Diffusion tensor imaging in the early phase of schizophrenia
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Citation for published version (APA):
Peters, B. D. (2010). Diffusion tensor imaging in the early phase of schizophrenia

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Polyunsaturated Fatty Acids and Brain White Matter Anisotropy in Recent-Onset Schizophrenia: a Preliminary Study


*This chapter was published in:* Prostaglandins, Leukotrienes and Essential Fatty Acids 2009 Jul; 81(1): 61–3

**Abstract**

Brain white matter myelin abnormalities and cell membrane fatty acid abnormalities have been implicated in schizophrenia and other psychiatric disorders. We investigated in young adults with a psychotic disorder (n=12) whether (poly)unsaturated fatty acid concentrations in erythrocyte membranes are related to an MRI measure of brain white matter, which depends on the degree of myelination. A significant correlation was found between total (poly)unsaturated fatty acid concentration and fractional anisotropy of a fronto-temporal white matter tract (r = 0.503, P = 0.048). Unsaturated fatty acids may be necessary for the myelinating activity of oligodendrocytes or for myelin maintenance. These results warrant further investigation.
4.1.1 Introduction

The pathophysiology of schizophrenia may involve a disturbance in myelination resulting in compromised connectivity between neurons and brain regions (Bartzokis, 2002). Myelin abnormalities in brain white matter have been found in vivo with T2 relaxation MRI, and abnormalities of oligodendrocytes, which form the myelin sheaths around axons, have been determined postmortemly (Flynn et al., 2003). Another MRI technique called diffusion tensor imaging (DTI) has also shown microstructural white matter abnormalities in schizophrenia (Kanaan et al. 2005). In DTI, anisotropic diffusion of water along the length of axons is measured as an indication of white matter integrity. Intact axonal membranes form the basis for white matter anisotropy (Beaulieu 2002), and myelination increases the degree of anisotropy (Sakuma et al., 1991).

Demyelination in peroxisomal disorders is related to unsaturated fatty acid deficiency, which can be corrected with dietary supplementation of fatty acids (Martinez and Vazquez, 1998). Myelin sheaths are formed from the membranes of oligodendrocytes and decreased membrane fatty acid concentrations may contribute to disturbed myelination. Decreased (poly)unsaturated fatty acid concentrations have been found in schizophrenia at illness onset (Assies et al., 2000) and prior to the start of medication treatment (Reddy et al., 2004). These abnormalities may reflect a pathophysiological mechanism in schizophrenia with both environmental and genetic contributions (Peet et al., 2006).

We hypothesized that in schizophrenia patients (poly)unsaturated fatty acid concentration in erythrocytes, which reflects membrane phospholipid metabolism in the brain (Richardson et al., 2001; Yao et al., 2002), is positively correlated to brain white matter anisotropy measured with DTI.

4.1.2 Patients and methods

Subjects

Twelve male patients (mean age 22.7 ± 2.7 years) from the Adolescent Clinic of the Academic Medical Center, Amsterdam, diagnosed with a recent onset of schizophrenia or related disorder, were included after the clinical condition had stabilized. All patients received antipsychotic med-
mication (median duration 20.6 weeks, range 2.7–83.7; median dose at MRI in haloperidol equivalents 2, range 0.67–7.3). Only one patient used a typical antipsychotic; six patients used olanzapine, three risperidone, and two clozapine. Exclusion criteria were: history of a neurological or endocrine disease, history of head trauma with loss of consciousness for more than 15 min, and mental retardation. This study was approved by the local and national medical ethics committees, and all patients gave written informed consent for study participation.

MRI acquisition and analysis

MR imaging was performed on a 1.5 T Siemens Vision, and included a 3-dimensional T₁-weighted imaging (1 mm³). For DTI imaging, a spin-echo EPI sequence was used with an extra 180° pulse and balanced diffusion sensitizing gradients to minimize artefacts induced by eddy currents (Reese et al. 2003). Other imaging parameters were: diffusion sensitivities of b=0 and b=1000 s/mm², voxelsize 6.5×2×2 mm, TE 109 ms, and 2 acquisitions with six icosahedric diffusion directions (Akkerman 2003). Sixteen axial slices were collected. A neuroradiologist assessed the conventional MR images to rule out gross brain abnormalities. Fractional anisotropy (FA) images were calculated as previously described (Akkerman 2003). One group-average fractional anisotropy (FA) image of all subjects was created by applying a non-linear spatial normalization to the FA images (Woods et al., 1998) to register them to the Montreal Neurological Institute MNI152 brain only T₁-template.

White matter regions of interest (ROIs) were manually defined relative to this average image: (1) splenium of the corpus callosum, (2) frontal, (3) parieto-occipital (adjacent to the splenium of the corpus callosum), (4) anterior internal capsule, (5) uncinate fasciculus, and (6) arcuate fasciculus. All ROIs were bilateral except the splenium of the corpus callosum. After 10 mm smoothing, the average FA within each of the ROIs was calculated for each of the subjects.

Blood sample collection and analysis of fatty acids

Venous blood samples were collected at the Academic Medical Center. The mean duration between time of MRI scanning and time of taking the blood sample was 7.1 months (± 4.5). Plasma was sepa-
rated within 4 hours of collection and stored at −80°C until analysis. (Poly)Unsaturated fatty acids (pufas) in erythrocytes, i.e. ω-3 (C18:3w3, C18:4w3, C20:5w3, C22:5w3, C22:6w3), ω-5 (C14:1w5), ω-6 (C18:2w6, C18:3w6, C20:2w6, C20:3w6, C20:4w6, C22:2w6, C22:4w6, C22:5w6), ω-7 (C16:1w7, C18:1w7, C20:1w7), and ω-9 (C16:1w9, C18:1w9, C20:1w9, C20:3w9, C22:1w9, C24:1w9), were analysed by capillary gas chromatography as their methyl esters. A 50 μl sample was added to 1 ml of a 3 M methanolic HCl solution and the lipids were hydrolysed at 90°C for 4 hours, achieving simultaneous methylation of the liberated fatty acids. After cooling, the fatty acid methyl esters were extracted with 2 ml hexane. Following evaporation of the solvent, the fatty acid methyl esters were separated on a capillary free fatty acid phase (FFAP) column. All concentrations were calculated with reference to the internal standard 18-methylnonadecanoic acid.

Statistical analysis

The total of the pufa concentrations was correlated to the fractional anisotropy of each of the six white matter ROIs with Spearman's rho, one-tailed. To assess the influence of antipsychotic medication, we correlated duration and dose of antipsychotics with FA and total pufa concentration.

4.1.3 Results and discussion

Total pufa concentration (mean 287.0 ± 81.7 pmol/10^6 cells) was positively correlated to fractional anisotropy in the bilateral uncinate fasciculus ($r = 0.503$, $P = 0.048$; see figure 4.1.1). No significant correlations were observed for the other ROIs.

These findings give support to our hypothesis that membrane (poly)unsaturated fatty acid concentration is related to white matter microstructure in schizophrenia. Decreased (poly)unsaturated fatty acid concentrations may negatively influence myelination. The pathophysiological relation between pufas and white matter microstructure is speculative. Myelin lipids are not rich in fatty acids (O'Brien and Sampson, 1965), but membranes of neurons and oligodendrocytes do contain phospholipids rich in pufas (Poduslo 1975). Martinez and Vazquez (1998) have therefore hypothesized that unsaturated fatty acids may be necessary for
the myelinating activity of oligodendrocytes or for myelin maintenance. A relation between n-3 fatty acids and myelin formation has been shown in rats (Bourre 1984).

The fact that the total pufa concentration was only correlated to FA in the uncinate fasciculus was unexpected. The uncinate fasciculus connects the anterior temporal lobe with the orbitofrontal lobe, and the orbitofrontal lobe may be particularly involved in fatty acid-related brain abnormalities (McNamara et al., 2007).

In figure 4.1.1, extrapolation of the straight line of best fit indicates an intercept on the ordinate at a value of over 300. This suggests that, hypothetically, at a pufa concentration of zero there would still be considerable fractional anisotropy. We interpret this finding to confirm that fractional anisotropy depends only partially on membrane pufa concentrations and their proposed effect on myelination. Myelination is determined by mul-

Figure 4.1.1: Correlation between erythrocyte total (poly)unsaturated fatty acid concentration and fractional anisotropy in the bilateral uncinate fasciculus (Spearmann’s rho: 0.503; \( P = 0.048 \)).
multiple factors, including the coherence of fiber tracts and the structural integrity of fibers, their diameter and packing density (Beaulieu, 2002).

It has been found that reduced pufa concentrations, particularly omega-3 fatty acids, can normalize after chronic treatment with atypical antipsychotics (e.g. Evans et al., 2003; McNamara et al., 2007). However, we found no relation between duration of antipsychotic medication use and total pufa concentration. This may be related to the relatively short time our patients had been treated with antipsychotics. In addition, there was no relation between duration or dose of antipsychotic medication and FA. Previous DTI studies have correlated dosage of antipsychotics to FA values. FA was not found to correlate with medication dose in four studies (Foong et al. 2000; Kubicki et al., 2002; Kubicki et al., 2003; Kumra et al. 2005), while in three studies there was a significant correlation (Minami et al., 2003; Okugawa et al., 2004; Rotarska-Jagiela et al 2007). Kanaan et al. (2009) found no DTI differences between age-matched chronically and briefly medicated patients. Thus, although current findings are somewhat conflicting, most evidence favours little or no effect of antipsychotic medication on fractional anisotropy. Future longitudinal studies in first-episode, neuroleptic naive patients can provide further insight in the effect of antipsychotic medication on erythrocyte pufa composition and white matter anisotropy.

Our findings are limited by the small number of subjects. Schizophrenia is a heterogeneous disorder, and our sample may not be representative of all patients. Furthermore, with a bonferroni correction our results would not be significant. We chose not to perform a bonferroni correction because our correlations were hypothesis driven and chosen a priori. However, in addition to this, the size of Spearmann’s rho in the uncinate fasciculus indicates a substantial correlation.

Another possible limitation is the delay between MRI scanning and taking of the blood samples. Pufa concentrations may fluctuate over time, for example due to dietary changes. On the other hand, pufa abnormalities in schizophrenia can be considered to be an independent pathophysiological factor apart from environmental influences (Reddy et al., 2004). We therefore assumed that pufa-related white matter anisotropy alterations would show stability over time. In support of this, post-hoc partial correlations correcting for time between MRI and blood sample collection produced virtually the same results. In addition, a longitudinal study found a stable relation between fatty acid erythrocyte membrane content and cognitive functioning at 4-year follow-up in elderly subjects (Whalley et al., 2008). As mentioned above, future longitudinal studies could fur-
ther assess the temporal dynamics of membrane pufa composition and brain white matter anisotropy.

Finally, we had no information available regarding the diets of our patients. About 80% of the patients smoked nicotine cigarettes. Assessing the underlying causes of pufa abnormalities was however not the aim of our study. Decreased pufa concentrations in schizophrenia may (partially) result from unhealthy dietary habits and smoking, which in this way could cause white matter abnormalities. Larger studies will have to assess whether the relation between pufas and white matter integrity is related to factors such as diet and smoking.

Despite the limitations of this study, these results warrant further DTI (and T2 relaxation MRI) studies in larger patient samples. If further investigations confirm our hypothesis, then the effect of dietary supplementation of fatty acids on brain white matter microstructure would be an important subsequent focus of interest in schizophrenia.

4.1.4 References


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