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A randomized controlled study of the efficacy of six-month supplementation with concentrated fish oil rich in omega-3 polyunsaturated fatty acids in first episode schizophrenia

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ABSTRACT

Short-term clinical trials of omega-3 polyunsaturated fatty acids (n-3 PUFA) as add-on therapy in patients with schizophrenia revealed mixed results. The majority of these studies used an 8- to 12-week intervention based on ethyl-eicosapentaenoic acid. A randomized placebo-controlled trial was designed to compare the efficacy of 26-week intervention, composed of either 2.2 g/day of n-3 PUFA, or olive oil placebo, with regard to symptom severity in first-episode schizophrenia patients. Seventy-one patients (aged 16–35) were enrolled in the study and randomly assigned to the study arms. The primary outcome measure of the clinical evaluation was schizophrenia symptom severity change measured by the Positive and Negative Syndrome Scale (PANSS). Mixed models repeated measures analysis revealed significant differences between the study arms regarding total PANSS score change favouring n-3 PUFA ($p = 0.016$; effect size (ES) = 0.29). A fifty-percent improvement in symptom severity was achieved significantly more frequently in the n-3 PUFA group than in the placebo group (69.4 vs 40.0%; $p = 0.017$). N-3 PUFA intervention was also associated with an improvement in general psychopathology, measured by means of PANSS ($p = 0.009$; ES = 0.32), depressive symptoms ($p = 0.006$; ES = 0.34), the level of functioning ($p = 0.01$; ES = 0.31) and clinical global impression ($p = 0.046$; ES = 0.29). The findings suggest that 6-month intervention with n-3 PUFA may be a valuable add-on therapy able to decrease the intensity of symptoms and improve the level of functioning in first-episode schizophrenia patients.

1. Introduction

Polyunsaturated n-3 fatty acids (n-3 PUFA) are the major constituents of the neuronal membranes that modulate a broad range of biological mechanisms and pathways, such as membrane fluidity, dopaminergic, serotonergic, glutamatergic and cholinergic neurotransmission, neuroinflammation, apoptosis and senescence, gene expression, synaptic plasticity and function (Abedi and Sahari, 2014). Moreover, n-3 PUFA play a pivotal role at various stages of brain development, especially the embryonic, prenatal and early postnatal stages (McNamara, 2013). N-3 PUFA are thus crucial for neurodevelopment, neurodegeneration and biological mechanisms of behaviour regulation, all processes known to be disrupted in schizophrenia and suggested as playing a pivotal role in schizophrenia pathogenesis (Berger et al., 2002; McNamara and Carlson, 2006).

Disturbances of PUFA metabolism were repeatedly observed in patients with schizophrenia (Hoen et al., 2013), which led to the formulation of the "membrane hypothesis" originally postulating that dietary deficiencies or increased turn-over of PUFA leads to prostaglandin deficiency syndrome related to decreased availability of eicosanoids, i.e. derivatives of mainly arachidonic acid (AA) and eicosapentaenoic acid (EPA) (Horrobin, 1998). Deficiencies of PUFA in red blood cells were observed in patients at different stages of

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disease, even in drug-naive individuals (Reddy et al., 2004). Magnetic resonance spectroscopy studies revealed accelerated loss of long-chain PUFA from neuronal membranes, which was attributed to increased activity of phospholipase A2 (Berger et al., 2002). The above observations and the results of epidemiological studies showing the relationship between PUFA deficiency and the severity of disease led to open-label and randomized clinical trials (RCTs) assessing the efficacy of n-3 PUFA in schizophrenia. To date, 9 RCTs have been carried out to assess the efficacy of n-3 PUFA in patients with schizophrenia. The results of trials were mixed. Four of them showed that n-3 PUFA had greater efficacy over placebo as the primary measure (Peet et al., 2001; Emsley et al., 2002; Jamilian et al., 2014); four of them did not show significant differences between groups (Fenton et al., 2001; Peet and Horrobin, 2002; Emsley et al., 2006; Berger et al., 2007); and one study (Bentsen et al., 2013) reported even a significant increase in symptom severity in the n-3 PUFA group in comparison with placebo. Meta-analyses carried out before the last positive study was published did not support n-3 PUFA efficacy in schizophrenia (Joy et al., 2006; Fusar-Poli and Berger, 2012). Only two of the RCTs enrolled patients with first-episode schizophrenia (Peet et al., 2001; Berger et al., 2007). One study assessed the efficacy of n-3 PUFA added-on to antipsychotics (Berger et al., 2007) and the other (Peet et al., 2001) as a sole treatment for schizophrenia. Peet et al. (2001) reported positive results and Berger et al. (2007) did not observe n-3 PUFA effect on the primary efficacy outcome measure. The intervention period in the majority of the studies was 8–12 weeks and the longest period was 16 weeks. No longer-term studies have been reported so far. Most studies used EPA or ethyl ester of EPA (E-EPA) as the intervention and different kinds of placebo oils.

Docosahexaenoic acid (DHA) is the principal n-3 PUFA present in the grey matter of the mammalian brain and comprises approximately 10–20% of total fatty acid composition of the adult frontal cortex (Carver et al., 2001). The concentration of DHA sharply increases during critical stages of brain development when connections between the frontal cortex and limbic system are formed (Carver et al., 2001). There is accumulating evidence that DHA is crucial for neurodevelopment (McNamara, 2013) and may exhibit neurotrophic (Rapoport et al., 2007; Sable et al., 2013; Bach et al., 2014) and neuroprotective (Hogyes et al., 2003; McNamara et al., 2015) effects in the brain. Docosahexaenoic acid is also involved in several cellular processes that modulate inflammation and apoptosis, both directly and indirectly via resolvins and neuroprotectins – the active derivatives produced in the metabolism of DHA (Bradbury, 2011; Calder, 2013). A study by Amminger et al. (2010) further supports the role of DHA in the developing brain, as this study provided preliminary support for using the mixture of n-3 PUFA (EPA and DHA) in populations of help-seeking adolescents and young adults at high clinical risk of psychosis (Ultra High Risk group). The authors showed that 12-week intervention composed of 700 mg of EPA and 480 mg of DHA reduces transition rate into psychotic episode during a 12-month follow-up. Mixtures of n-3 PUFA containing high concentration of EPA and DHA had not been used so far in populations of patients with first-episode schizophrenia.

Thus, n-3 PUFA is known to demonstrate a preventive effect in UHR individuals, evidence has accumulated on the crucial role of DHA in neurodevelopment and neuroprotection, and previous short-term supplementation studies have returned diverse results. Hence, the aim of the present study is to assess the efficacy of the long-term intervention of concentrated marine fish oil rich in EPA and DHA in reducing symptomatology in patients with first-episode schizophrenia.

2. Methods

2.1. Participant sample

The study population was composed of inpatients admitted to the Psychiatric Clinics of the Central Teaching Hospital, Medical University of Lodz and the wards of Babinski Hospital in Lodz, Poland. Patients were enrolled consecutively as they were admitted to the hospitals. Eligible patients were (1) aged 16–35; (2) diagnosed with first-episode schizophrenia according to the International Classification of Diseases 10th version (ICD-10); which is an obligatory classification of mental disorders in Poland. Diagnosis was confirmed by the mini neuropsychiatric interview plus (MINI plus) (Sheehan et al., 1998). Patients were excluded (1) if more than two years had passed since the onset of positive symptoms; (2) if the patient had bleeding disorders; (3) was using n-3 PUFA supplements within 8 weeks or (4) was using anticoagulants for any reason; (5) was diagnosed with drug-induced psychosis, first-episode mania, organic disorders presenting with psychotic symptoms or intellectual disability; (6) if the patient had a history of head injury with loss of consciousness, or any acute or unstable medical condition or one that could influence the results of the trial or affect their ability to take part in the trial; (7) if the patient was participating in another study.

Two hundred and three patients were screened for eligibility. One hundred thirty-two (65%) patients were excluded: 20 (9.9%) due to involuntary status or incapacity, 87 (42.9%) not meeting inclusion or meeting exclusion criteria, 22 (10.8%) for not providing informed consent. Seventy-one patients met inclusion criteria and consented to the study (Fig. 1). The first participant was included in November 2011 and the last participant completed the trial in March 2015.

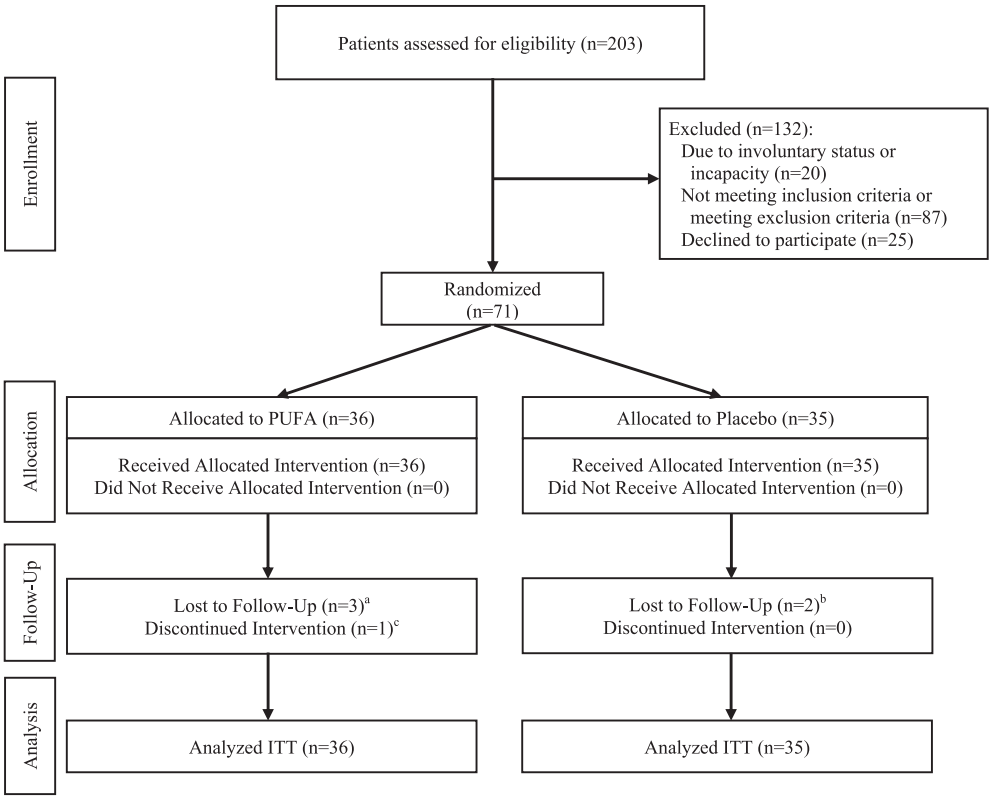
The trial procedures were explained verbally and in writing to all eligible patients. All participants provided written informed consent prior to study enrolment. Parental or guardian consent was obtained for participants under 18 years of age. The study was conducted in accordance with the Declaration of Helsinki and the study protocol was approved by the Ethics Committee of the Medical University of Lodz.

2.2. Study design

A randomized, double-blind, placebo-controlled, parallel-group 26-week augmentation trial of either concentrated fish oil rich in n-3 PUFA (2.2 g per day of EPA + DHA), or olive oil placebo, added on to an adjustable dose of antipsychotic medication was performed. The background antipsychotic therapy and concomitant medications were chosen and titrated according to the Polish standards of pharmacotherapy of mental disorders (Jarema, 2011). The rationale for the study and the study protocol is characterized in detail elsewhere (Pawelczyk et al., 2015). This study has been registered at Clinical Trials.gov with the following number: NCT02210962.

2.3. Randomisation and blinding

Random assignment to EPA + DHA or placebo was stratified using age (3 strata: 16–22; 23–29; 30–35 years), as age at onset is related to treatment response and schizophrenia prognosis (Carbon and Correll, 2014). Moreover, age at onset is also significantly related to the second important prognostic factor in schizophrenia, i.e. duration of untreated psychosis (Perkins et al., 2005). Stratified randomization was used to achieve higher between group comparability. A computer-generated random sequence based on block randomized design, with three age strata comprising block lengths of four within each, was kept in a remote secure location



[a] – Lost to follow-up: formally withdrew consent (N=1), did not attend follow-up assessments (N=2)
[b] – Lost to follow-up: did not attend follow-up assessments (N=2)
[c] - discontinued intervention before first follow-up visit and withdrew consent (N=1)
Abbreviations: PUFA – polyunsaturated fatty acids, ITT – intention-to-treat

Fig. 1. CONSORT flow diagram.

and administered by an independent third party until termination of the study and collection of all study data. Patients, parents, stuff involved in administering intervention, assessing the outcomes or entering data were blind to group assignments.

2.4. Study intervention

The active treatment was yellow gel capsules filled with concentrated fish oil containing 0.33 g of EPA and 0.22 g of DHA in each capsule. The daily dose of 4 capsules provided 2.2 g of n-3 PUFA, i.e.: 1.32 g/day of EPA plus 0.88 g/day of DHA. Due to limited efficacy of the marine fish oil concentration processes, each capsule of active intervention contained also small amounts of other fatty acids: saturated (C16:0, C18:0) - 35.5 mg; monounsaturated: (C22:1; C18:1n9, C20:1n9, C18:1n7) - 100.7 mg; n-6 PUFA (C18:2n6, C18:3n6, C20:4n6) - 27.5 mg; other n-3 PUFA (C18:3n3, C18:4n3, C20:4n3, C21:5n3; C22:5n3) - 104.4 mg. Olive oil was chosen as placebo because it contains mainly monounsaturated fatty acids and only small amounts of polyunsaturated fatty acids. Placebo capsules were prepared to match the active treatment in appearance and flavour. The placebo contained also a scant amount of fish oil to provide a comparable taste and smell of the different capsules. Both placebo and active capsules contained an antioxidant, i.e. 0.2% alpha-tocopherol (vitamin E). The rationale behind the choice of the study medication, its dose and composition, and kind of placebo used was based on the previous studies and is characterized in detail elsewhere (Pawelczyk et al., 2015). The study medication (concentrated fish oil and placebo) was provided by Marinex International Sp. z o.o. and shipped from Scandinavian Laboratories, Inc. Mt. Bethel, PA, USA. It was packed into numbered bottles and sent to the store of the Central Teaching Hospital of the Medical University of Lodz, Poland. Each bottle contained a fixed number of capsules of study medication or an equal amount of an olive oil placebo. Adherence to study intervention was monitored through patient/parent self-report and pill count at each medication appointment. The term EPA + DHA will be used for short through the rest of the paper to denote the active intervention composed of concentrated fish oil rich in EPA + DHA.

To increase the external validity of the study results and conform with the mentioned above guidelines of therapy, the use of benzodiazepines, Z-drugs, injectable forms of antipsychotics, antidepressants, mood stabilizers and anticholinergic medications was allowed if clinically indicated. Background antipsychotic and concomitant medication use was monitored throughout the study. The use of special diets or supplements, including other n-3 PUFAs, was not permitted throughout the study. Participants were assessed by a registered dietitian at the beginning of the study and advised to adhere to a balanced diet for the duration of the study.

2.5. Outcome measures

Clinical scales were used to assess several domains of symptom

severity and patient functioning at baseline and planed follow-up visits. After randomization, participants received weekly assessments for 4 weeks and then at week 6, 8, 16 and 26. The severity of schizophrenia symptoms was assessed using the Positive and Negative Syndrome Scale (Kay et al., 1987). Depressive features were measured by the Calgary Depression Scale for Schizophrenia (CDSS) (Addington et al., 1992). Patient functioning status was measured by means of Global Assessment of Functioning (GAF) scale (Jones et al., 1995). The Clinical Global Impressions scale was used to measure symptom severity (CGI-S) (Guy, 1976). The rationale behind the choice of clinical scales and their psychometric properties is characterized in more detail elsewhere (Pawelczyk et al., 2015).

The primary outcome measure was the magnitude of change in PANSS total scores between baseline and 26 weeks. Apart from presenting outcome as a continuous variable, primary outcome was also expressed in a binary fashion: i.e. as the comparison of the number of responders in each intervention group. Twenty-five percent and 50% decreases in PANSS total score were considered cut-offs for clinically significant symptom reduction (Leucht et al., 2007). The percentage change of scores was calculated according to Leucht et al. (2007; Leucht, 2014), based on the principle that a patient with no psychopathological symptoms scores 1 in all PANSS scale items. The formula for calculating change was as follows: $[(Pt2 - N) - (Pt1 - N)/(Pt1 - N)] \cdot 100$, where Pt1 represents the PANSS subscale score at the beginning of the study, Pt2 is the value at the end of the study and N is the number of PANSS subscale items. $N = 7$ for PANSS positive and negative subscales, $N = 16$ for the general psychopathology PANSS subscale and $N = 30$ for the total PANSS score. Patients who achieve at least a 25% reduction in the total PANSS score were regarded as responders. Those who achieve a 50% decrease were regarded as much improved (Leucht, 2014).

Secondary outcome measures included the changes between values at baseline and after 26 weeks of intervention in the PANSS subscale scores (positive, negative, and general psychopathology), CGI, GAF and CDSS scores.

All adverse events volunteered or observed during the study were recorded, together with their severity and duration during all assessment visits. Tolerability was measured with the Udvalg for Kliniske Undersøgelser Side Effect Rating Scale (UKU) (Lingjaerde et al., 1987).

2.6. Chlorpromazine dose equivalents

Daily doses of antipsychotics used were converted into chlorpromazine equivalents using equivalency table provided by Gardner et al. (2010). According to the recent meta-analysis (Patel et al., 2013), the above-mentioned method appears to provide the most complete estimation of equivalencies of the reviewed methods. Chlorpromazine equivalent cumulative exposure to antipsychotics was calculated according to the formula provided by Andreasen et al. (2010) and expressed in terms of the number of dose-years of 100 mg of chlorpromazine daily.

2.7. Interrater reliability

Each patient was rated by the same assessor at baseline and the following assessments. All the raters were experienced clinicians who were extensively trained in the scoring methods used in the study as part of the first Polish program for people at high clinical risk of psychosis development (PORT), which is described in detail elsewhere (Kotlicka-Antczak et al., 2015). The raters achieved good reliability scores for administration of the clinical scales (coefficient of agreement >0.82) and were all within 20% of the standard scores. Reliability was reassessed every 4 months to avoid drift of assessments.

2.8. Power calculation

Previous studies have suggested medium to large effect sizes in favour of n-3 fatty acids (Peet et al., 2001; Emsley et al., 2002). On the basis of previous studies, a nontrivial correlation was assumed between baseline and outcome scores at follow-up, and it was hypothesized that baseline scores will explain at least 25% of the variation in outcome measures. Power calculation revealed that 36 participants per study arm will result in 80% power to detect medium effect size (Cohen's $d = 0.3$) at a significance level of 0.05 (two sided).

2.9. Statistical methods and analytic plan

All analyses were performed on an intent-to-treat (ITT) basis. Distributions of continuous variables were assessed using the Shapiro–Wilk test. Comparisons between treatment groups at baseline for continuous variables were conducted using Student's t-test or the Mann–Whitney U-test depending on the distribution of the dependent variables. Differences in categorical variables were analysed using the Chi-square test or Fisher's exact test depending on the met assumptions.

As the missing data in the present study was the result of patient withdrawal or missed assessments, it cannot be regarded as missing completely at random and must be modelled (Friedman et al., 2010). To deal with missing values in our ITT sample, a conservative approach was used assuming that the severity of symptoms and functioning would have been maintained at the level that was observed during the last visit the patient was assessed (last observation carried forward, LOCF).

According to the study protocol, it was intended to perform one-way analysis of covariance (ANCOVA) to assess the primary outcome measure. The analytical method was changed due to the recent criticism of ANCOVA as a possible source of bias in clinical trials (Kraemer, 2015). The changes in clinical scores were assessed using a mixed model for repeated measures (MMRM) that included fixed-effect terms for intervention, visit, baseline score, and an intervention-by-visit interaction term, using scaled identity covariance structure for within-patient correlation. Differences between the treatment groups were reported using least-squares (LS) means with standard error (SE). No adjustment was made for multiple comparisons with respect to *post hoc* analyses. Cohen d effect sizes were calculated as the difference in LS mean change scores between treatment and placebo divided by the model estimate of the pooled standard deviation. The scores of the UKU Side Effect Rating Scale were analysed using change from baseline – a method which takes into account the initial level of symptoms present due to antipsychotic medication use before study initiation. The UKU is a 48-item rating scale scored with the following values: 0 (not or doubtfully present), 1 (mild), 2 (moderate) and 3 (severe). Thus, the change in score from baseline can range from −3 (maximal improvement) to +3 (maximal worsening). The frequencies of patients with changed scores in each study arm were compared using Fisher's exact test. All statistical tests were 2-tailed, with statistical significance set at alpha = 0.05.

3. Results

3.1. Study sample

Seventy-one individuals were enrolled in the study: 36 randomly assigned to the EPA + DHA group and 35 to placebo. The treatment groups were similar in terms of demographic variables

and baseline characteristics (Table 1). One of the 36 (1.8%) participants from the EPA + DHA group discontinued the intervention prematurely and withdrew consent. Three patients of 36 (8.3%) from EPA + DHA group were lost to follow-up and did not attend follow-up assessments. Two patients of 35 (5.7%) from the placebo group were lost to follow-up: one moved out of the area and another did not attend any follow-up assessments. The 26-week follow-up intervention was completed by 65 participants: 32 (88.9%) from EPA + DHA group and 33 (94.3%) from placebo group (Fig. 1). The difference in drop-out rate between groups was not statistically significant (Fisher exact test; $p = 0.674$). At the time of enrolment 43 participants (60.6%) were antipsychotic naive and 17 had fewer than 9 days of medication. Among those medicated, the mean duration of antipsychotic therapy was 14 days (SE = 3.3). All but 5 patients had been treated with antipsychotics for less than 6 weeks before enrolment. Study groups were not significantly different according to the frequency of antipsychotic-naive patients enrolled (Chi square test; $Ch^2 = 1.139$; $p = 0.286$). The groups were not different in terms of duration of antipsychotic therapy prior to trial inclusion (Mann–Whitney U test; $Z = 1.201$; $p = 0.230$). The groups were not significantly different in terms of baseline chlorpromazine equivalent dose (Table 1). Daily consumption of energy and PUFA was determined at baseline using the Polish version of the Food Frequency Questionnaire (Dehghan et al., 2012). Dietary consumption of energy and PUFA was not significantly different between the groups at baseline (Table 1).

3.2. Outcome measures

Mixed model for repeated measures (MMRM) analysis was used to assess differences between intervention groups in clinical score changes in the course of the study.

3.2.1. Primary outcome measure

The analysis of contrasts from MMRM revealed significant differences between groups regarding primary outcome symptom

Table 1
Baseline characteristics of participants.

Characteristic	EPA + DHA (N = 36)	Placebo (N = 35)	p Value
Age, mean (SD)	23.2 (4.8)	23.3 (4.8)	0.937
Male sex, N (%)	19 (52.8)	23 (65.7)	0.268
Duration of untreated psychosis, mean (SD), mo	3.1 (4.2)	2.7 (3.5)	0.702
Family history of schizophrenia, N (%)	13 (36)	14 (40)	0.736
Education level, N (%)			
elementary	12 (33)	9 (26)	
vocational	0 (0)	3 (8)	
secondary	18 (50)	14 (40)	0.092
bachelor degree	5 (14)	3 (9)	
master degree	1 (3)	6 (17)	
Years of education, mean (SD)	12.9 (2.7)	13.8 (3.1)	0.229
Marital status, N (%)			
married	2 (6)	2 (6)	0.346
single	34 (94)	31 (89)	
divorced	0 (0)	2 (5)	
Place of living, N (%)			
alone	5 (14)	4 (11)	0.573
with family	30 (83)	31 (89)	
dormitory	1 (3)	0 (0)	
Employment, N (%)			
employed	4 (11.1)	7 (20.0)	0.475
not employed	17 (47.2)	18 (51.4)	
sheltered workshops	1 (3)	0 (0)	
during education	14 (38.9)	10 (28.6)	
Tobacco use, N (%)	14 (39)	15 (43)	0.734
Energy consumption, mean (SD) [kCal]	2279.19 (982.49)	2328.32 (753.59)	0.833
PUFA consumption, mean (SD) [g]	14.68 (7.45)	13.82 (5.84)	0.625
CDSS score, mean (SD)	8.78 (5.13)	8.11 (5.52)	0.602
CGI-S score, mean (SD)	5.89 (0.75)	5.86 (0.77)	0.861
GAF score, mean (SD)	26.17 (8.8)	26.91 (9.42)	0.731
PANSS score, mean (SD)			
Positive	25.64 (5.21)	25.34 (5.83)	0.296
Negative	23.14 (6.13)	22.77 (5.96)	0.799
General	49.64 (7.53)	48.69 (7.02)	0.583
Total	98.4 (13.22)	96.8 (12.01)	0.592
Antipsychotic-naïve patients, N (%)	24 (66.7)	19 (54.19)	0.286
Antipsychotics, N (%)			0.889
Aripiprazole	1 (2.8)	2 (5.7)	
Risperidone	5 (13.9	4 (11.4)	
Olanzapine	2 (5.6)	4 (11.4)	
Quetiapine	3 (8.3)	4 (11.4)	
Sulpride	0 (0.0)	1 (2.9)	
Amisulpride	1 (2.8)	1 (2.9)	
CPZ equivalent dose at baseline[a], median (IQR) [mg]	0 (187.5)	0 (300)	0.256
CPZ equivalent dose at baseline[b], mean (SD) [mg]	263.16 (128.76)	292.81 (195.62)	0.669

CDSS – The Calgary Depression Scale for Schizophrenia. CGI-S – The Clinical Global Impressions Severity Scale. PANSS – The Positive and Negative Syndrome Scale. GAF – Global Assessment of Functioning Scale; N – number of observations in a population, SD – standard variation, mo -month, yr -year; CPZ – chlorpromazine; PUFA – polyunsaturated fatty acids.

[a] Entire population.

[b] Participants on antipsychotics at baseline; SD – standard deviation; IQR – interquartile range.

severity measured by means of total PANSS score change. Mean change from baseline in total PANSS score at month 6 was significantly higher in the EPA + DHA group in comparison with the placebo group. The magnitude of the observed effect can be considered as small according to Cohen (1988).

The association between 26-week EPA + DHA add-on therapy and 25% improvement rate in total PANSS score was not statistically significant. However, 50% improvement was achieved significantly more frequently in the EPA + DHA than in the placebo group (Table 3). The number needed to treat (NNT) with n-3 PUFA rich fish oil to achieve at least a 50% reduction in total PANSS during the 26-week period in patients diagnosed with first-episode schizophrenia was 4 (95% CI, 2–14).

3.2.2. Secondary outcome measures

The analysis of contrasts from MMRM revealed significant differences between groups regarding secondary outcome measures, i.e. symptom severity and level of functioning. Least squares mean change from baseline in clinical scale scores (CDSS, general psychopathology PANSS, GAF, CGI-S) at month 6 significantly favoured EPA + DHA group over placebo using MMRM analysis. No significant differences between groups were observed regarding the positive and negative PANSS score changes. The observed effects can be considered as small in case of CGI-S and GAF score change and moderate in case of CDSS and general psychopathology PANSS score change (Cohen, 1988). The least squares mean changes and mean differences in change scores between groups at month 6 are presented in Table 2.

3.2.3. Response rates at different time points

Significant differences in the frequency of patients achieving a response defined as at least a 25% improvement in total PANSS were observed after 6 weeks of intervention. A significantly higher proportion of patients in the EPA + DHA group (33.3%) achieved at least a 25% improvement than in the placebo group (5.7%). No significant differences between study groups were observed at other time points regarding at least 25% improvement. When response was defined as at least a 50% improvement in total PANSS score, the frequency of participants achieving response differed significantly between groups only at the end of the intervention (26 weeks), which was previously described in section 3.2.1. The rates of participants achieving response at different time points is summarized in Table 3 and the proportions are shown in Fig. 2.

3.3. Adherence and background medication

The mean rate for adherence with study intervention, based on pill count and self-report, was 83.2% (SD = 17.4) in the EPA + DHA group and 78.9% (SD = 16.6) in the placebo group (Student t-test; t = 1.096; p = 0.277). No significant differences between the study groups were found regarding chlorpromazine equivalent cumulative exposure to antipsychotics in different periods of the study. Data concerning dose-years of antipsychotic cumulative exposure is presented in Table 4. Concomitant medication use after randomization included the following compounds in the EPA + DHA and placebo groups respectively: benzodiazepines in 25 (69.4%) vs. 18 (51.4%) participants (Fisher exact test; p = 0.149); antidepressants in 7 (19.4%) vs. 6 (17.1%) participants (p = 1.0); mood stabilizers in 4 (11.1%) vs. 4 (11.4%) participants (p = 1.0); anticholinergics in 6 (16.7%) vs. 3 (8.6%) participants (p = 0.478).

3.4. Adverse events

A statistically significant difference was observed between the study groups regarding the change from baseline of UKU scale item 3.6 (constipation). The result of the Fisher's exact test favoured the EPA + DHA group over the placebo group (p = 0.021), i.e. a significantly higher frequency of patients achieving improvement in UKU constipation score was observed in the EPA + DHA group. Fisher's exact test did not reveal significant differences between the study groups with regard to all other changes in UKU scale items. The frequencies of patients with changes in UKU scale item 3.6 are shown in Table 5.

4. Discussion

The reported study appears to be the first long-term randomized, placebo-controlled trial of the efficacy of augmentation with n-3 PUFA rich concentrated fish oil in first-episode schizophrenia. Six-month intervention with marine fish oil containing 2.2 g/d of EPA + DHA significantly reduced the severity of schizophrenia symptomatology measured by means of PANSS. Moreover, 26-week n-3 PUFA intervention increased the number of patients achieving at least 50% improvement in symptom severity measured by means of total PANSS score but did not influence the response rate, i.e. at least 25% improvement (Tables 2 and 3). The NNT of 4 means that 4 patients should be supplemented with n-3 PUFA rich fish oil for 26 weeks apart from antipsychotic therapy to achieve at least 50% reduction in symptom severity in 1 patient. According to Leucht (2014) 50% reduction in rating scales should be used as cut-off for response in schizophrenia trials for acutely ill, nonrefractory patients and a 25% cut-off should be used for treatment-resistant patients to identify clinically meaningful improvement. Moreover, it was observed that the addition of EPA + DHA to

Table 2
Change in clinical scores across study arms.

Variable change	Baseline to week 26, mean (SE)		LS mean difference[a] (95% CI)	Effect size[b]
	EPA + DHA (N = 36)	Placebo (N = 35)		
CDSS	−2.74 (0.39)	−1.16 (0.39)	−1.58 (−2.7 to −0.47)**	0.34
PANSS				
Positive	−6.69 (0.46)	−5.61 (0.47)	−1.09 (−2.41 to 0.23)	–
Negative	−2.12 (0.45)	−1.43 (0.46)	−0.69 (−1.97 to 0.6)	–
General	−10.46 (0.81)	−7.32 (0.83)	−3.14 (−5.46 to −0.83)**	0.32
Total	19.27 (1.38)	−14.42 (1.4)	−4.84 (−8.77 to −0.92)*	0,29
GAF	17.22 (1.3)	12.4 (1.32)	4.89 (1.19–8.58)*	0,31
CGI-S	1.34 (0.11)	1.02 (0.12)	−0.32 (−0.64–0.01)*	0,24

Abbreviations: CDSS – The Calgary Depression Scale for Schizophrenia; CGI-S – The Clinical Global Impressions Severity Scale, PANSS – The Positive and Negative Syndrome Scale; GAF – Global Assessment of Functioning Scale; N – number of observations in a population; SE – standard error of the mean; LS – least squares.
* – $p < 0.05$; ** – $p < 0.01$.
[a] Based on the contrast from mixed models repeated-measures analysis.
[b] Difference in change from baseline in units of standard deviations of change.

Table 3
Rate of at least 25% and 50% improvement observed at different time points of add-on intervention with EPA + DHA or placebo in first-episode schizophrenia patients.

Timepoint	Percentage (N)		p Value[a]	Relative risk[b] (95% CI)	Absolute benefit increase, % (95% CI)	NNT (95% CI)
	Placebo (N = 35)	EPA + DHA (N = 36)				
Total PANSS score improvement ≥ 25% (Responders)						
Week 2	0.0 (0)	0.0 (0)	–	–	–	–
Week 4	0.0 (0)	5.6 (2)	0.493	–	5.56 (−1.93 to 13.04)	–
Week 6	5.7 (2)	33.3 (12)	0.003	5.83 (1.41–24.2)	27.62 (10.41–44.83)	4 (2–10)
Week 8	74.3 (26)	77.8 (28)	0.786	1.05 (0.81–1.36)	3.49 (−16.36 to 23.34)	–
Week 26	85.7 (30)	94.4 (34)	0.26	1.1 (0.94–1.29)	8.73 (−5.07 to 22.53)	–
Total PANSS score improvement ≥ 50% (Much improved)						
Week 2	0.0	0.0	–	–	–	–
Week 4	0.0	0.0	–	–	–	–
Week 6	0.0	0.0	–	–	–	–
Week 8	5.7 (2)	8.3 (3)	1.0	1.46 (0.26–8.21)	2.62 (−9.24 to 14.48)	–
Week 26	40.0 (14)	69.4 (25)	0.017	1.74 (1.1–2.75)	29.44 (7.31–51.58)	4 (2–14)

PANSS – The Positive and Negative Syndrome Scale; N – number of observations in a population.
[a] Determined using Fisher exact test; p – two-tailed asymptotic probability for chi-square test; CI – confidence interval.
[b] Favourable outcome was observed more frequently in the experimental group, thus relative risk denotes the relative benefit.

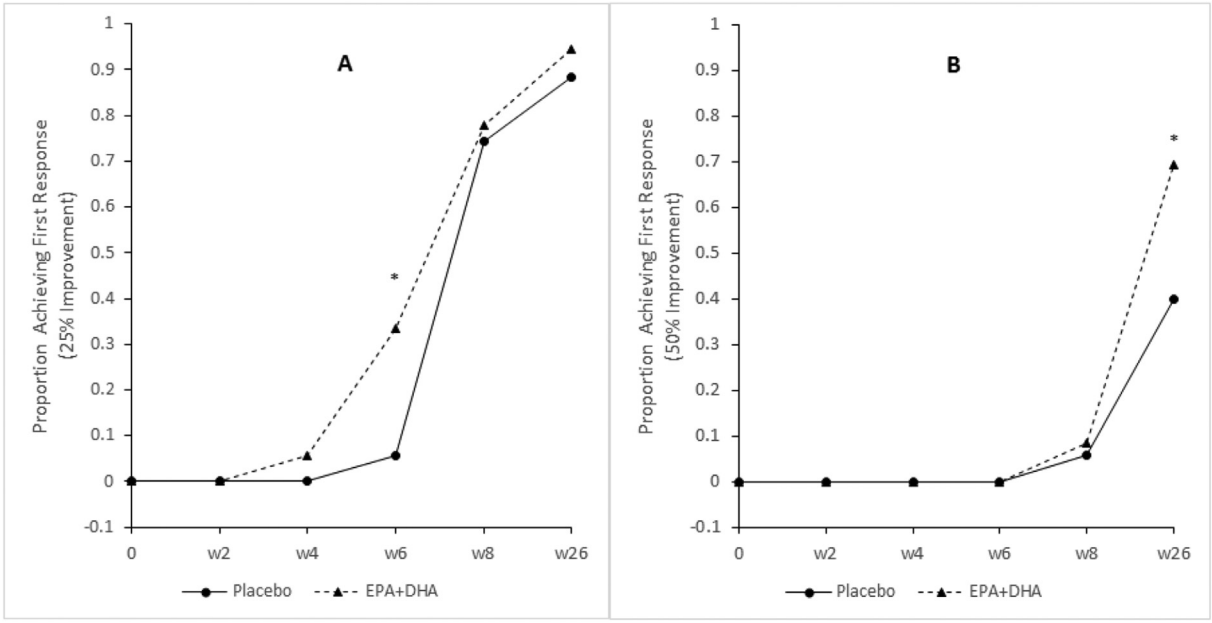


Fig. 2. Proportions of patients achieving at least 25% (A) and 50% (B) improvement in total PANSS score. Significant differences between groups were indicated (*).

Table 4
Chlorpromazine equivalent cumulative exposure to antipsychotics in dose-years (DY) in the study groups: EPA + DHA (N = 36) and placebo (N = 35).

Study period	Mean	Median	Minimum	Maximum	SD	p Value[a]
Baseline – week 4						
EPA + DHA	0.282	0.259	0.09	0.69	0.136	0.876
Placebo	0.295	0.23	0.06	0.67	0.181	
Week 4–8						
EPA + DHA	0.385	0.345	0.11	0.94	0.178	0.844
Placebo	0.419	0.307	0.15	0.92	0.242	
Week 8–26						
EPA + DHA	1.701	1.535	0.52	4.44	0.876	0.885
Placebo	1.882	1.524	0.34	4.14	1.091	
Baseline – week 26						
EPA + DHA	2.367	2.182	0.96	4.89	0.944	0.968
Placebo	2.596	2.143	0.59	5.75	1.435	

[a] Based on Mann–Whitney U test; SD – standard deviation; 1 DY is equivalent to therapy with 100 mg of chlorpromazine for one year (Andreasen et al., 2010).

pharmacotherapy with antipsychotics was related to an earlier response, defined as at least a 25% improvement in total PANSS score. The significant improvement was also observed in several secondary outcome measures: general psychopathology subscale of PANSS, severity of depressive symptoms, level of functioning and clinical global impression. The magnitudes of group differences ranged from small (total PANSS, CGI-S and GAF score change) to moderate (CSSS and general psychopathology PANSS score change). The high consent rate (74%) and relatively low withdrawal proportion during the treatment period, only 6 out of 71 patients, indicate that the n-3 PUFA rich fish oil was accepted and well tolerated by this population.

The finding that long-term add-on therapy with n-3 PUFA rich fish oil reduces symptom severity and increases response rate, improves level of functioning and decreases the intensity of depressive symptoms gives hope for patients diagnosed with first-episode schizophrenia, since n-3 PUFA supplements are well tolerated and accepted by patients. Two previous studies have investigated the efficacy of n-3 PUFA in first-episode schizophrenia

Table 5
Cross-tabulation of significant UKU change scores.

AE/group	N	Change score[a], % (N)							p Value
		−3	−2	−1	0	+1	+2	+3	
Constipation									
Placebo	35	2.86 (1)	0 (0)	8.57 (3)	54.29 (19)	17.14 (6)	11.43 (4)	5.71 (2)	0.021[b]
EPA + DHA	36	8.33 (3)	13.89 (5)	22.22 (8)	47.22 (17)	5.56 (2)	2.78 (1)	0 (0)	

Abbreviations: AE – adverse effect; UKU – Udvalg for Kliniske Undersøgelser Side Effect Rating Scale, EPA – eicosapentaenoic acid, DHA – docosahexaenoic acid, N – number of participants.
[a] Change score computed as follow-up score minus baseline score; -3 = marked improvement, -2 = moderate improvement, -1 = minimal improvement, 0 = no change, +1 = minimal worsening, +2 = moderate worsening, +3 = marked worsening.
[b] Assessed using Fisher's exact test.

(Peet et al., 2001; Berger et al., 2007). Peet et al. conducted the study in first episode schizophrenia patients who were unmedicated or had been medicated for a short period. The results showed that 12-week intervention with EPA enriched oil (2 g/d) as sole treatment for schizophrenia had significant effects on symptom severity, the need to initiate antipsychotic therapy after the emergence of psychotic symptoms and the duration of that therapy. Patients receiving EPA also demonstrated lower levels of positive psychotic symptoms and total PANSS score by the end of the study. A responder analysis, taking out 50% improvement as the criterion, revealed a significant association between active intervention and the response rate. Another study that investigated first-episode schizophrenia patients was carried out by Berger et al. (2007). The study assessed EPA augmentation together with antipsychotic medication on the severity of symptoms and the response rate in patients with first episode psychosis. No significant differences in symptom severity change were observed at the end of 3-month therapy with 2 g/day of EPA ethyl ester added to flexible dose of antipsychotic medication. The authors observed similar influence of E-EPA on the dose of antipsychotic medication used. Patients treated with the active intervention needed lower doses of antipsychotics and reported less extrapyramidal and sexual side effects than patients receiving placebo.

Studies in chronic schizophrenia have given much more mixed results. Seven randomized controlled trials comparing eicosapentaenoic acid with placebo as supplemental treatment to antipsychotics in chronic schizophrenia have been reported. Four of these studies report that EPA had a positive effect on primary or secondary efficacy outcome measures (Peet et al., 2001; Peet and Horrobin, 2002; Emsley et al., 2002; Jamilian et al., 2014), two studies report no significant differences between EPA and placebo (Fenton et al., 2001; Emsley et al., 2006) and one study reported the increase in symptomatology related to EPA add-on therapy (Bentsen et al., 2013). In that last study the harmful effect of EPA was reversed when antioxidant medication (vitamin C + E) was given additionally. A recent meta-analysis of RCTs revealed no beneficial effect of EPA augmentation on symptom severity in schizophrenia (Fusar-Poli and Berger, 2012). The most recent RCT of n-3 PUFA efficacy was carried out in an Iranian population of patients diagnosed with schizophrenia (Jamilian et al., 2014). That study was published after the last meta-analysis and yielded positive results, which are similar to the findings of the present study. Significant reductions in total and general PANSS scores were observed after 8-week intervention with n-3 PUFA (1 g/day). No significant differences were seen between groups in positive and negative PANSS score.

One of the reasons responsible for the positive results obtained in the present study may be related to the effect of disease stage. Supplementation with n-3 PUFA may demonstrate higher efficacy in populations of individuals at high risk of psychosis and early psychosis than in patients with chronic schizophrenia. A 12-week RCT conducted in individuals at high clinical risk of schizophrenia provides preliminary evidence that intervention composed of 1.2 g of PUFA (i.e. EPA + DHA) could prevent transition to first-episode psychosis (Amminger et al., 2010). Furthermore, another study notes that PUFA metabolism disturbances are present relatively early in the course of schizophrenia and diminish over time as the disease progresses, and that treatment with antipsychotic medications was able to correct aberrant PUFA levels in first-episode schizophrenia patients, but not in a group of patients with chronic schizophrenia (McEvoy et al., 2013). This suggests that intervention with n-3 PUFA can be more effective at early stages of the disease, when irreversible neurobiological changes are not yet established.

Another reason for the n-3 PUFA efficacy observed in the present study may be associated with its different design: (a) the intervention period was more than two times longer; (b) a fish oil with high concentration of both EPA + DHA was used; (c) the study population was composed of inpatients diagnosed with first-episode schizophrenia admitted to psychiatric ward due to exacerbation or emergence of psychotic symptoms. Berger et al. (2007) did not observe n-3 PUFA add-on therapy to have significant effects on symptom severity regarding total PANSS score in patients with first-episode psychosis who were moderately ill. However, a higher and nearly significant effect was present in patients with non-affective psychosis. Hence, the differences in inclusion criteria (first-episode psychosis vs first-episode schizophrenia) and the difference in symptom intensity could also be responsible for the significant result observed in the present study, but not reported by Berger et al. It is well established that long term supplementation is needed to fill the body stores of n-3 PUFA and to incorporate these compounds into cellular membranes.

The greatest improvement in the present study was observed in depressive symptomatology (Cohen's d = 0.34) that can be classified as moderate effect (Cohen, 1988). This observation is in line with the previous studies that analysed the effect of n-3 PUFA on depressive symptoms. A recent meta-analysis concluded that the use of n-3 PUFA is effective in patients with diagnosis of major depression (MDD) and in depressive patients without diagnosis of MDD (Grosso et al., 2014). However, the studies investigating the effects of n-3 PUFA on depressive symptoms as a secondary outcome in patients with schizophrenia reported inconclusive results (Fenton et al., 2001; Peet and Horrobin, 2002). The observation that n-3 PUFA-rich fish oil add-on therapy was associated with significant reduction of depressive symptoms contrasts with that of Amminger et al. (2010) who did not observe significant effect of n-3 PUFA on depressive symptomatology in individuals at high clinical risk of psychosis. The difference regarding depressive symptoms improvement between the result observed in the present and previous studies may be attributed to the following factors: different population, clinical scales used, length, dose and composition of the active intervention. Two of the mentioned studies

investigated chronic schizophrenia patients (Fenton et al., 2001; Peet and Horrobin, 2002). The intervention periods were12 (Peet and Horrobin, 2002; Amminger et al., 2010) and 16 weeks (Fenton et al., 2001). Amminger et al. (2010) used lower dose of EPA + DHA mixture (700 mg of EPA plus 480 mg of DHA) and two other studies used ethyl ester of EPA 3 g/d (Fenton et al., 2001) and 1, 2, or 4 g/d (Peet and Horrobin, 2002). The Montgomery–Åsberg Depression Rating Scale (MADRS) was used in the previous three studies, while the present study utilized the Calgary Depression Scale for Schizophrenia (CDSS). MADRS was designed to be used for depressive symptom assessment in major depression but CDSS was devised for use in schizophrenia patients and may be considered as more valid and more specific for discriminating depressive symptoms from negative symptoms of schizophrenia (Liu et al., 2009).

There is accumulating evidence from rodent, primate and human studies that both EPA and DHA play a crucial role in neurodevelopment via modulation of oxidative defence, neuroinflammation, directly and indirectly stimulating both the neuroplastic and neuroprotective mechanisms of the brain (Calder, 2012; McNamara et al., 2015). Thus, the potential underlying mechanism of the therapeutic action of n-3 PUFA observed in the present study may be neuroprotective, via modulation of the antioxidative intracellular defence (Hammamieh et al., 2014), antinflammatory via modulation of nuclear factor kappa B (NFkB) pathway (Calder, 2013), neuroplastic via stimulation of synthesis of neurotrophins (Gama et al., 2012) or antiapoptotic (Brown et al., 2013). The neuroprotective hypothesis is further supported by a recent study that showed a higher red blood cell concentration of EPA + DHA corresponding to larger total brain and hippocampal volumes in a sample of 1111 postmenopausal women after an 8-year follow-up (Pottala et al., 2014). The observed improvement in symptomatology may also be the result of a direct interaction between EPA or DHA and glutamatergic neurotransmission (Patten et al., 2013; Keleshian et al., 2014). In line with such a hypothesis, a magnetic resonance spectroscopy study in first-episode subjects has confirmed that EPA augmentation modulates glutathione and the glutamine/glutamate cycle in early psychosis, with some of the metabolic brain changes being correlated with negative symptom improvement (Berger et al., 2008).

The observed improvement in psychopathology and patient functioning raises hope for patients diagnosed with first-episode schizophrenia that add-on therapy with concentrated fish oil may result in a valuable change in disease outcomes, especially because n-3 PUFA are safe, well accepted by patients and relatively cheap. Moreover, there is accumulating evidence that n-3 PUFA exerts other beneficial effects that are highly preferable in patients with schizophrenia, i.e. reduction in antipsychotic cumulative dose needed to control psychotic symptoms and improvement of antipsychotic tolerability with less constipation and fewer extrapyramidal side effects (Berger et al., 2007), improvement of cognitive performance (Luchtman and Song, 2013) and metabolic profile (Savinova et al., 2015), reduction of the risk of diabetes mellitus (Takkunen et al., 2015), cardiac arrhythmia (Rix et al., 2014), sudden cardiac death (Nestel et al., 2015) and all-cause mortality (Villegas et al., 2015).

However, as this is the first long-term n-3 PUFA intervention study carried out in the population of patients with early schizophrenia, the results should be accepted cautiously. The present study has some limitations that need to be considered before formulating conclusions. The main limitation is lack of the objective measure of adherence, since it was not possible to assess the concentration of n-3 PUFA in the red blood cells of study participants. To account for this, pill counts were taken and data concerning medication adherence was collected during every study visit. However, the randomization and blinding used in the study design indicates that the possible bias introduced at this level could be distributed equally between the study arms. However, the effect of the intervention may be decreased because of possible adherence problems, which could not be detected objectively. Another limitation is the 26-week intervention period. As some patients may need more time to achieve improvement, the observed effect may be underestimated. The strengths of the study include its randomized, placebo-controlled design, blinding, inter-rater reliability testing, the composition of n-3 PUFA used, i.e. a 1.5:1 mixture of EPA and DHA, which has not yet been used in patients with first-episode schizophrenia, the dosage of PUFA supplementation that was higher than in previous studies and low enough to assure safety of intervention. The blinding method used in the present study was based on masking the smell and taste of the comparable interventions, thus maintaining the secrecy of the randomisation status throughout the study both with respect to participants and study stuff including those assessing the outcomes. However, the success of the blinding was not formally assessed, which might be another limitation of the study. The randomization stratified by age was used to achieve between-group comparability of certain baseline characteristics within the study population. This technique may well have protected the results from imbalanced randomization, which could be an even more important limitation for the study than the bias introduced by stratified randomization, particularly when a relatively small sample size was used. Moreover, a computer-generated random sequence administered by an independent party might reduce that possible bias. The use of stratified randomization generally could increase the power of our study and by reduction of between-group variability, could allow a study of a given size to detect smaller group differences in response variables or to detect a specified difference with fewer participants.

Further studies based on larger samples of patients are warranted because the results achieved in the present study regarding changes in both the positive PANSS subscale and the negative symptom domain show a trend toward improvement associated with n-3 PUFA add-on therapy. More studies are needed to describe the biological mechanism underlying the effects of n-3 PUFA action in patients with first-episode schizophrenia. It was found that the persistence of depressive symptoms in patients with schizophrenia is an important predictor of recurrence in schizophrenia (Tollefson et al., 1999; Jin et al., 2001), thus the effect observed in the present study encourage design of long-term n-3 PUFA studies focused on relapse prevention as a primary outcome measure.

There is accumulating evidence that n-3 PUFA supplementation can be an effective intervention in a wide range of psychiatric conditions, and the possible biological mechanisms of those effects are known. The NNT of 4 (95% CI 2 to 14) to achieve at least a 50% improvement with concentrated fish oil supplementation carried out for 26 weeks added-on to antipsychotics implies a clinically relevant effect (Andrade, 2015). However, the effect size for the efficacy of n-3 PUFA as add-on therapy in reducing symptom severity in schizophrenia observed in the present study (ES = 0.29) is lower in magnitude than the values reported elsewhere for second generation antipsychotic medications (ES = 0.51) (Leucht et al., 2009).

5. Conclusion

In conclusion, improvement in psychopathology and the level of functioning observed in the present study, together with the other beneficial effects mentioned above, reasonably suggest that n-3 PUFA may constitute a valuable add-on intervention, which should be further evaluated in first-episode schizophrenia patients.

Contributors

TP designed the study, wrote the protocol, was responsible for adult patient recruitment, clinical assessments and drafting the manuscript. MG-G was responsible for the recruitment and clinical assessments of youth participants. TP and MG-G collected informed consent. ET was responsible for conducting and interpreting dietary assessments. MK-A and AP were responsible for running the PORT program, which allowed the raters to be trained in using rating scales. TP, MG-G and AP were responsible for interpreting the results of the study and formulating conclusions. AP took part in study design, drafting the manuscript and collecting data. All authors contributed and approved the final manuscript.

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Conflict of interests

The authors declare that they have no conflict of interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jpsychires.2015.11.013.

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