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Original article

Effects of omega-3 polyunsaturated fatty acids on fibrosis, endothelial function and myocardial performance, in ischemic heart failure patients

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SUMMARY

Background & aims: Polyunsaturated fatty acids (PUFAs) may affect the cardiovascular system with a multiplicity of mechanisms. We assessed the effects of omega-3 PUFAs supplements on inflammation, fibrosis, left ventricle performance and endothelial function of ischemic heart failure (HF) patients.
Methods: In this double-blind, placebo controlled, cross-over trial we enrolled 31 patients with ischemic HF, followed by a 6-week wash-out period. Omega-3 PUFAs (2 g daily, 8 weeks) were administered PO in the intervention arm. Left ventricle ejection fraction (EF), global longitudinal strain and the ratio E/e' (early ventricular filling to early mitral annulus velocities)were measured. Endothelial function was evaluated by flow mediated dilation and myocardial fibrosis by soluble ST2. High sensitive C Reactive protein (hsCRP) levels were measured as an inflammatory marker.
Results: Treatment with omega-3 PUFA, compared to placebo, improved: left ventricle EF (percent increased by 4.7% vs 1.7%); global longitudinal strain (decreased by −10.6% vs −2.3%); the E/e' ratio (decreased by −9.47% vs −2.1%); ST2 levels (decreased by −4.53% vs −2.37%); flow mediated dilation (percent increased by 44% vs. 11% and hsCRP levels (decreased by −6.13% vs 4.35%) ($p < 0.05$ for all).
Conclusion: Short term treatment with omega-3 PUFAs in subjects with stable ischemic HF improved inflammatory and fibrotic status as well as endothelial function in parallel with systolic and diastolic performance of left ventricle. These findings provide further insights regarding the impact of omega-3 PUFAs administration on left ventricle performance indices, systemic inflammation and fibrosis biomarkers in patients with ischemic HF.

1. Introduction

Heart failure (HF) is a multifaceted disease affecting 1–2% of the adult population imposing diagnostic and therapeutic challenges [1]. Despite developments in the interventional management of coronary artery disease, ischemic etiology is the most frequent cause of HF with reduced ejection fraction [2]. Even with contemporary, optimal medical treatment, more than 20% of patients experience cardiovascular events, such as cardiovascular mortality and HF hospitalizations [3]. Considering adjunctive therapy with omega-3 polyunsaturated fatty acids (PUFAs) is recommended for these patients [1].

Omega-3 PUFAs may affect the cardiovascular system through a multiplicity of mechanisms, such as alteration of ion channel and cellular membrane properties, induction of anti-inflammatory effects, heart rate and arterial pressure decrease, and vascular function as well as left ventricle (LV) diastolic filling improvement [6,7]. The GISSI-HF (Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico–Heart Failure) trial has shown that in patients with chronic HF of ischemic etiology and especially in those

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with LV ejection fraction (LVEF) <40%, treatment with 1gr/day omega-3 PUFAs reduces morbidity and mortality [8].

There is lack of evidence, however, on the pathophysiologic mechanisms underlying functional improvement in HF patients treated with omega-3 PUFAs. Omega-3 PUFA supplementation may exert a beneficial effect on diastolic function [4], which has gained research interest since functional capacity and morbidity cannot be entirely determined by indices of LV systolic performance [1]. Furthermore, omega-3 PUFAs may exert their beneficial effects through endothelial dysfunction improvement by restoring nitric oxide bioavailability [5]. Nitric oxide, peripheral vascular endothelial function and cardiac endothelium (endocardium and intramyocardial capillaries) may be implicated in HF development, progression and clinical appearance while efforts to restore endothelial function are under investigation [6]. Finally, an emerging association between omega-3 PUFAs and biomarkers of myocardial fibrosis, which have been proposed as an additive stratification tool in patients with HF [7], has gained scientific interest [8].

Therefore, in the present study we tested the effects of short term treatment with omega-PUFAs on ST2 levels, endothelial function and LV systolic and diastolic performance in ischemic HF patients.

2. Methods

2.1. Study design

In this double-blind, placebo controlled, cross-over trial, which took place from September 2014 to March 2016, we considered eligible for enrollment patients aged 18–80 years old, with chronic HF of ischemic etiology, LV systolic dysfunction (LVEF<40%), on optimal medical treatment in the maximum tolerated doses per current guidelines [9] for at least 6 months (Table 1), at stable clinical condition for at least 3 months and with no symptoms or signs suggestive of myocardial ischemia. Coronary artery disease was specified based on coronary angiography performed at the time of cardiomyopathy workup with at least one vessel disease with >75% narrowing of the luminal diameter or based on the history of previous myocardial infarction.

We excluded patients with significant valvular or congenital heart disease, persistent or permanent atrial fibrillation, with severely impaired functional capacity New York Heart Association (NYHA) - class IV or minimal symptoms NYHA class I. We also excluded patients with advanced renal disease - estimated glomerular filtration rate 30 $\leq$ ml/min/1.73 m^2, chronic lung disease, advanced liver disease, and severe systematic disease or active malignancy.

After a screening visit including medical history and physical examination, blood sample was drawn for electrolytes, liver and renal function testing, as well as blood count. Following electrocardiographic and echocardiographic evaluation, eligible participants, after signing an informed consent form, were allocated in a random fashion into 2 groups, according to a computer-generated sequence. Patient allocation was concealed from all study participants with sealed, opaque envelopes.

The first group (Group A) received an oral treatment with omega-3 PUFAs (dose of 2 g, 46% eicosapentaenoic acid −38% docosahexaenoic acid) for a period of 8 weeks. The second group (Group B) received an oral treatment with placebo (olive oil) of undistinguishable appearance for a similar 8 weeks' period. The active drug preparation and placebo were administrated once daily and were prepared as identical formulations (capsules). Both active drug preparation and placebo were quality checked by the manufacturer for omega-3 fatty acid content with a deviation for eicosapentaenoic and docosahexaenoic acids less than ±2%. Follow up was performed at day 56. The same patients were called back for the second part of the study, following a 6 weeks' wash-out period. At that visit, the patients underwent the same measurements, but those participants who received omega-3 PUFAs in the first leg of the study were now treated with placebo, and vice versa, in a randomized double-blind, cross-over design (Fig. 1). Patients were instructed to maintain a balanced diet and to avoid significant changes in the dietary habits throughout the trial.

Table 1
Patient's demographic and clinical characteristics.

	Total number of patients	Group A (treated with ω-3 PUFAs) (n = 15)	Group B (Placebo) (n = 16)	P value
Age (years)	67 ± 6	66 ± 4	67 ± 7	0.62
Male Gender (%)	72	70	74	0.73
Weight (Kg)	80 ± 12	76 ± 13	83 ± 8	0.08
Body mass index (Kgr/m2)	27.2 ± 3.7	26.3 ± 3.9	28.2 ± 3.5	0.14
NT-pro-BNP(pg/ml)	832 (140–1103)	1008 (150–1435)	778 (111–1123)	0.45
NYHA				0.54
Class II	65	62	69	
Class III	35	38	31	
Left ventricle ejection fraction (%)	29 ± 8	30 ± 7	27 ± 8	0.27
Systolic blood pressure (mmHg)	123 ± 13	119 ± 13	126 ± 12	0.13
Diastolic blood pressure (mmHg)	80 ± 9	77 ± 9	83 ± 8	0.36
Diabetes mellitus (%)	29	27	32	0.51
Heart Rate (beats per minute)	68 ± 10	65 ± 7	71 ± 11	0.08
Diuretics (%)	81	81	80	0.92
ACE inhibitor or ARB (%)	87	93	75	0.25
b-blocker (%)	77	81	73	0.59
MRA (%)	71	62	80	0.28
Ivabradine (%)	25	25	27	0.92
Statins (%)	68	80	56	0.16
Total cholesterol (mg/dl)	181 ± 30	177 ± 24	184 ± 35	0.57
LDL Cholesterol (mg/dl)	106 ± 27	105 ± 18	107 ± 35	0.90
HDL cholesterol (mg/dl)	43 ± 10	42 ± 9	44 ± 11	0.56
Triglycerides (mg/dl)	157 ± 44	150 ± 33	165 ± 53	0.38

P-values refers to the difference between Group A and B.
NT-pro-BNP: N-terminal pro b-type natriuretic peptide; PUFAs: Polyunsaturated Fatty Acids; ACE: Angiotensin converting enzyme; ARB: Angiotensin receptor blocker; MRA: mineralocorticoid receptor.
Antagonist; HDL: High Density lipoprotein.

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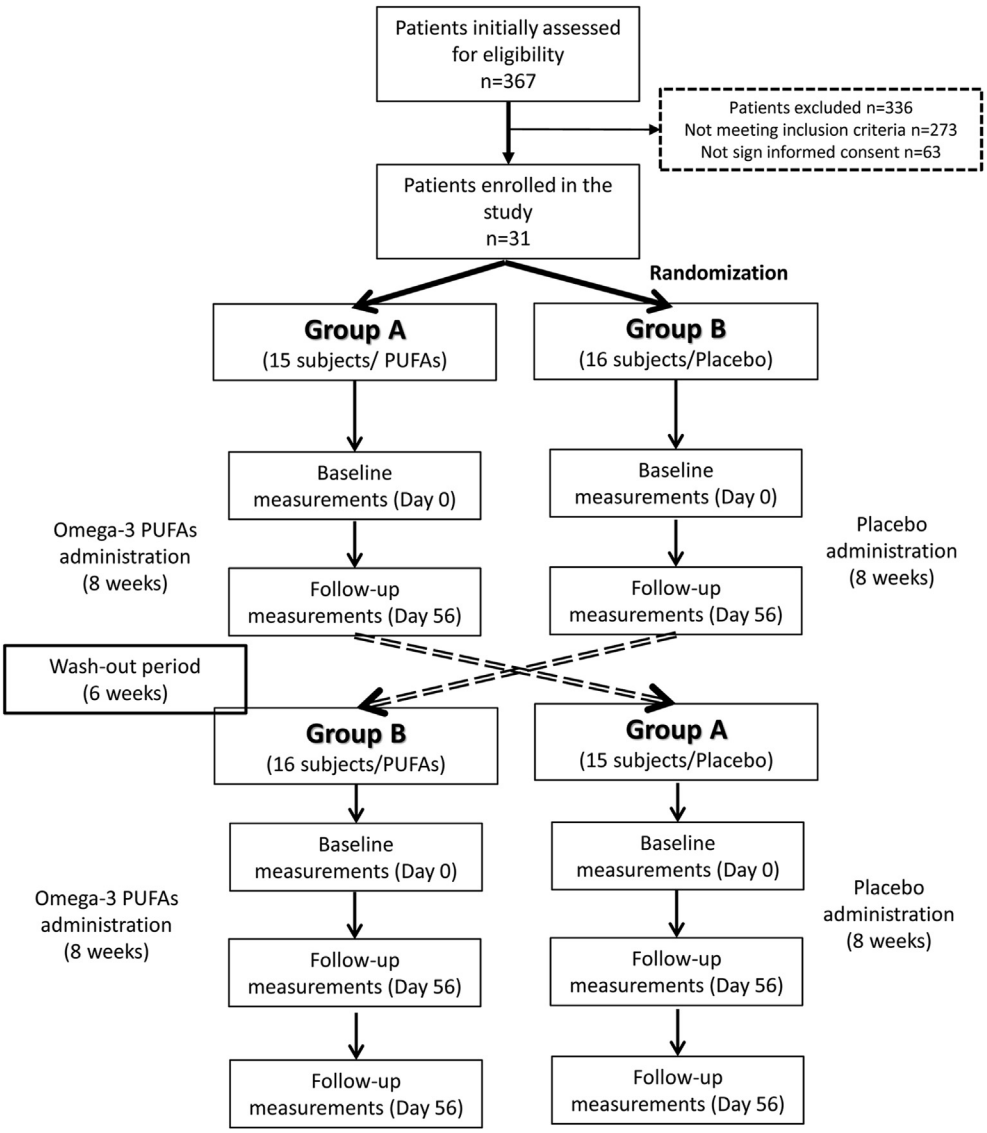

Fig. 1. Flow-chart of the study design. Selection of the study population. Randomization and allocation of the study population in two groups. PUFAs: polyunsaturated fatty acids; n: Subject Number.

Functional capacity classification by NYHA status was determined at baseline. Endothelial function was also assessed by estimating flow mediated dilation (FMD) and endothelial independent dilation (EID). All measurements were performed at baseline, at the end of the 8 weeks' treatment period, before placebo treatment as well as at the end of the 8 weeks' placebo period.

2.2. Endothelial function assessment

Assessment of endothelial function was performed by means of FMD and EID in the brachial artery, as previously described [10]. All patients were in a fasting state for at least 12 h prior to the study. In addition, patients were asked to withdraw from all vasoactive medications for a 12-h period before the study took place. All measurements were conducted in a quiet, temperature-controlled room. Following a 10 min rest, the right brachial artery was initially assessed 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. Reactive hyperemia was induced by placing a pneumatic cuff on the forearm in a distal position in relation to the ultrasound probe, which was then inflated to suprasystolic pressure for 5 min. Following the release of cuff-induced ischemia, brachial artery diameter was determined in a manual manner with electronic calipers (as the average value of numerous diameter measurements along a segment of the vessel) at the limit of the media adventitia interfaces, every 15 s for a 2 min period. FMD was calculated as the percentage change of brachial artery diameter from baseline measurement to the diameter 60 s after cuff release. EID was measured after 10 min rest; patients received single sublingual spray of glyceryl trinitrate (400 mg) and additional arterial diameter measurement was made between 2 and 5 min. EID was calculated as the percentage change of vessel diameter from rest to the maximum vessel diameter after nitrate administration. To avoid systemic bias, all examinations were carried out by the same examiner and the same observer, who was blinded to the image sequence assignment, measured images.

2.3. Echocardiographic evaluation

A General Electric Vivid E9 ultrasound machine (GE Vingmed Ultrasound AS, Horten, Norway) with a 2.5-MHz M5Sc transducer

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was used for all echocardiographic examinations, including 2-dimensional and Doppler images as well as myocardial deformation images. According to the American Society of Echocardiography and the European Association of Cardiovascular Imaging [11,12] the following parameters were measured: LV end-diastolic and end-systolic diameter as well as LV end-diastolic and end-systolic volume; LVEF (%) was calculated by Simpson's biplane method; Mitral diastolic inflow velocities peak velocity of early ventricular filling [E-wave], peak velocity of late ventricular filling [A-wave], E/A ratio, and average medial and lateral early ventricular filling mitral annulus velocities (average e') and the ratio of E-wave to average e' (E/e') were also measured [13]. Left ventricle global longitudinal strain (LVGLS) was evaluated based on speckle tracking software.

2.4. Biochemical analysis

Blood samples were drawn by venipuncture after a fasting 12 h state from each participant in each visit, between 8.00 and 10.00 a.m. Collecting of serum/plasma and storing at −80 °C, until their assay, followed the centrifugation of blood samples at 3000 rpm. ST2 plasma levels were determined with commercial enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer (R&D systems Inc, Minneapolis, MN, USA) instructions. The lower detection limit of the assay was 5.1 pg/ml, respectively. The intra and inter assay coefficient of variation was approximately 5% and 6% respectively. Serum levels of Nitrogen terminal pro B type natriuretic peptide (NT-pro BNP) were measured with an electrochemiluminescence immunoassay (ECLIA) kit according to the manufacturer. Additionally, high sensitive C Reactive protein (hsCRP) levels were measured by nephelometry (BNII Siemens System) as a marker of inflammatory status.

2.5. Bioethics

All participants received thorough information regarding the aims of the study and written informed consent was received before entering the study. The study received approval and was registered by the Greek National Organization for Medicines (EOF) with the approval code NIS-7-01-10 on the 9th of March 2010. It was carried out in accordance with the Declaration of Helsinki (1989).

2.6. Statistical analysis

All variables were tested for normal distribution of the data. Continuous variables with normal distribution were expressed as means ± standard deviation, skewed variables were expressed as median with interquartile range and categorical variables as valid percentages. Student's *t*-test, Mann–Whitney and chi-square tests were used as appropriate to test for differences between subjects categorized in Groups A and B. Percent change of each examined variable in each treatment arm (omega-3 PUFAs or Placebo) was calculated as the difference of the examined variable at the end of the treatment period from the baseline value divided by the baseline value and multiplied by 100. Paired sample t test was used to test for differences before and after each treatment arm as well as for differences in the percent changes in each treatment arm. Pearson's r coefficient was used to test the correlation between normally distributed quantitative variables. All reported p-values were based on two-sided tests. Exact values of $p < 0.05$ were considered statistically significant. Data analysis was performed with SPSS software, version 18.0 (SPSS Inc., Chicago, IL).

The sample size was selected based on an a priori power calculation. The effect of omega-3 PUFAs administration on LVEF improvement was hypothesized to achieve an effect size of 0.5. Accordingly, with a type I error of 0.05, power analysis revealed that for a 2-tailed paired data Student t test a sample size of 27 individuals is adequate to achieve a power of >80%.

3. Results

3.1. Demographic and clinical characteristics of the study population

The baseline characteristics of the participants are shown in Table 1. Participants' mean age was 67 ± 6 years, 72% of the participants were male. Most of the patients (65%) were in NYHA functional class II, while mean LVEF was 29 ± 8%. There were no differences in demographic, clinical characteristics and lipid parameters between subjects randomized to groups A and B.

3.2. Effects of omega-3 PUFAs on left ventricle systolic function

In the omega-3 PUFAs arm of the study there was a significant percent increase in LVEF at the end of the treatment period, compared to baseline (4.7%, $p < 0.001$). In the Placebo arm of the study there was no significant change in LVEF, compared to baseline (1.7%, $p = 0.37$). Similarly, in the omega-3 PUFAs arm of the study there was a significant change in LVGLS at the end of the treatment period, compared to baseline (−10.6%, $p < 0.001$); whereas in the Placebo arm of the study there was no significant change in LVGLS, compared to baseline (−2.3%, $p = 0.10$).

We also found significant differences in the observed changes of LVEF and LVGLS from baseline to the end of the treatment period between the 2 treatment arms (omega-3 PUFAs and Placebo). Specifically, changes were higher in the omega-3 PUFAs arm compared to placebo ($p = 0.05$ and $p = 0.02$, for LVEF and LVGLS, respectively) as it is shown in Fig. 2 (panels A and B, respectively).

As it is shown in Table 2 at baseline there was no difference in the examined values (LVEF, LVGLS, LV end-diastolic and end-systolic diameter, LV end-diastolic and end-systolic volume) between the two treatment arms. Omega-3 PUFAs administration achieved a significant improvement in the aforementioned parameters that was not observed with Placebo.

Interestingly, when the median value of NT-proBNP at baseline (832 pg/ml) was used to differentiate patients according to loading conditions we observed that the improvement in LVEF with omega-3 PUFAs treatment was significantly higher in patients with NT-proBNP levels below median values (Fig. 3).

3.3. Effects of omega-3 PUFAs on diastolic function

At baseline, there were no significant differences in the examined parameters of diastolic function between the omega-3 PUFAs arm and the Placebo arm (Table 3). Omega-3 PUFAs administration caused a significant improvement in the E/e' ratio (−9.47%, $p = 0.005$), increased the average e' by 16.2% ($p < 0.001$) and reduced LA volume by −1.99% ($p = 0.03$). In contrast, treatment with Placebo had no significant impact on the E/e' ratio (1.92%, $p = 0.66$), average e' (−2.1%, $p = 0.16$) and LA volume (0.19%, $p = 0.30$) (Table 3 and Fig. 4).

3.4. Effects of omega-3 PUFAs on ST2 and hsCRP

In the omega-3 PUFAs arm, there was a significant decrease in ST2 levels at the end of the treatment period compared to baseline (22211 ± 11260 pg/ml vs. 23506 ± 12695 pg/ml, $p = 0.02$) with an estimated percent decrease of −4.53%. In the Placebo arm, there was no significant change in ST2 levels at the end of the treatment

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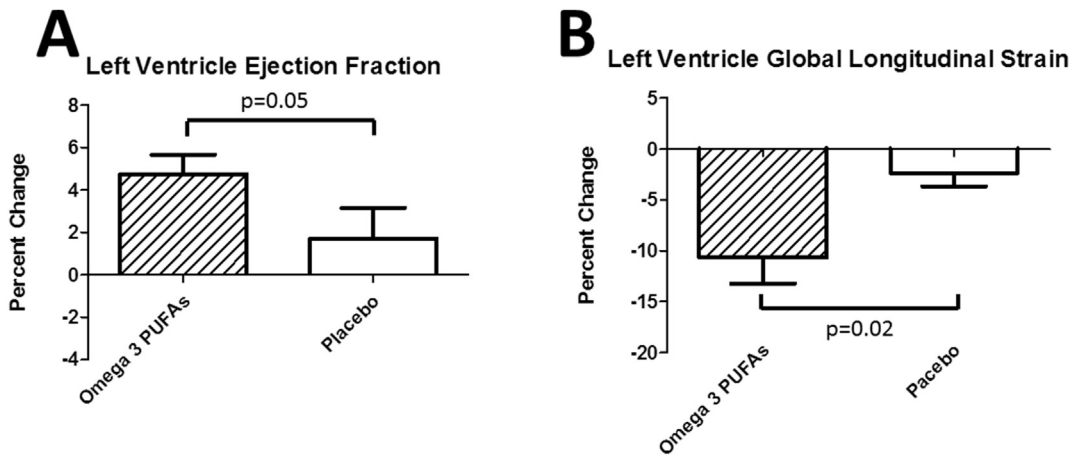

Fig. 2. Percent changes in Left Ventricle Ejection Fraction (Panel A) and Left Ventricle Global Longitudinal Strain (Panel B). **Panel A**: Comparisons between the mean percent changes in Left Ventricle Ejection Fraction from baseline to the end of the treatment period (8 weeks) in the Omega-3 PUFAs and Placebo arm. **Panel B**: Comparisons between the mean percent changes in Left Ventricle Global Longitudinal Strain from baseline to the end of the treatment period (8 weeks) in the Omega-3 PUFAs and Placebo arm. PUFAs: Polyunsaturated Fatty Acids.

Table 2
Effects of Omega-3 PUFAs on left ventricle systolic function.

	Omega-3 PUFAs			Placebo		
	Before treatment (baseline)	End of treatment period	p-value	Before treatment (baseline)	End of treatment period	p-value
LVEF (%)	28.6 ± 7.8	29.8 ± 7.9	<0.001	28.9 ± 7.7	29.2 ± 7.3	0.37
LVGLS	−13.3 ± 2.3	−14.6 ± 2.2	<0.001	−13.2 ± 2.0	−13.6 ± 2.5	0.10
LVEDD (mm)	56 ± 6	54 ± 6	<0.001	56 ± 6	56 ± 6	0.46
LVESD (mm)	47 ± 7	45 ± 7	<0.001	47 ± 7	47 ± 7	0.97
LVEDV (ml)	118 ± 23	113 ± 22	0.004	117 ± 23	120 ± 23	0.19
LVESV (ml)	81 ± 21	79 ± 21	<0.001	81 ± 21	82 ± 21	0.06

LVEF: Left ventricle ejection fraction; LVGLS: Left ventricle global longitudinal strain; LVEDD: Left ventricle end diastolic diameter; LVESD: Left ventricle end systolic diameter; LVEDV: Left ventricle end diastolic volume; LVESV: Left ventricle end systolic volume. PUFAs: Polyunsaturated Fatty Acids.
P-values are based on pair t test.

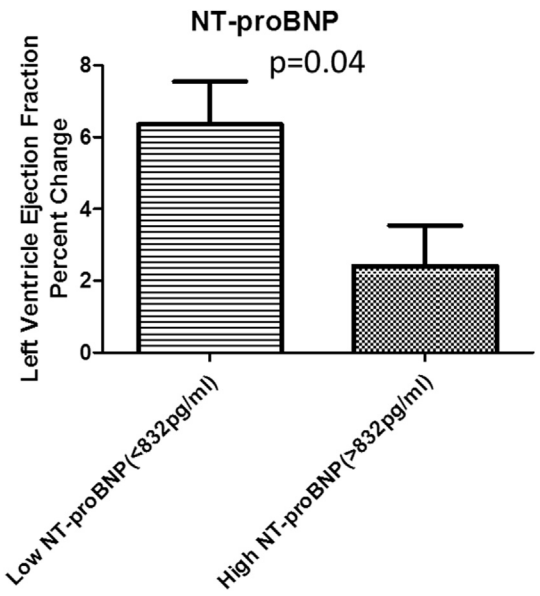

Fig. 3. Percent changes in Left Ventricle Ejection Fraction. Comparisons between the mean percent changes in Left Ventricle Ejection Fraction from baseline to the end of the treatment period (8 weeks) in the Omega-3 PUFAs between subjects with low and high NT-proBNP levels. NT-proBNP: Nitrogen terminal pro B type natriuretic peptide.

period compared to baseline (22958 ± 11855 pg/ml vs. 23301 ± 11414 pg/ml, p = 0.38) with an estimated percent change of −2.37% (Fig. 5, panel A). At baseline, there was no difference in ST2 levels between the 2 treatment arms (p = 0.50).

Concerning hsCRP levels, in the omega-3 PUFAs arm, there was a significant decrease at the end of the treatment period compared to baseline [3.00 (2.25–5.60)mg/L vs. 3.40 (2.35–7.75)mg/L, p = 0.02] with an estimated percent decrease of −6.13%. In the Placebo arm, there was no significant change in hsCRP levels at the end of the treatment period compared to baseline [4.10 (2.80–5.60)mg/L vs. 3.70 (2.650–5.76)mg/L, p = 0.21] with an estimated percent change of 4.36% (Fig. 5, panel B). At baseline, there was no difference in hsCRP levels between the 2 treatment arms (p = 0.23).

3.5. Effects of omega-3 PUFAs on endothelial function

At baseline there was no difference in FMD and EID values between the omega-3 PUFAs arm and the Placebo arm (Table 4). Treatment with omega-3 PUFAs caused a significant percent improvement in FMD values (44%, p = 0.03) while Placebo treatment had no significant impact on FMD values (percent change 11%, p = 0.64) at the end of the treatment period. EID has not been changed with either omega-3 PUFAs or Placebo (Table 4, Fig. 6). Interestingly, in the arm receiving omega-3 PUFAs treatment, the improvement in FMD was associated with the improvement in the E/e' ratio (r = −0.48, p = 0.005) (Fig. 7).

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Table 3
Effects of Omega-3 PUFAs on diastolic function.

	Omega-3 PUFAs			Placebo		
	Before treatment (baseline)	End of treatment period	p-value	Before treatment (baseline)	End of treatment period	p-value
Mitral E/A ratio	1.18 ± 0.80	1.16 ± 0.76	0.77	1.20 ± 0.79	1.18 ± 0.69	0.64
E/e' ratio	11.3 ± 2.9	10.0 ± 2.7	0.005	11.2 ± 2.7	11.3 ± 2.5	0.66
Mitral E-wave (cm/sec)	72 ± 21	73 ± 21	0.21	73 ± 22	72 ± 20	0.42
Mitral A-wave (cm/sec)	73 ± 25	74 ± 25	0.16	73 ± 25	76 ± 25	0.08
Average e' (cm/sec)	6.5 ± 1.5	7.4 ± 1.4	<0.001	6.6 ± 1.3	6.4 ± 1.3	0.16
LA diameter (mm)	41.6 ± 5.2	40.6 ± 5.2	<0.001	41.9 ± 5.7	42.1 ± 5.0	0.66
LA volume (ml)	60 ± 16	58 ± 16	0.03	60 ± 16	60 ± 16	0.58
RVSP (mmHg)	59 ± 10	61 ± 11	0.09	61 ± 10	60 ± 10	0.30

A: Peak velocity of late ventricular filling; Average e': Average medial and lateral early ventricular filling mitral annulus velocities; E: Peak velocity of early ventricular filling; e': average tissue Doppler early filling velocity; E/e': E-wave to average e'; LA: Left Atrium; PUFAs: Polyunsaturated Fatty acids; RVSP: Right ventricle systolic pressure.

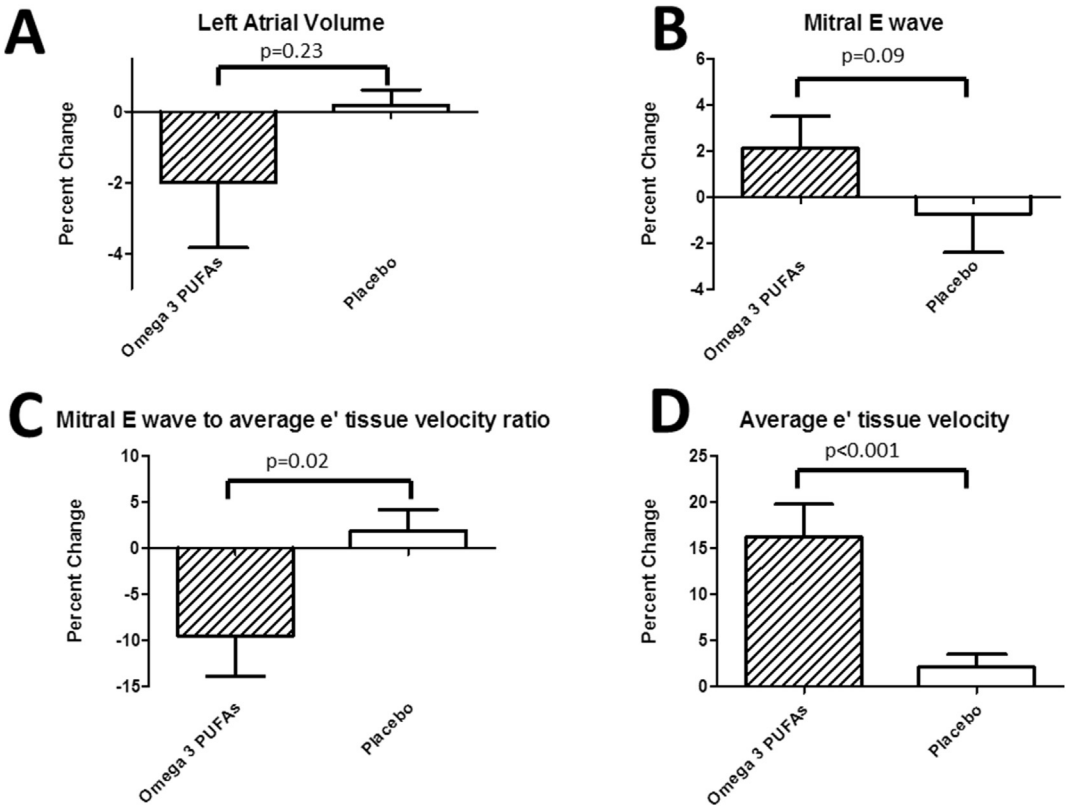

Fig. 4. Percent changes in Left Atrial Volume (Panel A), Mitral E wave (Panel B), Mitral E wave to average e tone tissue velocity ratio (Panel C) and Average e tone tissue velocity (Panel D). **Panel A**: Comparisons between Left Atrial Volume from baseline to the end of the treatment period (8 weeks) in the Omega-3 PUFAs and Placebo arm. **Panel B**: Comparisons between Mitral E wave from baseline to the end of the treatment period (8 weeks) in the Omega-3 PUFAs and Placebo arm. **Panel C**: Comparisons between Mitral E wave to average e tone tissue velocity ratio from baseline to the end of the treatment period (8 weeks) in the Omega-3 PUFAs and Placebo arm. **Panel D**: Comparisons between Average e tone tissue velocity from baseline to the end of the treatment period (8 weeks) in the Omega-3 PUFAs and Placebo arm. PUFAs: Polyunsaturated Fatty Acids.

4. Discussion

In the current study, we found that in stable patients with ischemic HF, short term treatment (8 weeks) with omega-3 PUFAs on top of optimal medical treatment, improved LV systolic and diastolic performance estimated by LVEF, global longitudinal strain and the ratio of mitral inflow E wave to the average medial and lateral early ventricular filling mitral annulus velocities. Interestingly these changes were observed in parallel with changes in endothelial function and serum levels of ST2, an established marker of inflammation and myocardial fibrosis. We also noticed that systolic performance was improved especially in subjects with better loading conditions and lower NT-proBNP levels. Furthermore, we found an association of endothelial function improvement with LV diastolic performance and the E/e' ratio which expresses LV end diastolic pressure; a finding highlighting the possible role of endothelial dysfunction in the pathophysiologic mechanisms of HF progression and which gives further insights in the potential beneficial mechanisms of omega-3 PUFAs in patients with HF of ischemic etiology.

4.1. Omega-3 PUFAs and left ventricle function

Experimental data may support our findings concerning LV systolic and diastolic performance. In male rats with congestive HF, supplementation with docosahexaenoic acid may modify heart fatty acid composition and can ameliorate congestive HF [14]. Moreover, omega-3 PUFAs ameliorated cardiac fibrosis and cardiac dysfunction

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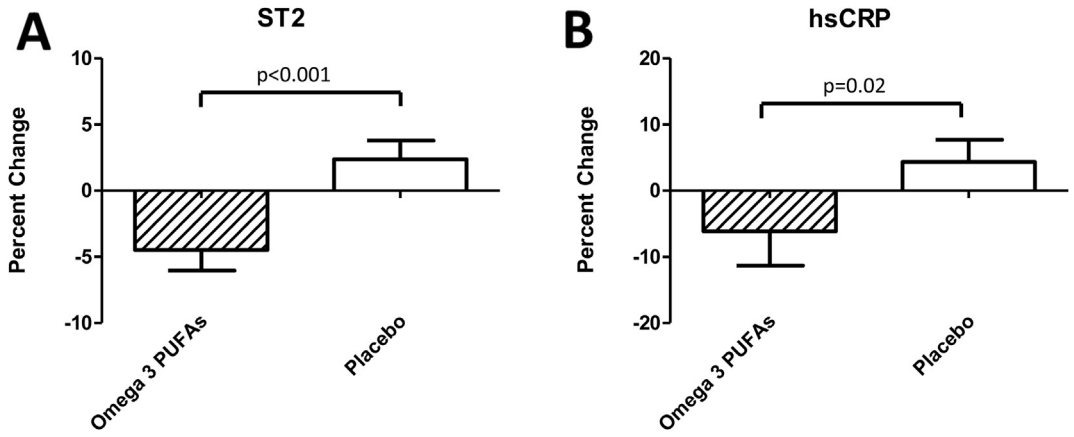

Fig. 5. Percent changes in ST2 and hsCRP. **Panel A**: Comparisons between ST2 from baseline to the end of the treatment period (8 weeks) in the Omega-3 PUFAs and Placebo arm. **Panel B**: Comparisons between hsCRP from baseline to the end of the treatment period (8 weeks) in the Omega-3 PUFAs and Placebo arm. PUFAs: Polyunsaturated Fatty Acids; hsCRP: High sensitive C reactive protein.

Table 4
Effects of Omega-3 PUFAs on endothelial function.

	Omega-3 PUFAs			Placebo		
	Before treatment (baseline)	End of treatment period	p-value	Before treatment (baseline)	End of treatment period	p-value
FMD (%)	4.32 ± 2.99	5.31 ± 2.26	0.03	4.40 ± 2.39	4.41 ± 2.03	0.64
EID (%)	12.58 ± 2.95	11.43 ± 3.71	0.17	11.18 ± 3.95	11.04 ± 3.75	0.99

EID: Endothelial independent dilation; FMD: Flow Mediated Dilation; PUFAs: Polyunsaturated Fatty acids.

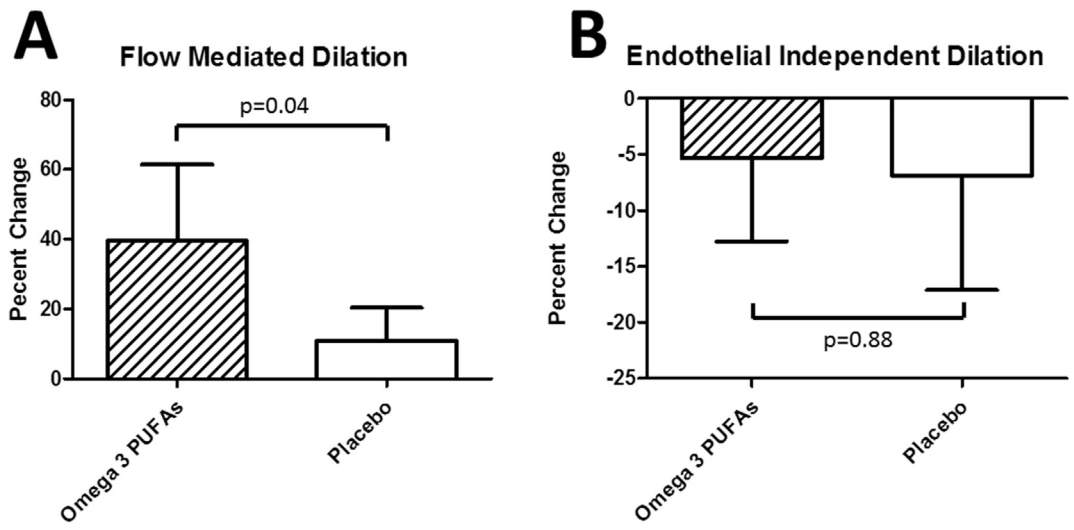

Fig. 6. Percent changes in Flow Mediated Dilation (Panel A) and Endothelial Independent Dilation (Panel B). **Panel A**: Comparisons between Flow Mediated Dilation from baseline to the end of the treatment period (8 weeks) in the omega-3 PUFAs and Placebo arm. **Panel B**: Comparisons between Endothelial Independent Dilation from baseline to the end of the treatment period (8 weeks) in the Omega-3 PUFAs and Placebo arm. PUFAs: Polyunsaturated Fatty Acids.

in mice after aortic constriction, by blocking transforming growth factor-β1-induced phospho-Smad2/3 nuclear translocation through activation of the cyclic GMP/protein kinase G pathway in cardiac fibroblasts [15]. In the same model, omega-3 PUFAs led to an increase in endothelial nitric oxide synthase and nitric oxide production [15]. Additionally, ventricular myocytes from pigs fed with fish oil showed a decrease in sarcoplasmic reticulum calcium content which may explain alterations in diastolic function [16].

Clinical studies are in line with our evidence regarding the role of omega-3 PUFAs in systolic and diastolic LV performance of subjects with HF. Chrysohoou et al. have found favorable effects of omega-3 PUFAs in right ventricle function and diastolic filling pressures [4]. Furthermore, in patients with dilated cardiomyopathy, omega-3 PUFAs for 12 months improved LV systolic performance and diastolic function in parallel with improvement in functional capacity [17]. Recently, it was also shown that in patients presented with acute myocardial infarction, additive treatment with omega-3 PUFAs was associated with reduction of adverse LV remodeling, noninfarct myocardial fibrosis, and serum biomarkers of systemic inflammation [18].

Of note, in our study the most significant improvement in terms of LVEF was observed in patients expressing low baseline NT-

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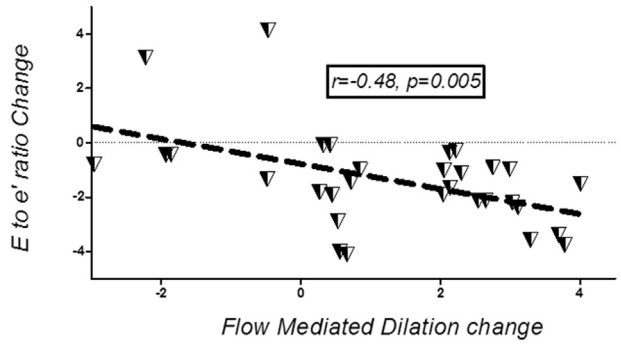

Fig. 7. Scatter dot of the association between change in E to e tone ratio and FMD value from baseline to the end of the treatment period in the Omega 3 Polyunsaturated Fatty Acids treatment arm.

proBNP levels and better loading conditions. Increased loading condition and NT-proBNP may enhance adverse left ventricle remodeling [19] intercepting any potential benefits of omega-3 PUFAs. This observation may help to differentiate an ischemic HF patient population likely to benefit by adding omega-3 PUFAs on standard pharmaceutical treatment.

4.2. Endothelial function, heart failure and omega-3 PUFAs

Endothelial dysfunction is an inherent feature of heart failure especially in subjects with ischemic heart disease [1] while diastolic dysfunction may be at least partially attributed to impaired endothelial function [20]. Importantly, endothelial incompetence with concomitant peripheral hypo-perfusion may limit functional capacity, cause exercise intolerance and increases LV afterload [21] and it may also affect prognosis especially in cases with diastolic dysfunction [22]. In addition, in patients with ischemic HF endothelial dysfunction may exacerbate insufficiency of coronary circulation [6]. Moreover, endothelial microvascular dysfunction is associated with LV wall stress in patients with cardiac fibrosis [23].

Omega-3 PUFAs, may lead to an improvement of endothelial function in various disease settings (e.g. diabetes mellitus, metabolic syndrome, hypertension) by enhancing endothelial nitric oxide synthesis, attenuating inflammation and cytokine production and suppressing thromboxane A2 [24–26]. Previous studies in patients with HF of non-ischemic etiology have shown that omega-3 PUFAs may improve endothelial function in parallel with interleukin 6 attenuation [27]. To the same direction in ischemic HF patients use of omega-3 PUFAs improves endothelium-dependent vasodilation which may be mediated by a decrease in the level of malondialdehyde and peroxynitrate and by an increased activity of antioxidant enzymes.

Based on the data presented in the current study omega-3 PUFAs may beneficially affect endothelial function in ischemic HF patients. Indeed, improvement of endothelial function was interdependent with LV diastolic performance and was also accompanied by changes in LV systolic performance, LV geometric characteristics and ST2 levels.

4.3. Omega-3 PUFAs and myocardial fibrosis

Myocardial extracellular matrix synthesis and especially myocardial collagen and fibrosis have been evolved as significant determinants of LV remodeling and prognosis of patients with HF [28] while myocardial fibrosis is associated not only with impaired systolic performance but also with diastolic performance and relaxation properties of the left ventricle [29]. Soluble ST2 has evolved as a useful biomarker integrating inflammation, fibrosis, and cardiac stress, which may predict hospitalization and death in patients with HF with an additive to natriuretic peptide levels prognostic ability [7,30,31]. Docosahexaenoic acid may reverse Angiotensin II-induced microRNA-21 expression, RECK suppression, matrix metalloproteinase 2 induction, and cardiac fibroblast migration [32]. Moreover, omega-3 PUFAs act protectively in pressure overload-induced myocardial hypertrophy in rats via modulation of oxidative stress and apoptosis [33]. It has also previously reported that omega-3 PUFAs treatment starting immediately after myocardial infraction and for a period of 6 months can decrease levels of ST2 [18]. We additionally observed that in patients with stable ischemic HF short term treatment with omega-3 PUFAs may improve ST2 levels in parallel with echocardiographic indices of LV systolic and diastolic performance supporting the active role of omega-3 PUFAs on myocardial fibrosis and remodeling. This evidence further indicates the possible role of pharmacologic interventions aiming to improve extracellular matrix remodeling in patients with HF.

4.4. Omega-3 PUFAs the role of inflammation

The mechanisms involved in the favorable impact of omega-3 PUFAs in patients with HF and especially concerning the improvement of endothelial function and left ventricle performance are not fully elucidated. Inflammation is not only involved in endothelial dysfunction [34] but is also associated with the development and progression of HF [35,36]. Moreover, there is evidence linking increased levels of CRP with worse left ventricle systolic performance in patients with HF [37]. Adding omega-3 PUFAs, as a supplementary treatment in patients with cardiovascular risk factors, may induce the inhibition of the production of inflammatory and fibrotic mediators, among which interleukins, CRP, tumor necrosis factor alpha, matrix metalloproteinases 2 and 9, and tissue inhibitors of metalloproteinase are included [26,38]. In our study population of ischemic HF patients, omega-3 PUFAs decreased hsCRP levels in parallel with the improvement of systolic performance indices and of endothelial function. These findings may further clarify the beneficial mechanisms of PUFAs in patients with HF.

4.5. Limitations

A major limitation of this study is the small number of the included subjects. This is an exploratory analysis, and our findings can be only considered as hypothesis-generating, needing confirmation by larger studies. Moreover, we cannot exclude the possibility same secondary endpoints to have been missing due to the limited sample size. Another limitation of this study is that there are no data on the baseline omega-3 index (the status of eicosapentaenoic and docosahexaenoic acid in erythrocytes membranes) and the impact of treatment in omega-3 status which may add valid to the interpretation of the results. Nevertheless, using a crossover design we were able to achieve sufficient statistical power for the analysis of our results. Second, the omega-3 PUFA dose used in this study was greater than the dose used in the GISSI-HF trial, which remains the definitive study regarding the use of omega-3 PUFAs in HF. Similarly high omega-3 PUFA doses have been, however, previously used [17], and in fact a significant dose–response relationship has been observed in the beneficial effects of omega-3 PUFAs on LV remodeling [18].

5. Conclusion

Short term treatment with omega-3 PUFAs supplements in subjects with stable HF of ischemic etiology improved endothelial

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function, biomarkers of myocardial fibrosis as well as LV systolic and diastolic performance. These findings emphasize the significant role of omega-3 PUFAs on the cardiovascular system, underlining the significant interaction between endothelial function, myocardial fibrosis and LV function, thereby suggesting a potential for wider omega-3 PUFA supplementation in ischemic HF patients. Should the validity of these findings be corroborated by larger studies, omega-3 PUFA treatment may be a valid option not only in symptomatic HF patients as suggested by the GISSI-HF trial, but also in asymptomatic patients to counter the adverse remodeling process.

Conflict of interest

Nothing to disclose

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