Smoking, Gender, and Dietary Influences on Erythrocyte Essential Fatty Acid Composition among Patients with Schizophrenia or Schizoaffective Disorder

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Background: *Prior reports of decreased levels of essential fatty acids among schizophrenic patients have generated several hypotheses proposing inherent abnormalities in phospholipid and fatty acid metabolism and have provided the basis for treatment trials; however, these essential fatty acid aberrations may be attributable to uncontrolled factors, such as smoking, rather than abnormalities inherent to schizophrenia.*

Methods: *Erythrocyte fatty acid compositions were quantified in 72 medicated schizophrenic or schizoaffective patients both at baseline and after 16 weeks of supplementation with 3 g/day of either ethyl-eicosapentaenoic acid or placebo. Current smoking status, gender, dietary survey, and Montgomery Asburg Depression Rating Scale, Repeatable Battery for the Assessment of Neuropsychological Status, Abnormal Involuntary Movement Scale, and Positive and Negative Syndrome Scale scores were assessed.*

Results: *Schizophrenic patients who smoked had lower baseline erythrocyte docosahexaenoic acid percent (2.98 ± .7 vs. 3.59 ± 1.2,* $p < .005$*) and eicosapentaenoic acid (EPA) percent (.39 ± .13 vs. .47 ± .22,* $p < .05$*), compared with nonsmokers, with a significant gender interaction (*$p < .01$*) in multivariate analyses of variance. Baseline arachidonic acid did not differ. Smokers reported lower dietary intake (percent total fat) of linolenic acid (*$F = 10.1$*,* $p < .003$*) compared with nonsmokers. Nonsmoking women reported greater dietary intake of EPA compared with smoking men or nonsmokers of either gender.*

Conclusions: *Smoking status, gender, and dietary intake significantly predicted erythrocyte polyunsaturated fatty acid status among schizophrenic patients. No evidence was found for subgroups of schizophrenia or relationships to specific symptom severity on the basis of erythrocyte fatty acids. Prior reports of abnormalities of essential fatty acid metabolism among schizophrenic patients may have been an artifact of patients' smoking behavior and differences in dietary intake of ω-3 fatty acids.* Biol Psychiatry 2003;53:431–441 © *2003 Society of Biological Psychiatry*

Key Words: Smoking, schizophrenia, docosahexaenoic acid, eicosapentaenoic acid, arachidonic acid, omega-3 fatty acids

Introduction

Several hypotheses have been advanced that genetic disturbances in phospholipid and prostaglandin metabolism may contribute to schizophrenic etiology and severity (for reviews see Fenton et al 2000; Rotrosen and Wolkin 1987). The observation that abnormalities in ω-3 essential fatty acid may play a critical role (Assies et al 2001; Horrobin 1998; Peet et al 2001) has been supported by reports of three types of essential fatty acid aberrations among schizophrenic patients. First, several authors have reported lower concentrations of erythrocyte essential fatty acids among schizophrenic patients as compared with control subjects (Assies et al 2001; Peet et al 1995; Yao et al 1994b). A second set of findings has correlated lower erythrocyte essential fatty acid concentrations with greater severity of negative symptoms (Glen et al 1994; Yao et al 1994a). Third, findings of bimodal distributions of arachidonic acid (AA or 20:4n6), eicosapentaenoic acid (EPA or 20:5n3), and docosahexaenoic acid (DHA or 22:6n3) concentrations among schizophrenic patients have raised the possibility that distinct subgroups of schizophrenia could be identified based on abnormalities in lipid composition (Glen et al 1994; Peet et al 1994); however, these reports of lower tissue essential fatty acid compositions and correlations between essential fatty acid compositions and symptom type or severity have been highly inconsistent (Fenton et al 2000). For example, the same group has reported finding elevated (Horrobin et al 1989; Vaddadi et

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Received December 31, 2001; revised July 2, 2002; accepted July 10, 2002.

0006-3223/03/$30.00
doi:10.1016/S0002-3223(03)01549-4

al 1986), reduced (Vaddadi et al 1989), and no difference (Vaddadi et al 1996) in schizophrenic patients' erythrocyte DHA as compared with controls. Finally, the proposition that correcting essential fatty acid perturbations may be therapeutic has been supported by recent reports of the clinical efficacy of ω-3 essential fatty acids from case studies, open clinical trials (Mellor et al 1995; Peet et al 1996; Puri and Richardson 1998) and from two pilot, randomized, placebo-controlled trials of ethyl ester EPA (Peet et al 2001); however, a larger trial failed to replicate these findings (Fenton et al 2001).

One potential explanation for discrepancies between these reports may have been the existence of unique subgroups within schizophrenia on the basis of essential fatty acid compositions, as is suggested by findings of bimodal distributions of polyunsaturated essential fatty acids among schizophrenic patients. A second possibility is that schizophrenic patients may consume a diet that differs from control subjects with respect to ω-6 and ω-3 essential fatty acids. A third reason for discrepancies may be that schizophrenic patients have disproportionately high rates of smoking (Diwan et al 1998), almost 90% among schizophrenic patients, compared with 33% of general populations and 45%–70% of psychiatric populations (for review, see Lohr and Flynn 1992). It has long been established that that pro-oxidants degrade polyunsaturated essential fatty acids (Porter 1984). Each puff of cigarette smoke contains more than 10^{15} organic radicals (Pryor 1997), so it is not surprising that cigarette smoking has been associated with decreased serum concentrations of polyunsaturated fatty acids (Simon et al 1996) and increased production of lipid peroxidation products, such as F_2-isoprostanes (Morrow et al 1995). Thus, suggestions of genetic disturbances in schizophrenic patients' phospholipid and essential fatty acid metabolism may be premature, as all but one of previously reported studies have not adequately controlled for confounds such as the patients' smoking habits or differences in dietary intake of essential fatty acids.

We previously conducted a 16-week, double-blind, placebo-controlled test of 3 g/day of ethyl ester EPA among 87 patients meeting criteria for schizophrenia or schizoaffective disorder with significant residual symptoms. As reported (Fenton et al 2001), we found no evidence of efficacy for ethyl ester EPA over placebo in improving residual symptoms, cognitive impairments, or any other psychometric end point; however, this data set also provided an opportunity to attempt replication of previously reported findings of fatty acid abnormalities among schizophrenic patients and examine possible confounding factors. Erythrocyte fatty acid data were collected while simultaneously assessing for dietary intake, alcohol consumption (Pawlosky and Salem Jr. 1999), current smoking status, antipsychotic medication, gender, age, psychopathology, subdiagnostic classification, and duration of illness. In addition, we examined correlational relationships between changes in selected essential fatty acids over 16 weeks of supplementation and duration of illness, as well as changes in psychometric parameters. Thus, we were able to test the hypotheses that distinct subgroups exist within schizophrenia, which are discernible by fatty acid composition, that erythrocyte fatty acid composition correlates with symptom type and severity, and that differences in dietary intake of essential fatty acids or in smoking status contributed to lower compositions of essential fatty acids.

Methods and Materials

Subjects

Eighty-seven outpatients between the ages of 18 and 65 years, who met DSM-IV criteria for schizophrenia or schizoaffective disorder based on clinical evaluation and review of illness course by one of two board-certified psychiatrists, were entered into the treatment study. This analysis included 72 subjects who both completed the study and for whom fatty acid data were available. Additional inclusion criteria were: 1) no changes in antipsychotic medication anticipated or in the prior 30 days; 2) pharmacologic treatment that conformed to U.S. Agency for Health Care Policy and Research Schizophrenia Patient Outcomes Research Team (Lehman and Steinwachs 1998) recommendations #1, 2, 7, 8, 9, 12, 17, and 18; 3) significant residual symptoms (defined as either one or more Positive and Negative Symptom Scale [PANSS] score greater than 4, or total PANSS score greater than 45, with three or greater on at least three positive or negative items) (Kay et al 1987). Patients were excluded if they met criteria for substance dependence or mental retardation, had a bleeding disorder, or were taking fish oil supplements, anticoagulants, cholestyramine, or clofibrate antilipemic agents. Current smoking status and numbers of cigarettes and other tobacco products used per day were determined by self-report. Written, informed consent was obtained from subjects after a complete description of the study, as approved by the Institutional Review Boards of Sheppard Pratt Hospital, Chestnut Lodge, and the National Institute on Alcohol Abuse and Alcoholism.

Patients were assessed with the PANSS (Kay et al 1987), Abnormal Involuntary Movement Scale (AIMS) (National Institutes of Mental Health 1976), Simpson-Angus Scale (Simpson and Angus 1970), Montgomery Asburg Depression Rating Scale (MADRAS) (Montgomery and Asberg 1979) and Clinical Global Impression Scale (National Institutes of Mental Health 1985). Assessments were repeated blind to treatment at weeks 1, 2, 4, 8, 12, and 16. The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) (Randolph et al 1998) was administered at baseline and week 16 by a research psychologist and/or nurse. Adverse events were elicited with open-ended questioning at each study visit. Patients were given no dietary instructions other than to avoid changes in nutritional supplement

use during the study, and baseline diet and current smoking status were assessed using the Willet Dietary Survey (Willett et al 1985). Pre- and posttreatment fasting erythrocyte fatty acid compositions were determined by extraction from washed erythrocytes, methylation (Morrison and Smith, 1959), and quantification using capillary gas chromatography (HP6890) as previously described (Moriguchi et al 2000). Erythrocyte fatty acid compositions were calculated in terms of percent total fat and the ratio between AA and EPA (AA/EPA).

Following baseline assessment, patients were randomized to receive six identical capsules per day containing 4 mg of vitamin E and either 500 mg ethyl-EPA or mineral oil placebo supplied by Laxdale, Ltd. Pharmaceuticals (Stirling, UK). Three grams EPA daily was chosen as a dose of ω-3 fatty acid within the range reported effective in open-label study (Fenton et al 2000) and generally recognized as safe (Department of Health and Human Services 1997). The vitamin E was combined with EPA at a dose unlikely to have physiologic effects, to retard spoilage of EPA. Patients were instructed to take six capsules daily with meals; either three capsules twice a day or all six capsules at dinner. Adherence was monitored by pill count and erythrocyte fatty acid quantification. Patients were unable to distinguish the tasteless and odorless ethyl-EPA from placebo.

Statistical Methods

Data were examined for normality of distribution and outliers using frequency distribution plots. All outlying data points greater than four standard deviations from the mean were removed from all following analyses. This resulted in the removal of two data points from daily alcohol consumption and one from baseline erythrocyte EPA. In addition, one data point was removed from analyses of cigarettes smoked per day, as the patient smoked only one cigarette per day, but also smoked 10 cigars and 1 pipe per day. Group differences were determined using analysis of variance (ANOVA) and multivariate ANOVA (MANOVA) comparisons or logistic regression, as appropriate. The level of significance for analyses of baseline variables was set at $p \leq .05$, as each analysis was associated with a specific unidirectional hypothesis. Correlational relationships between erythrocyte fatty acid compositions and psychometric measures, dietary intake data, alcohol consumption, and numbers of cigarettes smoked were examined using simple Pearson Product Moment regression analyses. Changes in erythrocyte fatty acid composition and psychometric parameters were calculated as the baseline value subtracted from the week 16 value. The relationship between change in fatty acid composition and change in psychometric measures were examined using simple regression analyses. To test specific hypotheses of fatty acid abnormalities and to limit errors of multiple testing, only EPA, DHA, AA, and the ratio between AA and EPA (AA/EPA) were examined in detail. The level of significance for correlational analyses of baseline data and analyses that compared changes in fatty acid composition following supplementation to symptom response were adjusted for multiple testing due to the exploratory nature of these analyses. Data were analyzed using Statview 5.0.1 (SAS Institute, Inc., Cary, NC).

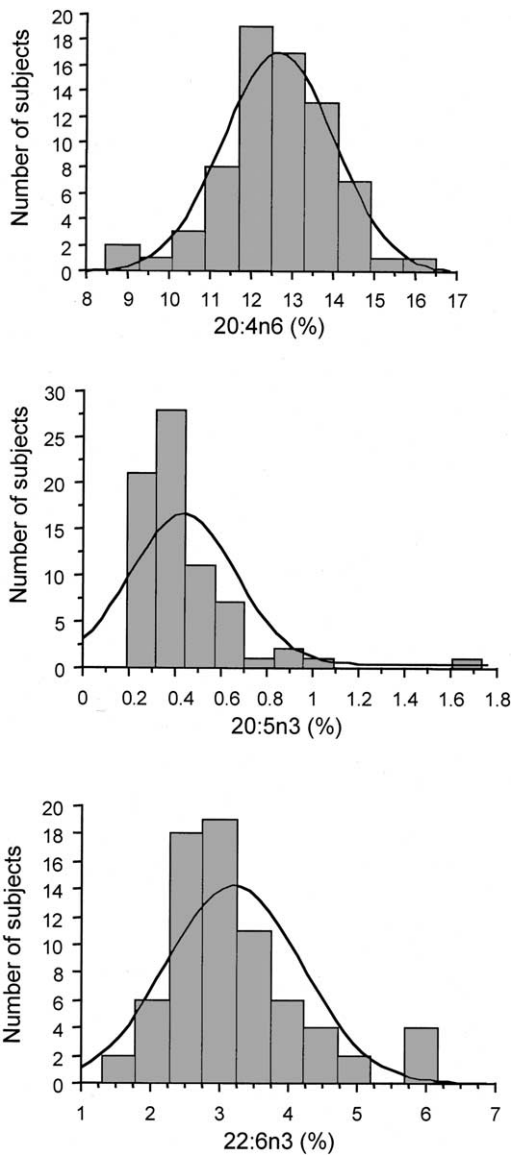

Figure 1. Frequency distributions of erythrocyte arachidonic acid (20:4n6), eicosapentaenoic acid (20:5n3), and docosahexaenoic acid (22:6n3) at baseline. Bars indicate the number of subjects with each range of erythrocyte fatty acid. 20:4n6 (%), 20:5 n3 (%), and 22:6n3 (%) are expressed as percent of total erythrocyte fatty acids. Normal comparison curves are included. No bimodal distributions of subjects are apparent.

Results

Fatty Acids at Baseline

In contrast to prior reports, there was no evidence of baseline bimodal distributions of erythrocyte EPA, DHA, or AA compositions (Figure 1). The following factors were tested as potential contributors EPA, DHA, AA, or AA/EPA erythrocyte compositions: age; duration of illness; alcohol intake (g/day); baseline psychopathology (as

Table 1. Subject Characteristics and Medication Use among Smoking and Nonsmoking Schizophrenic Patients

	Smokers (n = 45)	Nonsmokers (n = 27)
Male	30 (67)	17 (63)
Female	15 (33)	10 (37)
Caucasian	38 (84)	23 (85)
Other Ethnicity	7 (16)	4 (15)
High School Education or Higher	42 (93)	23 (85)
Using Clozapine	13 (29)	9 (33)
Other Atypical Antipsychotics	29 (64)	17 (63)
Two or More Antipsychotics	15 (3)	3 (11)[a]
Ethyl-ester EPA Treatment Group	22 (49)	14 (52)
Mineral Oil Treatment Group	23 (51)	13 (48)
Age (y)	39.0 ± 9.6	42.8 ± 10.0
Duration of Illness (y)	17.7 ± 9.8	21.7 ± 9.7
PANSS (Total)	78.8 ± 18.1	72.4 ± 14.5
PANSS (Positive subscale)	20.7 ± 5.4	19.2 ± 3.7
PANSS (Negative subscale)	18.9 ± 6.3	17.3 ± 5.7
PANSS (General psychopathology)	39.2 ± 10.4	35.9 ± 7.6
MADRAS	9.2 ± 6.6	7.7 ± 4.5
AIMS	3.5 ± 6.0	3.4 ± 6.4
RBANS	71.9 ± 14.0	72.3 ± 15.9

Values are expressed as n (%) or mean ± SD.

EPA, eicosapentaenoic acid; PANSS, Positive and Negative Symptom Scale; MADRAS, Montgomery Asberg Depression Rating Scale; AIMS, Abnormal Involuntary Movement Scale; RBANS, Repeatable Battery for the Assessment of Neuropsychological Status.

Smoking and nonsmoking schizophrenic patients do not differ in analysis of variance testing in any of these parameters.

[a]Indicates lower odds ratio by logistic regression testing (OR = .25, CI = .06–.96, $p < .05$).

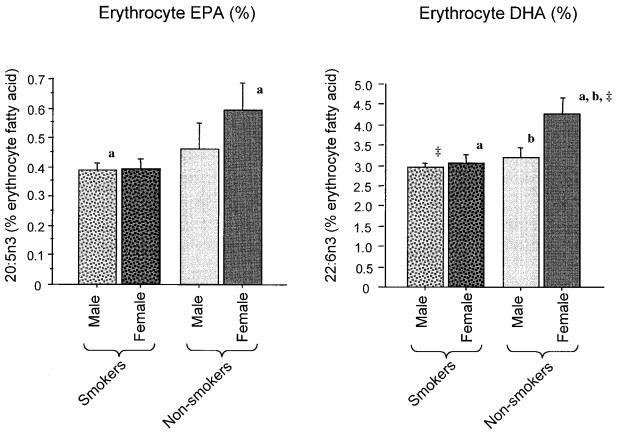

Figure 2. Erythrocyte fatty acid composition of schizophrenic patients at baseline. Bars indicate means and standard errors of the percent of 20:5n3 eicosapentaenoic acid (EPA) and 22:6n3 docosahexaenoic acid (DHA) in erythrocytes from schizophrenic subjects at baseline. [a,b] indicates that values with identical letters differ at $p < .05$ using Mann–Whitney testing. ‡, values with an identical letter significantly different at $p < .002$ using Mann–Whitney testing.

measured via positive PANSS score, negative PANSS score, general PANSS score, total PANSS score, MADRAS score, AIMS score, and RBANS score); dietary fat consumption of linolenic acid (LNA or 18:3n3), EPA, DHA, linoleic acid (LA or 18:2n6), and AA. Dietary fatty acid intake was analyzed both as absolute amount (g/day) and percent of total fat intake (% fat). Subgroup comparisons were made upon division of the sample by the following factors: subdiagnostic classification (paranoid, disorganized, catatonic, undifferentiated, residual, bipolar, depressive, or mixed type); medication type (clozapine, other atypical antipsychotic, or multiple antipsychotics); current smoking status; gender. Of these continuous and categorical factors, only gender and current smoking status were significantly related to fatty acid compositions: DHA percent was reduced in smokers compared with nonsmokers (3.0 ± .71 vs. 3.6 ± 1.2, $F = 6.87$, $p < .01$) and male patients had both lower DHA percent (3.0 ± .85 vs. 3.5 ± .1, $F = 4.55$, $p < .05$) and EPA percent (.42 ± .23 vs. .48 ± .22, $F = 4.89$, $p < .05$) compared with female patients. Smokers and nonsmokers did not differ by demographic characteristics or severity of psychopathology or medication use with the exception of the use of two or more antipsychotics being greater among smokers (Table 1). Examination via MANOVA of the interaction between the effects of gender and current smoking status on erythrocyte fatty acid compositions revealed that gender and smoking, as well as their interaction, were significant for both EPA and DHA, such that nonsmoking women had the highest fatty acid compositions (Figure 2). Erythrocyte AA composition did not differ by smoking status or gender.

Male and female patients were analyzed separately by smoking status. Among female patients, nonsmokers had greater erythrocyte EPA percent (.59 ± .29 vs. .40 ± .13, $F = 5.7$, $p < .05$) and DHA percent (4.27 ± 1.19 vs. 3.07 ± .79, $F = 9.2$, $p < .01$) compared with smokers. Among male patients, EPA percent, DHA percent, or AA percent did not differ when comparing smokers with nonsmokers. An analysis of the gender effect revealed that EPA percent (.59 ± .29 vs. .39 ± .11, $F = 7.5$, $p < .05$) and DHA percent (4.3 ± 1.2 vs. 3.0 ± .83, $F = 10.4$, $p < .005$) were elevated in female nonsmokers compared with male nonsmokers. Male and female smokers did not differ in their fatty acid compositions. The number of cigarettes smoked per day by male (22 ± 17) and female (17 ± 13) smokers did not differ, indicating that the gender specificity of the smoking effect was not due to differences in smoking severity. Among smokers (n = 45), the number of cigarettes smoked per day were not correlated with erythrocyte EPA, DHA, AA, or AA/EPA compositions.

Multivariate ANOVAs were conducted to determine the effects of gender and current smoking status on dietary intake of LNA, EPA, DHA, LA, and AA, expressed both as absolute amount (g/day) and as percentage of total fat

Table 2. Dietary Fat Intake and Cigarette and Alcohol Use among Smoking and Nonsmoking Schizophrenic Patients

	Smokers		Nonsmokers	
	Male (n = 30)	Female (n = 15)	Male (n = 17)	Female (n = 10)
Cigarettes Smoked (number/day)	23 ± 16	19 ± 11	0 ± 0[c]	0 ± 0[c]
Alcohol Consumption (g/d)	.2 ± .4	.3 ± .5	.3 ± .5	.3 ± .5
Dietary Fat Intake				
Total Fat in Diet (g/d)	83 ± 42	73 ± 29	84 ± 44	81 ± 81
Saturated (% fat)	35.2 ± 5.2[a]	36.9 ± 4.2	37.5 ± 6.5	32.2 ± 2.8[a]
Monounsaturated (% fat)	38.1 ± 3.4[a]	36.7 ± 1.8	35.8 ± 3.7[a]	37.6 ± 4.5
Polyunsaturated (% fat)	18.3 ± 4.2[a]	17.5 ± 3.6	18.6 ± 5.9	21.6 ± 4.0[a]
Dietary 18:2n6 (% fat)	15.4 ± 4.5	14.6 ± 3.4	15.6 ± 5.6	17.3 ± 5.0
Dietary 20:4n6 (% fat)	.2 ± .1	.2 ± .1[a]	.1 ± .1[a]	.2 ± .1
Dietary 18:3n3 (% fat)	1.5 ± .6[a,d]	1.6 ± .3[a]	1.8 ± .6[b]	2.1 ± .7[b,d]
Dietary 20:5n3 (% fat)	.11 ± .2[a,c]	.19 ± .2[c]	.17 ± .3	.54 ± .8[a]
Dietary 22:6n3 (% fat)	.22 ± .3[a]	.37 ± .3	.32 ± .4	.9 ± 1.3[a]

Values are expressed as mean ± SD; (% fat) dietary intake of (the specific dietary fat/total dietary fat) × 100
[a,b]Values are significantly different at $p < .05$ using Mann-Whitney testing
[c]Values are significantly different at $p < .0005$ using Mann-Whitney testing
[d]Values with an identical letter significantly different at $p < 0.05$ using Mann-Whitney testing

consumption. Dietary intakes of these polyunsaturated fatty acids did not differ when comparing male with female patients or smokers to nonsmokers when expressed in absolute amounts of intake per day. When expressed as a percentage of total fat intake (% fat), nonsmoking schizophrenic patients had greater consumption of LNA but did not differ for EPA, DHA, or AA (Table 2). The consumption of EPA (% fat) (.4 ± .6 vs. .1 ± .2, $F = 6.17$, $p < .05$) and DHA (% fat) (.7 ± .9 vs. .2 ± .3, $F = 6.43$, $p < .05$) was greater in female compared with male patients. No differences in AA intake (% fat) were found when comparing smoking status or gender.

Simple linear Spearman rank correlations were used to test for correlations between the dietary intake of the polyunsaturated fatty acids and erythrocyte fatty acid compositions. The dietary intake of EPA (% fat) predicted erythrocyte EPA for all subjects (Table 3) ($n = 72$, $r = .28$, $F = 5.8$, $p < .02$), for male patients only ($n = 47$, $r = .42$, $F = 9.7$, $p < .004$) and for nonsmoking male patients ($n = 17$, $r = .73$, $F = 17.0$, $p < .0009$). The dietary intake of DHA (% fat) predicted erythrocyte DHA among male patients only ($n = 47$, $r = .40$, $F = 8.1$, $p < .007$) and among nonsmoking male patients ($n = 17$, $r = .70$, $F = 13.9$, $p < .002$). Significant correlations were not found for any other polyunsaturated fatty acid or among other subgroups.

Fatty Acids following 16 Weeks of EPA Supplementation

Means and standard deviations of all erythrocyte fatty acids at baseline and after 16 weeks of supplementation for both treatment groups are listed in Table 4. The percentage of smokers and nonsmokers did not differ when comparing placebo and treatment groups in logistic regression analyses. Following supplementation, the EPA

Table 3. Baseline Erythrocyte Fatty Acids Comparing Smoking and Nonsmoking Schizophrenic Patients

Fatty acids (%)	Smokers (n = 45)	Nonsmokers (n = 27)	F	p
Nonessential				
14:0	.6 ± .2	.6 ± .2	—	ns
16:0	18.8 ± 2.3	18.0 ± 1.3	—	ns
18:0	10.7 ± 1.6	10.4 ± 1.0	—	ns
20:0	.2 ± .03	.1 ± .07	15.6	>.0005
24:0	1.6 ± .3	1.5 ± .3	—	ns
14:1	.2 ± .1	.2 ± .1	—	ns
16:1	.6 ± .3	.5 ± .2	—	ns
18:1n9	11.1 ± 1.4	11.1 ± .8	—	ns
18:1n7	1.5 ± .3	1.4 ± .2	—	ns
24:1n9	7.1 ± 2.0	8.0 ± 4.7	—	ns
n-6 PUFA				
18:2n6	9.5 ± 1.5	9.7 ± 1.4	—	ns
18:3n6	.05 ± .05	.04 ± .05	—	ns
20:2n6	.2 ± .05	.2 ± .08	—	ns
20:3n6	1.4 ± .3	1.5 ± .3	—	ns
20:4n6	12.7 ± 1.4	12.6 ± 1.3	—	ns
22:4n6	3.5 ± .6	3.4 ± .6	—	ns
22:5n6	.7 ± .2	.6 ± .2	—	ns
n-3 PUFA				
18:3n3	.09 ± .04	.08 ± .07	—	ns
20:5n3	.4 ± .1	.5 ± .2	—	ns
22:5n3	1.8 ± .3	1.9 ± .3	4.7	>.05
22:6n3	3.0 ± .7	3.6 ± 1.2	6.9	>.05

Data are expressed as the mean ± SD of the area percent for each fatty acid, compared with the total erythrocyte fatty acids. Analysis of variance comparisons are indicated by the corresponding F and p values for each fatty acid.
PUFA, polyunsaturated fatty acids.

Table 4. Erythrocyte Fatty Acids at Baseline and after 16 Weeks of 3 g/day Ethyl-Ester EPA or Mineral Oil

Fatty Acids (area %)	Baseline (n = 78)	Ethyl Ester-EPA (n = 36)	Mineral Oil (n = 36)	F	p
Nonessential					
14:0	.6 ± .2	.5 ± .2	.5 ± .2	—	ns
16:0	18.5 ± 2.0	17.7 ± 1.5	17.6 ± 1.4	—	ns
18:0	10.6 ± 1.4	10.5 ± 1.0	10.6 ± 1.0	—	ns
20:0	.1 ± .05	.2 ± .05	.2 ± .06	—	ns
24:0	1.5 ± .3	1.8 ± .4	1.6 ± .5	—	ns
14:1	.2 ± .1	.2 ± .1	.2 ± .1	—	ns
16:1	.5 ± .2	.5 ± .2	.5 ± .2	—	ns
18:1n9	11.1 ± 1.2	10.9 ± 1.0	11.0 ± 1.1	—	ns
18:1n7	1.4 ± .3	1.4 ± .3	1.4 ± .2	—	ns
24:1n9	7.4 ± 3.3	8.00 ± 3.2	8.2 ± 2.9	—	ns
n-6 PUFA					
18:2n6	9.6 ± 1.5	9.0 ± 1.2	9.4 ± 1.7	—	ns
18:3n6	.05 ± .05	.05 ± .05	.07 ± .06	—	ns
20:2n6	.2 ± .06	.2 ± .06	.3 ± .07	—	ns
20:3n6	1.4 ± .3	1.2 ± .3	1.4 ± .3	21.1	<.0001
20:4n6	12.6 ± 1.3	10.9 ± 1.3	12.6 ± 1.0	37.4	<.0001
22:4n6	3.5 ± .6	2.1 ± .5	3.3 ± .5	111.0	<.0001
22:5n6	.7 ± .2	.3 ± .1	.6 ± .2	110.3	<.0001
n-3 PUFA					
18:3n3	.09 ± .05	.1 ± .05	.1 ± .06	—	ns
20:5n3	.4 ± .2[a]	2.7 ± 1.1	.6 ± .3[a]	121.8	<.0001
22:5n3	1.9 ± .3	4.2 ± 1.00	2.0 ± .3	159.7	<.0001
22:6n3	3.2 ± 1.0	3.0 ± .6	3.4 ± 1.0	—	ns

Data are expressed as the mean ± SD of the area percent for each fatty acid of the total erythrocyte fatty acids. Analysis of variance comparisons of treatment and placebo groups at 16 weeks are indicated by the corresponding F and p values for each fatty acid. Treatment and placebo groups did not differ at baseline.

EPA, eicosapentaenoic acid; PUFA, polyunsaturated fatty acids.

[a]Groups differ at $t = 2.1$, $p < .05$.

treatment group had higher percent compositions than the placebo group of EPA and 22:5n3, and lower percent compositions of 20:3n6, AA, 22:4n6, and 22:5n6. Docosahexaenoic acid was not elevated in the EPA treatment group, but rather there was a nonsignificant trend toward a reduction in DHA ($F = 2.84$, $p < .10$). In the placebo group, EPA was elevated at 16 weeks compared with baseline ($t = 2.1$, $p < .05$). Within our EPA-treated group, gender or current smoking status did not influence changes in EPA, DHA, and AA.

In exploratory analyses we examined the potential for therapeutic efficacy of ω-3 fats in schizophrenia by examining correlations between changes (final–baseline) in erythrocyte EPA, DHA, AA, and the AA/EPA ratio and changes in psychometric parameters among EPA-treated patients (Table 5). Changes in DHA composition were negatively correlated to changes in positive symptoms ($r = -.46$, $p < .01$) and positively correlated to changes in involuntary movement ($r = .35$, $p < .01$); however, these results must be interpreted with caution because of the moderate power of the correlations, and because it is difficult to extrapolate from these results to treatment trials in which DHA may become significantly elevated follow-

Table 5. Correlational Relationships between Changes in Symptom Severity and Changes in Erythrocyte Fatty Acid Composition, from Baseline to 3 Months

	20:5n3[a]	22:6n3[a]	20:4n6[a]	AA/EPA[a]
PANSS Positive Subscale[a]	.15	−.46[b]	−.08	.27
PANSS Negative Subscale[a]	.15	−.14	−.04	.25
PANSS General Psychopathology[a]	.11	−.28	−.11	.26
PANSS Total[a]	.16	−.36[c]	−.10	.31
MADRAS[a]	.38[c]	.07	.04	.15
AIMS[a]	−.10	.35[c]	.14	.07
RBANS[a]	−.14	−.02	−.02	−.22

Subjects are from the ethyl-ester eicosapentaenoic acid treatment group (n = 36).

Values shown are correlational coefficients (r) of simple Pearson regression analyses.

PANSS, Positive and Negative Symptom Scale; MADRAS, Montgomery Asberg Depression Rating Scale; AIMS, Abnormal Involuntary Movement Scale; RBANS, Repeatable Battery for the Assessment of Neuropsychological Status; AA/EPA, the ratio of 20:4n6 (%) to 20:5n3 (%); AA, arachidonic acid; EPA, eicosapentaenoic acid.

[a]Indicates the difference in the parameter correcting for baseline (16-week value minus baseline value) for each fatty acid expressed as percent of total erythrocyte fatty acids.

[b]$p < .01$, significant after adjusting for multiple testing

[c]$p < .05$, significance not adjusted for multiple testing

ing intervention. Given the relatively long history of schizophrenia in our patient sample (19 years), we were concerned that our patients' extended duration of illness may have contributed to our lack of clinical effect; however, patients' duration of illness was not significantly related to changes in EPA, DHA, AA, AA/EPA ratio, or changes in any of our psychometric parameters following EPA supplementation.

Discussion

Among these patients with chronic schizophrenia and schizoaffective disorders, smoking status, gender, and differences in dietary intake of essential fatty acids were the primary determinants of erythrocyte fatty acid compositions. The following continuous variables did not correlate with erythrocyte EPA, DHA, AA, and AA/EPA composition at baseline: age, duration of illness, alcohol intake, numbers of cigarettes smoked (among smokers), and measures of baseline psychopathology. Female nonsmokers had the highest percent compositions of EPA and DHA in baseline erythrocytes when compared with male nonsmokers and to both male and female smokers. It is likely that differences in dietary intake of ω-3 fatty acids contributed to these findings, as female nonsmokers reported greater dietary intakes of LNA (% fat) compared with male nonsmokers and compared with both male and female smokers. Both female smokers and nonsmokers reported greater dietary intakes of EPA (% fat) than their male counterparts. These findings are consistent with numerous reports of the effects of smoking and gender on differences in food selection, with nonsmokers tending to report eating healthier diets containing more vegetables and fewer saturated fats (D'Avanzo et al 1997; Thompson et al 1995; for review, see Koo 1997). Specific differences in essential fatty acid intake include reports that smoking women reported consuming more LNA (% fat) than nonsmoking women (D'Avanzo et al 1997) and that smoking cessation was associated with short-term increases in LA consumption and both short- and long-term increases in EPA consumption (Thompson et al 1995). Thus, it is not appropriate to conclude that the low levels of erythrocyte EPA percent and DHA percent among smoking schizophrenic patients was solely and directly due to the effects of smoking itself on lipid metabolism, but may be associated with differences in dietary intake among smokers.

To our knowledge, this is the first study that has used an outpatient dietary survey to quantify the dietary habits of schizophrenic patients, although Peet et al (1996) have attempted to quantify the dietary intake of hospitalized schizophrenic patients. Because of the inherent difficulties of accurate dietary recall and the only fair ability of dietary surveys to predict erythrocyte fatty acid compositions (Romon et al 1995), it is possible that dietary surveys may not be appropriate for use with schizophrenic populations; however, when smoking status and gender were controlled, the reported dietary intakes of EPA and DHA were strongly correlated to erythrocyte measures (e.g., nonsmoking male patients, $r = .73$ and $r = .70$, respectively). These results give confidence that dietary surveys can be accurately used among schizophrenic patients, as these correlations are stronger than those reported from subjects without psychiatric illnesses (Romon et al 1995). We also note that the dietary intake of ω-3 fats differed on the basis of gender and current smoking status only when intake was expressed as a percent of total fat consumption, which corrects for differences in total caloric intake, and not when expressed as absolute amount (g/day). This finding is consistent with prior studies of dietary intake and adipose tissue compositions, which have also found that percent of total fat intake is a stronger predictor of tissue composition compared with absolute intake (Hunter et al 1992; London et al 1991).

Most of the previous reports of reduced polyunsaturated fatty acids among schizophrenic patients did not report smoking status in either the patient or control groups (Glen et al 1994; Horrobin et al 1989; Peet et al 1995; Vaddadi et al 1989; Yao 1994a, 1994b). Two studies reported smoking habits only among the schizophrenic patients, but not the comparison population (Assies et al 2001; Doris et al 1998). The only study that we could identify that reported smoking habits in both patients and control subjects found reductions in schizophrenic patients' postmortem brain phospholipid polyunsaturates that appeared to be driven by reductions in AA (Yao et al 2000). It is likely that these studies had a disproportionately large number of schizophrenic smokers (90%) when compared with the general population (33%) (Diwan et al 1998). Here, we report that smoking schizophrenic patients had lower erythrocyte EPA percent and DHA percent compositions and lower dietary intakes of LNA, EPA, and DHA. In the light of these findings, we conclude that the proposition (Peet et al 1999) that schizophrenic patients have inherent abnormalities in the metabolism or catabolism of essential fatty acids may be an artifact of differences in dietary fat intake or the disproportionate numbers of smokers among the schizophrenic patients compared with control subjects.

No evidence of unique subgroups within schizophrenia was found on the basis of erythrocyte fatty acid composition after comparing our sample on the basis of type of medication used or subdiagnostic classification. In contrast to previous reports, we also found no evidence of a bimodal distribution of erythrocyte EPA, DHA, or AA among these patients. We note that prior studies reporting bimodal distri-

butions of polyunsaturated fatty acids among schizophrenic patients did not control for patients' current smoking status (Glen et al 1994; Peet et al 1994, 1995). Thus, the findings reported here raise the question as to whether all prior reports of lowered or bimodally distributed polyunsaturated fatty acid compositions among schizophrenic patients may be entirely owing to subgroups of smokers or subjects with greater dietary intakes of seafood.

One interesting finding in this study was that the DHA composition of erythrocytes did not change despite 16 weeks of EPA supplementation; however, this is not likely to be evidence of a metabolic abnormality of DHA metabolism among schizophrenic patients. In virtually all studies identified in PubMed searches, in which pure (>95%) EPA was given to normal human subjects, DHA did not increase in serum (Grimsgaard et al 1997, 1998), platelets (Hirai et al 1989; von Schacky and Weber 1985), or plasma (Mori et al 1999; von Schacky and Weber 1985). The only contrary report was a single case study, in which not only erythrocyte DHA and EPA, but also AA were dramatically elevated following 1 month of EPA supplementation (Puri et al 2000).

Finally, prior reports of other abnormalities in phospholipid metabolism among schizophrenic patients need to be critically examined to determine if the numbers of smokers were appropriately balanced in the schizophrenic and control populations. Smoking causes numerous abnormalities of phospholipid metabolism and catabolism, including dose–response effects on serum lipids, including triglycerides and phospholipid dense low-density and high-density lipoprotein concentrations, which have been clearly documented in a meta-analysis of 54 studies (Craig et al 1989). Cigarette smoking has been reported to increase insulin resistance, upregulate hormone-sensitive lipase, and increase interleukin-6 inflammatory responses, with the cumulative result of producing an atherogenic dyslipidemic state (Talmud and Humphries 2001). Smoking also increases in lysophosphatidylcholine (Talmud and Humphries 2001), which may explain the report of increased serum lysophosphatidylcholine (Pangerl et al 1991) among schizophrenic patients, as smoking was not controlled.

The following studies have reported either increased phospholipase A_2 activity (Albers et al 1993; Bennett et al 1991; Doris et al 1998; Gattaz et al 1987, 1990; Noponen et al 1993; Peet et al 1998; Ross et al 1997, 1999) or decreased activity (Katila et al 1997) among schizophrenic patients, but none reported the number of smokers in the patient and comparison groups. Smoking increases phospholipase A_2 activity through at least two mechanisms: 1) free radicals from cigarette smoke peroxidize vulnerable polyunsaturated fatty acids, which are cleaved and repaired by a variety of cytosolic and membrane phospholipase A_2s (Beckman et al 1987; Nigam and Schewe 2000); and 2) nicotine and cotinine directly activates phospholipase A_2s in retina (Sastry and Hemontolor 1998) and placenta (Sastry et al 1998). The interpretation of peripheral measures of phospholipase A_2s in psychiatric disorders is further complicated by the finding that nicotine inhibits phospholipase A_2 activity in brain slices (Marin et al 1997). Thus, it is unclear what ultimate effects smoking would have on clinical measures of phospholipid metabolism, with its dual effects of increased oxidative burden and direct regulation of phospholipase A_2 activity.

Unfortunately, magnetic resonance spectroscopy studies that have described abnormalities in phospholipid metabolism among schizophrenic patients have not reported the numbers of smokers in the patient and control groups (Deicken et al 1994, 1995; Fujimoto et al 1992; Fukuzako et al 1996, 1999a, 1999b; Gattaz and Brunner 1996; Hinsberger et al 1997; Kato et al 1995; Keshavan et al 1993; Komoroski et al 2001; O'Callaghan et al 1991; Pettegrew et al 1991, 1993; Ross et al 1997; Shioiri et al 2000; Stanley et al 1994, 1995; Volz et al 1999, 2000). To our knowledge, there is only one report of phospholipid abnormalities among schizophrenic patients that balanced the number of smokers in the subject and control groups (Yao et al 2000). Their finding, that AA was lower in autopsied brains of schizophrenic patients but not among control subjects did not appear to be due to smoking but may have been due to the chronic use of neuroleptics. Four sets of findings underlie the proposition that schizophrenia is a genetic disorder of fatty acid or lipid metabolism (Peet et al 1999): 1) decreased DHA in erythrocytes; 2) increased phospholipase A_2 activity; 3) increased phosphodiesters measured with magnetic resonance spectroscopy; and 4) lowered antioxidant status (Mahadick et al 2001). It is possible that the disproportionate numbers of smoking schizophrenic patients and not inherent genetic abnormalities in phospholipid metabolism underlies each of these findings. For example, increased rates of AA turnover and recycling mediated by phospholipase A_2 has been proposed as a genetic abnormality among schizophrenic patients (Horrobin 2002), but the rate of turnover and recycling is in part determined by the dietary availability of DHA and AA and their oxidative degradation (Rapoport et al 2001). In summary, the data presented here cannot determine if there is or is not an inherent metabolic abnormality in lipid metabolism among schizophrenic patients; however, these findings indicate that differences in dietary intake and smoking behaviors should be adequately controlled to test this proposition.

This work was supported by the Stanley Foundation, Bethesda, MD; the National Institute on Alcohol Abuse and Alcoholism, Rockville, MD; and a gift of materials from Laxdale Ltd, Stirling, UK.

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