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ORIGINAL PAPER

Changes in erythrocyte membrane fatty acids during a clinical trial of eicosapentaenoic acid (EPA) supplementation in schizophrenia

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Abstract In a previously reported double-blind, placebo-controlled trial of eicosapentaenoic acid (EPA) as supplemental treatment in 40 patients with schizophrenia, we found significant improvement in symptoms as measured by the Positive and Negative Syndrome Scale (PANSS) compared to placebo (Emsley et al. 2002). Here we report changes in fatty acid composition of erythrocyte membranes

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in the same sample (n=16 in each group). After 12 weeks of receiving EPA, levels of several saturated and mono-unsaturated fatty acids decreased significantly while levels of n-3 fatty acids increased significantly compared to the placebo group. Increases of n-3 and n-6 fatty acids in the erythrocyte membranes were greater in subjects who improved more than 20% on overall symptoms. Changes in fatty acids correlated significantly with improvement in PANSS sub-scale scores, more so in females than in males. Docosahexaenoic acid (DHA) (22:6*n-3*) levels increased less than expected, suggesting a possible defect in synthesis or incorporation of DHA into membranes in schizophrenia. Improvement in dyskinesia correlated significantly with an increase in alpha-linolenic acid (18:3*n-3*; p=0.03), and a decrease in 20:1*n-9* (p=0.005).

Keywords Schizophrenia · Fatty acids · Eicosapentaenoic acid · Dyskinesia

Introduction

Lipids, including phospholipids and essential polyunsaturated fatty acids (PUFAs), play important structural and functional roles in cell membranes. In the brain they are involved in the formation and remodeling of dendrites and synapses, while the growth cones of neurons are enriched in phospholipase enzymes which release arachidonic acid (AA) and other PUFAs from phospholipids for cell signaling (Horrobin 1999). The aetiology of schizophrenia has been linked to an altered metabolism of lipids in neuronal membranes resulting from an increase in the activity of phospholipase A_2 (PLA_2) (Gattaz et al. 1990).

The PLA_2 enzymes are normally involved in neurotransmitter release, long-term potentiation, growth and differentiation, and membrane repair, but under pathological conditions the high levels of lipid metabolites generated are involved in neuroinflammation, oxidative stress and neuronal injury (Farooqui et al. 2006).

Specifically, it is hypothesized that in schizophrenia there is an accelerated rate of loss of PUFAs, notably AA (20:4*n-6*), eicosapentaenoic acid (EPA; 20:5*n-3*) and docosahexaenoic acid (DHA; 22:6*n-3*) from the membranes. When present to a mild degree, this increased rate of loss may be compensated for by increased incorporation of fatty acids (FAs) from the diet, but a change in membrane composition will occur if there are deficiencies in the enzymes involved in phospholipid synthesis, i.e. the elongases, desaturases, and acyltransferases, or if there is an insufficient supply of essential PUFAs in the diet (Peet 2008). Environmental factors such as dietary lipid intake may alter the expression of genes for schizophrenia, resulting in a favourable outcome and course of schizophrenia in communities with a higher intake of essential PUFAs (Christensen and Christensen 1988), while insufficient intake of PUFAs may aggravate symptoms of schizophrenia in individuals with a genetic predisposition to the illness.

A relationship between schizophrenia and PUFAs is supported by results obtained in recent studies. Sumiyoshi et al. (2008) showed that decreased levels of PUFAs contributed to impaired social cognition, as represented by event schema, in patients with schizophrenia, while Condray et al. (2008) found an association between cell membrane FAs and semantic memory and language in schizophrenia, with dopamine

modulation. Ohara (2007) outlined a possible association between reduced PUFAs and the dopamine hypothesis of schizophrenia from animal studies: Whereas dietary n-3 fatty acid deficiency caused a reduction of the dopamine concentration and number of D2-receptors in frontal lobes, these parameters were unchanged or increased in the limbic system.

Studies have reported reduced levels of PUFAs extracted from post-mortem brain tissue from patients with schizophrenia, correlating with PUFA levels in erythrocyte membranes (Yao et al. 2000; Peet 2008). Erythrocyte membranes are therefore useful models of the FA composition of brain membranes (Peet 2008). Several clinical trials have been conducted to investigate the effects of supplementation with PUFAs on symptoms of schizophrenia as well as on the incorporation of dietary fatty acids into cell membranes (Emsley et al. 2003). Supplementation with EPA-enriched oil produced significantly better clinical results than when the oil was enriched with DHA (Peet et al. 2001), leading to the hypothesis that the inhibition of PLA_2 by EPA, previously reported by Finnen and Lovell (1991), is important in producing the improvement. Whilst most randomised, placebo controlled trials using EPA as supplemental medication have shown significant benefit for EPA compared to placebo (Peet 2008), a study by Fenton et al. (2001) did not show reduction in the symptomatology of schizophrenia when patients were treated with EPA compared to placebo. These authors suggested that smoking may be a confounding factor in EPA trials. However, a later study by Reddy et al. (2004) in neuroleptic-naïve, first episode schizophrenic patients showed that membrane PUFA losses were not related to age, gender, smoking status or cotinine levels, and suggested that these deficits may be trait related. Khan et al. (2002) indicated that lower membrane AA and DHA most likely predated the illness and probably contributed to the onset.

In our own previously reported double blind controlled trial of 40 schizophrenic patients using EPA as add-on therapy (Emsley et al. 2002) the EPA treated subjects showed significantly greater improvement in Positive and Negative Syndrome Scale (PANSS) total scores at 12 weeks than subjects on placebo. We also found a greater reduction in Extrapyramidal Symptom Rating Scale (ESRS) dyskinesia scores for the EPA-treated subjects. We now report on the changes in FA composition in erythrocyte membranes in these subjects, and the associations thereof with changes in symptom scores.

Materials and methods

Subjects were eligible for inclusion if they were aged 18 to 65 years and met Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV, American Psychiatric Association 1994) criteria for schizophrenia. Only patients with persistent positive symptoms and a PANSS total score of>50 were considered. Patients were on a fixed dose of antipsychotic and concomitant medication for at least 6 months prior to randomization as reported previously (Emsley et al. 2002). Exclusion criteria comprised substance abuse, neurological disorder (other than medication-induced movement disorders) and other significant medical conditions.

The study was approved by the ethics committee of the University of Stellenbosch, South Africa. After providing written, informed consent, patients

were randomized to receive either ethyl-EPA 3 g/day, or an identical capsule containing liquid paraffin as placebo for 12 weeks (n=16 in each group). Baseline demographic and clinical variables were similar for the two groups. The age and illness duration were 46.2 years (SD=10.6) and 23.1 years (SD=8.5) for the EPA group, and 43.6 years (SD=13.9) and 22.2 years (SD=12.4) for the placebo group. Antipsychotic doses (chlorpromazine equivalents) were 1011 mg/day (SD=532) for the EPA group and 931 mg/day (SD=652) for the placebo group. No additional medication was prescribed throughout the trial, except for occasional analgesics for headache and lorazepam for insomnia.

Dietary survey

A dietician reviewed the dietary intake of each subject at baseline and throughout the study. EPA intake was calculated according to standard food supplementation tables provided by the South African Medical Research Council (Kruger et al., 1991).

Fatty acid concentrations in erythrocyte membranes

Blood samples were obtained from 16 patients in both the EPA and placebo groups before and after the trial for the determination of erythrocyte membrane FAs. Blood was collected in vacuum tubes containing EDTA as anticoagulant. The erythrocytes were washed twice with 0.15 mol/L NaCl by centrifugation at 1,800 × g for 15 min at 4°C to remove the buffy coat. Lipids were extracted with chloroform/methanol (2:1; v/v) according to a modified method of Folch et al. (1957). All of the extraction solvents contained 0.01% butylated hydroxytoluene (BHT) as an antioxidant. Heptadecanoic acid (C17:0) was used as internal standard to quantify the individual FAs. Neutral lipids were separated from total phospholipids by TLC on pre-coated silica gel 60 plates (10 x 10 cm) without a fluorescent indicator (Art. 1.05721, Merck, Darmstadt, Germany) using the solvent system petroleum benzine (bp 40–60°C)/diethyl ether (peroxide free)/acetic acid (90:30:1, by vol). The lipid bands were visualized with longwave ultraviolet light after spraying the plates with chloroform/methanol (1:1, by vol) containing BBOT (2,5-bis-(5′-tert.-butylbenzoxazolyl-[2′])thiophene; 10 mg/100 mL; Sigma Chemical Co.). The total phospholipid band was scraped off and analysed for FA composition as described previously (Van Jaarsveld et al. 2000). Briefly, FA methyl esters (FAME) were analyzed by gas-liquid chromatography (GLC Varian Model 3300 equipped with flame ionization detection) using 30 m fused silica megabore DB-225 columns of 0.53 mm internal diameter (J&W Scientific, Folsom, CA). Gas flow rates were: hydrogen, 25 ml/min; air, 250 ml/min; and hydrogen (carrier gas), 5–8 ml/min. Temperature programming was linear at 4°C/min, initial temperature 160°C, final temperature 220°C, injector temperature 240°C, and detector temperature 250°C. The FAME were identified by comparison of the retention times to those of a standard FAME mixture (Nu-Chek-Prep Inc., Elysian, Minnesota).

Statistics

The majority of the analyses were performed using analysis of covariance to determine differences between the EPA and placebo groups. The covariate variables

taken into account were age and years of education. In some cases Bonferroni post-hoc tests were done to test for differences in sub-groups. Due to the small sample sizes, residuals were inspected in all cases to identify influential observations, and reported as such. For determining correlations between variables, the non-parametric Spearman rank correlations were calculated. In some cases, paired Student t-tests were done to determine differences between levels of FAs before and after supplementation. A significance level of 0.05 was used as guideline for determining significant differences.

Results

Dietary survey

According to the dietary survey, subjects consumed fish on average 3 times per week, but this consisted mostly of hake, which contains low levels of EPA. Pilchards (containing 0.53 g EPA/100 g) and mackerel (containing 0.8 g EPA/100 g) were consumed on average once in 3 weeks. The suggested intake of EPA + DHA is 0.5–1 g /day (Gebauer et al. 2006). The average dietary intake of omega-3 FAs was 218± 215 mg/day in the EPA group, and 230±228 mg/day in the placebo group, while dietary EPA intake in the EPA group was 74±79 mg/day and in the placebo group 71±75 mg/day. This constitutes a very low level of dietary EPA intake.

Fatty acid concentrations in erythrocyte membranes

The erythrocyte membrane FA concentrations for the two groups (expressed as µg/100 µg) are summarized in Table 1. Significant changes are denoted by * ($p<0.05$) and ** ($p<0.001$). There was a significant reduction in saturated ($p<0.001$) and mono-unsaturated ($p<0.01$) FAs in the EPA group, with the greatest decrease (49.32%) recorded for eicosaenoic acid (20:1*n-9*) in the EPA group (a reduction occurred in 13 out of 16 patients, while 3 remained unchanged). In the placebo group 20:1*n-9* decreased in 7 patients and increased in 9 patients. The reduction of 20:1*n-9* levels in the EPA group was highly significant ($p<0.001$). While the baseline concentrations of 20:1*n-9* in membranes were generally not high (Table 1), further reductions were significantly correlated with improvement in PANSS scores and ESRS dyskinesia scores (Table 4). Reductions of saturated and mono-unsaturated FAs occurred in the placebo group as well, however the changes were not significant.

Total PUFAs increased significantly ($p<0.03$) in the EPA group, (but not in the placebo group), the greatest changes occurring in the n-3 FAs. (Signifcant increase in total n-3 FAs: $p<0.001$). As expected for the EPA group, there was a highly significant increase in EPA (20:5*n-3*) concentration ($p<0.001$) after 12 weeks (Table 1). There was a non-significant increase in EPA in the placebo group (Table 1). The difference between the two groups was highly significant ($p<001$). An immediate elongation product of EPA, docosapentaenoic acid (DPA; 22:5*n-3*) also increased significantly in the erythrocyte membranes of the EPA group ($p<0.001$; Table 1) and non-significantly in the placebo group. Docosahexaenoic acid (DHA; 22:6n-3), which is in turn

Table 1 Baseline concentrations (μg/100 μg) of fatty acids in erythrocyte membranes, endpoint concentrations and percentage changes after 12 weeks of treatment with EPA (3 g/day) or placebo. Percentage changes were calculated using (baseline—endpoint)/baseline × 100. Saturated and mono-unsaturated FAs were generally reduced, while the n-3 fatty acids increased. Significant differences ($p<$ 0.001) were found between the EPA and placebo groups for EPA (20:5n-3) and DPA (22:5n-3) (shown in bold), but not for DHA (22:6n-3)

	EPAGroup(n=16)			Placebogroup(n=16)		
	Baseline	Endpoint	%Change	Baseline	Endpoint	%Change
14:0	0.4±0.15	0.35±0.20	-12.43	0.38±0.17	0.3±0.11	-20.22
16:0	29.72±5.54	26.62±3.98	-10.43	29.25±5.51	27.09±4.27	-7.39
16:1	0.16±0.07	0.14±0.08	-12.16	0.19±0.11	0.15±0.09	-20.03
18:0	18.89±2.29	18.32±2.21	-3.02	18.71±2.40	18.25±1.74	-2.47
18:1*n-9*	13.69±1.60	13.33±1.16	-2.63	14.04±1.36	13.73±1.38	-2.21
20:0	0.67±0.27	0.52±0.14	-23.82*	0.62±0.17	0.53±0.18	-14.53
20:1*n-9*	0.22±0.11	0.12±0.04	-49.32**	0.21±0.08	0.23±0.17	9.72
24:0	8.82±2.93	6.7±2.43	-24.03*	7.53±2.19	6.41±1.72	-14.88
24:1*n-9*	5.23±1.18	4.27±1.57	-18.36*	5.15±1.40	4.46±1.55	-13.39
18:2*n-6*	6.1±3.00	7.21±1.97	18.02	6.97±2.96	8.02±2.20	15.07
18:3*n-6*	0.03±0.05	0.02±0.02	-30.48	0.03±0.04	0.02±0.02	-33.07
20:2*n-6*	0.22±0.10	0.21±0.06	-8.97	0.36±0.28	0.31±0.11	-13.93
20:3*n-6*	0.72±0.57	0.93±0.40	30.72	0.85±0.61	1.25±0.76	48.5
20:4*n-6*	6.15±4.68	7.54±3.03	22.59	6.82±4.92	8.90±4.08	30.66
22:4*n-6*	1.55±1.02	1.42±0.35	-20	1.71±1.27	2.13±1.13	24.6
18:3*n-3*	0.12±0.07	0.13±0.07	8.49	0.15±0.16	0.09±0.07	-39.77
20:5*n-3*	0.23±0.20	2.97±1.69	**1189.99****	0.28±0.15	0.40±0.23	46.63
22:5*n-3*	0.75±0.67	3.2±1.74	**328.03****	0.82±0.79	1.30±0.78	58.54
22:6*n-3*	2.65±2.19	3.52±2.15	32.85	2.69±1.83	3.92±2.09	45.67

Significant % change from baseline *($p<0.05$); ** ($p<0.001$)

synthesised from DPA, showed a smaller, non-significant increase from baseline to endpoint in the EPA group.

With respect to the n-6 FAs, no significant changes were measured. Gamma-linolenic acid (GLA;18:3*n-6*) decreased and dihomo-gamma-linolenic acid (DGLA; 20:3*n-6*) increased in both groups (Table 1). Adrenic acid (22:4*n-6*) was decreased in the EPA group compared to an increase in the placebo group. AA (20:4*n-6*) increased in both groups, although not significantly. In our trial changes in the n-6 FAs did not correlate with changes in schizophrenic symptoms (Table 4).

Individual differences in symptom improvement

At the end of the trial it was found that of the patients who had taken EPA there were 4 subjects who improved more than 20% on the PANSS total score. In these subjects (2 females and 2 males) the increase in membrane n-3 FA concentrations (Table 2) was greater compared to the total EPA group (Table 1). AA also increased more in

Table 2 Percentage changes in some membrane fatty acids in 4 subjects taking EPA (2 males and 2 females) who showed symptom improvement of more than 20%. Increases in EPA, DPA and DHA were greater in these subjects than in the total EPA group (Table 1)

		Baseline	%Change
AA	20:4n-6	7.15±5.56	46.94
EPA	20:5*n-3*	0.29±0.29	1,210.45
DPA	22:5*n-3*	0.98±0.94	383.83
DHA	22:6*n-3*	3.06±2.83	66.06

these subjects (46.94%) compared to the total EPA group (22.59%). In addition, these subjects achieved a favourable ratio of PUFAs to saturated fatty acids (SATS) in their erythrocyte membranes (Fig. 1), leading to a bell-shaped curve of disease outcome related to the ratio of membrane fatty acids. Two patients were outliers and were not taken into account on the graph in Fig. 1. However, when they were included the bell-shaped effect was still evident.

Males vs females

We investigated the changes in fatty acids in females and males separately (Table 3) because of previously reported gender differences (Horrobin1999). Females incorporated more EPA into their membranes than the males (1,553.98 vs 1,123.02%) and produced more DPA (351.19 vs 320.91%), more DHA (51.0 vs 27.52%) and more AA (25.61 vs 21.63%). EPA and DPA increased significantly in males, but DHA did not (Table 3). The number of females in the EPA group was too small (n=4) to show significance.

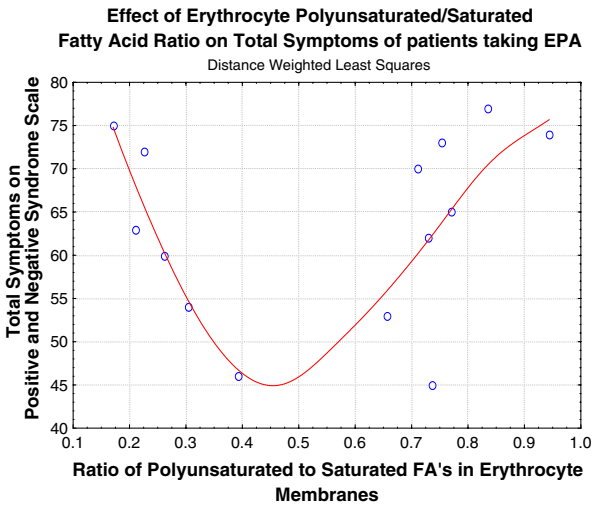

Fig. 1 Patients taking EPA (n=16) responded differently to EPA uptake into erythrocyte membranes. In 14 of the 16 subjects, a bell-shaped curve was obtained when the ratio of PUFAs to saturated FAs was plotted against PANSS Total Symptoms. Two patients were outliers and were not included on the graph

Table 3 Percentage changes of some fatty acids in erythrocyte membranes in females and males in the EPA group after 12 weeks of treatment with EPA. The increases in EPA, DPA and DHA were greater in females taking EPA than in males. EPA and DPA increased significantly in males, but DHA did not increase significantly

		Females(n=4)		Males(n=12)	
		%Change	p	%Change	p
AA	20:4*n-6*	25.61	1.000	21.63	0.286
EPA	20:5*n-3*	1,553.98	0.068	1,123.02	0.003
DPA	22:5*n-3*	351.19	0.068	320.91	0.004
DHA	22:6*n-3*	51	1.000	27.52	0.424

Change in the ratio of AA to EPA

The change in AA/EPA ratio for the patients taking EPA (-31.33) was similar to Fenton et al.'s result (-29.4), but the patients taking placebo differed: we calculated -0.06 (i.e. very little difference) while in Fenton et al.'s group the value was -4.0, suggesting that their controls also incorporated some EPA into their erythrocyte membranes (Fenton et al. 2001).

Correlations between fatty acid concentrations in erythrocyte membranes and symptoms of schizophrenia

Spearman Rank correlations between changes in erythrocyte membrane FAs and patient symptoms, measured by the sub-scales of the PANSS, are shown in Table 4. Some significant correlations between symptom improvements and PUFA concentrations (shown in bold) were found for PANSS Total scores, Positive Symptoms (e.g. hallucinations and thought disorder), Negative Symptoms (e.g. poverty of speech and emotional withdrawal) as well as dyskinesia scores. General Psychopathology Symptoms (e.g. anxiety and depression) showed a trend towards significance (p=0.06) in the "all patients" group.

Significant correlations were obtained in the females more often than in males even though the number of females (n=11) was less than the males (n=21), because the erythrocyte membranes of the females reacted more uniformly than those of the males in each case. A typical graph is shown in Fig. 2 (correlation between the improvement in Total Symptoms versus increased EPA levels). In the case of the females the points representing the individuals are distributed close to the line, whereas in the males the points are scattered.

Discussion

In the present study it was found that ingestion of EPA (3 g/day) caused significant changes in the FA profile of erythrocyte membranes in subjects with schizophrenia. The saturated FAs and mono-unsaturated FAs were probably displaced from the

Table 4 Spearman correlations between changes in fatty acids in erythrocyte membranes and changes (Δ) in patient symptoms (Subscales of the PANSS) for males and females, as well as for the total group (All). Significant correlations are shown in bold

Pair of variables	Male (n=21)		Female (n=11)		All (n=32)	
	R	p-value	R	p-value	R	p-value
Saturates						
18:0&Δnegativesymptoms	0.04	0.849	-0.74	**0.009**	-0.24	0.181
18:0&Δtotalsymptoms	-0.20	0.394	-0.61	**0.047**	-0.28	0.127
20:0&Δtotalsymptoms	-0.34	0.128	-0.40	0.223	-0.37	**0.040**
20:0&Δpositivesymptoms	-0.30	0.184	-0.63	**0.038**	-0.42	**0.018**
Monounsaturates						
18:1&Δpositivesymptoms	-0.22	0.349	-0.66	**0.027**	-0.32	0.075
20:1&Δpositivesymptoms	0.46	**0.036**	-0.50	0.116	0.07	0.688
20:1&Δnegativesymptoms	-0.24	0.305	-0.66	**0.028**	-0.39	**0.027**
20:1&Δdyskinesia	-0.61	**0.004**	-0.32	0.340	-0.48	**0.005**
24:1&Δdyskinesia	-0.49	**0.024**	0.21	0.538	-0.34	0.059
n-3fattyacids						
18:3*n-3*&Δdyskinesia	0.36	0.111	0.33	0.324	0.38	**0.030**
20:5&Δgeneralsymptoms	0.39	0.080	0.42	0.201	0.33	0.060
20:5&Δtotalsymptoms	0.43	0.050	0.75	**0.007**	0.46	**0.008**
20:5&Δpositivesymptoms	0.19	0.411	0.63	**0.038**	0.31	0.084
20:5&Δnegativesymptoms	0.54	**0.012**	0.67	**0.025**	0.51	**0.003**
22:5&Δtotalsymptoms	0.48	**0.029**	0.60	0.051	0.48	**0.005**
22:5&Δnegativesymptoms	0.56	**0.008**	0.60	0.052	0.59	**0.000**

membranes by EPA, although it cannot be ruled out that the endogenous synthesis of FAs changed with EPA supplementation. Furthermore, the changes of FAs in erythrocyte membranes correlated with clinical improvement in the patients as measured by the sub-scales of the PANSS. Although the number of patients used for the study is relatively small and many variables were analysed, these results are of interest in the context of similar findings from previous reports, and may provide a platform for more detailed prospective experiments. Reduced concentrations of PUFAs in schizophrenia have previously been demonstrated by several researchers (Peet 2008). Khan et al. (2002) demonstrated differences in erythrocyte membranes of schizophrenic patients compared to normal controls, both in never-medicated first-episode patients and after years of antipsychotic treatment. Similar changes in PUFAs in erythrocyte membranes also correlated with *in vivo* changes in neuronal membrane phospholipids as determined by Magnetic Resonance Spectroscopy (Yao et al. 2002), suggesting a direct correlation of composition of membrane lipids with brain function.

In the present study the concentrations of PUFAs in erythrocyte membranes increased significantly in patients taking EPA, while a non-significant increase in

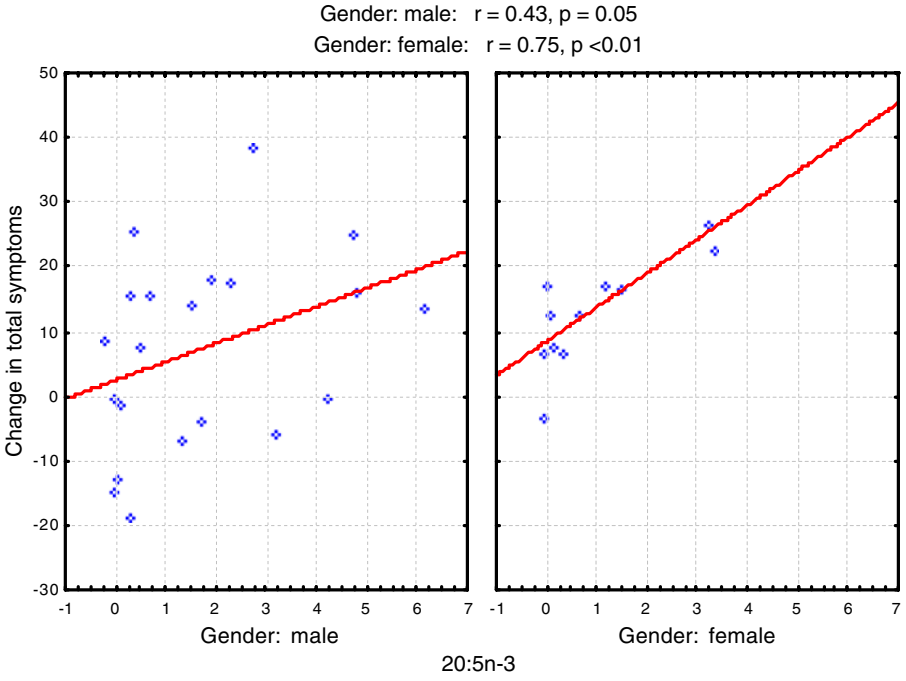

Fig. 2 Response of males and females to EPA supplementation. Typical graph of correlation of improvement in PANSS Total Symptoms versus EPA levels. In the case of the females the points representing the individuals are distributed close to the line, whereas in the males the points are scattered

PUFAs was found in the placebo group. Four patients improved more than 20% on overall symptoms following EPA supplementation, while the increase in their membrane n-3 FA concentrations was greater than in the total EPA group. In these patients, a balance was achieved in the ratio of total PUFAs to saturated fatty acids in the erythrocyte membranes (Fig. 1). The bell-shaped graph suggests that patients did not metabolise and incorporate dietary FAs to the same extent. Further studies would be needed to elucidate whether genetic differences have an effect on these parameters.

Unexpectedly, one of the patients in the placebo group also improved more than 20% on overall symptoms, while exhibiting increased levels of n-3 and n-6 FAs in his erythrocyte membranes (results not shown). This could possibly be attributed to the fact that when informed consent was obtained from the patients, the role of EPA in schizophrenia was discussed with them. The concept was undoubtedly reinforced when the dietician completed the dietary questionnaires. It may be therefore that patients from the control group could have increased their fish intake during the study, especially the patients who lived at home. It would be difficult to overcome such an occurrence in a trial such as this, since information cannot be withheld from the patients. We speculate that this may also have contributed to the failure of the trial by Fenton et al. (2001) to show significant differences between patients on EPA and placebo, since the change in EPA/AA ratio (-4.0) in their control group indicates

that their controls incorporated some EPA into their red cell membranes. The fact that half of our patients were inpatients consuming a standard diet may have reduced that possibility in our trial.

In a previous trial by Horrobin et al. (2002), increased AA and total n-6 FAs correlated with symptom improvement, while the concentration of EPA taken by the patients had a substantial effect on the final concentrations in erythrocyte membranes. Whereas 1 and 2 g/day of EPA increased AA in the membranes, 4 g of EPA per day caused a decrease of AA. In our trial the patients took 3 g/day, which may explain why AA did not increase significantly in our EPA group. However, in the patients who improved more than 20%, the increase in AA was greater than in the total EPA group (Table 2).

The statistical correlations found in our trial connecting improvement in symptoms of schizophrenia with concentrations of FAs in erythrocyte membranes (which in turn have been shown to correlate with *in vivo* brain lipids as shown in the study by Yao et al. 2002) confirms the relationship between membrane structure and function of the brain. As a whole group (n=32), the increase in membrane EPA correlated significantly with the change in PANSS Negative Symptom Score and with the change in PANSS Total Score, but not with the PANSS Positive Score or PANSS General Psychopathology Score, although there was a trend towards significance in the latter (Table 4).

The change in dyskinesia score did not correlate with an increase in EPA, but with an increase in 18:3*n-3*, and highly significantly with the decrease in 20:1*n-9* in all patients. A follow-up study using 2 g/day of EPA did not repeat the significant improvement in tardive dyskinesia (TD) in patients compared to placebo (Emsley et al. 2006), although a modest benefit was suggested in patients with more recent onset of TD. Some previous studies similarly suggested a relationship between membrane FAs, schizophrenia symptoms and dyskinesia. Mellor et al. (1995) found that a greater intake of EPA in the normal diet was associated with fewer positive symptoms as well as less TD. Vaddadi et al. (1996) showed that patients with TD had significantly lower n-3 and n-6 FA concentrations in their erythrocyte membranes compared with healthy controls and psychiatric controls without TD. It was postulated that the majority of chronic schizophrenics had an inherent deficit in incorporating essential FAs into neuronal membranes, thus affecting brain development and increasing their vulnerability to develop super-sensitivity to neuroleptics and therefore, TD.

The problem of incorporating PUFAs into membranes was confirmed in the present study in which an accumulation of DPA (22:5*n-3*) but unresponsiveness of DHA (22:6*n-3*) was found (Table 1). Since DHA is synthesised from DPA through a process of elongation and delta-6 desaturation, and finally beta-oxidation in the peroxisomes, this may indicate a dysfunction either in the synthesis of DHA, or in the incorporation of DHA into cell membranes. The latter possibility may explain the ineffectiveness of DHA supplementation in schizophrenia.

Horrobin (1999) previously reported that AA and DHA are more readily synthesised from their precursors and more readily incorporated into phospholipids in females than in males. In the present study we found similar results (Table 3). The mean age of the female patients was 43.1±14.9 years, range 18 – 64. Six of the eleven females were menopausal, suggesting that estrogen may not be the only

factor rendering the females more responsive to dietary EPA as seen by their improvement in symptoms, compared to males (Table 4).

It has been proposed that the abnormalities in fatty acid metabolism in schizophrenia are caused by an increased activity of PLA_2. The release of AA by PLA_2 is the rate limiting step in the generation of eicosanoids and platelet activating factor, which play critical roles in the initation and modulation of inflammation and oxidative stress (Farooqui et al. 2006). Enhanced oxidative stress has been demonstrated in the aetiology and outcome of schizophrenia. Khan et al. (2002) showed that lower PUFA levels in their patients were associated with significantly higher levels of TBARS, a measure of lipid peroxidation. Ranjekar et al. (2003) demonstrated decreased levels of antioxidant defense enzymes, together with decreased levels of EPA and DHA in Indian schizophrenic and bipolar mood disorder patients compared with controls, although a study by Peet et al. (2004) did not find a similar reduction in EPA and DHA in schizophrenic subjects in India and Malasia. Puri et al. (2008) demonstrated highly significantly increased levels of ethane, a marker of n-3 lipid peroxidation, in the breath of patients with schizophrenia.

In patients treated with haloperidol, which has been suggested to increase oxidative stress, additional treatment with PUFAs, vitamins E and C resulted in a significant improvement in symptoms of schizophrenia as well as the Barnes Akathisia Rating Scale, and ameliorated the side-effects of haloperidol (Sivrioglu et al. 2007). Yao et al. (2004) found that EPA supplementation enhanced serotonin responsivity and inhibited ADP-induced platelet aggregation in patients with chronic schizophrenia, and suggested that EPA may mediate its therapeutic effects via modulation of the 5-HT receptor complex, since there was a significant inverse correlation between 5-HT responsivity and psychosis severity in unmedicated patients.

Atypical antipsychotic medication may increase the levels of PUFAs in cell membranes. Respiridone treatment increased erythrocyte and prefrontal cortex n-3 FAs in a rat model (McNamara et al. 2009). Evans et al. (2003) found reduced levels of antioxidant enzymes, associated with increased plasma lipid peroxides and reduced membrane PUFAs in 16 first-episode psychotic patients at baseline, which normalised after 6 months of antipsychotic treatment (risperidone and olanzapine). These authors advised the augmentation of antipsychotic treatment by supplementation with a combination of antioxidants and n-3 FAs.

Other dietary factors have also recently been implicated in the aetiology and outcome of schizophrenia, notably folate and vitamin B12, as well as hyperhomocysteinemia (Levine et al. 2006). Supplementation with folic acid, vitamin B12 and vitamin B6 showed favourable outcomes in patients. Kemperman et al. (2006) found both n-3 FA deficiencies and hyperhomocysteinemia in their patient cohort. Peet (2008) has emphasised the poor nutritional status of patients, leading to multiple nutritional deficiencies which may aggravate mental disorders. Furthermore, it has become evident that the poor diet of subjects with schizophrenia in Western communities may lead to deficits of essential nutrients (Kemperman et al. 2006), thereby providing (or enhancing) environmental triggers for metabolic syndrome and cardiovascular disease (De Hert et al. 2009; von Hausswolff-Juhlin et al. 2009) in people with a genetic predisposition to schizophrenia. In the present study, the dietary survey also exposed the low n-3 dietary intake by the patients in our study.

Conclusion

The results of the present study demonstrate the uptake of EPA into erythrocyte membranes, with concomitant changes in other FAs. The improvement in symptoms of the patients taking the EPA was associated with changes in membrane FAs. However, supplementation with EPA did not result in significantly higher concentrations of DHA, indicating that there may be genetic defects in the enzymes responsible for the conversion of EPA to DHA. The results also support our previously reported clinical findings (Emsley et al. 2002) suggesting that EPA may be an effective and well-tolerated add-on treatment in schizophrenia.

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