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Abnormalities in the Fatty Acid Composition of the Postmortem Orbitofrontal Cortex of Schizophrenic Patients: Gender Differences and Partial Normalization with Antipsychotic Medications

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Abstract

Previous studies have observed significant abnormalities in the fatty acid composition of peripheral tissues from drug-naïve first-episode schizophrenic (SZ) patients relative to normal controls, including deficits in omega-3 and omega-6 polyunsaturated fatty acids, which are partially normalized following chronic antipsychotic treatment. We hypothesized that postmortem cortical tissue from patients with SZ would also exhibit deficits in cortical docosahexaenoic acid (DHA, 22:6*n*-3) and arachidonic acid (AA; 20:4*n*-6) relative to normal controls, and that these deficits would be greater in drug-free SZ patients. We determined the total fatty acid composition of postmortem orbitofrontal cortex (OFC) (Brodmann area 10) from drug-free and antipsychotic-treated SZ patients (n=21) and age-matched normal controls (n=26) by gas chromatography. After correction for multiple comparisons, significantly lower DHA (−20%) concentrations, and significantly greater vaccenic acid (VA) (+12.5) concentrations, were found in the OFC of SZ patients relative to normal controls. Relative to age-matched same-gender controls, OFC DHA deficits, and elevated AA:DHA, oleic acid:DHA and docosapentaenoic acid (22:5*n*-6):DHA ratios, were found in male but not female SZ patients. SZ patients that died of cardiovascular-related disease exhibited lower DHA (−31%) and AA (−19%) concentrations, and greater OA (+20%) and VA (+17%) concentrations, relative to normal controls that also died of cardiovascular-related disease. OFC DHA and AA deficits, and elevations in oleic acid and vaccenic acid, were numerically greatest in drug-free SZ patients and were partially normalized in SZ patients treated with antipsychotic medications (atypical > typical). Fatty acid abnormalities could not be wholly attributed to lifestyle or postmortem tissue variables. These findings add to a growing body of evidence implicating omega-3 fatty acid deficiency as well as the OFC in the pathoaetiology of SZ, and suggest that abnormalities in OFC fatty acid composition may be gender-specific and partially normalized by antipsychotic medications.

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Keywords

Schizophrenia; Postmortem brain; Docosahexaenoic acid (DHA); Arachidonic acid; Prefrontal cortex; Antipsychotic

1. Introduction

The principal omega-3 polyunsaturated fatty acid in the mammalian brain, docosahexaenoic acid (DHA, 22:6*n*-3) comprises ~15% of total fatty acid composition, and the principal omega-6 polyunsaturated fatty acid arachidonic acid (AA, 20:4*n*-6) comprises ~10% of total fatty acid composition. Both DHA and AA preferentially accumulate in synaptic membranes where they exert opposing effects on the phosphoinositide-protein kinase C signal transduction pathway and multiple clinically-relevant down-stream neurochemical processes (*reviewed in* McNamara et al., 2006b). Because mammals cannot synthesize omega-3 or omega-6 polyunsaturated fatty acids *de novo*, they are entirely dependent on dietary sources to procure and maintain adequate peripheral and central tissue concentrations. Cross-national and cross-sectional epidemiological surveys (Christensen & Christensen, 1988; Mellor et al., 1995; Peet et al., 2003) and intervention trials (Arvindakshan et al., 2003; Emsley et al., 2002; Mellor et al., 1995; Peet et al., 2001; Peet & Horrobin, 2002) suggest that dietary omega-3 fatty acid deficiency may increase symptom severity in SZ patients. Moreover, drug-naive first-episode psychotic patients exhibit significant deficits in omega-3 and omega-6 fatty acid concentrations in their red blood cell (RBC) membranes, suggesting that omega-3 and omega-6 fatty acid deficits *precede* illness onset, and both DHA and AA concentrations are partially normalized following chronic antipsychotic treatment leading to symptomatic improvement (Arvindakshan et al., 2003; Evans et al., 2003; Khan et al., 2002; Reddy et al., 2004).

Primate studies have demonstrated that RBC and cortical DHA concentrations both decrease in response to dietary deficits in omega-3 fatty acid intake, albeit at different rates (RBC≫>Cortex; Anderson et al., 2005; Connor et al., 1990). To date, three studies have investigated the fatty acid composition of postmortem brain tissue from SZ patients. Horrobin et al. (1991) found that DHA and AA concentrations were lower in the cholesterol ester fraction of the frontal cortex of antipsychotic-treated SZ patients. Yao et al. (2000) found that AA (−14%), but not DHA (−11%), concentrations were significantly lower in the postmortem caudate nucleus of antipsychotic-treated SZ patients. Landen et al. (2002) did not find significant alterations in individual fatty acid concentrations, including DHA or AA, in the postmortem cingulate cortex of antipsychotic-treated SZ patients. These postmortem studies have four notable limitations: (1) SZ patients were being treated with antipsychotic medications that have been found to partially normalize DHA and AA deficits in peripheral tissues of SZ patients (Arvindakshan et al., 2003; Evans et al., 2003; Khan et al., 2002), (2) normal controls were predominantly elderly subjects, and fatty acid concentrations have been found to vary as a function of age at death in human postmortem brain tissue (Carver et al., 2001; Gershbein et al., 1985; McNamara et al., 2006a), (3) these studies combined male and female SZ patients, and gender differences in brain fatty acid composition (Gershbein et al., 1985; McNamara et al., 2006a) and SZ epidemiology and psychopathology (Hafner, 2003) have been reported, and (4) these studies employed a relatively small number of SZ patients (n=7–11), and may have been underpowered to detect moderate changes in fatty acid concentrations.

In the present study, we determined the total fatty acid composition of postmortem orbitofrontal cortex (Brodmann area 10) from drug-free and antipsychotic-treated male and female SZ patients (n=21) and age-matched male and female healthy controls with no history of psychiatric illness (n=26). The OFC was selected as the region of interest because it has reciprocal connections with the amygdala, hippocampus, nucleus accumbens, and

hypothalamus (Kringelbach & Rolls, 2004), and is thought to play an important role in cognitive and emotional processes relevant to SZ psychopathology (Bechara, 2004; Kringelbach, 2005; London et al., 2000). Previous neuroimaging studies have observed cortical thinning and/or reductions in OFC volume in patients with SZ relative to normal controls (Andreasen et al., 1997; Crespo-Facorro et al., 2000; Goldstein et al., 1999; Gur et al., 2000; Kuperberg et al., 2003; Pantelis et al., 2003), and postmortem studies have found alterations in the expression of multiple genes in the SZ OFC suggestive of synaptic pathology (Akil et al., 1999; Garey et al., 2006; Glantz & Lewis, 1997; Karson et al., 1999; Knable et al., 2004; Meador-Woodruff et al., 1997; Torrey et al., 2005). Our primary hypothesis was that DHA and AA concentrations would be significantly lower in the postmortem OFC of SZ patients, and that these deficits would be greater in drug-free SZ patients.

2. Methods

2.1 Postmortem brain tissues

Frozen, unfixed, postmortem OFC (Brodmann area 10) from normal (no psychiatric illness) male and female controls (n=26) and age-matched male and female patients with DSM-IV defined SZ (n=21) were used. Brain tissue was generously provided by the Stanley Research Foundation Neuropathology Consortium (Torrey et al., 2000) and the Harvard Brain Tissue Resource Center. Axis I DSM-IV diagnoses were made independently by senior psychiatrists based on medical records and interviews with family members. A comparison of subject and tissue variables is presented in Table 1. At time of death, n=3 SZ patients were drug-free, n=9 SZ patients were receiving typical antipsychotic medications (fluphenazine: n=1, thiothixene: n=1, chlorpromazine: n=2, thioridazine: n=2, haloperidol: n=3), and n=9 SZ patients were receiving atypical antipsychotic medications (olanzapine: n=1, risperidone: n=3, clozapine: n=5). There were n=5 SZ patients additionally receiving mood-stabilizers (valproic acid, carbamazepine, or lithium), and n=5 SZ patients additionally receiving antidepressant medications.

2.2. Gas chromatography

The method for saponification and methylation of fatty acids for gas chromatographic (GC) analysis follows that originally reported by Mecalfe et al (1966). Frozen ~100 mg cortical samples (predominantly gray matter) were placed in a 20 ml glass vial into which 4 ml of 0.5N methanolic sodium hydroxide was added, and the sample heated at 80°C for 5 min. Following a 10 min cooling period, 3 ml of BF3 in methanol was added to methylate the sample. After an additional five minutes of heating in the water bath (80°C), the sample vial was allowed to cool, and 2 ml of a saturated solution (6.2 M) of sodium chloride and 10 ml of hexane was added. The samples were then mixed by vortex for one minute. The hexane fraction was then transferred into a 20 ml vial containing 10 mg of sodium sulfate to dry the sample. The hexane solution was then removed for GC analysis. An injection volume of 1 μL of the hexane solution was analyzed. Samples were analyzed with a Shimadzu GC-17A GC equipped with auto-injector (Shimadzu Scientific Instruments Inc., Columbia MD). Analysis of fatty acid methyl esters is based on areas calculated with Shimadzu Class VP 4.3 software. The column was a DB-23 (123–2332): 30m (length), I.D. (mm) 0.32 wide bore, film thickness of 0.25 μM (J&W Scientific, Folsom CA). Fatty acid identification was determined using retention times of authenticated fatty acid methyl ester standards (Matreya LLC Inc., Pleasant Gap PA). The GC conditions were: column temperature ramping by holding at 120ºC for one minute followed by an increase of 5ºC/min from 120–240ºC. The temperature of the injector and flame ionization detector was 250ºC. A split (8:1) injection mode was used. The carrier gas was helium with a column flow rate of 2.5 ml/min. All samples were processed by a technician blinded to treatment conditions.

In our GC analysis, we set the threshold at an area of 1000, which corresponds to 1 μg/mL of sample. The amount injected on the GC therefore allows us to detect 1 ng injected into the instrument, and we are able to detect 10 μg of an individual fatty acid in a 100 mg sample of tissue. At the outset of the experiment, analyses were conducted to determine between-sample reliability, and it was determined that our assays had an overall Cronbach's reliability coefficient alpha of 0.935, and alphas ≥0.60 for individual fatty acids, indicating very good between-sample reliability. Accordingly, statistical (Student's *t*-tests, 2-tail) comparison of duplicate values did not find significant differences for any fatty acid (ps>0.05). Based on this finding and limited tissue quantity, only a subset of brain samples (n=15 normals, n=15 SZ patients) were run in duplicate. Duplicate values were averaged for all subsequent analyses.

2.3. Statistical analysis

We restricted our primary analysis to the principal saturated fatty acids (palmitic, stearic, myristic), monounsaturated fatty acids (oleic acid, OA, vaccenic acid, VA), omega-6 polyunsaturated fatty acids (arachidonic acid, AA; docosatetraenoic acid, DTA; docosapentaenoic acid, DPA), and omega-3 polyunsaturated fatty acid (docosahexaenoic acid, DHA). Together these 9 fatty acids comprise ~90% of total fatty acids in postmortem brain tissue, and the remaining 10% is comprised of fatty acids that individually represent >2% of total fatty acids. Analyses of variance (ANOVA) and covariance (ANCOVA) were performed using SAS (Version 9.1, SAS Institute Cary, NC) PROC MIXED procedure. The null hypothesis that fatty acid concentrations do not differ by illness state (Normal, SZ) was tested as the interaction term Illness x Fatty acid in a two-factor ANOVA. Post-hoc tests of simple effects were performed using the Bonferroni correction with a group-wise error rate of $\alpha = 0.05$ to evaluate illness state effects for individual fatty acids ($\alpha = 0.05/9 = 0.0056$). An ANCOVA repeated the steps above, adding Age (continuous), Gender (Male, Female), Treatment (typical antipsychotic, atypical antipsychotic, drug-free) and second-order interaction terms of all variables, after which stepwise regression using p=0.10 as a cutoff point determined which terms remained in the final model. Post-hoc tests of simple effects using the Bonferroni correction were also performed on the final ANCOVA model, with a group-wise error rate of $\alpha = 0.05$ to evaluate illness state effects for individual fatty acids ($\alpha = 0.05/9 = 0.0056$). Pearson product moment correlation analyses were performed using GB-STAT (Dynamic Microsystems, Inc., Silver Springs MD) to determine the interrelationship between fatty acid concentrations and subject characteristics (age at onset of illness, duration of illness, age at time of death) and tissue variables (brain pH, brain weight, postmortem interval, and days in freezer storage). All tests were 2-tailed and performed at $\alpha = 0.05$. Exploratory analyses of continuous variables were conducted using Student's *t*-tests (2-tailed, $\alpha = 0.05$). Effect size was calculated using Cohen's *d*, with small, medium, and large effect sizes being equivalent to *d*-values of 0.30, 0.50, and 0.80, respectively.

3. Results

3.1. OFC fatty acid composition in normal controls

In the OFC of normal controls, concentrations of individual saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids (Fig. 1) are consistent with those previously reported for postmortem frontal cortex from a larger cohort (N=38) of normal adult male and female subjects (Carver et al., 2001). Normal females exhibited higher OFC AA (+10%, p=0.04) and DTA (+4%, p=0.02) concentrations relative to normal males. In the OFC of normal controls, DHA ($r = -0.43$, p=0.02), AA ($r = -0.44$, p=0.02), DPA ($r = -0.54$, p=0.004), and stearic acid ($r = -0.64$, p=0.0004) concentrations were negatively correlated, and OA ($r = +0.52$, p=0.006) and VA ($r = +0.40$, p=0.04) concentrations were positively correlated, with age at death.

3.2. OFC fatty acid composition in normals vs. SZ patients

The overall ANOVA found a significant Illness x Fatty Acid Interaction, $F(8,377)=3.87$, $p=0.0002$, and a significant main effect of Fatty Acid, $F(8,377)=255$, $p \leq 0.0001$, but not Illness $F(1,377)=2.46$, $p=0.117$. A significant Age x Fatty Acid Interaction, $F(8,377)=6.35$, $p \leq 0.0001$, was found, and the main effect of Age, $F(1,377)=1.14$, $p=0.286$, and the Illness x Age Interaction, $F(1,377)=0.20$, $p=0.659$, were not significant. After correction for multiple comparisons, significantly lower DHA (−20%, p=0.0051) concentrations, and significantly greater vaccenic acid (VA) (+12.5, p=0.003) concentrations, were found in the OFC of SZ patients relative to normal controls (Fig. 1). Negative trends for AA (−10%, p=0.012), palmitic acid (−9%, p=0.01), and stearic acid (−4%, p=0.04), and a positive trend for oleic acid (OA) (+11%, p=0.03), were observed in the OFC of SZ patients relative to normal controls (Fig. 1). In the OFC of SZ patients, DHA concentrations were inversely correlated with OA concentrations ($r = -0.93$, $p<0.0001$), VA concentrations ($r = -0.83$, $p<0.0001$), and docosatetraenoic acid concentrations ($r = -0.46$, $p=0.001$), and were positively correlated with AA ($r = +0.86$, $p<0.0001$), palmitic acid ($r = +0.81$, $p<0.0001$), and stearic acid ($r = +0.74$, $p<0.0001$) concentrations. These findings suggest that DHA loss is predominantly compensated for by elevations in OA and VA. Elevations in the AA:DHA (+17%, p=0.01), OA:DHA (+41%, p=0.01), DPA:DHA (+28%, p=0.09), and palmitic acid:VA (−19%, p=0.003) ratios were observed in SZ patients relative to normal controls (Fig. 2). Total saturates (∑ MA+PA+SA) (−6%, p=0.01) and total polyunsaturates (∑AA+DPA+DT+DHA) (−11%, p=0.01) were lower, and total monounsaturates (∑OA+VA) were higher (+11%, p=0.02), in SZ patients relative to normal controls. The ∑saturate: ∑polyunsaturate (+7%, p=0.04) and ∑monunsaturate: ∑polyunsaturate (+24%, p=0.01) ratios were higher, and the ∑saturate: ∑monounsaturate (−15%, p=0.01) ratio lower, in SZ patients relative to normal controls.

SZ patients that died of cardiovascular-related disease (n=8) exhibited lower DHA (−31%, p=0.002) and AA (−19%, p=0.002) concentrations, and greater OA (+20%, p=0.006) and VA (+17%, p=0.003) concentrations, relative to normal controls that also died of cardiovascular-related disease (n=17). However, SZ patients that committed suicide (n=5) did not exhibit significantly lower DHA (−17%, p=0.07) or AA (−4%, p=0.57) concentrations, or greater OA (+6%, p=0.43) and VA (+7%, p=0.27) concentrations, relative to normal controls that died of cardiovascular-related disease (n=17).

We found a significant Illness x Fatty Acid x Gender Interaction, $F(8,377)=5.37$, $p \leq 0.0001$, whereas the main effect of Gender, $F(1,377)=0.07$, $p=0.793$, and the Illness x Gender Interaction, $F(1,377)=0.97$, $p=0.325$, were not significant. OFC DHA concentrations did not differ significantly between normal male and female controls (p=0.158), or between SZ male and female patients (p=0.213). However, relative to age-matched same-gender controls, male SZ patients exhibited greater OFC DHA deficits (−27%, p=0.001) than did female SZ patients (−2%, p=0.91)(Fig. 3A). Similar OFC AA deficits were observed in male (−11%, p=0.03) and female (−7%, p=0.31) SZ patients, and the resulting AA:DHA ratio was greater in male SZ patients (+25%, p=0.003) than female SZ patients (−4%, p=0.57) relative to age-matched same-gender controls (Fig. 3B). Conversely, male SZ patients exhibited greater OFC OA concentrations (+15%, p=0.01) than female SZ patients (+0.5%, p=0.95), and the resulting OA:DHA ratio was greater in male SZ patients (+52%, p=0.01) than female SZ patients (+5%, p=0.83) (Fig. 3C). OFC DPA concentrations did not differ between male and female SZ patients (p=0.055), and the resulting DPA:DHA ratio was greater in male (+39%, p=0.04) than female (−15%, p=0.19) SZ patients relative to age-matched same-gender controls (Fig. 3D).

3.3. Effect of antipsychotic medications on OFC fatty acid composition in SZ patients

Analysis by Treatment (Normals, n=26; atypical antipsychotic, n=9; typical antipsychotic, n=9; drug-free, n=3) found a significant Treatment x Fatty Acid Interaction, $F(24,315)=2.07$,

p=0.0028, and significant Age x Treatment x Fatty Acid, F(24,315)=2.67, $p \leq 0.0001$, and Gender x Treatment x Fatty Acid, F(24,315)=1.72, p=0.02, Interactions. After adjustment for Age and Gender, the OFC DHA deficit observed in *drug-free* SZ patients (−33% vs. normal controls, p=0.008) was numerically greater than the DHA deficit observed in SZ patients treated with typical (−26% vs. normal controls, p=0.003) or atypical (−8% vs. normal controls, p=0.84) antipsychotic medications (Fig. 4A). OFC DHA concentrations in drug-free SZ patients did not differ significantly from OFC DHA concentrations in SZ patients treated with typical (p=0.73) or atypical (p=0.21) antipsychotic medications. The OFC AA deficit observed in drug-free SZ patients (−26% vs. normal controls, p=0.006) was numerically greater than the AA deficit observed in SZ patients treated with typical (−12% vs. normal controls, p=0.002) or atypical (−3% vs. normal controls, p=0.92) antipsychotic medications (Fig. 4B). OFC AA concentrations in drug-free SZ patients did not differ significantly from OFC AA concentrations in SZ patients treated with typical antipsychotic medications (p=0.21), but were significantly lower relative to SZ patients treated with atypical antipsychotic medications (p=0.01). The OFC AA:DHA ratio observed in drug-free SZ patients (+29% vs. normal controls, p=0.01) was greater than the ratio observed in SZ patients treated with typical (+21% vs. normal controls, p=0.003) or atypical (+7% vs. normal controls, p=0.14) antipsychotic medications (Fig. 4C). The OFC AA:DHA ratio in drug-free SZ patients did not differ significantly from the OFC AA:DHA ratio in SZ patients treated with typical (p=0.70) or atypical (p=0.25) antipsychotic medications. Finally, the OFC PA deficit observed in drug-free SZ patients (−11% vs. normal controls, p=0.003) was numerically greater than the PA deficits observed in SZ patients treated with atypical antipsychotic medications (−1% vs. normal controls, p=1.00), and were similar to SZ patients treated with typical antipsychotic medications (−17% vs. normal controls, p=0.0003).

Additionally, elevated OFC VA concentrations observed in drug-free SZ patients (+21% vs. normal controls, p=0.049) were numerically greater than the elevation observed in SZ patients treated with typical (+14% vs. normal controls, p=0.003) or atypical (+5% vs. normal controls, p=0.70) antipsychotic medications (Fig. 4D). OFC VA concentrations in drug-free SZ patients did not differ significantly from OFC AA concentrations in SZ patients treated with typical antipsychotic medications (p=0.40), but were greater relative to SZ patients treated with atypical antipsychotic medications (p=0.01). Similarly, elevated OFC OA concentrations observed in drug-free SZ patients (+24% vs. normal controls, p=0.0003) were numerically greater than the elevation observed in SZ patients treated with typical (+13% vs. normal controls, p=0.003) or atypical (+3% vs. normal controls, p=0.99) antipsychotic medications.

The negative correlation between age at death and OFC DHA concentrations in normal controls ($r = -0.43$, p=0.02) was also observed in *typical* antipsychotic-treated SZ patients ($r = -0.68$, p=0.04) but not in *atypical* antipsychotic-treated SZ patients ($r = +0.49$, p=0.17)(Fig. 5). Similarly, the negative correlation between age at death and OFC AA concentrations in normal controls ($r = -0.44$, p=0.02) was also observed in *typical* antipsychotic-treated SZ patients ($r = -0.79$, p=0.01) but not in *atypical* antipsychotic-treated SZ patients ($r = +0.23$, p=0.55)(Fig. 5). Conversely, the positive correlation between age at death and OFC OA concentrations found in normal controls ($r = +0.52$, p=0.006) was also observed in *typical* antipsychotic-treated SZ patients ($r = +0.72$, p=0.02) but not in *atypical* antipsychotic-treated SZ patients ($r = -0.37$, p=0.32). Similarly, the positive correlation between age at death and OFC VA concentrations found in normal controls ($r = +0.40$, p=0.04) was also observed in *typical* antipsychotic-treated SZ patients ($r = +0.77$, p=0.01) but not in *atypical* antipsychotic-treated SZ patients ($r = -0.32$, p=0.40).

The duration of illness was negatively correlated with OFC DHA concentrations in *typical* antipsychotic-treated SZ patients ($r = -0.67$, p=0.04), but was positively correlated in *atypical* antipsychotic-treated SZ patients ($r = +0.88$, p=0.05). Similarly, the duration of illness

was negatively correlated with OFC AA concentrations in *typical* antipsychotic-treated SZ patients ($r = -0.71$, p=0.03), but was positively correlated in *atypical* antipsychotic-treated SZ patients ($r = +0.61$, p=0.05). Conversely, the duration of illness was *positively* correlated with OFC OA concentrations in *typical* antipsychotic-treated SZ patients($r = +0.62$, p=0.07), but was negatively correlated in *atypical* antipsychotic-treated SZ patients ($r = -0.88$, p=0.05). Similarly, the duration of illness was positively correlated with OFC VA concentrations in *typical* antipsychotic-treated SZ patients ($r = +0.84$, p=0.004), but was negatively correlated in *atypical* antipsychotic-treated SZ patients ($r = -0.83$, p=0.17).

3.4. Effects of lifestyle variables on OFC fatty acid concentrations

OFC DHA (p=0.264), AA (p=0.313), OA (p=0.347), and VA (p=0.121) concentrations did not differ between SZ patients positively identified as cigarette smokers (n=5) relative to SZ patients that did not smoke (n=7). OFC DHA (p=0.197), AA (p=0.241), and OA (p=0.239) concentrations did not differ between normals+SZ patients that smoked cigarettes (n=9) relative to normals+SZ patients that did not smoke (n=8), whereas OFC VA concentrations were higher in normals+SZ smokers (+11%, p=0.02). OFC DHA (p=0.676), AA (p=0.806), OA (p=0.908), and VA (p=0.916) concentrations did not differ between SZ patients with low alcohol abuse severity (n=9) relative to SZ patients with high alcohol abuse severity (n=4). OFC DHA (p=0.179), AA (p=0.445), OA (p=0.427), and VA (p=0.249) concentrations did not differ between normals+SZ patients with low alcohol abuse severity (n=21) relative to SZ patients with high alcohol abuse severity (n=5). OFC DHA (p=0.663), AA (p=0.368), OA (p=0.445), and VA (p=0.601) concentrations did not differ between SZ patients with low substance abuse severity (n=11) relative to SZ patients with high substance abuse severity (n=4). OFC DHA (p=0.869), AA (p=0.896), OA (p=0.773), and VA (p=0.923) concentrations did not differ between normals+SZ patients with low substance abuse severity (n=23) relative to normals+SZ patients with high substance abuse severity (n=4).

3.5. Effects of patient and postmortem tissue variables on OFC fatty acid composition

Among all SZ patients (n=21), there were no significant correlations between DHA concentrations and age at death ($r = -0.15$, p=0.51), age at onset of illness ($r = 0.10$, p=0.71), duration of illness ($r = -0.29$, p=0.26), fluphenazine mg equivalents ($r = +0.42$, p=0.12), brain pH ($r = +0.22$, p=0.34), brain weight ($r = -0.38$, p=0.08), postmortem interval ($r = -0.27$, p=0.25), or freezer storage duration ($r = -0.39$, p=0.15), nor were there significant correlations between other individual fatty acid concentrations and these variables. Separate analyses of fluphenazine mg equivalents in SZ patients treated with typical ($r = +0.19$, p=0.65) or atypical ($r = +0.74$, p=0.25) did not find significant correlations. Within normal controls, there were no significant correlations between OFC DHA concentrations and brain pH ($r = +0.25$, p=0.22), brain weight ($r = -0.05$, p=0.79), postmortem interval ($r = -0.14$, p=0.49), or freezer storage duration ($r = -0.12$, p=0.69), nor were there significant correlations between other fatty acid concentrations and these variables. The absence of correlations between freezer storage duration and DHA concentrations in notable in view of the greater freezer storage duration in SZ patients relative to normal controls (Table 1).

4. Discussion

Based on previous findings of DHA and AA deficits in the RBC of drug-naïve first-episode SZ patients, and primate studies suggesting a correlation between RBC and cortical DHA and AA concentrations, we hypothesized that the postmortem OFC from SZ patients would exhibit deficits in DHA and AA. In the present study, we found that OFC DHA concentrations (−20%), but not AA concentrations (−10%), were significantly lower in combined drug-free + antipsychotic-treated male and female SZ patients relative to age-matched male and female normal controls after correction for multiple comparisons. These fatty acid alterations were

associated with elevations in AA:DHA (+17%) and OA:DHA (+41%) ratios, and a reduction in the PA:VA (−19%) ratio. Analysis by gender found that male SZ patients, but not female SZ patients, exhibited significantly lower OFC DHA concentrations, and elevated AA:DHA, OA:DHA, and DPA:DHA ratios, relative to age-matched same-gender controls. Elevated membrane AA:DHA and OA:DHA ratios are significant in view of previous *in vitro* studies finding that DHA inhibits, and AA and OA stimulate, protein kinase C (PKC), a major synaptic signal transduction molecule activated by phosphoinositide-coupled receptors (*reviewed in* McNamara et al., 2006b), and previous studies finding elevated indices of phosphoinositide metabolism in the postmortem prefrontal cortex of SZ patients (Jope et al., 1998; Lin et al., 1999). SZ patients that died of cardiovascular-related disease exhibited lower DHA (−31%) and AA (−19%) concentrations, and greater OA (+20%) and VA (+17%) concentrations, relative to normal controls that also died of cardiovascular-related disease. OFC DHA deficits were not significantly correlated with postmortem tissue variables, and could not be wholly attributed to alcohol abuse severity, substance abuse severity, or cigarette smoking.

The present postmortem study has four important limitations. First, there is no information available regarding the diets of SZ patients or normal controls in the months preceding their death in order to investigate its contribution to the present findings. Indeed, the precise cause of OFC DHA deficits in SZ patients cannot be determined from the present study, and may be attributable to a combination of state factors (diet, substance, alcohol and tobacco abuse) and/or trait factors, including deficits in brain DHA transport mechanisms and/or impaired DHA biosynthesis from dietary precursors. Second, positive and negative symptom severity at the time of death is not known, and may correlate with DHA concentrations (e.g., Mellor et al., 1995). Third, because SZ is a heterogeneous disorder, the small number of SZ patients, particularly in the drug-free group (n=3), may not be representative of all patients with SZ. Fourth, RBCs were not available to determine the interrelationship between peripheral and central tissue fatty acid composition (e.g., Carver et al., 2001).

In the present study, male SZ patients exhibited greater OFC DHA deficits (−27%) than did female SZ patients (−2%) relative to age-matched same-gender controls. This gender difference is consistent with epidemiological data indicating a gender difference in age at onset and illness course among SZ patients (*reviewed in* Hafner, 2003). Moreover, the conversion of α-linolenic acid to DHA is positively regulated by estrogen (Burdge & Wooten, 2002; Giltay et al., 2004), and a recent study found reduced levels of circulating estrogen levels in male first-episode psychotic patients (Huber et al., 2005). In contrast with the present findings, we previously found that OFC DHA deficits were greater in female (−32%) than male patients (−16%) with unipolar depression relative to age-matched same-gender controls (McNamara et al., 2006a), a pattern also consistent with epidemiological data indicating higher (2:1) prevalence rates of unipolar depression among females versus males (Kuehner et al., 2003). Elucidation of the interrelationship between circulating estrogen levels, tissue DHA concentrations, and symptom type and severity may provide important insight into the mechanisms conferring gender differences in psychopathology.

A previous study of adult monozygotic twin pairs discordant for SZ found that affected twins had significantly lower plasma DHA concentrations than unaffected twins (Bates et al., 1992), suggesting environmental rather than genetic determinants. For example, SZ patients smoke cigarettes at higher rates than the general population (McCloughen, 2003), and cigarette smoking is associated with elevated indices of lipid peroxidation and lower DHA and AA concentrations in peripheral tissues of female, but not male, SZ patients (Hibbeln et al., 2003) and in normal subjects (Leng et al., 1994). However, in the present study greater OFC DHA deficits were observed in male than female SZ patients, and we did not observe differences in OFC DHA concentrations between SZ patients that smoked cigarettes relative to those that did not smoke cigarettes, or between normal+SZ patients that smoked relative to

normal+SZ patients that did not smoke. In a separate analysis, there were also no differences in OFC DHA concentrations in combined normals, SZ, bipolar, and depressed patients that smoked (n=22) relative to normals, SZ, bipolar, and depressed patients that did not smoke (n=17) (p=0.981)(McNamara, unpublished observation). Together these data suggest that cigarette smoking cannot wholly account for deficits in OFC DHA concentrations in SZ patients, but may nevertheless be a contributing factor in conjunction with other lifestyle factors. We also found that there were no differences between OFC DHA concentrations in SZ patients with low versus high alcohol abuse severity. However, in a separate analysis it was found that OFC DHA concentrations were significantly lower in combined normals, SZ, bipolar, and depressed patients with high alcohol abuse severity (n=17) relative to combined normals, SZ, bipolar, and depressed patients with low alcohol abuse severity (n=36) (−25%, p=0.006) (McNamara, unpublished observation). This finding is consistent with a previous primate study finding 20–30% reductions in cortical DHA concentrations following chronic alcohol intake (Pawlosky et al., 2001), and suggests that alcohol abuse may also contribute in part to DHA deficits in the human OFC.

In the present study, OFC DHA and AA deficits, and elevated OA and VA concentrations, were numerically greater in drug-free SZ patients relative to SZ patients treated with antipsychotic medications (atypical > typical). The latter finding is consistent with the previous finding that DHA deficits in RBC of drug-naive SZ patients are partially normalized following chronic antipsychotic treatment (Arvindakshan et al., 2003; Evans et al., 2003; Khan et al., 2002), as well as the more moderate DHA deficits (Yao et al., 2000), or no differences in DHA or AA concentrations (Landen et al., 2002), in postmortem brain tissues of *antipsychotic-treated* SZ patients relative to normal controls. Moreover, these findings suggest that the OFC DHA deficits observed in antipsychotic-treated SZ patients are not a *consequence* of chronic antipsychotic exposure which is consistent with preclinical findings (Levant et al., 2006; Parikh et al., 2003). Furthermore, the OFC AA deficit (−10%) observed in combined drug-free and antipsychotic-treated SZ patients (n=21) is similar to that found by Yao et al. (2000) in postmortem caudate nucleus of antipsychotic-treated SZ patient (−14%), and the 10% AA deficit reported by Horrobin et al. (1991). Together these findings suggest that antipsychotic treatments that are efficacious in reducing symptom severity in SZ patients partially normalize peripheral as well as central tissue fatty acid abnormalities in drug-free SZ patients. Future studies examining the fatty acid composition of central or peripheral tissues from SZ patients will therefore need to account for antipsychotic treatment as a confounding variable.

Antipsychotic medications have a high affinity for dopamine D_2 and/or serotonin 5-$HT_{2A/C}$ receptors, and both serotonin 5-$HT_{2A/C}$ receptors (Basselin et al., 2005a; Garcia & Kim, 1997; Qu et al., 2003) and dopamine D_2 receptors (Basselin et al., 2005b; Nilsson et al., 1998; Piomelli et al., 1991), are coupled to phospholipase A_2 (PLA_2), which mediates the cleavage of membrane-bound AA (calcium-dependent PLA_2) and DHA (calcium-independent PLA_2). It is off interest, therefore, that some studies (Gattaz et al., 1990, 1995; Lasch et al., 2003; Noponen et al., 1993; Ross et al., 1997; Smesny et al., 2005), but not all (Albers et al., 1993), have observed elevated PLA_2 activity in the serum/platelets of drug-free SZ patients which are attenuated following chronic antipsychotic treatment. Moreover, preclinical studies have found that chronic blockade of D_2 receptors with haloperidol significantly decreases PLA_2 activity in rat brain (Myers et al., 2001; Ross et al., 1999; Trzeciak et al., 1995). Furthermore, a postmortem study observed normal calcium-independent PLA_2 activity, and blunted calcium-stimulated PLA_2 activity, in the postmortem OFC (Brodmann area 10) of *antipsychotic-treated* SZ patients (Ross et al., 1999). Collectively, these findings suggest that elevations in 5-$HT_{2A/C}$ and/or dopamine D_2 receptor-generated PLA_2 activity may contribute to the observed abnormalities in fatty acid composition. It is of interest, therefore, that dietary-induced DHA deficits in rat frontal cortex that are comparable to those observed in the OFC of drug-free SZ patients are associated with both an elevation in the 5-HT_{2A}:D_2 receptor

binding density ratio (McNamara et al., 2006c) as well as elevations in calcium-dependent PLA_2 expression and activity (Rao et al., 2006). However, elevated PLA_2 activity and abnormalities in OFC fatty acid composition may also be a consequence of elevated lipid peroxidation, indices of which are increased in the plasma of drug-naïve first-episode SZ patients and reduced following chronic treatment with antipsychotic medications (Arvindakshan et al., 2003; Evans et al., 2003; Khan et al., 2002). However, a preclinical study found that elevations in lipid peroxidation following chronic treatment with haloperidol does not alter brain fatty acid composition, suggesting that these are dissociable events (Parikh et al., 2003).

Previous neuroimaging studies have observed cortical thinning and/or reductions in OFC volume in patients with SZ relative to normal controls (Andreasen et al., 1997; Crespo-Facorro et al., 2000; Goldstein et al., 1999; Gur et al., 2000; Kuperberg et al., 2003; Pantelis et al., 2003), and postmortem studies have found alterations in the expression of multiple genes in the SZ OFC suggestive of synaptic pathology or agenesis (Akil et al., 1999; Garey et al., 2006; Glantz & Lewis, 1997; Karson et al., 1999; Knable et al., 2004; Meador-Woodruff et al., 1997; Torrey et al., 2005). It is not known whether the observed OFC DHA deficits contribute to, or are a consequence of, OFC neuropathology. In support of DHA contributing to OFC cortical shrinkage or agenesis, preclinical studies have found that perinatal deficits in cortical DHA accrual are associated with reductions in neuronal arborization (Calderon & Kim, 2004) and neuronal size (Ahmad et al., 2002a,b) in rat brain. Moreover, OFC DHA concentrations (present results; McNamara et al., 2006a), OFC neuronal size (Cotter et al., 2005), and OFC volume (Tisserand et al., 2002) are all inversely correlated with age in normal subjects. Finally, preterm delivery, a risk factor for SZ (Ichiki et al., 2000), is associated with deficits in cortical DHA accrual (Clandinin et al., 1980), and children born preterm exhibit cortical volume reductions relative to term-born children (Peterson et al., 2000). However, a recent study found that the postmortem OFC of SZ patients does not exhibit reductions in neuronal size (Cotter et al., 2005), and because DHA preferentially accumulates in synaptic membranes (Suzuki et al., 1997), DHA deficits may simply reflect reductions in the number of synapses in the OFC of SZ patients. It will therefore be of interest to determine whether chronic omega-3 fatty acid treatment can normalize OFC volume deficits in SZ patients.

Preclinical studies have found that brain DHA concentrations are relatively resistance to loss following long-term dietary omega-3 fatty acid deficiency in the *adult* rat brain (Bourre et al., 1992; DeMar et al., 2004), suggesting that OFC DHA deficits may originally stem from perinatal deficits in DHA accrual that were not subsequently corrected by diet (*reviewed in* McNamara & Carlson, 2006). Because primate PFC DHA deficits can be normalized with long-term dietary omega-3 fatty acid treatment (Anderson et al., 2005), normalization of cortical DHA concentrations may contribute to reductions in symptom severity in SZ patients following chronic treatment with either omega-3 fatty acids (Arvindakshan et al., 2003; Emsley et al., 2002; Mellor et al., 1995; Peet et al., 2001; Peet & Horrobin, 2002) or antipsychotic medications (Arvindakshan et al., 2003; Evans et al., 2003; Khan et al., 2002). The present data therefore have implications for future clinical trials because they would suggest, for example, that drug-free male SZ patients would exhibit the greatest benefit from omega-3 fatty acid treatment whereas atypical antipsychotic-treated female SZ patients would exhibit the least benefit.

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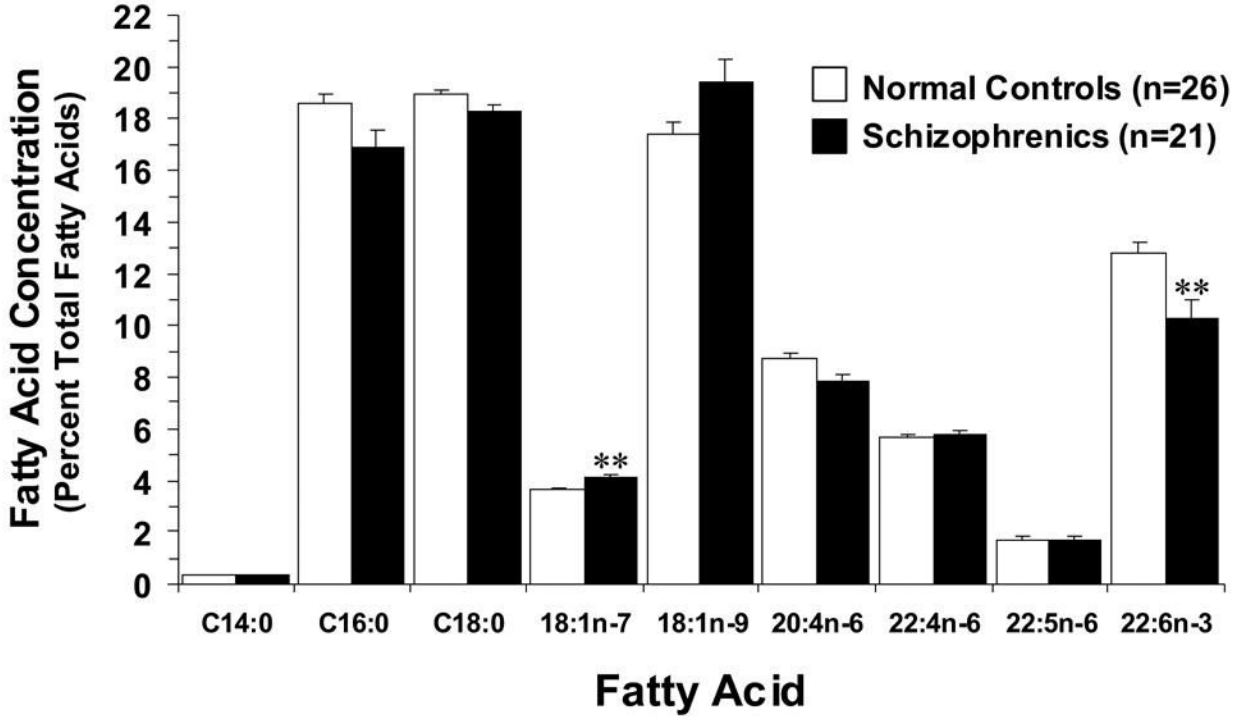

Figure 1.

Comparison of principal fatty acid concentrations (mean ± S.E.M. percent total fatty acid composition) in the OFC of male and female normal controls (N)(n=26) and drug-free and antipsychotic-treated male and female SZ patients (n=21). After correction for multiple comparisons (α =0.05/9), DHA (22:6*n*-3) concentrations were significantly lower (p=0.0051), and vaccenic acid (18:1*n*-7) concentrations significantly higher (p=0.003), in the OFC of SZ patients relative to normal controls (**$P \leq 0.0056$ vs. Normal controls). Saturated fatty acids (C14:0 - myristic acid; C16:0 - palmitic acid; C18:0 - stearic acid), monounsaturated fatty acids (18:1*n*-9 - oleic acid; 18:1*n*-7 - vaccenic acid), omega-6 polyunsaturated fatty acids arachidonic acid (20:4*n*-6), docosatetraenoic acid (22:4*n*-6), and docosapentaenoic acid (22:5*n*-6), and omega-3 fatty acid docosahexaenoic acid (22:6*n*-3 - DHA).

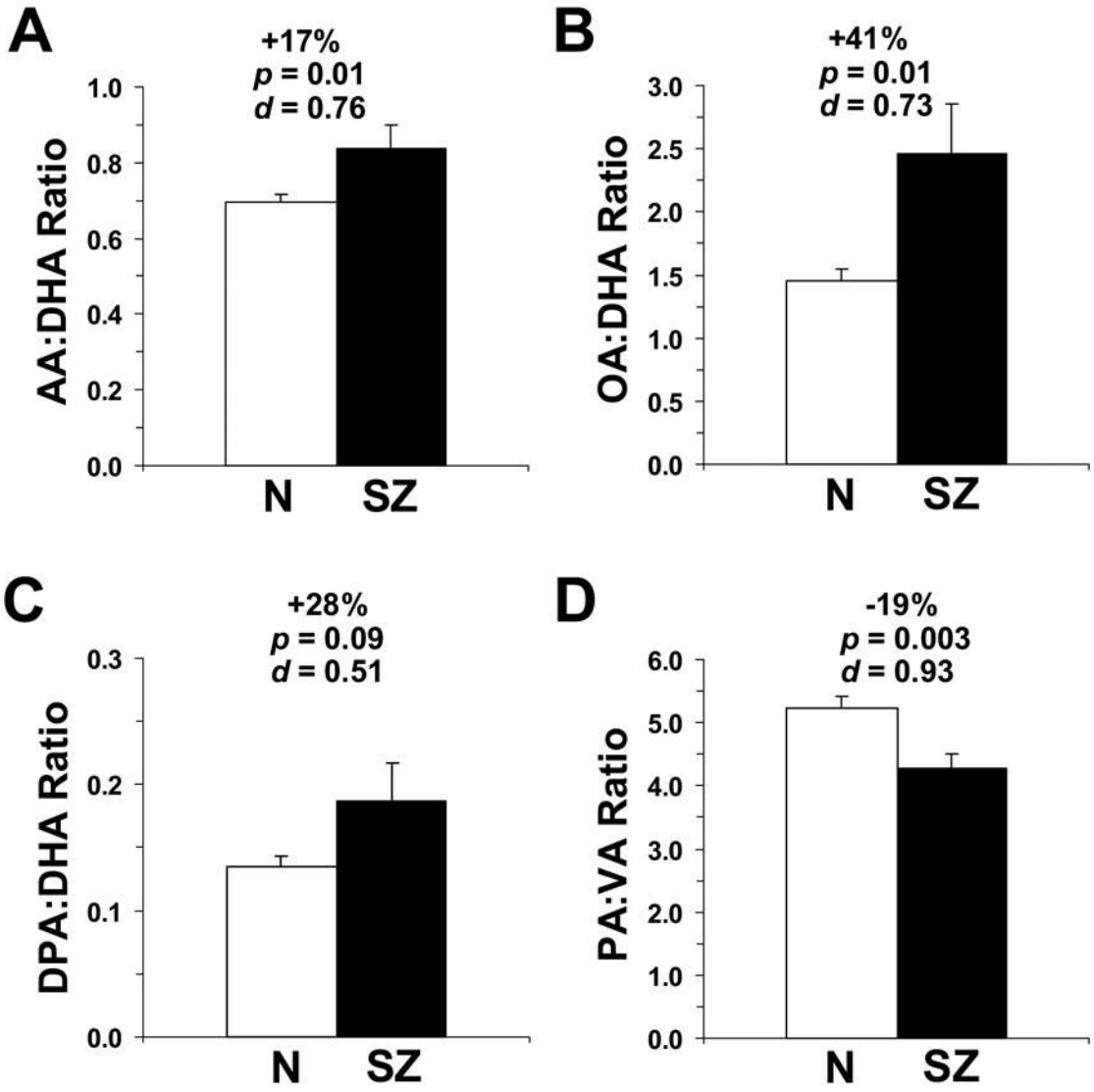

Figure 2.

Comparison of the arachidonic acid (AA):DHA ratio (**A**), oleic acid (OA):DHA ratio (**B**), docosapentaenoic acid (DPA):DHA ratio (**C**), and palmitic acid (PA):vaccenic acid (VA) ratio (**D**) in the OFC of normal controls (N)(n=26) and SZ patients (n=21). Data are expressed as mean ± S.E.M. percent total fatty acids. Effect size is expressed as mean percent difference from normal controls, associated p-values (Student *t*-test, two-tail), and Cohen's *d*-values.

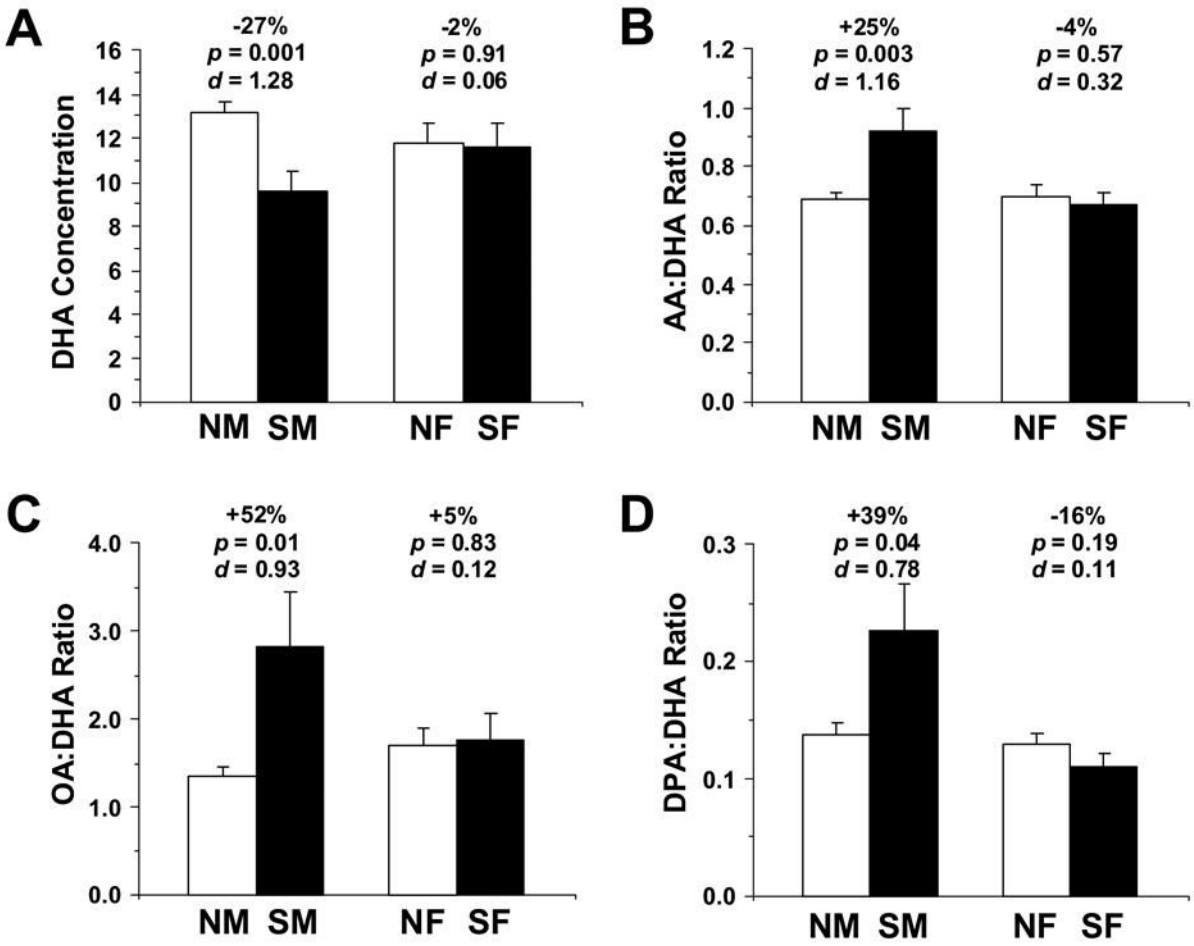

Figure 3.

Comparison of DHA concentrations (**A**), AA:DHA ratio (**B**), OA:DHA ratio (**C**), and DPA:DHA ratio (**D**) in the OFC of normal males (NM, n=19), normal females (NF, n=8), male SZ patients (n=9), and female SZ patients (n=6). Note that DHA concentrations are lower, and AA:DHA, OA:DHA, and DPA:DHA ratios are higher, in male SZ patients relative to female SZ patients relative to same-gender controls. Effect size is expressed as mean percent difference from normal controls, associated p-values (Student *t*-test, two-tail), and Cohen's *d*-values.

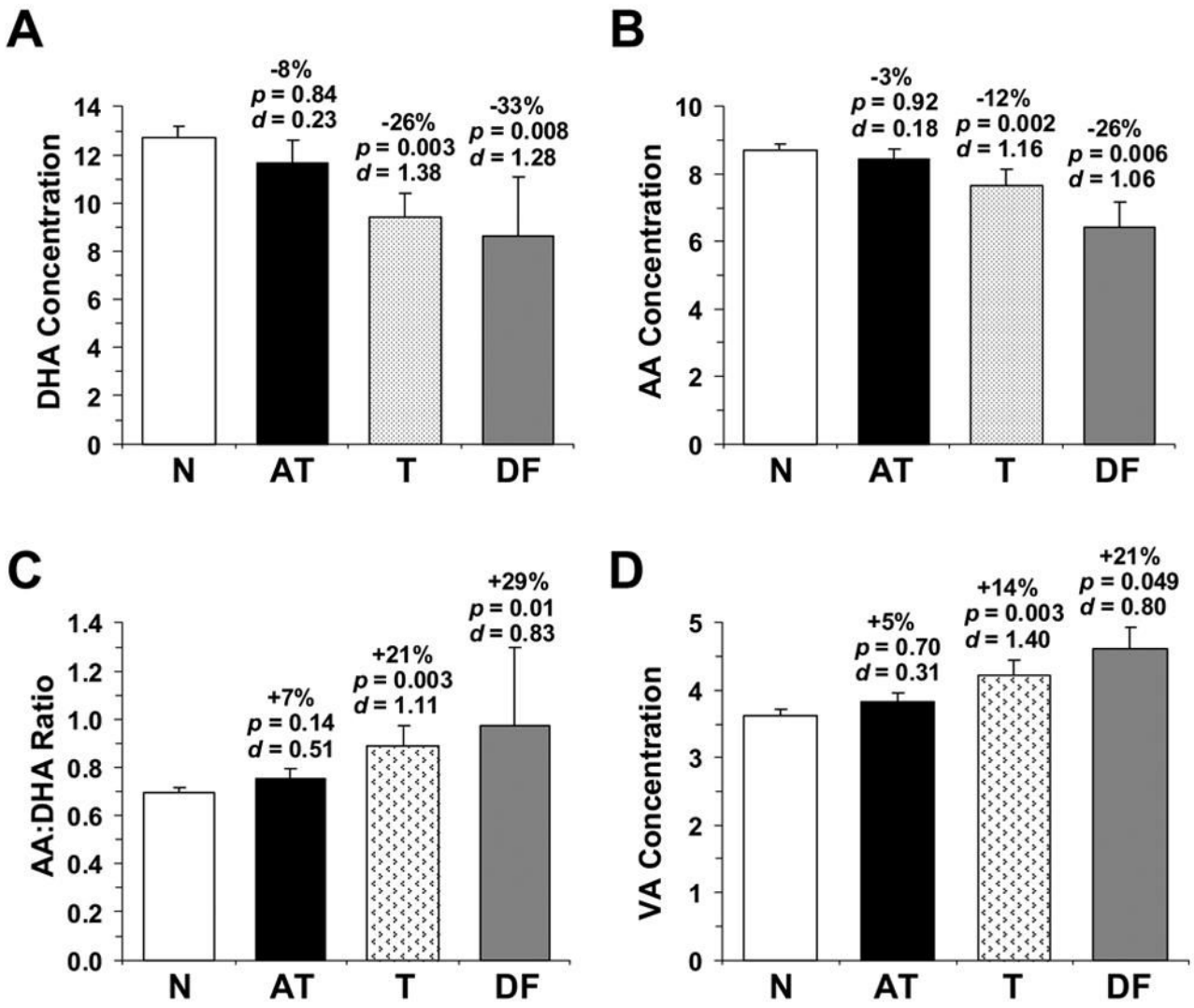

Figure 4.

OFC DHA concentrations (**A**), AA concentrations (**B**), AA:DHA ratio (**C**), and VA concentrations (**D**) (% total fatty acid composition) in normal controls (n=26), drug-free SZ patients (DF, n=3), and SZ patients treated with typical antipsychotic medications (T) (n=9) or atypical antipsychotic medications (AT) (n=9). Note that AA and DHA deficits, and elevated AA:DHA ratio and VA concentrations, are greater in drug-free SZ patients, and partially normalized in SZ patients treated with atypical antipsychotic medications, and to a lesser extent typical antipsychotic medications. Data are expressed as mean ± S.E.M. percent total fatty acids. Effect size is expressed as mean percent difference from normal controls and associated age- and gender-adjusted p-values and Cohen's *d*-values.

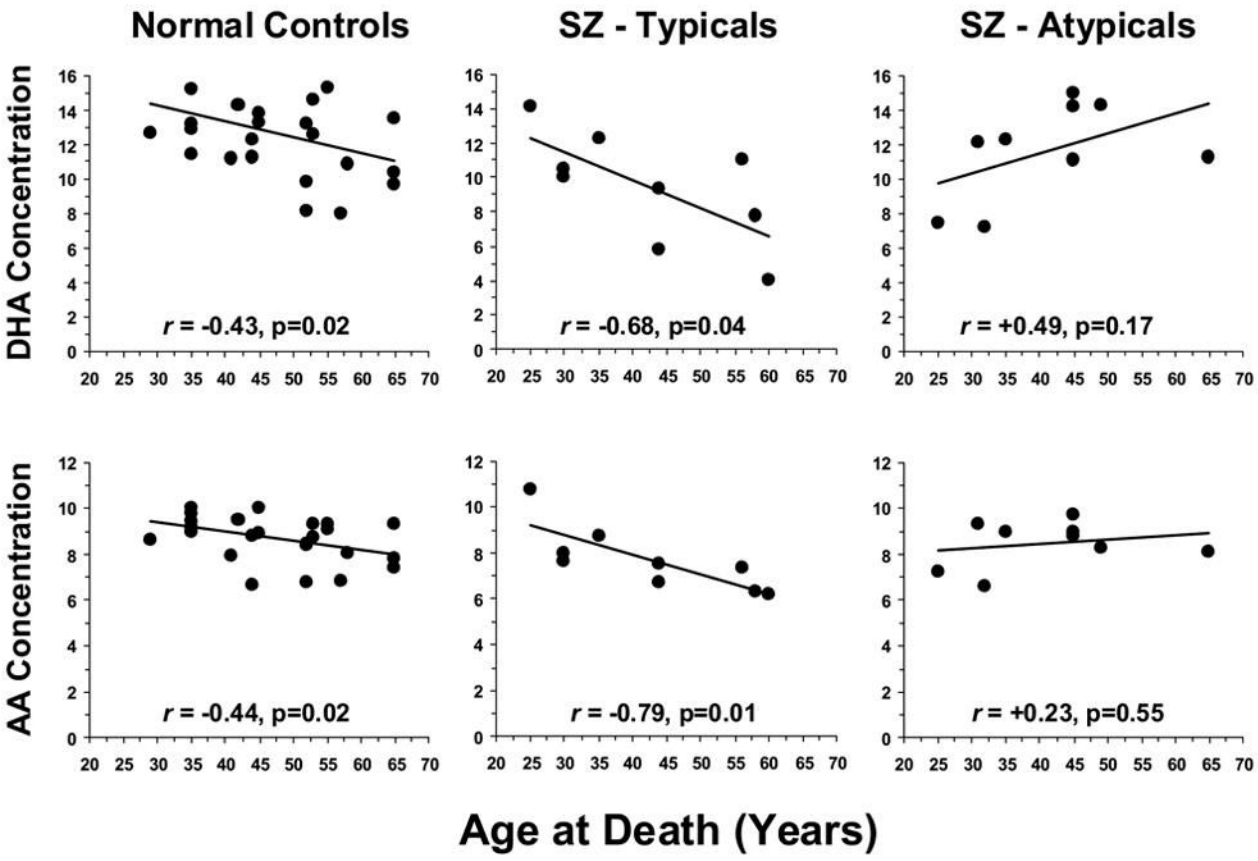

Figure 5.

Correlations between age at death and DHA and AA concentrations in normal controls, and SZ patients treated with typical (SZ – Typicals) or atypical antipsychotic (SZ - Atypicals) medications at time of death. Note that the negative correlations between age at death and OFC DHA and AA concentrations in normal controls are observed in SZ patients treated with *typical* antipsychotic medications but not in SZ patients treated with *atypical* antipsychotic medications.

Table 1

Comparison of Subject and Brain Tissue Characteristics

	Normal (n=26)	Schizophrenia (n=21)	*p*-value[1]
Patient Characteristics:			
Age at death, mean ± S.D. (range)	46.8 ± 10.7 (29–65)	44.2 ± 12.8 (25–65)	0.450
Male	46.2 ± 9.21 (35–62)	40.6 ± 12.4 (25–65)	0.149
Female	48.1 ± 14.3 (29–65)	51.4 ± 11.2 (30–62)	0.630
Gender	18M, 8F	14M, 7F	
Race[2]	19C,7UN	17C,4A	
Cause of death			
Suicide	0	5	
Cardiopulmonary	19	10	
Accident	3	3	
Other	4	3	
Age at disease onset (mean years ± S.D.)	-	23.2 ± 7.9	
Duration of disease (mean years ± S.D.)	-	20.3 ± 11.8	
Cigarette Smoker (yes/no)[3]	2/3	7/5	
Alcohol abuse severity (low/high)[3]	12/1	4/9	
Substance abuse severity (low/high)[3]	13/0	11/4	
Tissue Characteristics:			
Brain hemisphere	10R/16L	9R/12L	
Brain mass (mean grams ± S.D.)	1459 ± 161	1443 ± 27	0.704
Postmortem interval (mean hours ± S.D.)	23.6 ± 7.7	29.9 ± 14.0	0.076
Storage time (mean days ± S.D.)	330 ± 228	621 ± 233	0.002
Tissue pH (mean ± S.D.)	6.3 ± 0.3	6.2 ± 0.3	0.312

[1] Student's *t*-test (2-tail)

[2] C = Caucasian, A = Asian, UN = Unknown

[3] Smoking status, alcohol abuse severity, and substance abuse severity could not be ascertained for the remaining subjects