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Oxidative stress reduction related to the efficacy of n-3 polyunsaturated fatty acids in first episode schizophrenia: Secondary outcome analysis of the OFFER randomized trial

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ABSTRACT

Intervention studies of n-3 polyunsaturated fatty acids (n-3 PUFA) as add-on therapy in patients with schizophrenia have not examined changes in oxidative stress. A randomized placebo-controlled trial of a 26-week intervention composed of 2.2 g/day of n-3 PUFA was found to reduce symptom severity in first-episode schizophrenia patients. The present study is an extension of our previous report, whose secondary aim was to assess the association between the clinical effect of n-3 PUFA and changes in oxidative stress indices. Seventy-one patients aged 16–35 were enrolled in the study and randomly assigned to the study arms. Total plasma antioxidant capacity and 8-epi-isoprostane $F_{2\alpha}$ content were assessed at baseline and at weeks 8 and 26 of the study as secondary outcome measures. Significant changes in oxidative stress indices favouring the intervention group were observed: decreases in 8-isoprostane $F_{2\alpha}$ ($p < 0.001$) and increases in total plasma antioxidant capacity ($p < 0.001$). Significant correlations between changes in clinical scores relevant to symptom severity and changes in oxidative indices were observed. The results of the present study hence suggest that the efficacy of a six-month intervention with n-3 PUFA observed in first-episode schizophrenia may be related to improvement in oxidative stress indices.

1. Introduction

Oxidative stress is a disequilibrium between reactive oxygen species (ROS) and reactive nitrogen species (RNS) production and the activity of oxidative defence systems. A relative excess of ROS/RNS levels leads to oxidative damage to macromolecules such as nucleic acids, proteins and lipids, as well as changes in gene expression, intracellular signalling pathway modulation and deleterious changes in the activity of critical cell enzymes (e.g. caspases), which can lead to cellular death. Lipid peroxidation is a well-characterized effect of ROS that results in damage to the cell membrane, as well as to the membranes of cellular organelles such as mitochondria. Although the brain comprises about 2% of the human body weight, due to its high energy demand, it utilizes more than 20% of the consumed oxygen [1]. The brain is composed of PUFA, which is especially vulnerable to oxidative damage and peroxide formation because of the increased number of double carbon bonds in the molecule.

The Antioxidative Defence System (AODS) is generally divided into enzymatic and non-enzymatic parts, which together comprise its total antioxidant capacity (TAC). The enzymatic part of the brain's AODS is composed mainly of enzymes such as glutathione peroxidase (GSH-Px) and reductase, and its substrates: reduced glutathione (GSH) and its oxidized form, glutathione disulphide (GSSG). Other antioxidant enzymes present in the brain, i.e. catalase (CAT) and superoxide dismutase (SOD), work in concert with GSH-Px. The non-enzymatic part of the AODS is composed of α-tocopherol (vitamin E), bilirubin, albumin, uric acid, niacin, carotenoids and flavonoids [2]. The antioxidative defence of the brain is regarded as weak in relation to its energy needs and oxygen utilization [3]. Its high oxygen consumption, high double bond content in PUFA molecules and low content of AODS, together with a high metal content (iron, zinc, copper, manganese), make the brain especially vulnerable to oxidative damage. Alterations in AODS and the increased production of free radicals have been repeatedly reported in schizophrenia, both in preclinical and in clinical

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studies [1,4–7]. A recent meta-analysis [8] suggests that total plasma or serum antioxidant status, red blood cell (RBC) CAT and plasma nitrite levels may act as state markers in schizophrenia. In contrast, RBC SOD content can be regarded as a trait marker of schizophrenia, since it was found to be decreased in acutely relapsed inpatients, first-episode schizophrenia (FES), and stable outpatients. Abnormalities in the oxidative balance of the brain, characteristic of schizophrenia patients, leads to increased lipid peroxidation, with complex biochemical consequences [9].

Isoprostanes (IPs) are chemicals formed by the peroxidation of PUFA, which is catalysed by free radicals. F_2-isoprostanes (F_2-IPs) are formed by the oxidation of arachidonic acid and are further classified into four regioisomeric groups. One of the abundant isomers is 8-epi-isoprostane $F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$), whose concentration has been shown to be a sensitive and reliable index of lipid peroxidation *in vivo* [9]. Since the majority of AA in the brain is esterified in neuronal membranes and is present in the form of membrane phospholipids, IPs are initially formed esterified and are subsequently released by phospholipase A_2 (PLA_2). Oxidization of the AA molecule leads to the breakdown of double bonds and allosteric changes of phospholipids, thus resulting in the disruption of the physicochemical integrity of cell membranes. The 8-epi-$PGF_{2\alpha}$ molecule can act as a potent modulator of platelet aggregation and activator of intracellular signalling, and possesses potent biological activity as a proinflammatory mediator. Several studies have reported increased levels of 8-epi-$PGF_{2\alpha}$ in human diseases associated with oxidative stress, such as diabetes mellitus and cardiovascular disease [10]. The concentration of 8-epi-$PGF_{2\alpha}$ was also found to be increased in a plethora of psychiatric disorders [11] including first-episode psychosis [12] and schizophrenia [13]. Moreover, 8-epi-$PGF_{2\alpha}$ level has been found to be a more sensitive and more reliable index of lipid peroxidation in schizophrenia than the commonly-used thiobarbituric reactive substances (TBARS) level [13].

The discovery of polyunsaturated fatty acid deficiencies in schizophrenia patients, together with the postulated involvement of oxidative stress in the pathophysiology of the disease, led to the design of PUFA supplementation studies. Intervention studies using PUFA in patients with schizophrenia revealed mixed results [14]. These studies were rather short-term (8–16 weeks) and the majority used ethyl esters of EPA (ethyl-EPA) as the active intervention. In contrast, the present study is based on a different design: a long-term intervention of six months and an intervention composed of concentrated fish oil rich in EPA + DHA. In addition, the previous intervention studies did not report changes of the above oxidative stress indices throughout the study, which did not allow links to be made between the clinical effect of PUFA supplementation in FES patients and the changes of oxidative stress indices. The current study was designed to assess the efficacy of add-on intervention composed of n-3 PUFA in FES patients and to describe the possible mediators of that effect regarding the influence of PUFA on oxidative stress indices in plasma, i.e. 8-epi-$PGF_{2\alpha}$ and TAC.

2. Patients and methods

The present study is a secondary endpoint analysis of a randomized double-blind n-3 PUFA intervention trial, the comprehensive details of which have been published elsewhere (Trial registration: clinicaltrials.gov identifier NCT02210962). The detailed description of the study design, participant sample, inclusion and exclusion criteria, randomization process, power calculation, study intervention, primary and secondary outcome measures and estimation of chlorpromazine dose equivalents are given in detail elsewhere [15]. The results of the primary outcome analysis, interrater reliability, adherence to study medication and adverse effects analysis have been published previously [16] and are not replicated in the present paper.

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 the 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; (3) currently psychotic, as reflected by the presence of at least one psychotic symptom daily for more than one week. Diagnosis was confirmed by the mini neuropsychiatric interview plus (MINI plus) [17]. Seventy-one patients met the inclusion criteria and consented to the study. 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

The study constituted a randomized, double-blind, placebo-controlled, parallel-group 26 week augmentation trial of either 2.2 g per day of EPA + DHA, or olive oil placebo, added on to an adjustable dose of antipsychotic medication. The background antipsychotic therapy and concomitant medications were chosen and titrated according to the Polish standards of pharmacotherapy of mental disorders [18].

2.3. 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 four capsules provided 2.2 g of n-3 PUFA, i.e. 1.32 g/day of EPA plus 0.88 g/day of DHA. The placebo contained olive oil, which is composed of mainly monounsaturated fatty acids (73.9%) and only small amounts of polyunsaturated fatty acids (9.8%). 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 of the different capsules. Both placebo and active capsules contained an antioxidant, i.e. 0.2% alpha-tocopherol (vitamin E). The study medication (both 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.

To increase the external validity of the study results and conform with the mentioned above therapy guidelines, the use of benzodiazepines, Z-drugs, injectable forms of antipsychotics, antidepressants, mood stabilizers and anticholinergic medications was allowed if clinically indicated. Background antipsychotics 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.

2.4. Outcome measures

Clinical scales were used to assess several domains of symptom severity and patient functioning at baseline and planned follow-up

visits. After randomization, participants received weekly assessments for four weeks and then at weeks 6, 8, 16 and 26. The primary outcome measure was the magnitude of change in the Positive and Negative Syndrome Scale (PANSS) [19] total scores between baseline and 26 weeks. Secondary clinical outcome measures included the changes in the PANSS subscale scores (positive, negative, and general psychopathology), The Clinical Global Impressions scale (CGI) [20], the Global Assessment of Functioning (GAF) [21] and Calgary Depression Scale for Schizophrenia (CDSS) [22] scores between baseline and after 26 weeks of intervention. Oxidative stress indices and their association with the efficacy of n-3 PUFA in symptom severity and level of functioning was a secondary biochemical outcome measure of the OFFER trial.

2.5. Oxidative stress index assessments

Oxidative stress assessments were carried out at three time points: at the beginning of the study, i.e. before intervention (t_0), after 8 weeks (t_1) and after 26 weeks (t_2) of intervention. Venous blood for 8-epi-$PGF_{2\alpha}$ analysis was collected in tubes pre-coated with EDTA and containing 1 mg/mL whole blood of reduced glutathione (GSH) (Sigma Chemical Company, St Luis, MI, USA) as an antioxidant in order to prevent *in vitro* production of isoprostanes. Similarly, to prevent further *in vitro* production of isoprostanes, the separated plasma was transferred to storage tubes pre-coated with butylated hydroxytoluene (BHT) as an antioxidant, and stored at −70 °C until analysis. 8-epi-$PGF_{2\alpha}$ level was determined using an 8-isoprostane EIA kit (Cayman Chemical Company, Ann Arbor, MI, USA). The ability of this assay to measure 8-epi-$PGF_{2\alpha}$ was validated using a series of known amounts of 8-epi-$PGF_{2\alpha}$, and a high correlation was found between the known positive control concentrations and the values determined using the kit. The assay was performed according to the manufacturer's instructions. The product of the enzymatic reaction was determined spectrophotometrically at 412 nm.

The total antioxidant capacity (TAC) of the plasma samples was estimated by measuring the oxidation inhibition of the ABTS 2,2′-Azino-di-[3-ethylbenzthiazoline sulphonate] by antioxidants in the samples using the Antioxidant Assay kit (Cayman Chemical Company Ann Arbor, MI, USA). The amount of ABTS produced was monitored by reading the absorbance at 750 nm. Under the reaction conditions used, the antioxidant in the sample suppresses absorbance at 750 nm to a degree proportional to its concentration. The capacity of the antioxidants in the sample to prevent ABST oxidation was compared with that of Trolox, a water-soluble tocopherol analogue, and was expressed in Trolox equivalents.

2.6. Statistical methods

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

As the missing data in the present study occurred as a result of patient withdrawal or missed assessments, it cannot be regarded as missing completely at random, and must be modelled [23]. To deal with missing values in our ITT sample, a conservative approach was used, assuming that the results of oxidative stress measures would have been maintained at the level that was observed during the last visit the patient was assessed (last observation carried forward, LOCF).

The changes in biochemical 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's 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. All statistical tests were 2-tailed, with the statistical significance set at alpha = 0.05.

Table 1
Clinical sample characteristics.

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)	0.092
vocational	0 (0)	3 (8)	
secondary	18 (50)	14 (40)	
Bachelor's degree	5 (14)	3 (9)	
Master's degree	1 (3)	6 (17)	
Years of education, mean (SD)	12.9 (2.7)	13.8 (3.1)	0.229
Marital status, N (%)			0.346
married	2 (6)	2 (6)	
single	34 (94)	31 (89)	
divorced	0 (0)	2 (5)	
Place of living, N (%)			0.573
alone	5 (14)	4 (11)	
with family	30 (83)	31 (89)	
dormitory	1 (3)	0 (0)	
Employment, N (%)			0.475
employed	4 (11.1)	7 (20.0)	
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)	0731
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
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.

3. Results

3.1. Study sample

Seventy-one individuals were enrolled in the study: 36 randomly assigned to the EPA + DHA group, 35 to the placebo group. The treatment groups were similar in terms of demographic variables and baseline characteristics (Table 1). One of the 36 (1.8%) participants from EPA + DHA group discontinued the intervention prematurely and

withdrew his consent. Three patients of the 36 (8.3%) from the EPA + DHA group were lost to follow-up and did not attend follow-up assessments. Two patients of the 35 (5.7%) from the placebo group were lost to follow-up: one moved out of the area and the other did not attend any follow-up assessments. Therefore, the 26-week follow-up intervention was completed by 65 participants: 32 (88.9%) from the EPA + DHA group and 33 (94.3%) from the placebo group. The difference in drop-out rate between groups was not statistically significant (Fisher's exact test; p = 0.674).

At the time of enrolment, 43 participants (60.6%) were antipsychotic naive and 17 had fewer than nine days of medication. Among those medicated, the mean duration of antipsychotic therapy was 14 days (SE = 3.3). All but five patients were treated with antipsychotics for less than six weeks before enrolment. Study groups were not significantly different according to the frequency of antipsychotic-naive patients enrolled (Chi square test; $Chi^2 = 1.139$; 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), nor in terms of baseline chlorpromazine equivalent dose (Table 1). All patients received antipsychotics after 26-week intervention. Daily consumption of energy and PUFA was determined at baseline using the Polish version of the Food Frequency Questionnaire [24]. Dietary consumption of energy and PUFA were not significantly different between the groups at baseline (Table 1).

3.2. Oxidative stress indices

Mixed Model for Repeated Measures analysis (MMRM) was used to assess differences between groups regarding the plasma levels of 8-epi-$PGF_{2\alpha}$ and TAC. Decreases in 8-epi-$PGF_{2\alpha}$ concentrations were observed in both groups, and were significantly higher in the EPA + DHA group compared to placebo (Fig. 1). Significant increases in plasma TAC were observed in both groups (Fig. 2). The analysis of contrasts from MMRM revealed significant differences between groups regarding plasma TAC. Mean change of plasma TAC from baseline was significantly higher in EPA + DHA group then placebo. The observed effects can be considered as moderate (8-isoprostanes) and small (TAC). The least squares mean changes and mean differences between groups in change scores at month 6 are presented in Table 2.

Significant correlations were observed between the change from baseline to week 26 for plasma TAC and 8-epi-$PGF_{2\alpha}$, and score changes from baseline to week 26 for clinical measures. Correlation coefficients for significant associations are presented in Table 3. According to Cohen [25], the magnitude of the observed significant correlations can be regarded as large (TAC * CGI-S; 8-epi-$PGF_{2\alpha}$ * CDSS) and medium (the rest of significant associations listed in Table 3). The remaining

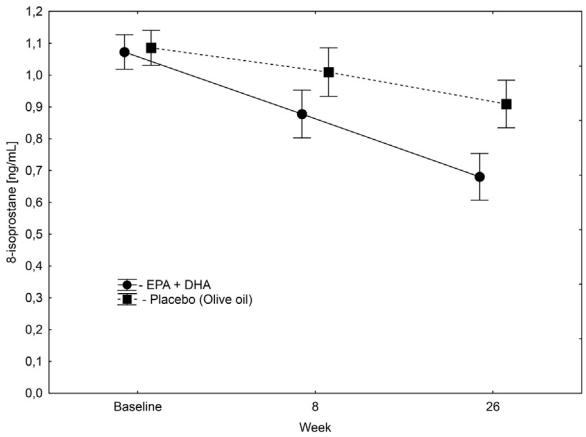

Fig. 1. 8-Isoprostane level at baseline, week 8 and week 26 in the study arms. Means and 95% confidence intervals.

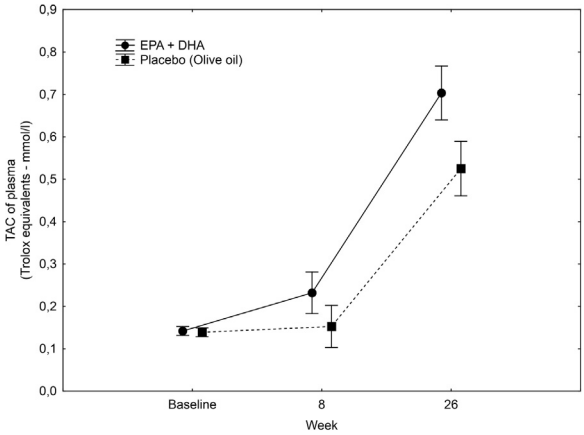

Fig. 2. Total anti-oxidative capacity of plasma at baseline, week 8 and week 26 in the study arms. Means and 95% confidence intervals.

Table 2
Change in biochemical measures across study arms.

Variable change	Baseline to week 26, mean (SE)		LS mean difference[a]	Effect size[b]
	PUFA (n = 36)	Placebo (n = 35)	(95% CI)	
8-isoprostanes F2alpha [ng/mL]	−0.29 (0.029)	−0.13 (0.03)	−0.17 (−0.25 to −0.08)***	0.44
Total antioxidative capacity (TAC) [mmol/l]	0.33 (0.02)	0.2 (0.021)	0.131 0.08–0.19)***	0.24

Abbreviations: N - number of observations in a population; SE - standard error of the mean; LS – least squares.
*** - p < 0.001.
[a] - based on the contrast from mixed models repeated-measures analysis of covariance.
[b] - difference in change from baseline in units of standard deviations of change.

Table 3
Significant correlations between changes from baseline to 26 weeks in the study population (N = 71).

Variable 1 change from baseline to 26 weeks	Variable 2 change from baseline to 26 weeks	Pearson's r	p value[a]
TAC	CDSS	−0.319	0.007
TAC	CGI-S	−0.701	< 0.001
8-isoprostanes	CDSS	0.77	< 0.001
8-isoprostanes	Negative PANSS score	0.257	0.03
8-isoprostanes	General PANSS score	0.285	0.016
8-isoprostanes	GAF	−0.336	0.004
8-isoprostanes	CGI-S	0.378	0.001

Abbreviations: TAC - plasma total anti-oxidant capacity; 8-isoprostanes – 8-epi-isoprostanes F2 alpha plasma concentration; 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.
[a] - two sided asymptotic test probability for Pearson's r correlation coefficient.

associations between the changes of the assessed variables were found to be insignificant and are not given in Table 3.

4. Discussion

4.1. Results summary

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 [16]. Significant improvements were also observed in several secondary clinical outcome measures: the general psychopathology subscale of the PANSS, severity of depressive symptoms, level of functioning and oxidative stress indices. The change in clinical scores was accompanied by a decrease in plasma level of 8-epi-$PGF_{2\alpha}$ and an increase in plasma TAC. The differences in observed changes of these oxidative stress indices significantly favoured the intervention group over the placebo group. Increases in plasma TAC were observed in both groups, but a significantly greater increase was seen in the EPA + DHA group. Similarly, while significant decreases in 8-epi-$PGF_{2\alpha}$ levels were observed in both groups during the study, the reduction was significantly greater in the EPA + DHA group than the placebo group. Moreover, significant correlations of medium to large magnitude were observed between the change in clinical measures score and the oxidative stress index.

The efficacy of n-3 PUFA observed in the present study is discussed elsewhere [16]. Therefore, the Discussion is focused on the biochemical correlates and possible mechanism of the effect of n-3 PUFA on changes in clinical score.

4.2. Comparison with previous studies

The increased production of ROS and decreased oxidative defence mechanisms observed in many animal models, *in vitro* studies and in clinical studies of patients diagnosed with either early or chronic schizophrenia indicate that oxidative stress contributes to the pathophysiology of mental disorders, including schizophrenia [26]. PUFA content and metabolism abnormalities have also been repeatedly observed in individuals at different stages of schizophrenia development, i.e. in ultra-high risk individuals, first-episode and chronic patients [27–29].

The present study is the first n-3 PUFA supplementation study carried out in patients with first-episode schizophrenia that assesses AA-derived lipid peroxide production and total antioxidant capacity of plasma. Previous studies assess the effect of n-3 PUFA supplementation on antioxidative enzyme activity, as well as lipid peroxide, vitamin E and GSH levels. In general, the results of the current study are in line with previous studies regarding relationships between oxidative stress and n-3 PUFA supplementation in schizophrenia patients. Two intervention studies with n-3 PUFA that assess oxidative stress markers have been reported so far: one open-label [30] and one RCT [31].

Sivrioglu et al. [30] reported the results of combined 4-month n-3 PUFA (360 mg EPA + 240 mg DHA/d), vitamin E (800 IU/d) and vitamin C (1000 mg/d) intervention on antioxidative enzyme activity and lipid peroxide levels in a small population (n = 17) of chronic schizophrenia patients treated with haloperidol for at least three months. A significant reduction in the severity of positive and negative symptoms was observed, which was accompanied by the decrease of RBC-SOD levels. The content of erythrocyte Malonyl Dialdehyde (MDA), a lipid peroxide level marker, and the level of whole blood GSH, an oxidative defence system marker, were not significantly different after 4-month intervention, possibly due to lack of statistical power. As SOD was shown to be increased in schizophrenia, which is considered compensatory to the increased production of ROS, the observed decrease in RBC-SOD supports previous observations that intervention composed of n-3 PUFA plus vitamins E and C has oxidative stress reducing properties.

A second study [31] analyses the data from the intervention RCT study reported by Amminger et al. which demonstrates the efficacy of n-3 PUFA in preventing transition to psychosis after a short [32] and longer term follow-up [31] in individuals at high clinical risk. The study assesses the effect of an active intervention composed of n-3 PUFA 1.2 g/d plus α-tocopherol (30.4 mg/d) or placebo (coconut oil plus α-tocopherol 30.4 mg/d) supplementation on α-, γ- and δ-tocopherol levels and different forms of GSH (total, oxidized, reduced) in erythrocyte membrane phospholipid lysate. Lipid peroxidation level was not measured in this study. Multivariate testing found the assessed effects to be insignificant, but a statistical trend was observed. Univariate analyses revealed significant increases in RBC-α-tocopherol in the intervention group, which was not present in the placebo group; n-3 PUFA was observed to have a significant effect on total RBC-GSH level, which was found to be decreased at the end of a 12-week intervention compared with baseline. The authors discuss the above results and conclude that in terms of the vitamin E protection system, n-3 PUFA intervention seems to directly support the antioxidative defence at membrane level but the effect of n-3 PUFA on total GSH level could demonstrate antioxidative effects, resulting in decreased demand for GSH. The authors postulate that the vitamin E and GSH mechanisms may account for the observed clinical effectiveness of n-3 PUFA administration in high clinical risk individuals. Both studies mentioned above seem to be consistent with the results of the current study, since favourable changes in oxidative defence were observed following n-3 PUFA intervention. The present study extends the understanding of the possible modulators of n-3 PUFA efficacy in first-episode schizophrenia by assessing the lipid peroxide levels related to arachidonic acid (8-epi-$PGF_{2\alpha}$ level), which was not reported previously.

The effects of n-3 PUFA supplementation were also assessed in healthy individuals. Kiecolt-Glaser et al. [33,34] assessed the effects of four-month supplementation with n-3 PUFA in healthy individuals on the indicators of inflammation and oxidative stress. In addition to a reduction in proinflammatory cytokine levels (TNFα and Il-6), n-3 PUFA intervention also significantly reduced AA-derived lipid peroxide levels (i.e. 8-epi-$PGF_{2\alpha}$). Our previous study [35] revealed that a 12-week intervention of supplementation with n-3 PUFA (1350 mg), antioxidants, vitamins E and D_3 in 66 healthy women aged 35–55 was associated with increases in telomerase levels, as well as favourable changes in antioxidative defence capacity (TAC) and oxidative stress indicators (MDA). Significantly increased plasma TAC level and RBC-SOD activity were noted, with slight effects on oxidative stress biomarkers in erythrocytes: MDA and 4-hydroxyalkene levels. Apart from the observed antioxidative effects, the tested supplement also showed anti-ageing activity.

4.3. Possible mechanism

We propose that the potential underlying mechanism of the therapeutic action of n-3 PUFA observed in the present study may be neuroprotective, acting via changes in physical and biochemical properties of cellular membranes [36,37], modulation of the inflammatory responses [7,38,39] and antioxidative intracellular defence system [40], and reduction of dopamine toxicity; it may also act by reducing inflammation via modulation of cytokine production [41]. The observed improvement in symptomatology may also be the result of a direct interaction between EPA or DHA and glutamatergic neurotransmission [42,43]. Interestingly, 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 changes in the brain being correlated with improvements in negative symptoms [44].

4.4. Clinical implications

The observed improvement in psychopathology and patient functioning level raises hope that add-on therapy with concentrated fish oil may result in a valuable change in disease outcomes for patients diagnosed with first-episode schizophrenia, especially because n-3 PUFAs are safe, well accepted by patients and relatively cheap. Moreover, there is accumulating evidence that n-3 PUFAs exert 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 [45], improvement of cognitive performance [46] and metabolic profile [47], and a reduction of the risk of diabetes mellitus [48], cardiac arrhythmia [49], sudden cardiac death [50] and all-cause mortality [51]. The current study provides an insight into possible mediators of the efficacy of n-3 PUFA in patients with first-episode schizophrenia.

4.5. Limitations

As this is the first long-term n-3 PUFA intervention study carried out in a 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 one being a 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. Nevertheless, the effect of the intervention may be decreased because of these possible adherence problems, which were difficult to detect objectively. Another limitation is the 26-week intervention period, as some patients may need more time to achieve improvement, resulting in the final effect being underestimated.

The strengths of the study include its randomized, placebo-controlled design, blinding and its inter-rater reliability testing. Another strength, and novel aspect, is the composition of n-3 PUFA used, i.e. a 3:2 mixture of EPA and DHA, which has not yet been used in patients with first-episode schizophrenia: this dosage of PUFA supplementation was higher than that used in previous studies and low enough to assure safety of intervention.

Further studies incorporating larger samples of patients are warranted, as the results achieved in the present study, based on changes in CGI-S score and both the positive and negative subscales of PANSS, indicate that n-3 PUFA add-on therapy may be associated with improvements in condition. More studies are needed to describe the biological mechanism underlying the effects of n-3 PUFA in patients with first-episode schizophrenia. Further studies should also include an analysis of antioxidative enzyme levels to obtain a greater insight into the possible role of n-3 PUFA in oxidative damage prevention.

4.6. Summary and conclusions

1. The efficacy of n-3 PUFA add-on therapy in reducing symptom severity in first-episode schizophrenia was related to oxidative stress indices.
2. N-3 PUFA supplementation was associated with a significant increase in plasma total antioxidant capacity and decrease in plasma content of 8-isoprostane $F_{2\alpha}$.
3. A significant correlation was observed between clinical improvement and favourable changes of the oxidative stress indices during add-on therapy with n-3 PUFA.

Conflict of interests

The authors declare that they have no conflict of interest.

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