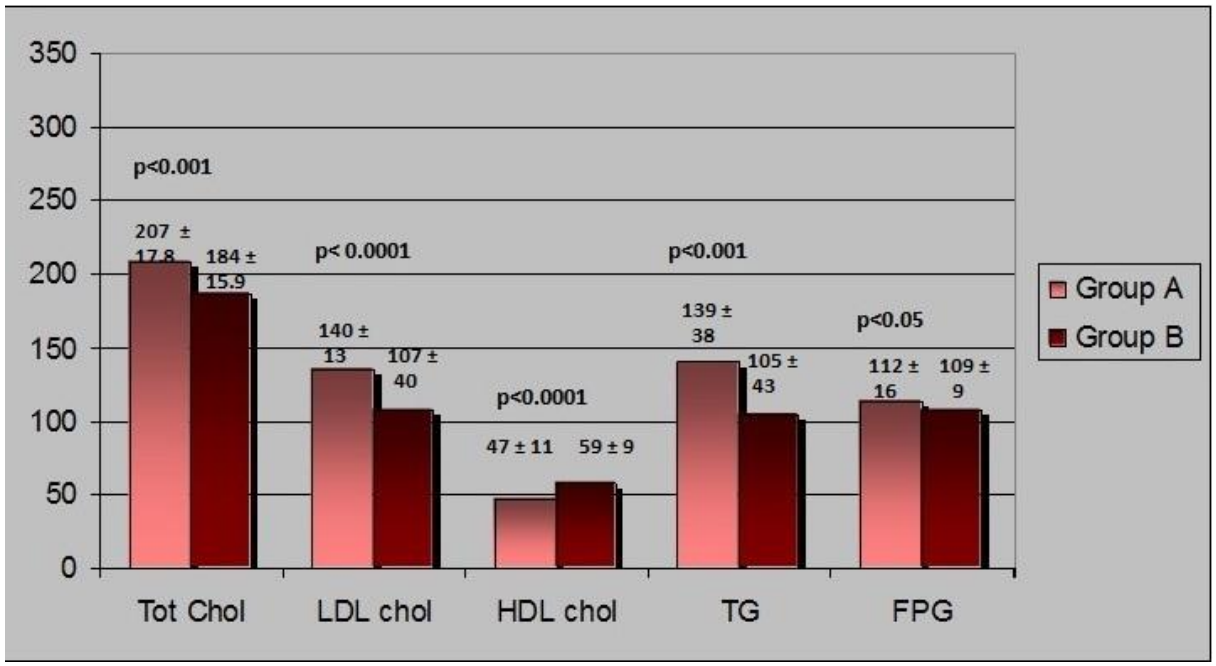

Figure 2. *Modification of metabolic parameters after 1 year.*

After 1 year of treatment, our results showed significant differences between the two groups for TG, LDL-cholesterol, HDL-cholesterol and total-cholesterol (Figure 2).

Regarding the levels of adipokines, at 6 months the results were clinically significant for the leptin to adiponectin ratio and also for leptin and adiponectin, by themselves.

The same trend was maintained after 1 year administration of omega-3 supplements, with the most significant results for leptin values and leptin to adiponectin ratio as shown in Table 3.

Regarding atherosclerosis progression, after 6 months the differences between groups were especially significant for IMT at both left and right common carotid artery as shown in Figure 3.

Table 3. Evolution of adipocytokines levels after 6 months and 1 year.

Parameters 6 months	Group A – Diet only 6 months	Group B – Diet + PUFA 6 months	P value 6 months
Leptin (ng/ml)	18 ± 4.7	16 ± 3.6	P < 0.001
Adiponectin (ng/ml)	9.46 ± 2.76	10.86 ± 2.68	P < 0.001
Leptin-to-Adiponectin Ratio	1.9 ± 1.7	1.47 ± 1.34	P < 0.001
Parameters 1 year	Group A – Diet only 1 year	Group B – Diet + PUFA 1 year	P value 1 year
Leptin (ng/ml)	17 ± 3.9	14 ± 2.8	P < 0.001
Adiponectin (ng/ml)	9.88 ± 2.7	12.25 ± 2.3	P < 0.002
Leptin-to-Adiponectin Ratio	1.72 ± 1.44	1.14 ± 0.84	P < 0.001

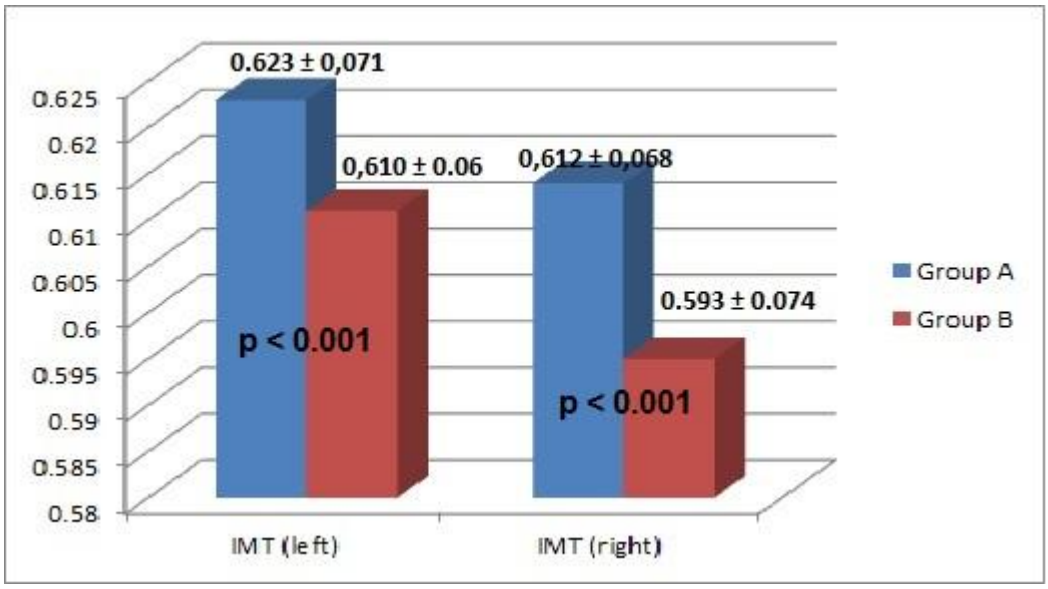

Figure 3. *Modification of IMT in left and right CCA after 6 months.*

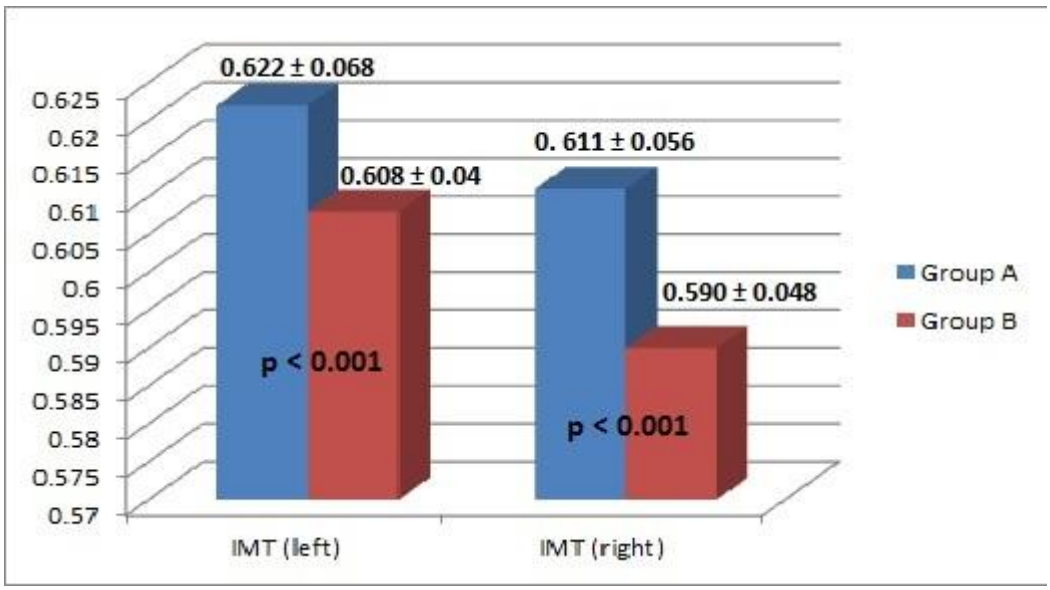

Figure 4. *Modification of IMT in left and right CCA after 1 year.*

As depicted in Figure 4, IMT was also significantly decreased in group B vs. group A at 1 year.

The level of oxidative stress, measured by FORT units decreased significantly more in the group that received PUFA-supplements after 6 months and also after 1 year, as shown in Table 4.

Table 4. Evolution of oxidative stress (FORT units) after 6 months and 1 year.

Parameters 6 months	Group A – Diet only 6 months	Group B – Diet + PUFA 6 months	P value 6 months
FORT units	321 ± 94	272 ± 78	P<0.0001
Parameters 1 year	Group A – Diet only 1 year	Group B – Diet + PUFA 1 year	P value 1 year
FORT units	294 ± 77	255 ± 62	P < 0.001

Finally, we performed a univariate analysis in order to assess the correlation between atherosclerosis progression (assessed by IMT change) and evolution of several clinical and biochemical parameters. The analysis showed a strong correlation between IMT and lipid profile, oxidative stress, adipocytokines (especially leptin to adiponectin ratio) and %BF, underlying the important role of omega-3 supplements on the delay of atherosclerosis and endothelial cells damage (Table 5).

Table 5. Correlation of IMT with different assessed variables.

Variables	IMT	
	R	P
%BF	0.22	0.001
WHR	0.03	0.02
Leptin	0.03	0.001
Adiponectin	0.04	0.05
Leptin/Adiponectin Ratio	0.06	0.001
Oxidative Stress (FormOx)	0.17	0.001
Triglycerides	-0.5	0.002
LDL-Cholesterol	0.67	0.0037
HDL-Cholesterol	0.49	0.01

Discussions

In our study on metabolic syndrome patients, we documented that treatment with 2 g daily of omega-3 polyunsaturated acids resulted in a decrease of triglycerides levels. Also, we observed favorable effects of this treatment on total, LDL and HDL cholesterol levels.

This effect of omega-3 PUFA on lipid fractions was also shown by previous studies [19-22], so that current guidelines recommend 2-4 g/day in patients with hypertriglyceridemia.

The key cause of cardiovascular diseases is atherosclerosis; inflammation and oxidative stress being essential factors in the formation of plaques. Extensive evidence from studies in animal models and data from human studies have underlined the role of oxidative stress in metabolic and cardiovascular disease. In this study, we showed a significant effect of omega-3 PUFA on oxidative stress assessed by FormOx. However, there are conflicting reports on the effects of these supplements on oxidative stress. While a study by Taylor et al. [23] demonstrated an increase in oxidative stress, several other studies showed that supplementation with omega-3 PUFA increased glutathione peroxidase activity, an antioxidant enzyme that reduces hydrogen peroxide [24]. Other studies sustain our findings, suggesting that omega-3 PUFA increase the oxidative stability of the liver, resulting in protection against oxidative stress [25].

Arterial stiffness is another common feature of the MetS. Several studies have documented favourable effects of omega-3 PUFA administration on arterial stiffness in healthy subjects, in smokers, and subjects with dyslipidemia and hypertension [26,27]. In this study we documented a favourable effect of omega-3 PUFA administration on intima-media-thickness (IMT), an effect recently confirmed by

Tousoulis et al [28], as well as several previous studies [29,30].

The metabolic syndrome is also characterized by chronic inflammation and imbalance between pro-inflammatory and anti-inflammatory cytokines. As shown in the MESA study [31], omega-3 PUFA may inhibit the production of inflammatory mediators such as C reactive protein, tumor necrosis factor alpha (TNF-α), and Interleukin-6 and may positively influence the production of leptin and adiponectin by adipose tissue. In our study, we documented a significant decrease in leptin levels and increase in adiponectin levels, which are associated with inhibition of pro-inflammatory cytokines.

Our study had several limitations. Thus, we might mention the need for further long-term, placebo-controlled studies. Also, our study did not show the effect of omega-3 PUFA on inflammatory markers, such as C reactive protein, TNF-α or IL-6.

Conclusions

Our study showed that the supplementation of diets with omega-3 PUFA can have beneficial effects on the cardiovascular risk of metabolic syndrome patients, resulting in a significant decrease of oxidative stress and atherosclerosis progression.

Because of their anti-inflammatory and antioxidant properties, omega-3 PUFA might help preventing the formation of atherosclerotic plaques and lower the risk of developing and progression of cardiovascular disease.

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