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Telomerase level increase is related to n-3 polyunsaturated fatty acid efficacy in first episode schizophrenia: Secondary outcome analysis of the OFFER randomized clinical trial

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ABSTRACT

Schizophrenia is associated with shortening of the lifespan mainly due to cardiovascular events, cancer and chronic obstructive pulmonary disease. Both telomere attrition and decrease of telomerase levels were observed in schizophrenia. Polyunsaturated fatty acids (PUFA) influence multiple biochemical mechanisms which are postulated to accelerate telomere shortening and limit the longevity of patients with schizophrenia. Intervention studies based on add-on therapy with n-3 polyunsaturated fatty acids (n-3 PUFA) in patients with schizophrenia did not assess the changes in telomerase levels. A randomized placebo-controlled trial named OFFER was designed to compare the efficacy of a 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. The secondary outcome measure of the study was to describe the association between the clinical effect of n-3 PUFA and changes in telomerase levels. Seventy-one patients aged 16–35 were enrolled in the study and randomly assigned to the study arms. The Positive and Negative Syndrome Scale (PANSS) was used to assess the change in symptom severity. Telomerase levels of peripheral blood mononuclear cells (PBMC) were assessed at three points: at baseline and at weeks 8 and 26 of the intervention. A significantly greater increase in PBMC telomerase levels in the intervention group compared to placebo was observed ($p < 0.001$). Changes in telomerase levels significantly and inversely correlated with improvement in depressive symptoms and severity of the illness. The efficacy of a six-month intervention with n-3 PUFA observed in first-episode schizophrenia may be related to an increase in telomerase levels.

1. Introduction

Telomere attrition (Rao et al., 2016a) and decreased levels of telomerase (TA) (Porton et al., 2008) have been shown in schizophrenia; however, this observation is not consistent (Darrow et al., 2016; Lindqvist et al., 2015). Telomere shortening has also been observed in individuals with ultra-high risk of psychosis (Maurya et al., 2017). Our previous study found that the recurrence of psychotic symptoms in schizophrenia, as well as their intensity and chronicity, is related to telomere attrition (Pawelczyk et al., 2015). Telomere length is regulated by various factors including telomerase activity, inflammatory cytokines and oxidative stress (Blackburn, 2005).

A mixture containing n-3 PUFA has been shown to increase TA activity in heathy female volunteers (Balcerczyk et al., 2014). TA levels have also been demonstrated to be influenced by n-3 PUFA supplementation in mice (Chen et al., 2017) and in human adenocarcinoma cells (Eitsuka et al., 2005). Telomere length, and possibly TA activity, may be differently regulated by dietary n-3/n-6 ratio, which may be associated with the opposing mechanisms of action of n-3 and n-6 PUFA derivatives regarding inflammation and oxidative stress (Chen et al., 2017). Our previous studies have found that diet of patients with schizophrenia and individuals with ultra-high risk of schizophrenia is characterized by an unfavorable n-3/n-6 PUFA ratio, with excess consumption of proinflammatory n-6 PUFA (Pawełczyk et al., 2015b).

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Studies indicate that the protective effects of n-3 PUFA supplementation may act by an increase in oxidative defense and a reduction of neuroinflammation, which in turn may influence TA levels and telomere length (Chen et al., 2017). Moreover, there is accumulating evidence that the mechanism of action of psychotropic agents, including antipsychotics, may involve TA activation (Bersani et al., 2015).

There is still a lack of knowledge of the influence of n-3 PUFA supplementation on TA levels in schizophrenia patients. Therefore, the aim of the present study was twofold: (a) to assess TA level changes in peripheral blood mononuclear cells (PBMC) after a 26-week period of supplementation with concentrated fish oil rich in n-3 PUFA and (b) to find out whether PBMC TA levels changes are related to improvement in psychopathology, disease severity and patient functioning in first-episode of schizophrenia.

2. Methods

The present study describes 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, medications used during the study, and change in cumulative dose of antipsychotics used are given in detail elsewhere (Pawełczyk et al., 2015a). The results of the primary outcome analysis, interrater reliability, adherence to study medication and adverse effects analysis have been published previously (Pawełczyk et al., 2016) and are not replicated in the present paper.

2.1. Study participants and procedures

All the participants were inpatients admitted to the Psychiatric Clinics of the Central Teaching Hospital, Medical University of Lodz and the wards of the Babinski Memorial Hospital, Lodz, Poland. Eligible patients were (1) aged 16–35 (2) and had been 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. The patients were excluded (1) if more than two years had passed since the first onset of positive symptoms, (2) if the patient had bleeding disorders, (3) the patent was using n-3 PUFA supplements within eight 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 a condition 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.

The study group comprised participants included for the randomized placebo-controlled trial (OFFER) (Pawełczyk et al., 2016), the aim of which was to investigate the efficacy of augmentation with concentrated fish oil containing 2.2 g of n-3 PUFA, i.e. eicosapentaenoic (1320 mg) and docosahexaenoic acid (880 mg) (EPA + DHA), in 71 drug-naive or early-treated first-episode schizophrenia patients. Of the 71 individuals enrolled in the study, 36 were randomly assigned to the EPA + DHA group and 35 to the placebo group. The treatment groups were similar in terms of demographic variables and baseline characteristics (Table 1).

A patient flow diagram showing the history of participant inclusion, exclusion and attrition at different stages of the study was presented in detail in a previous paper (Pawełczyk et al., 2016).

The trial procedures were explained verbally and in writing to all eligible individuals. All participants provided written informed consent prior to study enrollment. Consent was obtained from parents or guardians for participants under 18 years of age. The study was approved by the Ethics Committee of the Medical University of Lodz and was carried out in accordance with the Declaration of Helsinki.

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)	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 (%)			
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
Income category, % of average wages, N (%)			
< 10	2 (5.7)	1 (2.9)	0.939
10–20	8 (22.9)	10 (28.6)	
21–30	10 (28.6)	10 (28.6)	
31–40	8 (22.9)	5 (14.3)	
41–50	2 (5.7)	3 (8.6)	
51–60	4 (11.4)	4 (11.4)	
> 60	1 (2.9)	2 (5.7)	
CDSS score, mean (SD)	8.8 (5.13)	8.1 (5.5)	0.602
CGI-S score, mean (SD)	5.9 (0.8)	5.8 (0.7)	0.861
GAF score, mean (SD)	26.2 (8.8)	26.9 (9.4)	0.731
PANSS score, mean (SD)			
Positive	25.6 (5.2)	25.3 (5.8)	0.822
Negative	23.1 (6.1)	22.8 (6.0)	0.799
General	49.6 (7.5)	48.7 (7.0)	0.583
Total	98.4 (13.2)	96.8 (12.0)	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]	263.13 (128.76)	292.81 (195.62)	0.669
Energy consumption, mean (SD) [kCal]	2279.19 (982.49)	2328.32 (753.59)	0.833

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 participants in a group, N - number of observations in a study population, SD - standard variation, mo -month, yr -year; CPZ - chlorpromazine, IQR – interquartile range.

[a] Entire population.

[b] Participants on antipsychotics at baseline.

2.2. 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 flavor. 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), to prevent oxidation of fatty acids. 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-reporting and pill count at each medication appointment.

To increase the external validity of the study results and conform with guidelines of schizophrenia therapy, the use of benzodiazepines, *Z*-drugs, injectable forms of antipsychotics, antidepressants, mood stabilizers and anticholinergic medications was allowed if clinically indicated. The background use of antipsychotics and concomitant medication 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.

2.3. 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) (Kay et al., 1987) 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) (Guy, 1976), the Global Assessment of Functioning (GAF) (Jones et al., 1995) and Calgary Depression Scale for Schizophrenia (CDSS) (Addington et al., 1992) scores between baseline and after 26 weeks of intervention. One of the secondary biochemical outcome measures was the change in PBMC telomerase level. Telomerase concentration was assessed three times: at baseline, eight weeks and 26 weeks after initiation of study intervention.

2.4. Telomerase concentration assay

The Telomerase (TA) concentration of PBMCs was quantified using TE ELISA Kit (CUSABIO, China, CSB-E08021h) according to the manufacturer's protocol. Human PBMCs were isolated using a density gradient technique: density gradient solutions Ficoll Paque PLUS from GE Healthcare Life Sciences and Histopaque 1077 from Sigma Aldrich. The PMBC cells were suspended in 300 µl PBS, freeze-thawed two times to break the cell membranes and then used for TA quantification: 100 µl of Standard, Blank, or Sample were added per well, which were covered with an adhesive strip and incubated for two hours at 37 °C. Biotin-antibody working solution (100 µl) was added to each well and incubated for one hour at 37 °C. After washing for three times, 100 µl HRP-avidin working solution was added to each well, covered with a new adhesive strip, and incubated for one hour at 37 °C. After washing, 90 µl TMB Substrate was added to each well, and incubated for 15–30 min at 37 °C. The optical density of each well was determined using a microplate reader set to 450 nm.

2.5. Statistical analyses

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 performed using the Student's *t*-test or Mann-Whitney *U* test depending on the distribution of the dependent variables. Differences in categorical variables were analyzed using the Chi-square test or Fisher's exact test depending on the met assumptions. Kendall's tau b correlation coefficient with Bonferroni correction for multiple comparisons was used to assess the strength of relationship and significance of linear association between change from baseline of clinical scores and change of PBMC TA level during the study.

As the missing data in the present study was lost due to patient withdrawal or missed assessments, they cannot be regarded as missing completely at random and must be modeled (Friedman et al., 2010). To deal with missing values in our ITT sample, a conservative approach was used which assumed that TA concentration 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 telomerase level were assessed using a mixed model for repeated measures (MMRM) that included fixed-effect terms for intervention, visit, baseline score as a covariate, and an intervention-by-visit interaction term, using autoregressive heterogeneous covariance structure for within-patient correlation. Between treatment group differences were reported using least-squares (LS) means with standard error (SE). No adjustment was made for multiple comparisons with respect to post hoc analyses. Planned contrasts were performed to assess differences between study groups at two time points: t1 (8 weeks) and t2 (26 weeks). 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. All statistical tests were two-sided, 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 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 the EPA + DHA group discontinued the intervention prematurely and withdrew his consent. Three of the 36 patients (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 enrollment, 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 enrollment. 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 were treated with antipsychotics after the 26-week intervention. Daily consumption of energy and PUFA was determined at baseline using the Polish version of the Food Frequency Questionnaire (Dehghan et al., 2012). No significant difference was observed between the groups at baseline with regard to dietary consumption of energy and PUFA (Table 1).

3.2. Medication status during the study

No significant differences between the study groups were found regarding chlorpromazine equivalent cumulative exposure to antipsychotics in different periods of the study. Chlorpromazine equivalent cumulative exposure to antipsychotics calculated using method proposed by Andreasen et al. (2010) in dose-years in the study groups is presented in detail in the previous paper (Pawełczyk et al., 2016). 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 ($p = 0.478$).

3.3. Telomerase levels

Mixed Model for Repeated Measures analysis (MMRM) was used to assess differences between groups regarding the PBMC TA levels. Significant increases in TA concentrations were observed in both groups during the study (paired samples Student-*t*-test, EPA + DHA: $t = 13.494$, df = 35, $p < 0.001$; placebo: $t = 8.283$, df = 34, $p < 0.001$), and were significantly higher in the EPA + DHA group than placebo (Fig. 1). The analysis of contrasts from MMRM revealed significant differences between groups regarding PBMC TA levels. The mean change of TA level from baseline was significantly higher in EPA + DHA group than placebo. The observed effects can be considered as high. The least squares mean changes and mean differences between groups in change scores at week 26 compared to baseline are presented in Table 2. An analysis of contrasts between the study groups at different time points revealed lack of significant differences in TA levels at eight weeks ($t = 1.675$, df = 69, $p = 0.098$) and the presence of significant differences between the groups at 26 weeks ($t = 3.153$, d = 69, $p = 0.002$). However, numerical differences in TA levels between the groups were observed at week eight and reached a trend value ($p < 0.1$). Mean TA levels with 95% confidence intervals are presented in Fig. 1.

Sensitivity analysis revealed that the differences in PBMC TA levels between the study groups remained significant after controlling for additional potential confounding variables (Sex, Age and BMI). The results of MMRM analysis are presented in On-line Supplementary Table 1.

Significant negative correlations after controlling for multiple comparisons were observed between the change from baseline to week 26 for TA levels, and score changes from baseline to week 26 for the Calgary Depression Scale for Schizophrenia (CDSS) (Kendall's tau b correlation coefficient = −0.305; $p < 0.001$) and the Clinical Global Impressions - Severity scale (CGI-S) (Kendall's tau b correlation coefficient = −0.743; p < 0.001) in the whole population of the patients assessed ($n = 71$). Significant relationships between disease severity (CGI-S) and PBMC TA level were also observed when correlation coefficients were calculated separately for the two study groups (see On-line Supplementary Table 2). According to Cohen (Cohen, 1988), the magnitude of the observed significant correlations can be regarded as large (human TA*CGI-S) and medium (human TA*CDSS). The remaining associations between the changes of TA levels and changes of the other clinical assessments were found to be insignificant (for details see On-line Supplementary Table 2).

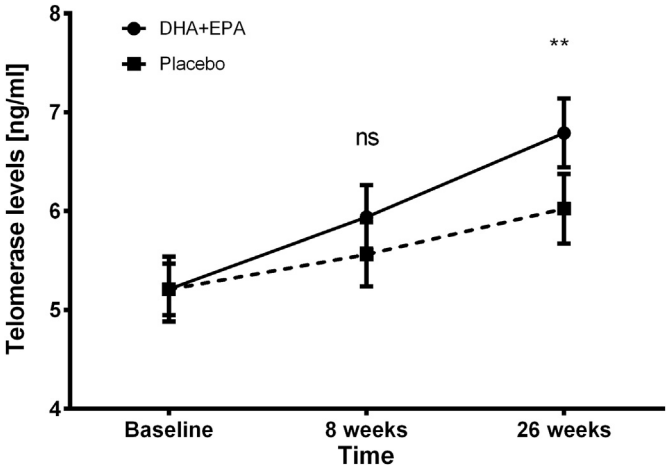

Fig. 1. Telomerase levels at baseline, week 8 and week 26 in the study arms. Means and 95% confidence intervals. Differences between groups: ns - not significant, ** - $p = 0.002$.

Table 2
Change in telomerase levels across study arms controlling for baseline PBMC telomerase levels.

Variable change	Baseline to week 26, mean (SE)		LS mean difference[a] (95% CI)	Effect size[b]
	EPA + DHA ($n = 36$)	Placebo ($n = 35$)		
Telomerase level (ng/ml)	1.466[c] (0.061)	0.8[c] (0.062)	0.666*** (0.494–0.837)	1.82

Abbreviations: SE - standard error, LS - least squares, CI - confidence interval.
*** $p < 0.001$.
[a] Based on the contrast from mixed models repeated-measures analysis.
[b] Difference in change from baseline in units of standard deviations of change.
[c] Estimated marginal means adjusted for telomerase level at baseline (HT0). Covariate appearing in the model is evaluated at the following value: HT0 = 5.21.

4. Discussion

Our findings show a significant increase of PBMC TA levels in patients with first-episode of schizophrenia treated with antipsychotics and concentrated fish oil enriched with EPA + DHA or placebo (olive oil) for 26 weeks. The mean level of increase was significantly higher in participants supplemented with EPA + DHA in comparison to placebo. Eight weeks after initiation of the intervention, the groups did not differ significantly according to TA concentration. Significant differences between the study groups were observed at 26 weeks; however, differences were observed at trend level after eight weeks of intervention. A significant correlation was observed between the change in TA levels and reduction in depressive symptoms and severity of disease. No significant correlation was present between change in total PANSS and its sub-scale scores and change in TA concentration of PBMC.

4.1. Comparison with previous studies

Previous studies have not assessed TA levels in patients with first-episode schizophrenia supplemented with concentrated fish-oils. Thus, no direct comparisons with previous studies could be made. However, there is accumulating evidence suggesting that telomeric and TA pathology play a role in the pathophysiology of mental disorders, including schizophrenia: the etiopathogenesis of which is known to be related to neurodegeneration (Plitman et al., 2014). An accelerated loss of telomeric DNA sequences has been reported in chronically stressed individuals (Daubenmier et al., 2012; Epel et al., 2010) as well as in those with anxiety, affective and psychotic disorders including schizophrenia (Lindqvist et al., 2015; Rao et al., 2016a). Telomere attrition has recently been found in individuals at ultra-high risk of psychosis (Maurya et al., 2017). Our previous study found that the severity of symptoms, recurrence rate and chronicity of schizophrenia are inversely related to telomere length (Pawelczyk et al., 2015).

Telomere stability is maintained, among others, by TA activity (Blackburn, 2005; von Zglinicki, 2002), and reduced TA levels have been found in schizophrenia, where they seem to be inversely related to severity and chronicity of the disease (Porton et al., 2008). Moreover, a recent study has found that the risk of paranoid schizophrenia development is related to specific variants of TERT influencing telomere length (Rao et al., 2016b). Risk reduction of the conversion to psychosis has become one of the respected targets of preventive strategies in Psychiatry over the last two decades. The prevention of schizophrenia has been addressed in various intervention trials, including those using n-3 PUFA.

4.2. Mechanism of action and clinical implications

There is continuing debate about the efficacy of n-3 PUFA in reducing the risk of schizophrenia development (Amminger et al., 2010; McGorry et al., 2016) and reducing disease symptomatology (Berger et al., 2007; Emsley et al., 2002; Pawełczyk et al., 2016; Peet et al., 2002). The mechanism of action of N-3 PUFA in schizophrenia is postulated to be related mainly to reduction of oxidative damage to neuronal membranes due to (a) anti-oxidative defense enzyme activation, (b) modulation of membrane fluidity, which affects binding of ligands to neurotransmitter receptors, (c) limiting inflammation due to the production of anti-inflammatory derivatives such as prostaglandins, lipoxins, protectins, resolvins and maresins (Calder, 2015; Kiecolt-Glaser et al., 2013; Serhan et al., 2015). Tests on animal models have shown that n-3 PUFA supplementation affects TA levels by reduction of inflammatory responses and oxidative stress caused by an imbalance between overproduction of free radicals and inhibition of defense mechanisms (Chen et al., 2017). PUFA, especially DHA and EPA, have been found to inhibit TA activity of neoplastic cells in vitro (Eitsuka et al., 2005) and in animal models of hepatic and testicular cancer via reduction of oncogenes *c-Myc* and *p53* expression, which are activated in response to oxidative damage (Chen et al., 2017). The relationship between DHA dose and TA activity in that study has been found not to be linear, with high and low doses of DHA leading to opposite effects on TA levels. Doses of n-3 PUFA within different range may be responsible for the disparity between the results obtained by Eitsuka et al. and in the present study.

N-3 PUFA may also influence the processes involved in senescence and aging. PBMC TA levels 25% higher than normal have been observed in healthy middle-age women supplemented with a complex compound containing n-3 PUFA (Balcerczyk et al., 2014). An analysis of sirtuin (SIRT1 and 2) expression in PBMCs has shown significant increases for both genes on a mRNA level. These genes are essential for senescence and regulation of oxidative stress (Godoy et al., 2014). Moreover, changes in TA concentration have been accompanied by increase in anti-oxidative enzyme activities (glutathione, sodium superoxide dismutase) and reduction of oxidative stress markers (Balcerczyk et al., 2014). The above changes also suggest that n-3 PUFA has anti-aging properties, which were also shown in the present study in a population of first-episode schizophrenia patients.

Most of the mechanisms described above may be responsible for the observed increase in TA levels in the placebo group. Both the EPA/DHA group and the placebo group were treated with antipsychotics throughout the study. The action of the antipsychotics was observed to be related to (a) oxidative stress modulation; (b) anti-inflammatory response modulation; (c) neuronal plasticity and changes in brain derived neurotrophic factor production (Flatow et al., 2013; Magalhães et al., 2016). These mechanisms may be responsible for the modulation of telomere length by antipsychotics. Telomere length was also observed to be related to the response to risperidone (Li et al., 2015) and antipsychotic efficacy (Savolainen et al., 2012). Thus, EPA/DHA supplementation could bolster the effects of antipsychotics, leading to an increase in TA levels in both study groups.

PBMC TA pathology and telomere attrition may have significant implications for patients. There is accumulating evidence that TA concentration and activity may be related to (a) schizophrenia ethiopathology and disease outcome (Porton et al., 2008) (b) antipsychotic action (Bersani et al., 2015) and efficacy (Li et al., 2015), (c) lengthening of the lifespan of schizophrenia patients (Lindqvist et al., 2015; Porton et al., 2008) and (d) a reduction in the prevalence of age-related diseases, such as atherosclerosis, their complications and cognitive dysfunctions among schizophrenia patients (Rentoukas et al., 2012). If replicated in further sufficiently powered, well-designed studies, the results demonstrated in the present study, showing an increase in TA levels related to n-3 PUFA supplementation, may offer hope to patients that preterm senescence can be slowed or restricted, and the outcome of the disease improved.

4.3. Limitations

The present study has some limitations that need to be considered before formulating conclusions, the main one being a lack of any 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. Secondly, the 26-week intervention period may be regarded as another limitation, as some patients may need more time to demonstrate TA changes. However, because this study measured peripheral rather than central nervous system levels of TA, it is unknown how the observed findings translate to brain changes or pathology. As a placebo, the study used olive oil, which contains mainly monounsaturated fatty acids and only small amounts of polyunsaturated fatty acids. Although olive oil is not an inert substance, it was chosen to avoid bioethical concerns associated with the use of a placebo composed of unsaturated fatty acids or containing high amounts of n-6 PUFA. Thus, the effect of n-3 PUFA observed in the present study might be decreased due to the presence of small amounts of PUFA in placebo.

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 but low enough to ensure safety of intervention.

Finally, there are possible risks related to increased TA levels in PBMC. TA is an enzyme that is upregulated in different kinds of neoplasms, and it has been studied as a potential target for anti-neoplastic strategies (Gladych et al., 2011). N-3 PUFA safety was assessed during the present study and no serious adverse events were observed, but the period of follow-up was a little too short to observe neoplastic growth as a possible side effect. Therefore, further studies are warranted that will address long-term safety of telomerase modulation as a potential pharmacological target in schizophrenia.

4.4. Conclusion

The present study found that 26-week supplementation with N-3 PUFA added to antipsychotics in first-episode schizophrenia patients resulted in a significant increase in PBMC TA levels. This increase was also observed in the second arm of the study where patients received placebo (olive oil) and antipsychotics. Changes of TA levels in the whole population were inversely related to the severity of disease and depressive symptoms. Taking into consideration the manifold and complex roles of TA, the results of the present study encourage exploration of the new research fields in patients with schizophrenia, such as the relationship of TA levels and activity to (a) excess mortality and shortened lifespan, (b) modulation of immune system impairments, and (c) processes of neurodegeneration and neurogenesis, especially in the hippocampi. Moreover, our findings indicate that some of the mechanisms of action of antipsychotics and n-3 PUFA may be shared, including the modulation of TA levels. Thus TA may constitute a novel

pharmacological target in schizophrenia that can be explored in further studies.

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Contributors

Authors TP and AP were responsible for literature searches, study design, patient enrollment, clinical assessments and drafting the manuscript. Author MG-G took part in literature searches, enrollment of patients and clinical assessment of patients. Authors ET and NŻ took part in study preparation and clinical assessments of patients. Author JS was responsible for preparation of biological material, biochemical analyses, management of data for statistical analyses and drafting parts of the manuscript. Author AP was responsible for enrollment of patients, clinical assessments, drafting and correcting the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

The authors declare that they have no conflicts of interest.

Ethical statement

The trial procedures were explained verbally and in writing to all eligible individuals. All participants provided written informed consent prior to study enrollment. Consent was obtained from parents or guardians for participants under 18 years of age. The study was approved by the Ethics Committee of the Medical University of Lodz and was carried out in accordance with the Declaration of Helsinki.

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