Journal of the American College of Cardiology Vol. 57, No. 7, 2011

Published by Elsevier Inc.
ISSN 0735-1097/$36.00
doi:10.1016/j.jacc.2010.11.017

EXPEDITED PUBLICATIONS

Effects of n-3 Polyunsaturated Fatty Acids on Left Ventricular Function and Functional Capacity in Patients With Dilated Cardiomyopathy

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Objectives This study was designed to test the effects of n-3 polyunsaturated fatty acids (PUFAs) on left ventricular (LV) systolic function in chronic heart failure (HF) due to nonischemic dilated cardiomyopathy (NICM).

Background One hundred thirty-three patients with NICM and minimal symptoms on standard therapy were randomized to 2 g of n-3 PUFAs or placebo. LV function and functional capacity were assessed prospectively by echocardiography and cardiopulmonary exercise testing at baseline and at 12 months after randomization.

Methods Patients with chronic HF due to NICM and minimal symptoms while receiving evidence-based therapy were enrolled. LV function and functional capacity were assessed prospectively by echocardiography, cardiopulmonary exercise test, and New York Heart Association functional class at baseline and at 12 months after randomization to either 2 g of n-3 PUFAs or placebo.

Results At 12 months after randomization, the n-3 PUFAs group and the placebo group differed significantly ($p < 0.001$) in regard to: 1) LV ejection fraction (increased by 10.4% and decreased by 5.0%, respectively); 2) peak VO_2 (increased by 6.2% and decreased by 4.5%, respectively); 3) exercise duration (increased by 7.5% and decreased by 4.8%, respectively); and 4) mean New York Heart Association functional class (decreased from 1.88 ± 0.33 to 1.61 ± 0.49 and increased from 1.83 ± 0.38 to 2.14 ± 0.65, respectively). The hospitalization rates for HF were 6% in the n-3 PUFAs and 30% in the placebo group ($p = 0.0002$).

Conclusions In patients with NICM and minimal symptoms in response to evidence-based medical therapy, n-3 PUFAs treatment increases LV systolic function and functional capacity and may reduce hospitalizations for HF. Given these promising results, larger studies are in order to confirm our findings. (J Am Coll Cardiol 2011;57:870–9)

Despite the improvement in morbidity and mortality achieved with the use of current treatments, heart failure (HF) is still associated with an overall poor prognosis and with high hospitalization rates (1). The results of the GISSI-HF (Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico–Heart Failure) trial indicate that in patients with chronic HF receiving evidence-based medical therapy and in New York Heart Association (NYHA) functional class II to IV, long-term treatment with n-3 polyunsaturated fatty acids (PUFAs) 1 g daily reduces mortality and hospitalizations for cardiovascular reasons, irrespective of etiology and left ventricular (LV) ejection fraction (EF) (2).

See page 880

Several potential mechanisms may underlie the beneficial effects of n-3 PUFAs in HF patients, including, but not limited to, antiarrhythmic and hemodynamic actions. In particular, experimental and clinical studies have shown that n-3 PUFAs may ameliorate endothelial function (3), blood pressure and heart rate (4), and arterial compliance (5).

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Manuscript received August 26, 2010; revised manuscript received November 1, 2010, accepted November 9, 2010.

Furthermore, in an animal model of HF, treatment with dietary eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) prevents the increase in LV end-diastolic and end-systolic volumes and the shift between alfa- and beta-myosin chains secondary to aortic banding (6). However, whether n-3 PUFAs may improve LV function in patients with HF remains unknown. The current investigation was therefore designed to test the hypothesis that treatment with n-3 PUFAs improves LV systolic function expressed as EF in patients with stable chronic HF secondary to a nonischemic dilated cardiomyopathy (NICM). Additionally, we sought to determine whether n-3 PUFAs also exert positive effects on LV diastolic function assessed by echocardiography, functional capacity expressed as peak VO_2 assessed by cardiopulmonary stress testing (CPET), and NYHA functional class.

Methods

Patient population. Patients aged between 18 and 75 years with a diagnosis of NICM, LV systolic dysfunction (defined as an EF ≤45%), and stable clinical conditions with minimal or no symptoms for at least 3 months on evidence-based medical treatment at maximum tolerated target doses for at least 6 months were considered eligible for the study. In all patients, the diagnosis of NICM was based on the absence of stenoses ≥50% on coronary artery angiography performed at the time of the workup of the cardiomyopathy. Furthermore, additional imaging tests had been performed in the presence of subsequent changes in clinical status or if symptoms suggestive of ischemia had occurred. The following criteria were grounds for exclusion: presence of symptoms or evidence of coronary artery disease diagnosed through noninvasive tests, peripheral arterial disease, presence of congenital or primary valvular heart disease, persistent atrial fibrillation, inability to perform bicycle ergometry for noncardiac causes, moderately to severely reduced functional capacity, NYHA functional class IV, poor acoustic windows limiting the ability to assess echocardiographic measurements, chronic lung disease, advanced renal disease (estimated glomerular filtration rate [eGFR] ≤30 ml/min/1.73 m^2), advanced liver disease; any disease limiting life expectancy to ≤1 year, contraindications to study drugs, and concomitant participation in other research studies.

Experimental design. The study had a double-blind, placebo-controlled, 2-arm design. Potential participants were recruited consecutively from the HF outpatient clinic of the University of Brescia and were scheduled to undergo a screening visit including history and physical exam, electrocardiogram (ECG), blood draw for complete blood count and chemistry panel for electrolytes and renal and liver function tests, and echocardiogram with Doppler. Within 2 weeks after the screening visit, all eligible patients underwent CPET, which was preceded by a training session to familiarize with the equipment. Participants were subsequently randomized to either 1.0-g gelatin capsules containing 850 to 882 mg of EPA and DHA ethyl esters in the average ratio (EPA/DHA) of 0.9:1.5 (OMACOR, Pronova Biopharma, Lysaker, Norway) or to 1.0-g gelatin capsules of placebo (olive oil) of identical appearance. The treatment dose was 5 capsules daily for the first month followed by 2 capsules daily for the rest of the study. Capsules were dispensed on a monthly basis in a glass bottle containing 80 capsules. Drug compliance was assessed by pill count at each monthly visit. Patients were considered noncompliant and withdrawn from the study if less than 80% of the expected number of capsules had been taken. Follow-up visits were scheduled at monthly intervals or at any time clinical events considered potentially significant by the study physician were reported. At the 12-month follow-up visit, physical exam, ECG, blood draw for complete blood count and chemistry panel including renal and liver function tests, echocardiogram with Doppler, and CPET were repeated. The study protocol was approved by the Institutional Review Board of the University of Brescia (Brescia, Italy). The study complied with the Declaration of Helsinki and the Guidelines for Good Clinical Practice. A written informed consent was obtained from each patient.

Abbreviations and Acronyms

ACE-I = angiotensin-converting enzyme inhibitor
ARB = angiotensin II receptor blocker
CPET = cardiopulmonary stress testing
DHA = docosahexaenoic acid
eGFR = estimated glomerular filtration rate
ECG = electrocardiogram
EPA = eicosapentaenoic acid
FFA = free fatty acid
HF = heart failure
HR = heart rate
IL = interleukin
LV = left ventricular
LVEF = left ventricular ejection fraction
NICM = nonischemic dilated cardiomyopathy
NYHA = New York Heart Association
PUFA = polyunsaturated fatty acid
TNF = tumor necrosis factor
VO_2 = oxygen uptake

Procedures. ECHOCARDIOGRAPHIC EVALUATION. The echocardiographic study, including 2-dimensional, M-mode, and Doppler imaging, was performed using a General Electric Vivid 7.0 (General Electric Healthcare, Milan, Italy) with a 2.5-MHz transducer. The following parameters were measured according to the professional standards defined by the American Society of Echocardiography and the European Association of Echocardiography (7): LV end-diastolic and -systolic diameter and LV end-diastolic and end-systolic volume; LVEF (%) calculated by Simpson's biplane method; shortening fraction (%); degree of mitral regurgitation by Doppler and color Doppler, scored on a scale from 0 to 4 (0 = none, 1 = trace, 2 = mild, 3 = moderate, and 4 = severe); left atrial diameter; LV posterior wall thickness and interventricular septal thickness; right atrial pressure; pulmonary artery systolic pressure; mitral diastolic inflow velocities (peak velocity of early ventricular filling [E-wave], peak velocity of late ventricular filling [A-wave], E/A ratio, and E-wave deceleration time); diastolic function score, graded on a scale from 1 to 4 (normal, impaired relaxation, pseudonormal, and restrictive), with higher scores indicating worse diastolic

function (8). Echocardiographic exams were performed by a single cardiologist (G.M.) and interpreted by a different cardiologist (A.M.), both blinded to patients' treatment.

CARDIOPULMONARY EXERCISE TESTING. Bicycle ergometer CPET was performed by trained staff under the supervision of 2 cardiologists; all patients were instructed and underwent a training session to familiarize with the equipment (Medical Graphics Italia, Milan, Italy). A light breakfast was allowed 2 h before the test. All tests were performed between 10 AM and 1 PM in a temperature-controlled room (21°C to 23°C), with standard 12-lead continuous ECG monitoring (Cardio Perfect, Medical Graphics Italia). Blood pressure was measured manually. A ramp protocol at a pedal speed of 60 revs/min with a linear increase in workload at a rate of 1 W every 6 s (10 W/min) was used. All tests were interrupted for symptoms (dyspnea and/or fatigue). Minute ventilation, oxygen uptake (VO_2), carbon dioxide output, and other cardiopulmonary variables were acquired breath-by-breath by pneumotachograph with bidirectional differential pressure (preVent Pneumotach, Medical Graphics, St. Paul, Minnesota). Resting VO_2 and heart rate (HR) were computed as the mean values recorded during the last 30 s of the resting period, whereas peak effort VO_2 was calculated as the mean values during the last 30 s of effort. Automatic calculation of the anaerobic threshold was obtained by the Wassermann or V-slope method (9). A CPET was considered adequate if the peak respiratory exchange ratio was higher than 1.05. Maximum HR predicted for age was calculated as: (220 − age). The percentage of the peak HR in relation to the maximal HR predicted for the age was calculated as: (peak HR/predicted HR) × 100. The percentage of maximum predicted VO_2 (% VO_{2max}) was calculated as the ratio between VO_{2peak} and theoretical VO_{2max}, according to the standard formula: [([50.72 − (0.372 × age)] × weight)/weight] for males and [([22.78 − (0.17 × age)] × (weight + 43))/weight] for females (10,11). CPET exams were performed and interpreted by 2 cardiologists (A.M. and G.M.) blinded to patients' treatment.

LABORATORY EXAMS. Blood samples were collected for the assessment of complete blood count, comprehensive chemistry panel, inflammatory cytokines including tumor necrosis factor (TNF)-alpha, interleukin (IL)-6 and IL-1, and serum free fatty acids (FFAs). Inflammatory cytokine levels were measured using venous blood collected in empty tubes and centrifuged within 1 h at 1,700 rpm at 4°C for 15 min. The collected serum was stored at −80°C for assessment by immunoenzymatic test with spectrophotometric determination (12). Plasma levels of n-3 and n-6 PUFAs were measured on blood samples drawn after ≥10 h of fasting, collected in K_3 ethylenediaminetetraacetic acid–containing tubes, centrifuged at 400 rpm at 4°C for 10 min, and subsequently stored at −20°C. Plasma FFAs including linoleic acid, arachidonic acid, alpha-linolenic acid, EPA, and DHA were analyzed by gas–liquid chromatography after derivation to methyl esters and expressed as percentage of total FFAs. Briefly, 50 μl of plasma were directly methylated with 1 ml of 3 N MeOH/HCl (Supelco, Bellafonte, Pennsylvania). After 1 h of incubation at 90°C, FFAs were extracted twice with 2 ml of water, 2 ml of a KCl-saturated solution, and 2 ml of n-hexane (13). The resulting fatty acid methyl esters were separated by gas–liquid chromatography (85.10; Dani Instruments S.p.a., Cologno Monzese, Italy) equipped with a capillary column (Omegawax 320, Supelco), flame ionization detector, and programmed temperature vaporizing injector. The interassay coefficients of variation were 0.9% and 1.3% for EPA and DHA, respectively. The omega-3 index was calculated from (EPA + DHA) per total FFA content.

Statistical analysis. Continuous variables are expressed as mean (SD). Categorical variables are presented as absolute and percent values and were compared by chi-square test. Analysis of covariance on the value detected at the 12-month follow-up with baseline value of the main variables as covariate was not possible owing to the statistical significance of the parallelism test. For each parameter, the analysis of the results was done following an intention-to-treat approach by means of the unpaired Student *t* test or Wilcoxon rank sum test as appropriate on the differences (before minus after the treatment). In case of a statistically significant interaction, within-group comparisons were carried out by means of an "a posteriori" test with the significance level reduced by means of the Bonferroni correction. Statistical significance was set at $p < 0.05$ (2-tailed).

The primary end point of the study was the change in LV systolic function expressed as LVEF between baseline and 12-month follow-up. Secondary end points included: 1) change in LV diastolic function assessed by echocardiography; 2) change in functional capacity expressed as peak VO_2; and 3) change in NYHA functional class between baseline and 12-month follow-up.

Sample size calculation was based on the difference between the change of LVEF in the treatment with n-3 PUFA 2 g daily in comparison with placebo. We hypothesized that n-3 PUFA administration would be associated with an improvement of effect size of 0.5 in LVEF (or 3% for a phenomenon variability, difference between baseline and 12-month follow-up of 6%). A sample of 65 patients in each group was calculated to have 80% power to detect such 0.5 effect size with $\alpha = 0.05$ (2-tailed) at the Student *t* test for unpaired data. Analyses were performed using SAS software (version 9.1, SAS Institute, Cary, North Carolina).

Results

The flow chart of the study is shown in Figure 1. A total of 133 patients took part in the study. The first patient was enrolled on November 5, 2007, and the last patient completed the study on June 30, 2009. The mean age of the study population was 63 ± 10 years; 13 (9.8%) of the participants were women; all patients were being treated

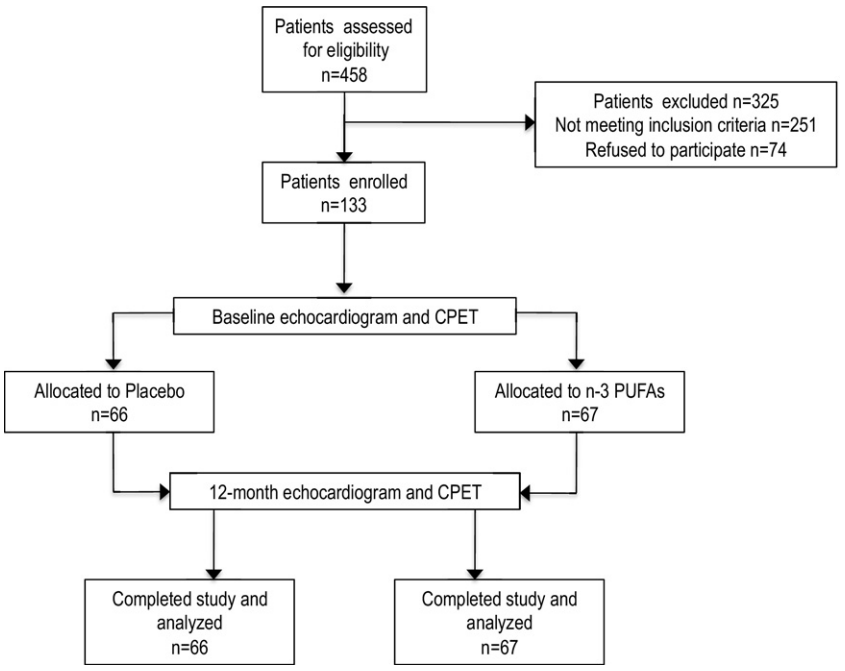

Figure 1 **Flow Diagram of the Phases of the Study**

The various phases of the study and the number of patients assessed for eligibility, actually enrolled, and randomized are shown.
CPET = cardiopulmonary stress testing; PUFA = polyunsaturated fatty acid.

with an angiotensin-converting enzyme inhibitor (ACE-I) or angiotensin II receptor blocker (ARB), a beta-blocker, and furosemide, and 80 patients (60.2%) were also receiving an aldosterone receptor blocker. In both arms, compliance levels to study treatment were above 80%, and no patient was withdrawn because of noncompliance. Baseline echocardiographic findings confirmed the presence of LV enlargement (mean LV end-diastolic diameter and LV end-diastolic volume of 68 ± 6 mm and 192 ± 44 mL, respectively) and moderate LV systolic dysfunction (mean LVEF 37 ± 6%). Cardiopulmonary exercise test showed a mean peak VO_2 of 19 ± 4 mL/kg/min and a mean percentage of maximum predicted VO_2 of 70 ± 11%.

The baseline demographic and clinical characteristics according to study groups are reported in Table 1.

Effects of treatments on circulating levels of n-3 PUFAs. At baseline, EPA + DHA accounted for 1.68 ± 0.43% and 1.76 ± 0.50% of circulating FFAs in the placebo and in the n-3 PUFAs group, respectively ($p = 0.363$). At the 12-month follow-up, no significant changes in EPA + DHA concentrations were observed in the patients on placebo (1.66 ± 0.41% vs. 1.68 ± 0.43%; $p = 0.082$). In contrast, a significant increase was observed in the patients treated with n-3 PUFAs (from 1.76 ± 0.50% to 4.31 ± 0.83%; $p < 0.001$). Thus at the end of the treatment period, n-3 PUFA levels were significantly higher in the active treatment than in the placebo group ($p < 0.001$).

Effects of n-3 PUFAs on ejection fraction and other parameters of LV systolic function. In patients randomized to n-3 PUFAs, a significant increase in LVEF (10.4 ± 9.5%; $p < 0.001$) was observed at the 12-month follow-up compared with baseline. In contrast, a statistically significant decrease of LVEF (−5.0 ± 3.8%; $p < 0.001$) was observed in the placebo group. The comparison of the changes of LVEF from baseline to 12-month follow-up between the 2 groups was statistically significant ($p < 0.001$) (Fig. 2) Treatment with n-3 PUFAs resulted in a reduction of LV diameters (−1.8%, $p < 0.0001$; and −4.5%, $p < 0.001$, for LV end-diastolic diameter and LV end-systolic diameter, respectively) and LV volumes (−2.5%, $p < 0.001$; and −7.5%, $p < 0.0001$, for LV end-diastolic volume and LV end-systolic volume, respectively). In contrast, in the placebo group, small increases in LV end-diastolic diameter (0.9%; $p < 0.001$), LV end-systolic diameter (0.86%; $p = 0.0001$), and LV end-systolic volume (1.03%; $p < 0.001$), but not in LV end-diastolic volume, were observed. The comparison of the changes in LV end-diastolic diameter, LV end-systolic diameter, LV end-diastolic volume, and LV end-systolic volume from baseline to the 12-month follow-up between the 2 groups was statistically significant ($p < 0.001$ for all) (Table 2).

Effects of n-3 PUFAs on diastolic function. In the patients treated with n-3 PUFAs, at the 12-month follow-up, the E-wave and A-wave velocities and the E/A ratio were similar

JACC Vol. 57, No. 7, 2011
February 15, 2011:870–9

Table 1 Baseline Demographic and Clinical Characteristics of Study Patients

	Placebo (n = 66)	n-3 PUFAs (n = 67)
Age, yrs	64 ± 9	61 ± 11
Male	56 (84.9)	64 (95.5)
Weight, kg	76.0 ± 7.5	76.9 ± 10.1
BMI, kg/m^2	25.7 ± 2.22	25.9 ± 2.3
Time since diagnosis, yrs	4.1 ± 1.5	4.2 ± 1.7
NYHA functional class		
Average	1.83 ± 0.38	1.88 ± 0.33
Class I	11 (16.7)	8 (11.9)
Class II	55 (83.3)	59 (88.05)
Diabetes	15 (23)	7 (10.4)
SBP, mm Hg	120.5 ± 12.2	119.5 ± 9.2
DBP, mm Hg	76.2 ± 5.1	76.0 ± 5.2
HR, beats/min	62 ± 4	62 ± 5
Creatinine, mg/dl	1.13 ± 0.23	1.10 ± 0.21
eGFR, ml/min	75.7 ± 27.5	81.4 ± 26.9
ICD	26 (39.4)	27 (40.3)
CRT	22 (33.3)	17 (25.4)
Medications		
ACE-I	59 (89.4)	52 (77.6)
ARBs	7 (10.6)	15 (22.4)
ACE-I/ARB	66 (100)	67 (100)
Aldosterone blockers	42 (63.6)	38 (56.7)
Beta-blockers	66 (100)	67 (100)
Amiodarone	35 (53)	27 (40.3)
Furosemide	66 (100)	67 (100)
Statins	11 (17)	7 (10)

Data are expressed as mean ± SD or n (%).

ACE-I = angiotensin-converting enzyme inhibitor; ARB = angiotensin II receptor blocker; CRT = cardiac resynchronization therapy; DBP = diastolic blood pressure; eGFR = estimated glomerular filtration rate; HR = heart rate; ICD = implantable cardioverter-defibrillator; NYHA = New York Heart Association; PUFA = polyunsaturated fatty acid; SBP = systolic blood pressure.

to baseline. However, the deceleration time was significantly increased (12.1%; $p < 0.0001$), and the diastolic function score was significantly decreased (−4.74%; $p = 0.004$), suggesting an improvement in diastolic function (Table 2). In the placebo group, compared with baseline, an increase in the E-wave velocity (12.5%; $p = 0.006$) with a nonsignificant change in the A-wave velocity and E/A ratio (3.9%, $p = 0.77$; and 14.9%, $p = 0.11$, respectively) were observed at the 12-month follow-up. However, a decrease of the deceleration time (−6.7%; $p < 0.001$) and an increase of the diastolic function score (10.1%; $p = 0.003$) were recorded in these patients, suggesting a worsening of diastolic function. The comparison of the changes of E-wave velocity and the E/A ratio from baseline to the 12-month follow-up between the 2 groups was statistically significant ($p = 0.023$ and $p = 0.038$, respectively), whereas no significant difference in the change of A-wave velocity was noted ($p = 0.714$). At the end of the treatment period, the diastolic function score was significantly lower in the n-3 PUFA group than in the placebo group ($p < 0.001$) (Table 2). Compared with baseline, at the 12-month follow-up, 80% of patients receiving placebo presented the same diastolic function score, whereas 18% had an increase and 2% had a decrease in this parameter. In the n-3 PUFA group, at the 12-month follow-up, 78% had no change in their baseline diastolic function score, whereas 19% had a reduction and 3% had an increase from the baseline value. When compared between groups, these changes were statistically significant ($p = 0.0002$).

Effects of n-3 PUFAs on exercise capacity. In the placebo group, compared with baseline, a significant decrease of peak VO_2 (−4.9%; $p < 0.001$), percent of predicted peak VO_2 (−2.9%; $p < 0.001$), length of exercise (−5.7%; $p < 0.001$), and workload (−5.3%; $p < 0.001$) were observed at the 12-month follow-up. In contrast, n-3 PUFA–treated patients showed a significant increase in peak VO_2 (6.2%; $p < 0.001$), percent of predicted peak VO_2 (4.3%; $p < 0.001$), length of exercise (7.7%; $p < 0.001$), and workload (7.1%; $p = 0.002$). The comparison of the changes of these parameters from baseline to the 12-month follow-up between the 2 groups was statistically significant (Table 3).

Effects of n-3 PUFAs on NYHA functional class. At the end of treatment, among patients receiving placebo, 47 (71.2%) remained in the same NYHA functional class, whereas 19 (28.8%) increased their NYHA functional class, suggestive of worsening clinical status. In the n-3 PUFA group, 49 patients (73.1%) maintained their initial NYHA functional class, whereas 18 (26.9%) were assigned to a lower NYHA functional class, indicating functional improvement. No patients had an increase of their NYHA functional class from baseline, suggesting that no patients experienced a functional decline during follow-up. When compared between groups, these changes were statistically significant ($p < 0.001$) (Fig. 3).

Effects of n-3 PUFAs on inflammatory cytokines and other metabolic parameters. After 12 months of placebo, levels of TNF-alpha, IL-6, and IL-1 were significantly increased from baseline. In contrast, the concentrations of

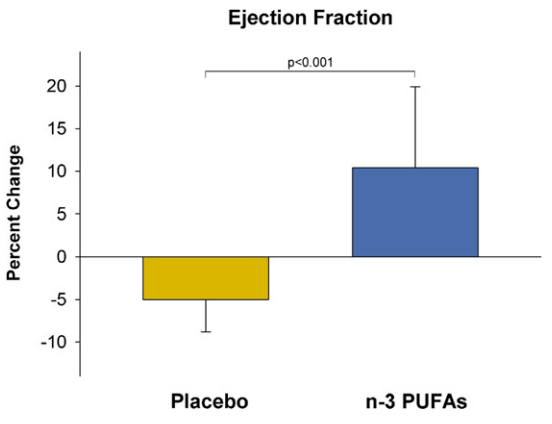

Figure 2 Changes in Ejection Fraction in the Study Groups

Comparison is shown between the mean percent changes in ejection fraction from baseline to 12-month follow-up recorded in the placebo and in the n-3 polyunsaturated fatty acids (PUFAs) group.

Table 2 Echocardiographic Parameters at Baseline and at 12-Month Follow-Up

	Placebo			n-3 PUFAs			
Variable	Baseline	12 Months	p Value	Baseline	12 Months	p Value	p Value*
LVEDD, mm	67.9 ± 4.5	68.4 ± 4.5	<0.001	67.4 ± 6.9	66.1 ± 6.7	<0.001	<0.001
LVESD, mm	53.9 ± 6.5	54.3 ± 6.4	<0.001	52.8 ± 7.4	50.4 ± 7.0	<0.001	<0.001
LVFS, %	21 ± 6	21 ± 6	0.59	22 ± 6	24 ± 6	<0.001	<0.001
LVEDV, ml	193 ± 26	194 ± 26	0.69	191 ± 56	186 ± 53	<0.001	<0.001
LVESV, ml	123 ± 25	124 ± 26	<0.001	123 ± 40	113 ± 35	<0.001	<0.001
LVEF, %	37 ± 6	35 ± 6	<0.001	36 ± 7	39 ± 6	<0.001	<0.001
PWT, mm	8.8 ± 1.0	8.8 ± 0.9	0.59	8.8 ± 1.0	8.7 ± 1.0	0.06	0.292
IVST, mm	8.8 ± 1.4	8.8 ± 1.1	0.48	8.9 ± 1.1	8.8 ± 1.1	0.13	0.172
LA size, mm	45 ± 5	45 ± 5	0.97	44 ± 5	43 ± 4	<0.001	<0.001
DT, ms	217 ± 38	200 ± 33	<0.001	219 ± 41	241 ± 33	<0.001	<0.001
DF score	2.17 ± 0.48	2.32 ± 0.47	<0.03	2.18 ± 0.42	2.01 ± 0.27	0.003	<0.001
PASP, mm Hg	29 ± 9	33 ± 7	<0.001	29 ± 8	27 ± 4	0.02	<0.001
MR score	1.71 ± 0.49	1.83 ± 0.57	0.09	1.61 ± 0.60	1.22 ± 0.62	<0.001	<0.001
E, cm/s	55 ± 20	61 ± 25	0.006	60 ± 28	55 ± 18	0.06	0.023
A, cm/s	65 ± 19	66 ± 18	0.77	70 ± 16	67 ± 20	0.96	0.714
E/A	0.90 ± 0.37	0.98 ± 0.40	0.11	0.89 ± 0.29	0.84 ± 0.19	0.17	0.038

Data are expressed as mean ± SD. *p values refer to the comparison of the changes in echocardiographic parameters observed in each group between baseline and 12-month follow-up.
A = peak velocity of late ventricular filling; DF = diastolic function; DT = deceleration time; E = peak velocity of early ventricular filling; IVST = interventricular septum thickness; LA = left atrium; LVEDD = left ventricular end-diastolic diameter; LVESD = left ventricular end-systolic diameter; LVFS = left ventricular fractional shortening; LVEDV = left ventricular end-diastolic volume; LVESV = left ventricular end-systolic volume; LVEF = left ventricular ejection fraction; MR = mitral regurgitation; PASP = pulmonary artery systolic pressure; PUFA = polyunsaturated fatty acid; PWT = posterior wall thickness.

these inflammatory cytokines were significantly decreased at the end of treatment with n-3 PUFAs. When compared between groups, these changes were statistically significant ($p < 0.001$) (Table 4).

Both in the placebo and active treatment group, no significant changes were noted in glucose and total cholesterol concentrations between baseline and the 12-month follow-up. A borderline significant decrease in triglyceride levels was observed in the n-3 PUFA–treated patients at the 12-month follow-up compared with baseline. When compared between groups, these changes were not significant (Table 4).

Predictors of changes in ejection fraction in n-3 PUFA–treated patients. To explore possible predictors of the response to active treatment, we performed univariate and multivariate analyses between changes in EF and: 1) time from diagnosis of NICM; 2) baseline EF; and 3) the baseline values of TNF-alpha, IL-6, and IL-1. On univariate analysis, cytokine levels were directly correlated, whereas time from diagnosis and baseline EF were inversely correlated, with changes in EF. When fitted in a multivariate model, only time from diagnosis and TNF-alpha retained their significance (Table 5).

Events at follow-up. Treatment with n-3 PUFAs was well tolerated. No serious adverse events were recorded in either treatment group, and no patients discontinued treatment throughout follow-up. No significant difference in all-cause hospitalization was observed between the placebo- and n-3 PUFA–treated patients. However, a significantly lower number of patients in the active treatment group required hospitalization for cardiovascular causes and/or worsening

Table 3 Cardiopulmonary Exercise Testing Parameters at Baseline and 12-Month Follow-Up

	Placebo			n-3 PUFAs			
Variable	Baseline	12 Months	p Value	Baseline	12 Months	p Value	p Value*
Time, min	10.6 ± 2.1	10.0 ± 2.0	<0.001	10.4 ± 1.9	11.2 ± 2.1	<0.001	0.0013
Workload, W	114 ± 31	108 ± 30	<0.001	113 ± 30	121 ± 32	0.002	<0.001
HR rest, beats/min	62 ± 4	64 ± 4	<0.001	62 ± 4	60 ± 3	<0.001	<0.001
HR max, beats/min	121 ± 18	117 ± 22	0.04	122 ± 17	125 ± 22	0.046	0.005
% HR max	78 ± 9	75 ± 13	0.04	77 ± 7	79 ± 10	0.047	0.006
Peak VO_2, ml/kg/min	18.3 ± 4.4	17.4 ± 4.2	<0.001	19.5 ± 3.8	20.7 ± 4.3	<0.001	<0.001
% VO_2 max	70 ± 13	68 ± 13	0.001	0.70 ± 0.09	0.73 ± 0.09	<0.001	0.0068
Rest SBP, mm Hg	120 ± 12	119 ± 11	0.13	119 ± 9	121 ± 6	0.049	0.015
Rest DBP, mm Hg	76 ± 5	76 ± 5	0.07	76 ± 5	75 ± 4	0.15	0.986
Peak SBP, mm Hg	149 ± 14	148 ± 14	0.02	149 ± 15	151 ± 11	0.20	0.026
Peak DBP, mm Hg	85 ± 8	85 ± 12	0.67	84 ± 7	83 ± 6	0.015	0.163

Data are expressed as mean ± SD. *p values refer to the comparison of the changes in cardiopulmonary stress testing parameters observed in each group between baseline and 12-month follow-up.
DBP = diastolic blood pressure; SBP = systolic blood pressure; VO_2 = oxygen uptake; other abbreviations as in Table 1.

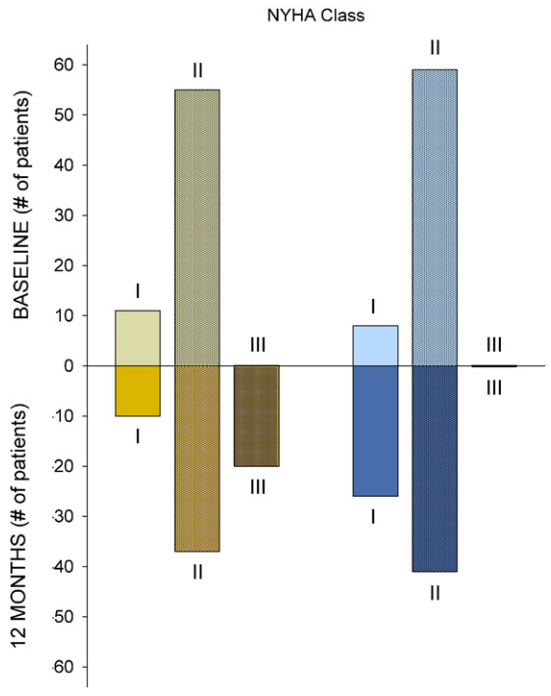

Figure 3 NYHA Functional Class Distribution in the Study Groups

Distribution of patients in the New York Heart Association (NYHA) functional classes at baseline and at 12-month follow-up in the placebo **(left gold bars)** and active treatment group **(right blue bars)**. Data are expressed as absolute number of patients.

HF (Table 6). Among patients receiving placebo, implantable cardioverter-defibrillator interrogation showed the occurrence of 9 appropriate shocks during follow-up, whereas in those receiving active treatment, only 2 events were recorded. The difference in the number of shocks between the 2 groups was statistically significant ($p = 0.026$). In the placebo group, 16 patients (24%) required an increase of furosemide dosage and 50 patients (76%) maintained the baseline dose, whereas among the n-3 PUFA–treated patients, the dose of furosemide was maintained in 41 patients (61%) and reduced in 26 patients (39%). No increase of diuretic dose was recorded in the n-3 PUFA group at the 12-month follow-up as compared with baseline. When compared between groups, these changes were statistically significant ($p < 0.001$). In both groups, no changes were observed in the dose of ACE-Is/ARBs and beta-blockers between baseline and the 12-month follow-up (Table 7). No patients underwent placement of a cardiac resynchronization therapy device during follow-up.

Table 5 Predictors of Changes of Ejection Fraction in n-3 PUFA–Treated Patients

	Univariate Analysis p Value	Multivariate Analysis p Value
Time from diagnosis	<0.001	<0.001
Baseline EF	<0.001	NS
Baseline TNFα	0.043	<0.001
Baseline IL-1	0.046	NS
Baseline IL-6	0.046	NS

EF = ejection fraction; NS = not significant; other abbreviations as in Table 4.

Discussion

The main findings of this study are that in patients with NICM, mildly reduced functional capacity, and in stable condition in response to maximal tolerated evidence-based therapy, 1-year treatment with n-3 PUFAs improves parameters of LV systolic and diastolic function, as well as functional capacity. Our data indicate that the increase in EF is accompanied by a small but significant reduction in diastolic and systolic dimensions and in the degree of mitral regurgitation and is correlated with the time from diagnosis and baseline TNF-alpha levels. The improvement of diastolic function is associated with a significant decrease in pulmonary artery systolic pressure and in left atrial dimension. From a functional standpoint, both peak VO_2 and NYHA functional class appear to be favorably affected by the treatment. These benefits are consistent with the decrease of the mean furosemide dose needed to maintain a congestion-free clinical status observed in the n-3 PUFA group and may account for the lower number of cardiovascular hospitalizations and hospitalizations for HF recorded during the 12-month follow-up period in these patients compared with those receiving placebo.

Our evidence of an improvement in EF associated with favorable LV remodeling is consistent with data from

Table 4 Serum Cytokine Levels and Metabolic Parameters at Baseline and 12-Month Follow-Up

	Placebo			n-3 PUFAs			
Variable	Baseline	12 Months	p Value	Baseline	12 Months	p Value	p Value*
TNF-alpha, pg/ml	21.7 ± 8.3	26.1 ± 10.9	<0.001	24.3 ± 13.9	13.5 ± 4.9	<0.001	<0.001
IL-6, pg/ml	10.1 ± 4.5	11.2 ± 5.4	<0.001	11.0 ± 6.0	3.53 ± 0.9	<0.001	<0.001
IL-1, pg/ml	459 ± 117	574 ± 215	<0.001	507 ± 208	366 ± 145	<0.001	<0.001
Blood glucose, mg/dL	109 ± 22	110 ± 20	0.20	104 ± 18	104 ± 13	0.54	0.19
Total cholesterol, mg/dL	187 ± 28	188 ± 24	0.09	187 ± 26	187 ± 24	0.76	0.71
Triglycerides, mg/dL	154 ± 76	155 ± 69	0.87	149 ± 62	143 ± 45	0.05	0.08

Data are expressed as mean ± SD. *p values refer to comparison of changes from baseline to 12-month follow-up between groups.
IL = interleukin; PUFA = polyunsaturated fatty acid; TNF = tumor necrosis factor.

Table 6 Hospitalizations During the 12-Month Follow-Up Period

	Placebo	n-3 PUFAs	p Value
All-cause hospitalization	28 (42.4)	18 (26.9)	0.0599
Non-CV hospitalization	2 (7.1)	8 (44.4)	0.052
CV hospitalization	26 (39.4)	10 (14.9)	0.0029
HF hospitalization	20 (30.3)	4 (5.9)	0.0002

Data are expressed as n (%).
CV = cardiovascular; HF = heart failure; PUFA = polyunsaturated fatty acid.

experimental studies conducted to investigate the potential mechanisms underlying the effects of n-3 PUFAs on the failing myocardium. In particular, in a Syrian hamster model of idiopathic dilated cardiomyopathy, long-term treatment with omega-3 fatty acids induced a significant enhancement of isometric and isotonic contractile properties of isolated papillary muscle and increased actomyosin cross-bridges number, force, and kinetics (14). In intact rats, chronic n-3 PUFA supplementation after aortic banding attenuated the increase in LV end-diastolic and end-systolic volumes, the decline of the velocity of circumferential shortening, and the reduction of LVEF, also preventing the switch in myosin heavy chain isoform from alpha to beta (6). Furthermore, in cardiomyopathic hamsters, n-3 PUFA administration led to a significant improvement in the peak rate of tension rise and peak rate of tension fall, which indicated a faster rate of muscle contraction and relaxation, respectively (15). These effects are likely related to the prevention of Ca^{2+} overload and to the decrease of sarcoplasmatic reticulum Ca^{2+} content (16). Taken together, these findings may suggest potential mechanisms underlying the amelioration of diastolic function parameters observed in our active treatment group. Furthermore, the enhanced exercise capacity recorded in the n-3 PUFA–treated patients is consistent with an improved cardiac performance, which is supported by the evidence that, in isolated rat hearts, n-3 PUFAs reduce myocardial O_2 consumption while sustaining cardiac output and external work, indicating an increase in the efficiency of O_2 use under conditions of constant HR, pre-load, and afterload (17). In our study, baseline TNF-alpha levels were 1 of the 2 independent predictors of n-3 PUFA–related increase in EF. Active treatment significantly decreased circulating concentrations of TNF-alpha, IL-1, and IL-6, confirming the experimental evidence that supplementation with EPA + DHA reduces inflammation (18), an effect possibly linked to an increase in circulating adiponectin (19) and to the modulation of transcription factors such as nuclear transcription factor kappa B (18).

The above mechanisms may account for the overall favorable effects on hemodynamic and functional parameters observed with n-3 PUFAs and are consistent also with epidemiological data. In particular, an analysis of a large cohort of adults enrolled in the Cardiovascular Health Study explored the associations between dietary intake of fish during the previous year and cardiac structure, function, and hemodynamics (20). In this study, consumption of tuna or other broiled or baked fish was associated with a lower HR, lower systemic vascular resistance, greater stroke volume, and higher E/A ratio. Additionally, the effect of n-3 PUFAs in HF may be related to an improvement of endothelial function, including a significant enhancement of endothelium-dependent vasodilatation (21) and a reduction in the vasoconstrictor response to angiotensin II (3). Thus, n-3 PUFAs may exert potentially beneficial effects on multiple pathophysiological abnormalities underlying the impairment of myocardial function and the progression of remodeling present in the failing heart. Importantly, in our study population, these actions appear to be independent from, and additive to, the standard neurohormonally and hemodynamically oriented treatments for HF.

Among patients randomized to n-3 PUFAs, approximately 27% showed an improvement in their myocardial function and functional capacity parameters, and the remaining participants maintained their initial class. In contrast, in the placebo group no patients improved functionally and about 30% showed a decline from their baseline condition. Therefore, n-3 PUFAs treatment appeared to favorably affect the progression of the disease and the decline of myocardial function in our population. Impor-

Table 7 Mean Dose of ACE-I/ARB and Beta-Blockers at Baseline and at 12-Month Follow-Up

	Placebo		n-3 PUFAs	
	Drug Dose (mg)		Drug Dose (mg)	
	Baseline	12 Months	Baseline	12 Months
ACE-i/ARB				
Enalapril	32.9 ± 8.8	32.5 ± 8.7	33.6 ± 9.5	33.6 ± 9.5
Ramipril	9.1 ± 2.0	9.1 ± 2.0	8.9 ± 2.1	8.9 ± 2.1
Losartan	87.5 ± 25	87.5 ± 25	80.0 ± 27.4	80.0 ± 27.4
Candesartan	32 ± 0	32 ± 0	28.8 ± 6.8	28.8 ± 6.8
Beta-blocker				
Carvedilol	41.9 ± 11.7	41.6 ± 11.5	42.0 ± 10.6	42.0 ± 10.6
Bisoprolol	8.0 ± 2.6	8.0 ± 2.6	9.3 ± 1.9	8.6 ± 2.5

Data are expressed as mean ± SD.
ACE-I = angiotensin-converting enzyme inhibitor; ARB = angiotensin II receptor blocker; PUFA = polyunsaturated fatty acid.

tantly, the time from diagnosis was inversely correlated with the improvement of EF observed in the active treatment group, suggesting that the beneficial effects of n-3 PUFAs may be more evident if they are administered early in the natural history of NICM. Furthermore, even if our investigation was not designed to explore the effects of n-3 PUFAs on prognosis, we observed a significantly lower number of hospitalizations for cardiovascular cause and for worsening HF, as well as a lower mean dose of furosemide, in the active treatment group compared with the placebo group, which is consistent with the results of the GISSI-HF trial (2). Despite significant differences in the study design and population characteristics, our findings may suggest that the functional benefits observed with n-3 PUFAs may contribute to an improvement in outcomes. Finally, n-3 PUFAs were very well tolerated, as reflected by a high compliance, the absence of serious adverse events, and the lack of drop-outs.

Study limitations. Our investigation was a single-center trial with a small sample size and a limited number of clinical events. Our evaluation of diastolic function was performed according to the standard recommendations published at the time of study design. Therefore, it did not include tissue Doppler measurements, which are currently considered an integral part of the echocardiographic assessment and staging. By design, our study population included patients with mild and moderate HF due to idiopathic dilated cardiomyopathy, with only moderately reduced LVEF, mildly impaired functional capacity, and an overall good short-term prognosis. This was reflected in the absence of mortality and in a limited number of admissions during follow-up among study participants. Therefore, our results cannot be generalized to HF patients with a different etiology and/or at more advanced stages of the disease. Second, all patients were treated with maximal-tolerated doses of evidence-based therapies, including furosemide, a beta-blocker, an ACE-I/ARB, an aldosterone receptor antagonist, and devices. It is not possible to infer from our data whether the effects of n-3 PUFAs would also be present in patients who are not receiving such treatments.

Conclusions

The results of this investigation indicate that in patients with chronic HF and reduced LVEF due to NICM, and minimal or no symptoms in response to evidence-based therapy at maximal tolerated target doses, the addition of n-3 PUFA 2 g daily for 1 year improves echocardiographic parameters of LV systolic and diastolic function, as well as functional capacity. These beneficial effects suggest that n-3 PUFAs may favorably affect cardiac remodeling and the decline of myocardial function in patients with HF and may account for the reduction in cardiovascular hospitalizations and hospitalizations for HF observed in our study. However, whether n-3 PUFAs exert similar effects in patients with HF caused by other etiologies, at more advanced stages, or who are not receiving evidence-based therapy remains to be verified.

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Key Words: cardiomyopathy ■ functional capacity ■ heart failure ■ nonischemic myocardial function ■ n-3 PUFAs ■ NYHA functional class.