Reduced Red Blood Cell Membrane Essential Polyunsaturated Fatty Acids in First Episode Schizophrenia at Neuroleptic-Naive Baseline

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Abstract

There is emerging evidence in schizophrenia of membrane abnormalities, primarily reductions in the essential omega-3 and omega-6 series of polyunsaturated fatty acids (PUFA). Because previous studies have largely been in chronic patients, it is not known whether these membrane abnormalities also occur early in illness. In the present study, red blood cell membrane fatty acid levels were determined by capillary gas chromatography from 24 neuroleptic-naive patients with first episode schizophrenia or schizoaffective disorder and 31 age-matched normal controls. Relative to normal subjects, patients had significant reductions in total PUFA (–13%) but not in monounsaturated or saturated fatty acids. Specifically, significant reductions were found in arachidonic acid (–18%), docosapentaenoic acid (–36%), and docosahexaenoic acid (–26%) concentrations. These reductions were not related to age, gender, smoking status, or cotinine levels. These results confirm previous findings of membrane deficits in schizophrenia and show that significant PUFA reductions occur early in the illness, prior to initiation of treatment, raising the possibility that these deficits are trait related. The findings also suggest that membrane fatty acid losses are quite specific to the highly unsaturated fatty acids.

Keywords: Red blood cell, fatty acid composition, polyunsaturated fatty acids, first episode schizophrenia.

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Several studies have identified a variety of deficits in membrane polyunsaturated fatty acids (PUFA) in patients with schizophrenia (Horrobin et al. 1991; Glen et al. 1994; Yao et al. 1994, 2000; Mahadik et al. 1996). The significance of these findings lies in the fact that PUFA are the major constituents of cell membrane phospholipids such as phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol, which make up over 80 percent of total phospholipids. The dynamic functional state of cellular, mitochondrial, and nuclear lipid bilayer membranes is dependent on the membrane's composition. As membrane constituents, PUFA have important biological roles: receptor binding, neurotransmission, signal transduction, and eicosanoid synthesis (Horrobin 1998). Thus, even small changes in key essential fatty acids that make up phospholipids can lead to a broad range of membrane dysfunctions. Deficits in membrane PUFA may underlie many biological, physiological, and clinical phenomena observed in some neuropsychiatric disorders, most notably schizophrenia (Horrobin 1996).

Essential fatty acids cannot be manufactured de novo in the body and must be provided by diet. The two types of essential PUFA, defined on the basis of the first double-bond position, are the omega-6 (or n-6) and omega-3 (or n-3) series. The PUFA are desaturated and elongated derivatives of linoleic acid (n-6) and α-linolenic acid (n-3) (figure 1). Neuronal membrane phospholipids contain substantial proportions of saturated fatty acids and PUFA, primarily arachidonic acid (AA), 20:4(n-6), and docosahexaenoic acid (DHA), 22:6(n-3). In addition to helping maintain normal membrane structure and function, PUFA also are critical in all aspects of normal brain development (Tacconi et al. 1997; Uauy et al. 2001). The developing brain in utero is entirely dependent on the maternal source of essential fatty acids.

Much of the evidence showing reductions in red blood cell (RBC) membrane fatty acid levels has come from studies in chronic schizophrenia patients (Yao and Reddy 2000), where a preponderance of the subjects were receiving antipsychotic agents. Thus, it has not been possible to determine whether the membrane fatty acid deficits are present early in the course of illness and there-

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Figure 1. An outline of the synthesis of highly unsaturated essential fatty acids

n-6 fatty acid series		n-3 fatty acid series
Linoleic acid 18:2(n-6)	*Δ6-desaturase*	α-Linolenic acid 18:3(n-3)
↓		↓
γ-Linoleic acid 18:3(n-6)		Stearidonic acid 18:4(n-3)
↓	*Elongase*	↓
Dihomo-γ-linoleic acid 20:3(n-6)		Eicosatetraenoic acid 20:4(n-3)
↓	*Δ5-desaturase*	↓
Arachidonic acid 20:4(n-6)		Eicosapentaenoic acid 20:5(n-3)
↓	*Elongase*	↓
Adrenic acid 22:4(n-6)		Docosapentaenoic acid 22:5(n-3)
↓	*Δ4-desaturase*	↓
Docosapentaenoic acid 22:5(n-6)		Docosahexaenoic acid 22:6(n-3)

fore are not a consequence of treatment or progression of the illness. We hypothesized that there would be reductions in RBC AA and DHA in first episode schizophrenia patients, prior to the initiation of treatment, when compared to normal control subjects.

Methods

Clinical Design. Twenty-four patients were recruited at first episode of psychosis after they provisionally met *DSM–IV* (APA 1994) criteria for schizophrenia, schizophreniform, or schizoaffective disorder based on the Structured Clinical Interview for *DSM–III–R* (Spitzer 1990). The initial diagnostic assessments were performed by experienced research clinicians. Initial and followup diagnoses were confirmed at a diagnostic conference attended by research faculty and staff and chaired by one of the authors (M.S.K.). Normal control subjects were recruited through local advertisement. We excluded subjects with previous exposure to antipsychotic agents, substance abuse during the preceding 6 months, systemic medical illness requiring treatment, and neurological disorders. Additional exclusion criteria for normal subjects included personal or family history of psychosis. Written informed consent was obtained from all subjects after full explanation of the study. The study was carried out in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of the University of Pittsburgh. Blood samples were obtained in patients immediately prior to the initiation of antipsychotic agents. The Brief Psychiatric Rating Scale (BPRS; Overall and Gorham 1962) and the Global Assessment Scale (GAS; Endicott et al. 1976) were administered at neuroleptic-naive baseline, blind to any information regarding the biochemical assays.

Twenty-four patients participated in the study. Twenty-one patients met *DSM–IV* criteria of schizophrenia disorder, and 3 patients met criteria for schizoaffective disorder. Thirty-one normal control subjects were recruited via advertisement in the local community. The majority of control subjects were not recruited from within the health care system. There were 17 male and 7 female patients, and 20 male and 11 female normal control subjects; there was no significant difference in gender distribution. The mean age of patients was 27.8 (standard deviation [SD] 7.8) years and of control subjects was 27.0 (SD 8.0) years. The onset of psychosis in patients was at a mean age of 24.8 (SD 8.6) years. The years of education was significantly lower in patients (13.3 ± 2.7 years) than control subjects (15.6 ± 4.0 years). Height, in meters, was identical between the groups at 1.7 ± 0.3 meters in controls and 1.7 ± 0.1 meters in patients. Weight, in kilograms, was 75.5 ± 21.3 in control subjects and 72.2 ±

12.6 in patients; this was not statistically significantly different between the groups. Body mass index (BMI) was 26.0 ± 4.8 in controls and 24.0 ± 3.3 in patients and was not statistically significantly different. Among patients, 11 (46%) smoked and 13 (54%) did not. Among control subjects, 2 (6%) of 31 smoked. We used levels of plasma cotinine, an index of smoking, in examining relations between smoking and RBC PUFA. Because so few normal control subjects smoked, we examined the potential effects of smoking on RBC PUFA in patients only.

Biochemical Measurement

Sample preparation. Freshly drawn blood with anticoagulant citrate dextrose was centrifuged at 750g for 7 minutes to remove platelet-rich plasma and leukocytes. Hemoglobin-free RBC ghost membranes were prepared by the method of Dodge et al. (1963). Lipid extraction of RBC ghost membranes was performed according to the procedure of Rose and Oklander (1965). Fatty acid methyl esters (FAME) were prepared using methanolic KOH reagent as described by Ichihara et al. (1996). Diheptadecanoyl lecithin (Matraya) was used as an internal standard.

Capillary gas chromatography. The method used to determine levels of RBC membrane fatty acids was essentially the same as described by Yao et al. (1994, 2000). In brief, all the FAME were analyzed on a Hewlett-Packard capillary gas chromatograph, Model 5890 Series II, equipped with a hydrogen flame ionization detector. A 30-meter, fused silica SP–2380 column with an inner diameter of 0.25 mm and a 0.20-μm film thickness (Supelco) was used. Each sample was run under a splitless injection mode with hydrogen as the carrier gas (30 mL/minute) and with an inlet pressure of 6.5 psi. Oven temperature was programmed under three stages: stage 1, from 50 to 150°C at a rate of 25°C/minute; stage 2, from 150 to 190°C at a rate of 4°C/minute; and stage 3, from 190 to 250°C at a rate of 6°C/minute, with a final time of 3 minutes at 250°C. Peaks on the chromatograms were identified by comparing the retention times with those of standard mixtures (Supelco) and were calculated by an Agilent ChemStation, Rev. A.09.03, using an internal standard mode.

Plasma cotinine level. Cotinine levels, an index of cigarette smoking, were assayed simultaneous to PUFA assays. Cotinine is the major metabolite of nicotine. The half-life of cotinine in blood is far longer than that of nicotine (Langone et al. 1973; Langone and Van Vunakis 1975). Moreover, cotinine levels remain fairly constant in individuals with regular tobacco consumption. Thus, plasma cotinine levels provide us with a better marker than nicotine for smoking status (Langone et al. 1973; Hall et al. 1984; Jarvis et al. 1987). The Diagnostic Reagents (DRI; Sunnyvale, CA) enzyme immunoassay kit is applied to measure plasma cotinine level. The DRI immunoassay is based on the competition between a cotinine-labeled enzyme glucose–6-phosphate dehydrogenase (G–6-PDH) and the free cotinine in the sample for a fixed number of cotinine-specific antibody binding sites. In the absence of cotinine, the cotinine-labeled G–6-PDH is bound to the antibody and the enzyme activity is inhibited. The G–6-PDH activity is measured spectrophotometrically at 340 nm by converting Nicotinamide Adenine Dinucleotide (NAD) to reduced NAD (NADH). The plasma sample is first centrifuged to remove any interfering debris. In a typical assay, 20 μL of sample is used. The standard curve is obtained from the cotinine calibrators provided by the DRI kit. A normal plasma sample spiked with a known amount of cotinine standard is used as a control for each assay. A Cobas Fara centrifugal analyzer (Roche Diagnostics Systems, Branchburg, NJ) is used to measure the enzymatic reaction rate at 37°C.

Statistics. The data are expressed as mean ± SD. Testing for normality was accomplished by normality plots and the Kolmogorov-Smirnov test, which quantifies the discrepancy between data distribution and an ideal Gaussian distribution. The Dallal and Wilkinson approximation to the Lilliefors method was used to compute the p values. The data pass the normality test if $p > 0.10$. Between-group comparisons were done using a 2-tailed Student t test. Correlation between variables was studied using Pearson correlation analysis. When data was non-normally distributed, nonparametric statistics were employed to analyze them. Correlations between smoking and RBC PUFA were examined in patients only because of very low rates of smoking (6%) in the normal control subjects.

Results

There were no significant differences in saturated, monounsaturated, and total fatty acid concentrations (nmol/mL packed RBC) between patients and control subjects (table 1). By contrast, patients had significant reduction (–13%) in total PUFA (table 1).

Among the PUFA assayed, statistically significant reductions in patients were observed with AA (–18%, relative to mean value of normal subjects), docosapentaenoic acid (–36%), and DHA (–26%) (table 1). There were no statistically significant correlations between age, age of onset of illness, and BMI on these measures. There were no gender effects on any of these PUFA measures.

Eleven of 24 patients (46%) and 2 of 31 normal subjects (6%) were smokers. The proportion of normal subjects who smoked was too low to permit statistical analyses. Therefore, relations between smoking, using plasma

Table 1. Fatty acids and essential PUFA distribution in red blood cell membranes of normal controls and first episode neuroleptic-naive schizophrenia patients

Fatty acids	Normal controls (n = 31), mean ± SD	Patients (n = 24), mean ± SD	p (2-tailed t tests)
Saturated	1,243 ± 287[1]	1,280 ± 303	*ns*
Monounsaturated	471 ± 97	477 ± 121	*ns*
Polyunsaturated[2]	896 ± 148	782 ± 193	0.020
Total fatty acids	2,611 ± 451	2,538 ± 519	*ns*
Linoleic acid, 18:2(n-6)	317 ± 712	311 ± 106	0.810
γ-Linoleic acid, 18:3(n-6)	40 ± 12	39 ± 17	0.747
Arachidonic acid, 20:4(n-6)	339 ± 61	278 ± 69	0.001
Adrenic acid, 22:4(n-6)	60 ± 18	59 ± 26	0.816
Docosapentaenoic acid, 22:5(n-3)	69 ± 37	44 ± 17	0.002
Docosahexaenoic acid, 22:6(n-3)	61 ± 24	45 ± 16	0.003

Note.—*ns* = nonsignificant; PUFA = polyunsaturated fatty acids; SD = standard deviation.

[1] nmol/mL packed red blood cells.

[2] Sum of fatty acids with more than one double bond.

cotinine level as an index for smoking, and PUFA were examined in only patients. There were no significant differences in PUFA levels between smoking and nonsmoking patients. There were no statistically significant correlations between plasma cotinine and PUFA levels.

Whether reductions in specific PUFA were due to alterations in the metabolic pathways (i.e., changes in the relative proportions of the fatty acids with a PUFA series), the following parameters were used as biochemical indexes of product-substrate relationship for elongation and desaturation in the PUFA pathways (figure 1): (1) ratio of 18:0/16:0—saturated fatty acids; (2) ratio of 22:6/22:5—the n-3 family; (3) ratios of 20:4/18:2, 20:4/20:3, and 22:4/20:4—the n-6 family; and (4) ratio of 18:1/18:0—the n-9 family. The ratio of 20:4(n-6)/18:2(n-6) was significantly lower in patients than in normal control subjects. In addition, the ratio of 18:0/16:0 was also significantly reduced in the patient group. No significant differences were observed in the ratios of 18:1/18:0, 20:4(n-6)/20:3(n-6), 22:4(n-6)/20:4(n-6), and 22:6(n-3)/22:5(n-3) between normal control subjects and schizophrenia patients.

To examine whether the levels of RBC PUFA in schizophrenia patients are related to their severity of psychopathology, we examined correlations between levels of 18:2(n-6), 20:4(n-6), 22:5(n-3), and 22:6(n-3) with various BPRS items and the GAS. There were no significant relations between PUFA measures and the BPRS or the GAS.

Discussion

Although there has been accumulating evidence for a variety of membrane PUFA deficits in schizophrenia, the evidence was based on studies of chronic patients who were being treated with antipsychotic agents, leaving open the question of whether the PUFA reductions were related to treatment effects. The current study demonstrates that there are indeed significant reductions in key RBC membrane fatty acids in schizophrenia patients at first episode with no prior exposure to antipsychotic drugs. Reductions in RBC membrane DHA and AA observed in this study are highly consistent with the findings of reductions in RBC AA and DHA in 20 never-medicated schizophrenia patients in India (Arvindakshan et al. 2003) and 22 never-medicated schizophrenia patients in the United States (Khan et al. 2002), as well as previous similar findings in chronic schizophrenia patients (Yao and Reddy 2000). The finding of reduced RBC membrane AA and DHA in both neuroleptic-naive and treated patients suggests that the membrane deficits that have been detected prior to treatment continue after initiation of antipsychotic treatment (Arvindakshan et al. 2003). Whether the mechanisms that lead to reduced PUFA early in the course of illness are the same as those operating later in illness is not known.

It is possible that differences between patients and healthy subjects in this study can be accounted for by environmental factors, such as diet, which is known to affect RBC membrane PUFA levels (Dougherty et al. 1987; Stanford et al. 1991). In a recent study of 81 patients with chronic schizophrenia, Strassnig et al. (2003) found higher total calorie intake and higher fat intake compared with norms for the U.S. population. Further analyses of the patients' diet indicated normal or higher intake of n-3 and n-6 PUFA (M. Strassnig 2003, unpublished). Whether the dietary findings in chronic patients with long treatment histories have the same

implications for first episode patients is not known. In the present study, however, there was a trend ($p = 0.07$) for a higher BMI in the normal control subjects (26 ± 4.8) than in the first episode patients (24.0 ± 3.3). However, BMI was not related to RBC n-3 and n-6 PUFA in either healthy controls or patients. The Western diet is particularly rich in AA (Simopoulos 2000). Furthermore, under normal physiological conditions, there is a selective retention of AA in phospholipids (Zhou and Nilsson 2001). Even if patients had altered their diet preceding entry to the study, it is unlikely that the diet would be deficient in AA. Furthermore, a dietary deficiency of 18:2(n-6) usually leads to a higher content of trienoic acids of the n-9 group, which can be synthesized endogenously (Holman 1973). An increased level of 20:3(n-9) was not demonstrated either in RBC ghost membranes of our schizophrenia patients or in plasma samples of other schizophrenia patient groups (Horrobin et al. 1989; Kaiya et al. 1991). Thus, the observed reductions in RBC essential PUFA in patients are less likely to be due to dietary modifications alone, although this cannot be ruled out, because we did not use a comprehensive analysis of dietary intake.

Cigarette smoking may affect PUFA levels (Pawlosky et al. 1999). Given the high rates of cigarette smoking in patients with schizophrenia, the potential confounding effect of smoking on PUFA measures in patients with schizophrenia cannot be ignored (Hibbeln et al. 2003). There are a number of mechanisms by which smoking can reduce membrane PUFA. Cigarette smoke is highly prooxidant (Church and Pryor 1985). PUFA are particularly vulnerable to free radical–mediated damage (Thomas 2000). Nicotine has been found to both induce (Sastry and Hemontolor 1998) and inhibit (Marin et al. 1997) phospholipase A_2 activity. Phospholipase A_2 has been implicated in schizophrenia as a mechanism that can lead to reduced membrane PUFA (Gattaz 1992; Noponen et al. 1993; Ross et al. 1997). The findings from the present study indicate that smoking may not significantly account for the reduced RBC membrane PUFA when cotinine is used as the index of smoking. Although the proportion of the first episode patients smoking (42%) in the present study is lower than the 70 percent to 90 percent reported for patients with schizophrenia in general, it nonetheless is greater than that observed in the general population (Lohr and Flynn 1992). By contrast, Hibbeln et al. (2003) found reductions in DHA and eicosapentaenoic acid (EPA), but not AA, in chronic schizophrenia patients who smoked compared to nonsmokers. However, it is not clear whether schizophrenia patients had reduced RBC PUFA levels independent of smoking status, because there was no normal control group. It is possible that smoking may have differential effects on PUFA depending on the phase of illness, treatment status, and smoking duration. Similarly, gender may confound the findings because of variable dietary, smoking, or physiological factors. However, there were no gender differences in PUFA levels.

Reductions in specific PUFA could be due to disturbances in the conversion of one fatty acid to another within the metabolic pathway (figure 1). Accumulation of a substrate would suggest that the enzyme (in this case, either a Δsaturase or elongase) may be the site of the problem. For example, the ratio of 20:4(n-6)/18:2(n-6) provides a biochemical index of product-substrate relationship in the n-6 PUFA pathway. The n-6 pathway consists of a series of desaturations and elongations. In normal RBC membranes, the levels of 18:3(n-6) and 22:5(n-6) are very minute. The major metabolic product of 18:2(n-6) is 20:4(n-6). Therefore, a decreased ratio of 20:4(n-6)/18:2(n-6) associated with a normal ratio of 22:4(n-6)/20:4(n-6) suggests a defect in the desaturation steps of the n-6 metabolic pathway. Formed by a final desaturation at Δ4 are 22:6(n-3) and 22:5(n-6). These reactions, however, follow elongations and Δ5 desaturations in both the n-3 and the n-6 series. The ratio of 22:6(n-3)/22:5(n-3) was not significantly different between schizophrenia patients and normal control subjects. Therefore, decreases in 22:5(n-3) and 22:6(n-3) may be caused, at least in part, by the decreased formation of 20:5(n-3), which is derived from 20:4(n-3) by the Δ5 reaction. Recently, dietary supplementation with EPA has shown promise in ameliorating some of the clinical symptoms of schizophrenia (Peet et al. 1996, 2001; Puri et al. 2000). Furthermore, it is interesting to note that the clinical response to EPA supplementation appears to parallel increases in RBC levels of AA (Horrobin et al. 2001).

Previous studies in recent-onset schizophrenia patients have shown reductions in fibroblast membrane AA as well as reductions in key membrane phospholipids (Mahadik et al. 1994). Specifically, these studies noted decreased RBC membrane phosphatidylethanolamine (Keshavan et al. 1993) and decreased fibroblast membrane phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, and phosphatidylinositol (Mahadik et al. 1996). Thus, the current findings are consistent with earlier reports of membrane phospholipid and fatty acid deficits occurring early in the course of schizophrenia. In a recent study of young treated schizophrenia patients with a mean duration of illness of less than 1 year, RBC membrane DHA and docosapentaenoic acid were significantly reduced, but not AA (Assies et al. 2001). It is noteworthy that treatment with atypical antipsychotics, such as olanzapine or clozapine, is associated with increases in AA in patients (Horrobin et al. 2001) and rats (Mahadik et al. 2001). Such treatment-related increases in AA may account for the lack of differences in AA levels between

patients and control subjects by Assies et al. (2001). Of the 19 patients in their study, 11 were being treated with olanzapine, 4 with risperidone, and 1 with clozapine.

The current findings are also consistent with the evidence to date implicating membrane deficits in schizophrenia. Reductions in RBC membrane AA, linoleic acid (the precursor of AA), and DHA have been reported by several investigators (Yao and Reddy 2000). Postmortem studies have shown reductions in neuronal membrane PUFA in the frontal cortex (Horrobin et al. 1991) and the caudate (Yao et al. 2002). Highly suggestive evidence of brain phospholipid abnormalities in schizophrenia comes from studies using magnetic resonance spectroscopy (MRS). Pettegrew et al. (1991) first demonstrated a significant reduction of phosphomonoesters (PME; phospholipid precursors) and significantly increased levels of phosphodiesters (phospholipid breakdown products) in frontal cortices of neuroleptic-naive first episode schizophrenia patients relative to controls. Other groups also reported similar findings of membrane phospholipid perturbations in both acutely and chronically ill patients (Keshavan et al. 2000).

Whether the reductions in RBC membrane PUFA are state related or trait related is important and cannot be definitively resolved by the present study. However, the finding of reduced membrane phospholipids in skin fibroblasts of first episode neuroleptic-naive schizophrenia patients lends support to the notion that membrane deficits are trait related (Mahadik et al. 1994). A further suggestion that membrane deficits are trait related comes from the finding that membrane phospholipid perturbations, observed via ^{31}P MRS, have been detected prior to the onset of illness (Keshavan et al. 1993). It is also evident that membrane deficits seen as early as at first episode of illness persist later into the illness, because similar reductions in PUFA are seen in chronic patients, as well as in the postmortem brain. A recent study comparing treatment-naive first episode patients and chronic schizophrenia patients, relative to normal control subjects, found significant reductions in AA and DHA in first episode patients but reductions in only DHA in chronic patients, albeit of a lesser magnitude (Arvindakshan et al. 2003). They suggest that the differences between the two groups are attributable to treatment effects. While this is plausible, the absence of a within-subject longitudinal assessment of treatment effects leaves the question unanswered. While it is possible that treatment can contribute to membrane deficits, this is unlikely to be the primary cause. Similarly, illness progression alone cannot account for the findings to date.

Membrane perturbations have been described in a number of other psychiatric conditions, such as depression (Edwards et al. 1998; Mamalakis et al. 2002), attention deficit hyperactivity disorder (ADHD; Stevens et al. 1995), and autism (Vancassel et al. 2001). The issue of whether the observed membrane PUFA reductions are specific to schizophrenia cannot be answered by this study. Treatment with PUFA supplementation has shown initial positive results in depression (Peet and Horrobin 2002), bipolar disorder (Stoll et al. 1999), borderline personality disorder (Zanarini and Frankenburg 2003), and ADHD (Richardson and Puri 2002). The findings from these studies appear to suggest that PUFA supplementation, primarily EPA, may be a nonspecific palliative agent. Even so, this in itself would be a valuable adjunct to current treatment of these conditions. However, it is possible that EPA may act differentially in each of the disorders for which it has been tried. EPA has an array of effects on lipid metabolism, such as blocking the actions of phospholipase A_2 and increasing membrane AA (Peet and Horrobin 2002). Membrane perturbations can be a result of oxidative stress (Yao and Reddy 2003). Various indexes of oxidative stress have been found in chronic and first episode schizophrenia patients (Yao and Reddy 2003; Reddy et al. 2003). Arvindakshan et al. (2003) found an inverse relationship between levels of lipid peroxides (index of free radical–mediated fatty acid damage) and RBC DHA and AA in never-medicated patients. Therefore, PUFA reductions in schizophrenia may in part be due to oxidative stress, while PUFA reductions in other disorders may have other pathophysiological mechanisms but still respond to PUFA supplementation.

The clinical implications of the membrane PUFA deficits in schizophrenia have not been fully characterized. RBC PUFA reductions have been associated with positive symptoms, negative symptoms, cognitive impairment, and tardive dyskinesia (see review by Reddy and Yao 1999). An alternative approach to examining the clinical relevance of PUFA deficits in schizophrenia has been to rectify these deficits by the use of fatty acid supplementation. If PUFA supplementation was found to be efficacious, then it could provide further evidence that PUFA deficits were of pathophysiological significance to schizophrenia. Early studies using a variety of PUFA preparations suggested that supplementation had general beneficial effects in patients with psychoses (Soulairac et al. 1983; Bourguignon et al. 1984; Wolkin et al. 1986). Vaddadi et al. (1989) showed a significant improvement of Wechsler Memory Scale scores and psychopathology scores in a group of psychiatric (primarily schizophrenia) patients. Subsequent studies have largely used EPA as the primary adjunctive agent. In an open-labeled study using EPA in schizophrenia patients, Mellor et al. (1995) showed significant improvement of scores on rating scales for both symptomatology and tardive dyskinesia. Puri et al. (2000) have reported sustained remission of positive

and negative symptoms over a 1-year period in a chronic schizophrenia patient treated with EPA alone. In another open-label study (Shah et al. 2001), improvement of total scores on the Positive and Negative Syndrome Scale (PANSS) was noted in chronic schizophrenia patients following neuroleptic treatment in conjunction with EPA supplementation, with maximal response to EPA occurring in the first 6 months of treatment. Furthermore, in some of these patients, reductions in antipsychotic dose was possible. Recent controlled trials have provided further evidence for the efficacy of EPA. Two small double-blind trials of n-3 PUFA supplementation were reported by Peet et al. (2001). In the first study of 45 patients, they compared EPA, DHA, and placebo and observed the greatest improvements in total PANSS, positive symptoms, and general psychopathology in antipsychotic-treated chronic schizophrenia patients with EPA, as compared to DHA and placebo. In the second study, they randomly administered either EPA or placebo to schizophrenia patients who were drug-free. By the end of the study, 12 out of 12 patients on placebo required administration of antipsychotics agents, and 8 out of 14 patients on EPA required antipsychotic drugs. Furthermore, patients taking EPA had significantly lower PANSS total scores at the end of the study. Peet and Horrobin (2002) conducted a randomized, double-blind, placebo-controlled dose-finding study of EPA (1, 2, and 4 g/day) administered for 12 weeks to 115 chronic schizophrenia patients being treated with clozapine (n = 31), typical (n = 48), and atypical antipsychotic agents (n = 36). The 2 g/day dose was most effective in reducing the symptoms. It is noteworthy that clinical improvement was associated primarily with increases in RBC AA but not EPA or DHA. By contrast, Fenton et al. (2001) have recently shown in a double-blind, placebo-controlled trial with 43 patients receiving EPA and 44 patients receiving placebo that there was no greater improvement in residual symptoms and cognitive impairment in those receiving 3 g/day of ethyl EPA as compared to those treated with placebo. Horrobin (2003) has argued that the dose of 3 g/day has been found to be not as efficacious as 2 g/day (Peet and Horrobin 2002). On the other hand, a randomized, double-blind, placebo-controlled study, also using 3 g/day EPA, found in 40 schizophrenia patients significant reductions in PANSS total score and reductions in tardive dyskinesia severity (Emsley et al. 2002). However, further analysis suggested that the reduction in PANSS score was associated with reduction in dyskinesia scores. The significance of this finding is not entirely clear. A broad analysis of the evidence suggests that, on balance, there is merit in continuing to examine whether PUFA supplementation can ameliorate membrane deficits and have beneficial clinical effects in patients with schizophrenia. It is possible that therapeutic benefits will be found when EPA is administered early in the course of illness. Early intervention appears to be critical in achieving better clinical outcomes (Wyatt 1995).

While the above findings suggest that membrane deficits may be relevant to understanding the pathophysiology of schizophrenia, it is unclear whether reductions in RBC PUFA reflect alterations in brain lipid metabolism. Preliminary evidence indicates that RBC AA levels are correlated with cortical PME levels determined by ^{31}P MRS (Yao et al. 2002), suggesting that decreased RBC membrane phospholipid PUFA may reflect a decrease in membrane synthesis of phospholipids in the brain. In addition, PUFA supplementation is associated with structural brain changes (Puri et al. 2000). In a study designed to examine the relative changes of phospholipids and PUFA in rat neural and RBC membrane, Carlson et al. (1986) found that a fatty acid–enriched diet in rats resulted in similar relative changes in phosphatidylethanolamine (PE) and phosphatidylcholine (PC) fatty acid in both neural and RBC membranes. In another study also designed to examine brain and RBC DHA concentration, Araya et al. (1994) found in the rat parallel increases in brain and RBC DHA concentrations. Yeh et al. (1998) found that AA supplementation in rats increased accretion of AA in the brain by 9 percent and in RBC by 25 percent. DHA supplementation increased phospholipid DHA by 24 percent and 54 percent in RBC. In a study of fatty acid–deficient juvenile rhesus monkeys fed a fish oil–rich diet for 129 weeks, DHA increased from week 1 to a maximum at week 26 in the frontal cortex; RBC phospholipid increased in parallel (Connor et al. 1990). Similarly, in 15-day-old piglets, DHA increased in the brain, RBC, and other tissues after the piglets consumed high-DHA milk (Arbuckle and Innis 1993). There appeared to be regional differences in PE AA accumulation after phospholipid-enriched formula was fed to piglets, with the greatest accumulation occurring in the temporal cortex relative to the frontal, parietal, or occipital regions (Goustard-Langelier et al. 1999). In a study of breast-fed and formula-fed term infants who later died, DHA in the cortex and RBC increased in parallel. Furthermore, better neurodevelopment was observed in breast-fed infants (Makrides et al. 1994). In a study of patients with generalized peroxisomal disorders, which are associated with DHA deficiency, it was found that treatment with DHA was associated with normalization of RBC DHA that paralleled the virtual normalization of myelin images on MRI (Martinez and Vazquez 1998). This animal and human evidence suggests that peripheral measures of PUFA may indeed be reflecting central PUFA metabolism. Taken together, these findings indicate that further investigation into the nature and treatment impli-

cations of membrane deficits in schizophrenia are warranted.

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