

The effects of an omega-3 fatty acid and vitamins E and C on neuropsychological functions in schizophrenia and related psychoses.

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There is ample evidence of disturbed membrane phospholipid metabolism and oxidative stress in schizophrenia. Trials with fatty acid and antioxidant supplementation have shown conflicting results as regards symptoms. This study is part of a larger multicenter, double-blind, placebo-controlled trial of Ethyl-Eicosapentaenoic Acid (EPA) and antioxidants (vitamins E and C) as add-on to antipsychotic drugs in younger patients with schizophrenia and related disorders.

In the present study, we tested the effect of trial drugs on cognitive functioning in a geographically defined subgroup of these patients. Both patients (n=53) and controls (n=20) completed a broad battery of neuropsychological tests and were tested at baseline and at week 16. PUFA levels were measured in red blood cell (RBC) membranes. The longitudinal effects were analysed by Linear Mixed Model (SPSS 12.0). We expected the following hierarchy of effects, from greater to lower: 1) combined EPA and antioxidant treatment, 2) EPA or active antioxidants alone and 3) double placebo.

In contrast to our expectations, performance on CPT and Stroop was impaired in the groups receiving single supplements. Antioxidants prolonged the Stroop- conflict time and EPA impaired sustained attention, as measured by CPT dprime. These effects were restricted to the low-PUFA group, according to the bimodal distribution of PUFA levels found in the larger study. Combining EPA and antioxidants had a non-significant beneficial effect on sustained attention in the low-PUFA group only, compared to placebo. There were no other significant effects of trial drugs on neuropsychological measures.

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1.0 Introduction

Worldwide, schizophrenia affects 1% of the population and describes a heterogenic group with a variety in symptoms, prognosis and functioning (Austin, 2005).

Schizophrenia is often chronic and affects people at a young age. The illness is also associated with low premorbid functioning and social difficulties. About three quarters of patients with schizophrenia suffer recurrent relapse and continued disability (Nadeem, McIntosh and Lawrie, 2004) and need treatment for the rest of their lives. Onset of first time psychosis in schizophrenia occurs typically in late adolescence or early adulthood (Mueser and Salyers, 2003; Perkins, Lieberman and Lewis, 2006). Time of onset is associated with course and outcome, where later onset of first time psychosis predicts less relapse and better prognosis (Mueser and Salyers, 2003). Prognosis is also influenced by other factors, such as; gender, marriage and social function (Perkins, Miller-Andersen and Lieberman, 2006). On average, women often spend less time in hospitals, have less severe psychopathology and show better social competence than men (Malt, Retterstøl and Dahl, 2003; Perkins et al., 2006). The clinical picture of schizophrenia contains “positive” and “negative” symptoms (Lindenmayer and Khan, 2006). Symptoms of “positive” nature are those of active, hallucinating and delusional type, whereas the “negative” symptoms describe the more passive traits, as blunted affect, lack of motivation, and self neglect (e.g. Austin, 2005; Lindenmayer and Khan, 2006). The last decades research on this clinical group have raised questions about the validity of the schizophrenia diagnose. The heterogeneity of this disorder laid ground for the assumption that there exist diverse subtypes in this group. It is likely that schizophrenia as currently defined involve several heterogeneous disease processes (Sullivan, Owen, O’Donovan and Freedman, 2006). The fact that subgroups have different course and prognosis creates the basis for classification systems (Malt et al., 2003).

Cognitive deficits are often reported as a core feature in individuals with schizophrenia and are seen to account for much of the impairment in their functioning (Green, 2006). People with schizophrenia often experience a cognitive decline associated with illness onset, but the level of cognitive functioning tends to remain relatively stable over time (Mueser and McGurk, 2004). The cognitive impairments in schizophrenia are often divided in three domains; verbal learning/memory, attention and executive functions.

Improving cognition is an important target for treatment because it has profound effect on psychosocial function.

Historically, patients with schizophrenia have been a stigmatized group, with little hope for sustained remission and recovery of functioning. Countering this perspective, new findings suggest that the illness can be favourably influenced by comprehensive and continuous treatment (Lieberman, Kopelowicz, Ventura and Gutkind, 2002). Research on prognosis has also implemented the heterogeneity of schizophrenia when focusing on good prognostic variables in subgroups of patients achieving full remission or a continually symptom-free condition (Torgalsbøen, 2005). This good prognosis group is characterized by a later debut, an acute onset with positive symptomatology, have a higher share of women and do not display the typical cognitive deficits and enlarged ventricles associated with a more chronic prognosis (Torgalsbøen, 2005; Keefe and Eesley, 2006).

Treatment of schizophrenia is currently based on administration of antipsychotic medications that reduces the most prevailing symptoms of schizophrenia, like anxiety, hallucinations, delusions and rage, but leaves the negative symptoms and cognitive deficits unchanged (Mahadik and Yao, 2006). Whereas 50%-68% of treated patients have long term favourable outcome, many obtain less than optimal results from current medications (Mahadik and Yao, 2006). Taken together with the array of morbidities following such medication, such as weight gain, lethargy, insulin resistance, cardiovascular problems and abnormal lipid metabolism, it leads to a decline in the patient's quality of life and poorer lifetime prognosis (Austin, 2005; Mahadik and Yao, 2006). This underpins the importance of looking into new aspects of treatment, like in this study, where the therapeutic effect of fatty acids and antioxidants on cognition is investigated.

1.1. The aetiology of schizophrenia

The aetiology of schizophrenia is complex and has a multifactorial origin. A number of explanations that range from genetic models to those that attribute the disease to social environment are set out. The aetiology is still not fully understood, but it seems reasonable to divide the contributing aetiological factors in three groups; biological, psychological and social (Malt et al., 2003).

Data of twin and adoption studies highlight the importance of both genetic and environmental factors in the development of the illness (Austin, 2005). The genetic relatedness to an affected family member has shown to correlate with the risk of developing schizophrenia (Hollis, 2005; Sullivan et al., 2006) and it has an estimated heritability of 60%-85% (Merikangas and Risch, 2003). Efforts to identify genes involved in schizophrenia are emerging, and evidence support some candidate genes, but no chromosomal loci or genes have been unequivocally demonstrated (Mueser and Salyers, 2003; Sullivan et al., 2006). Aetiological research also strives to discern biological markers in the pathogenesis of schizophrenia. Much focus has been directed at endophenotypes¹ that are intermediate between genetic predisposition and the clinical phenotype, but no markers of sufficient sensitivity and specificity have been found (Lewis, 2004; Sullivan et al., 2006).

Considerable evidence also supports the importance of environmental factors like abnormal fetal milieu and complications during pregnancy and/or delivery (Mahadik, Pillai, Joshi and Foster, 2006). Risk factors contributing to this are: prenatal and neonatal infections, famines, maternal malnutrition, alcohol and drug abuse, season of birth and acute life events (Nadeem et al., 2004; Mahadik et al., 2006). The precise contributions of these factors and the how they interact are not clear (Nadeem et al., 2004). A critical and emotionally over involved family atmosphere has also been implicated as a contributing factor in the development of schizophrenia (Breitborde, Lopez, Wickens, and Karno, 2007). This is conceptualized as “expressed emotion” (EE) in the literature. Parental hostility is found to predict relapse and to influence onset (Breitborde et al., 2007). Furthermore, sociodemographic factors are also suggested to have a causal role as schizophrenia is overrepresented in lower social classes. The “drift hypothesis” and the “social causation hypothesis” are two theories that are set out to explain this occurrence (Kringlen, 2001). The drift hypotheses propose that suffering from a major mental illness like schizophrenia affects occupational functioning and make them drift downward in social class. The social causation hypotheses propose that experiencing the type of stress that poverty brings; elicit the predisposition of schizophrenia more frequently (Kringlen, 2001).

¹ An endophenotype is an intermediate expression of the genetic expression of the genetic predisposition for a disease that is expressed endogenously, in difference to exogenous/observable, like behaviour (Lewis, 2004).

The leading model of schizophrenia during the past century has been the Kraepelinian notion of degenerative disease, conceptualised in the term *dementia praecox* (Keshavan, Gilbert and Diwadkar, 2006). During the past few decades research in neuropathology has made an extensive contribution to the understanding of cellular and molecular mechanisms of schizophrenia. In line with this, the view of schizophrenia as a “functional” psychosis has changed to the current paradigm of schizophrenia as a neurodevelopment disorder (Chaturvedi and Thakur, 2003). The view that abnormal neurodevelopment may underlie schizophrenia has been gaining acceptance in recent years. Observations of pre-morbid behavioural, neurocognitive, and minor physical anomalies together with evidence of risk factors to brain adversity before the clinical manifestation of the illness is regarded as the strongest, although indirect, evidence of disordered neurodevelopment in schizophrenia (Keshavan et al., 2006). Over the past two decades, at least three developmental formulations have been suggested (Hollis, 2005). One formulation postulate altered pre-or perinatal brain development, another suggests peri-adolescent development abnormalities and thirdly, there are theories arguing for progressive neuroregressive processes after illness onset (Keshavan et al., 2006). There are evidence supporting all three perspectives, and preliminary evidence also suggests that they are not mutually exclusive (Keshavan et al., 2006).

It has been increasingly support around the term “schizo-spectrum” disorders as a dimensional perspective on schizophrenia. This is also being referred to as “schizotaxia” in the literature, to describe the unexpressed genetic liability to schizophrenia (Hollis, 2005; Lewis, 2004). This dimensional theoretical approach can be understood in the light of the “diathesis-stress model”, where the amount of stress an individual experiences awakens the inherited diathesis to a smaller or greater extent. In this view, an individual with the same diathesis, but with different environmental experience can e.g. stay healthy; develop a schizotypal personality disorder or schizophrenia. The definitional boundaries of this spectrum however, are uncertain (Sullivan et al., 2006).

To summarise, schizophrenia is a heterogeneous disorder as for aetiology, pathogenesis and phenotype. Today it is common to view the disorder in a neurodevelopmental perspective and to conceptualise schizophrenia in the framework of the diathesis-stress

model. One main challenge in this field is to specify validated subgroups, first as regard to phenotypes, then as regards to pathogenesis and aetiology (Compton, 2004).

1.2 The pathophysiology of schizophrenia

Schizophrenia is associated with a broad range of neurodevelopmental, structural and behavioural abnormalities that often progress with or without treatment (Mahadik et al., 2006). Neuropathology can be defined as a consistent cellular abnormality found in the nervous system (Halliday, 2001). The main positive neuropathological findings in schizophrenia concern neuronal morphology, organisation and presynaptic and dendritic parameters (Harrison, 2004). The leading neurochemical hypothesis of schizophrenia has concerned the dopamine pathways, especially since current medications for schizophrenia work primarily through the blockade of the D2-receptor (Javitt and Laurelle, 2006). Schizophrenia is also associated with other biochemical abnormalities involving serotonin, glutamate, and gamma -aminobutyric acidergic systems (Malt et al., 2003; Javitt and Laurelle, 2006). The relationship between any neurochemical and structural abnormality remains unclear.

With advances in imaging techniques, there are now consistent reports of structural and functional differences in the brains of people with schizophrenia in comparison to controls (Halliday, 2001; Harrison, 2004). These include ventricular enlargement and decreased cortical- and hippocampal volume (Halliday, 2001). Several studies have also yielded decreased frontal lobe volumes in grey and white matter (Belger and Dichter, 2006). Volume decreases are found to have functional consequences, where for instance frontal lobe grey matter has been linked to severity of negative symptoms (Belger and Dichter, 2006). There is also observed expanded or reduced sulci, giving support to an aberrant brain development not only in the subcortical areas, but in cortex as well (Lingjærde, 2002). In addition, the frontal and temporal lobes and their thalamic relays also appear to be particularly affected in schizophrenia (Halliday, 2001). Some researchers suggest that the pathophysiology of schizophrenia reflects unusual/abnormal activity and integration of the components of distributed circuits in the brain. These circuits involve the prefrontal cortex, hippocampus and certain subcortical structures (Harrison, 2004). This points out that there seems to be “global changes” in brain-systems or circuits, rather than alterations in only one area of the brain (Malt et al.,

2003; Belger and Dichter, 2006). However, as mentioned above, the neuropathology of schizophrenia and how the pathology is expressed as phenotypes is still uncertain.

2.0 The cell membrane (phospholipid) hypothesis and oxidative stress

It has been known for a long time that diet have profound effect on physical and mental health; for example are certain dietary risk factors for physical health also risk factors for depression and cognitive impairment (Peet, 2001). Here, we primarily focus on the mechanisms of fatty acids and antioxidants. Polyunsaturated fatty acids (abbreviated as PUFA`s) and antioxidants are required for the maintenance of optimal health, but are only partly synthesized in the body and need supplies from dietary sources. Patients with schizophrenia often consume a poor diet due to the nature of negative symptoms which affects their ability to take care of their health. Recognition of the importance of nutrition for mental health together with hypotheses on altered cell metabolism (the phospholipid hypothesis) and impaired antioxidant defence system (AODS) in patients with schizophrenia, have generated a body of research on the therapeutic effect of antioxidants and fatty acids.

Observations of altered membrane phospholipid composition in people with schizophrenia have been reported for decades (Du Bois, Chao and Huang, 2005). These changes have been considered to be a result of a distorted metabolism (reduced synthesis and/or increased breakdown) of fatty acids. The membrane is consist of a collection of phospholipids (complex molecules containing phosphorus and essential fatty acids) as well as receptors and a number of other structures that are responsible for the cell-metabolism (for an illustration, see figure 1 in Appendix, 10.1). Theories about the neuronal membrane describes how essential fatty acids, such as arachidonic acid (AA) from the omega-6 family, and docosahexaenoic acid (DHA) from the omega-3 family, play important roles in neurotransmission once they are incorporated into the membrane (Fenton, Hibbeln and Knable, 2000). Phospholipid composition of PUFA`s is a premise for the dynamic state of all membranes, especially neuronal (Mahadik and Yao, 2006). The cell membrane hypothesis, also referred to as the phospholipid hypothesis, proposes that the neuropathology of schizophrenia is mediated by, or has its offspring in a dysfunctional neuronal metabolism. Horrobin (1977) was the first researcher proposing a link between schizophrenia and a dysfunctional cell membrane, more specifically a deficiency in chemicals called prostaglandins that are manufactured

from essential fatty acids. PUFA influence many important intracellular processes, like the activity of ion-channels, enzyme activity, transporters and receptors. A change in membrane phospholipid composition may therefore affect neurotransmission. In turn; these modifications can affect cognition and behaviour through multifaceted downstream biological effects (Fendri, Mechri, Khiari, Othman, Kerkeni and Gaha, 2006).

Several mechanisms causing the membrane pathology in schizophrenia has been proposed; 1) reduced levels of polyunsaturated fatty acids, 2) increased activity in phospholipase A2 and 3) oxidative stress (Skrede and Holmsen, 2003).

Lack of polyunsaturated fatty acids are consistently reported in red blood cells (RBC's)², brain and skin fibroblasts in chronic-medicated schizophrenic patients as well as never medicated patients (Assies, Lieveise, Peter, Wanders, Dingemans and Linsezen, 2001). Reduced levels of membrane PUFA is associated with other neuropsychiatric conditions including Attention Deficit (Hyperactivity) Disorder, Alzheimers`s Disease, Depression and psychopathology in general (Young and Conquer, 2005; McNamera, and Carlson, 2006). Similarly, the level of RBC PUFA`s has been found to inversely correlate with the severity of psychopathology, where increased PUFA`s correlate with reduced psychopathology (Mahadik, Evans and Lal, 2001).

Increased activity in the enzyme phospholipase A2 (PLA2), an enzyme breaking down phospholipids by splitting polyunsaturated fatty acids, have also been reported among patients with schizophrenia (Ross, Hudson, Erlich, Warsh and Kish, 1997). This activity can have two broad effects on the cell membrane: direct physical-chemical changes that influence proteins and enzymes, or through an imbalance in cell communication (Skrede and Holmsen, 2003). The low levels of PUFA`s combined with increased activity of the enzyme phospholipase A2 may be causally linked to the membrane pathology in schizophrenia. For example might increased levels of phospholipase A2 reflect a

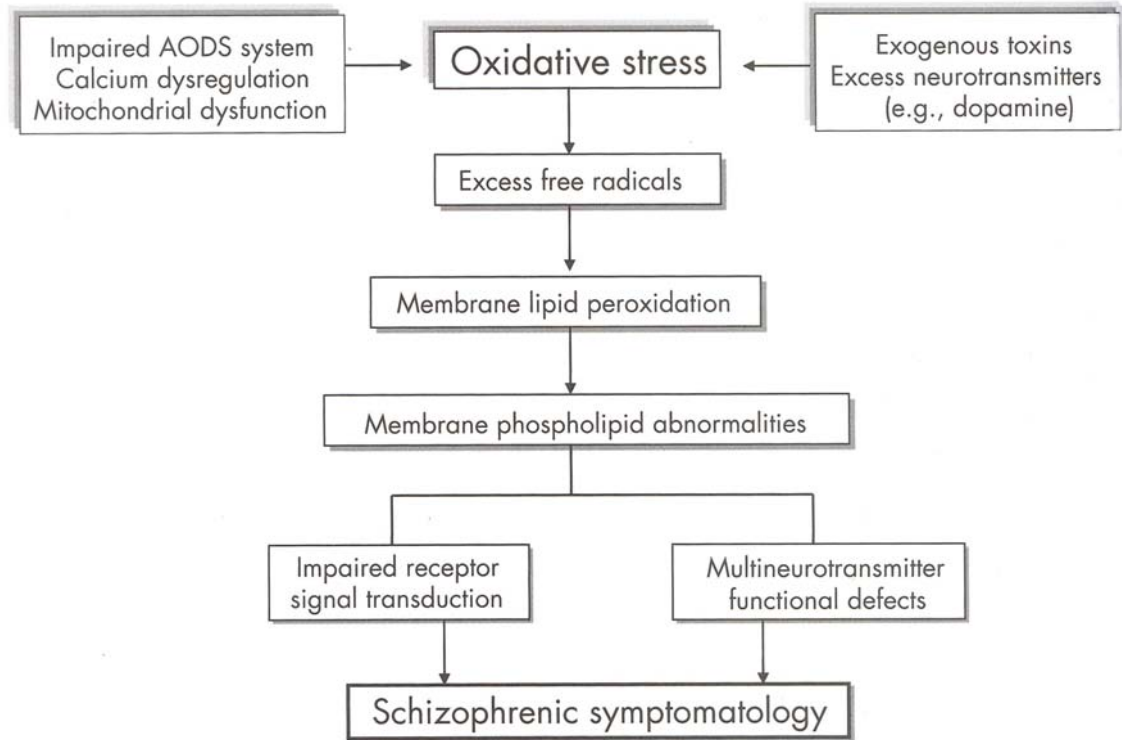
² Red blood cell (RBC) levels of PUFA are considered a reliable measure in reflecting the percent of total fatty acid, and the distribution of these in the CNS (Khan, Evans, Gunna, Scheffer, Parikh and Mahadik, 2002).

compensatory mechanism removing peroxidative PUFA's from the neuronal membrane (Skrede and Holmsen, 2003).

Another mechanism associated with this psychopathology in schizophrenia is the process of *oxidative stress*. Oxidative stress is defined as a state where there is unbalance between the generation of free radicals and the antioxidant defence system (AODS) (Fendri et al., 2006). Free radicals are a consequence of the cells interaction with oxygen as part of the energy producing process of the body. They are short-lived and are usually inactivated by antioxidants before they can damage genes, proteins or PUFA's (Mahadik and Yao, 2006). The AODS blocks the initiation of free radical chain reactions (Mahadik and Mukherjee, 1996). Both the antioxidant enzymes, already existing in the body, and antioxidants obtained from diet (non-enzyme antioxidants), are very important in the overall AODS. The non-enzymatic antioxidants (like vitamin E and C) inactivate free radicals by scavenging (Mahadik and Yao, 2006). The detection of abnormal antioxidant enzyme activity (Fendri et al., 2006), and lowered levels of plasma antioxidant proteins (Yao, Reddy and Kammen, 2001) supports a deficient antioxidant system (AODS) in schizophrenia, secondary to a free radical-mediated pathology caused by oxidative stress.

In sum, there is evidence of reduced levels of PUFA's, increased lipid peroxidation and an impaired antioxidant defence system (AODS) in patients with schizophrenia. This is consistent with high levels of oxidative stress. Antioxidant defence systems exist to protect against oxidative stress, but in schizophrenia there is evidence for a dysregulation of free radical metabolism (Reddy and Yao, 1996). It is suggested that the ongoing oxidative injuries can be prevented by dietary supplementation of antioxidants (e.g., vitamins E, C and A; flavons, beta-carotene, Q-enzyme etc.) and that the depleted membrane phospholipids can be adjusted by dietary supplementation of PUFA's (Mahadik and Mukherjee, 1996). Preliminary evidence of improved outcome of patients with schizophrenia given PUFA's and antioxidants indicates favourable effects of such dietary supplementation (Mahadik and Mukherjee, 1996). The flow diagram (figure 1) shows how the impaired antioxidant defence system (AODS) of oxidative stress may lead to membrane phospholipid peroxidative breakdown and the pathophysiological consequences that may contribute to development of schizophrenia symptomatology.

Figure 1



Source: Adapted from Yao et al. (2001).

2.1 Literature review

2.1.2 Fatty acid

Evidence in support of PUFA-deficiency as a risk factor for schizophrenia is provided by four primary areas of research: 1) cross-national epidemiological studies demonstrate improved course and outcome of schizophrenia in populations whose average national diets contains high levels of unsaturated fat compared to levels of saturated fat and total fat consumption (Christensen and Christensen, 1988), 2) reduced fatty acid (plasma and red blood cells) concentrations in patients with schizophrenia (Reddy, Keshavan and Yao, 2004), and other neuropsychiatric conditions (Young and Conquer, 2005), 3) randomized, double-blind, placebo-controlled clinical trials and case studies showing that dietary intake of EPA reduces symptom severity (Laugharne, Mellor and Peet 1996; Emsley, Myburgh, Oosthuizen and van Rensburg, 2002), and 4) studies presenting the

positive effects of DHA-intake on mental development of children (Werkman and Carlson, 1996; Lauritzen, Jorgensen, Hansen and Michalsen, 2002; Helland, Smith, Saarem, Saugstad and Drevon, 2003).

The association between cognitive functioning and fatty acids has not been comprehensively explored. One study by Fontani, Corradeschi, Felici, Alfatti, Migliorini and Lodi (2005) investigated the effect of omega-3 supplementation on some cognitive and physiological parameters in healthy subjects. The results showed that the mood-profile improved (e.g. reduced anger and anxiety states and increased vigour), and this was further associated with an effect on reactivity with a reduction of reaction time in the Go/No-Go and Sustained attention tests. The researchers concluded that omega-3 supplementation is associated with an improvement of attentional and physiological functions, particularly those involving complex cortical processing in healthy subjects. However, this study was not randomized and placebo controlled, and concerned healthy subjects.

Few researchers have investigated the effect of fatty acids and/or antioxidants on cognitive functions in schizophrenia. We searched for these kinds of studies in EBSCO, Pubmed, Psychinfo and Medline databases and were only able to find one article on this topic, executed by Fenton, Dickerson, Boronow, Hibbeln and Knable (2001). This is surprising, since cognitive functions are considered to be a fundamental deficit in schizophrenia.

The article by Fenton et al. (2001), investigated 87 patients with schizophrenia in a 16-week, double blind supplementation trial. The intervention group receiving 3 grams of EPA per day did not improve their cognitive performance compared to the group treated with placebo. This paper only explored the effect of fatty acid and only included traces (not active dose) of antioxidants-supplement. Fenton et al. (2001) suggest that dietary supplementation among less severely ill patients earlier in their illnesses and/or that a different EPA dose or treatment duration should be considered in further studies.

In order to explore the phospholipid hypothesis in schizophrenia Peet, Laugharne, Mellor and Ramchand, (1996) measured PUFA levels in patients. They found depletions in fatty acids, with the most significant reduction reported for AA and DHA.

This adds to the evidence on reduced PUFA levels in people with schizophrenia. Another study by Laugharne et al., (1996) gave subjects with a schizophrenia diagnoses ten grams of EPA per day, and found a significant decrease in scores on the Positive and Negative Syndrome Scales (PANSS) after six weeks. Moreover, the PUFA levels in patients' blood correlated directly with the improvement of their symptoms (Laugharne et al., 1996). A measure of tardive dyskinesia, a side effect of many anti-psychotic drugs, did also improve significantly with the EPA supplementation in this study.

More recently, a randomized, double-blind, placebo-controlled study by Emsley et al., (2002) investigated the effect of giving either three grams of EPA per day or placebo in addition to their usual anti-psychotic medications. The patients taking EPA showed a significant reduction of negative and positive symptoms of schizophrenia, as well as symptoms of tardive dyskinesia. Despite positive results like this, reviews going through published trials investigating PUFA's effect on schizophrenia are mixed. While some studies have found an effect of EPA on tardive dyskinesia and PANSS (Emsley, et al., 2002), others have failed to replicate this findings (Emsley, Niehaus, Koen, Oosthuizen, Turner, Carey, van Rensburg, Maritz and Murck, 2006). These equivocal results may be explained by a variety of variables like dose, age, duration of use, type of fatty acid and the heterogeneity of the schizophrenia population.

2.1.3 Antioxidants

Several studies have tried to disentangle the effects of antioxidants, fruit and vegetable consumption on cognitive functions. Studies show that both vitamin-E (Morris, Evans, Dienias, Tangney and Wilson, 2002), vegetable consumption (Morris, Evans, Tangney, Bienias and Wilson, 2006) and adjunctive use of E or C vitamin and/or multivitamin (Maxwell, Hicks, Hogan, Basran and Ebly, 2005) is associated with reduced risk for cognitive decline in elderly populations. One cross-sectional, naturalistic study investigated antioxidant use (vitamin E and C) in community-dwelling elderly women (Grodstein, Chen and Willett, 2003). This study showed that long-term, current users of vitamin E and C had better mean performance on cognitive tests, compared to women who had never used vitamin E or C (Grodstein et. al., 2003). The researches also found a tendency for higher mean scores with increasing duration of use. The benefits were also greater for women taking vitamin E and C in combination (Grodstein et al., 2003). However, Kang, Cook, Manson, Buring, and Grodstein (2006), did not find that long-

term use of vitamin E supplements gave cognitive benefits among healthy female subjects. In addition, a randomized double-blind placebo-controlled study of antioxidant vitamin supplementation in the elderly yielded no significant effect of supplementation on mood or cognitive function (Smith, Clark, Nutt, Hayward and Perry 1999; Smith, Clark, Nutt, Hayward and Perry, 2000).

In treatment of schizophrenia, vitamin E supplementation has proved to improve symptoms of tardive dyskinesia (TD) (Mahadik and Gowda, 1996). In a total of 14 studies, all but two (Adler, Rotrosen, Edson, Lavori, Lohr, Hitzemann and et al., 1999; Shriqui, Bradjewejn, Annable and Jones, 1992) found vitamin E beneficial in controlling some symptoms of TD and psychopathology (Mahadik et al., 2006). The effect of vitamin C in patients with schizophrenia is less explored, as we found only one study carried out by Dakhale, Khanzone, Khanzone, and Saoji (2005). This is the first study to investigate a single supplementation of vitamin C on forty patients with schizophrenia. Their prospective, double-blind and placebo-controlled study, revealed that vitamin C supplementation added on the patients typical antipsychotic medication reduced measures on oxidative stress, reversed the initially low levels of ascorbic acids (vitamin C) and improved scores on the Brief psychiatric rating scales (BPRS) score compared to placebo with atypical antipsychotics (Dakhale et al., 2005).

In summary, fatty acids have yielded mixed results in treatment of symptoms in schizophrenia. In a review of all randomized trials of fatty acids in the treatment of schizophrenia, Joy, Mumby-Croft and Joy (2006) conclude that the supplement of fatty acids reduces the need for antipsychotic medication and may improve their mental state, but that the use of fatty acids in schizophrenia is still experimental. The researches highlight the need for large well designed studies to ensure the effect of fatty acids in schizophrenia (Joy et al., 2006). In regard to antioxidants like vitamin E and C, epidemiological studies have not been able to establish a consistent relationship between antioxidant status and cognitive functioning in generally healthy subjects. However in schizophrenia, both antioxidants have shown to reduce psychopathology and symptoms of TD. Finally, the effects of fatty acids or antioxidants on cognitive functioning in schizophrenia are minimally explored. Our conclusion is that there is a shortage of research studying the association between cognition and dietary supplementation of fatty acids and antioxidants in individuals with schizophrenia

2.1.4 Interaction-effects

The only study using a combination of fatty acids and antioxidants in people with schizophrenia was done by Arvindakshan, Ghate, Ranjekar, Evans and Mahadik (2003). Arvindakshan et al., (2003) investigated the outcome on symptom-scales when patients with schizophrenia were given a *combination* of fatty acids and antioxidants (vitamin E and C). They found a significant reduction in psychopathology based on reduction in individual total BPRS, PANSS and general psychopathology -PANSS. Additionally, they found an increase in Henrich's Quality of Life (QOL) scale. Unfortunately, Fenton et al., (2001), only used traces of antioxidants in his study so we did not find any demonstrations of the interaction effect of antioxidants and fatty acids on cognition in individuals with schizophrenia.

In sum, no consistent pattern linking fatty acids and antioxidants to better cognitive outcome in schizophrenia has been identified (Addington, 2000). Studies investigating fatty acids in relation to deficits of the cell membrane and psychiatric disorders have pointed to several critical issues in designing and carrying out new studies (Mahadik and Evans, 1997, Fenton et al., 2000; Mahadik et al., 2001). One important aspect concerns the age of patients used as participants. This is essential because after 45 years of age the antioxidant defence declines rapidly, and adjunctive use of antioxidants may be critical. Secondly, duration of illness is also vital because some typical antipsychotics have been found to have a pro-oxidant property (Jeding, Evans, Akanmu, Dexter, Spencer and Aruoma et al., 1995). Thus, long-term use of typical antipsychotics may have entailed membrane pathology that may be difficult to correct. Other important factors are the type and dose of PUFA's given to the subjects. Thus, the combined use of the antioxidants and PUFA's may be necessary for optimal treatment of oxidative cell injury. Few studies reported so far involve the use of both supplements in patients with schizophrenia.

3.0 Schizophrenia and cognition

The pattern of cognitive deficits found in schizophrenic patients is widely accepted as a characteristic of schizophrenia (Lewis, 2004), yet it has never been included in any of the major diagnostic criteria systems. It is an ongoing debate whether the diagnostic criteria for schizophrenia should be reconsidered (Tsuang and Faraone, 2002; Lewis,

2004). It is argued that diagnostically it is an overreliance on psychotic symptoms, which are not specific to the schizophrenia diagnose. Some researchers even postulate schizophrenia to be a cognitive disorder (Green, Nuechterlein and Gold, 2004). Lewis (2004) proposes that cognitive deficits should be included for clinical and educational reasons, and as a way to identify vulnerable pre-psychotic children and adolescents. The cognitive deficits are well established by research, where families with an affected member are found to have the same pattern of abnormal cognitive functioning when tested neuropsychologically (Hollis, 2005). This rules out the perspective where cognitive deficits in schizophrenia is secondary to psychotic symptoms. Severely impaired performance on cognitive tests is the strongest evidence for the importance of cognitive deficits in schizophrenia. Stable cognitive functions might also be favourable to prevent relapse, or increase time between relapses. This is also why many specialised cognitive training-programs are under development (Ueland, 2005).

Cognitive impairments found in individuals with schizophrenia includes problems in speed of processing, attention/vigilance, working memory, verbal learning, visual learning, reasoning and problem solving, and social cognition (Keefe and Eesley, 2006). Green (2006) also suggests that these deficits can serve as an endophenotype for the illness in studies of genetics. Analyses of data from 150 patients with schizophrenia suggest that up to 98% of patients perform worse on cognitive tests than would be predicted by their parents education level (Keefe, Eesley and Poe, 2005). Consistent findings report a lowered IQ-score in individuals with schizophrenia (Keefe and Eesley, 2006). The average impairments can reach 2 standard deviations below the healthy control mean in several cognitive domains (Keefe and Eesley, 2006).

According to Bowie and Harvey (2006) deficits across neurocognitive domains such as attention, working memory, language skills, and executive functions tend to be moderate, with the most pronounced deficits found in verbal learning and memory. In general, the domains that are most consistently cited as being severely impaired in schizophrenia are in memory, attention/vigilance and executive functions (Malt et al., 2003). Next we will elaborate on these domains.

3.2 Verbal learning and memory

Patients with schizophrenia demonstrate memory deficits in a variety of different tasks. Deficits include problems with working memory, episodic and semantic memory, while implicit memory remains intact (Egeland, Sundet, Rund, Asbjørnsen, Hugdahl and Landrø et al., 2003). Knowledge of deficits in semantic memory is important because it is closely related to thought disturbances, which are regarded as a core symptom of schizophrenia. Memory can be divided into three parts; encoding, retention and retrieval. In terms of episodic memory, evidence indicates that encoding is the most vulnerable part of the memory process for patients with schizophrenia (Egeland et al, 2003). It is also clear that these deficits are not secondary to neuroleptic medication, attentional struggles or differences in subgroups (Egeland and Landrø, 2005). Research indicates that this finding does not reflect the general lowering in IQ amongst patients with schizophrenia (Egeland and Landrø, 2005).

The abilities involved in memory functioning include those associated with learning new information, retaining newly learned information over time and recognizing previously presented material (Keefe and Eesley, 2006). In general, patients show larger deficits in learning than in retention (Keefe and Eesley, 2006). The findings for recognition are vaguer, where relatively mild and large deficits have been reported. The tests used to measure learning typically involve the ability to learn lists of words or written passages. After five repeated trials of the same word list, most control subject can recall at least 13 of the words (total of 16), while patients with schizophrenia on average solely recall 9 (Keefe and Eesley, 2006). Additionally, patients with schizophrenia can recall about 5 words after the first trial, while healthy controls recall approximately 8 words. Hence, patients are impaired both in their ability to learn over time and in immediate recall compared to control subjects.

3.3 Attention

Many neurocognitive tests require vigilance functions, even if the test itself is not a measure of “pure” vigilance. Vigilance refers to the ability to maintain attention over time. The Continuous performance test (CPT) is a standard vigilance test used in several studies. Attentional vigilance and a reduced capacity to focus are considered both as a core symptom and as an underlying mechanism for other cognitive dysfunctions (Borg and Rund, 1999). Studies show that people with schizophrenia perform poorer than healthy controls on task of attention (Chen, Chang, Liu, Hwang and Hwu, 2004).

Deficits in sustained attention remain relatively unchanged through symptom fluctuations, illness stages, and were not changeable with neuroleptics when more difficult CPT versions were used (Liu, Hwu and Chen, 1997 and Hong, Kim, Koh, Koo, Kim and Lee et al., 2002). Span of Apprehension-tests (SPAN), Backward Masking test and different versions of the CPT are common assessments used to investigate performance in attention.

Attentional impairments are also seen in pre-psychotic people before they exhibit signs of schizophrenia (Chen et al., 2004). It has also been explored if non-psychotic relatives of schizophrenic and schizotypal probands have an elevated risk of deficits in sustained attention as measured by CPT. In their study Chen et al. (2004) concludes that relatives of both schizophrenic and schizotypal personality disorder show poorer performance in comparison to controls, but that the effect was more profound for the schizophrenic relatives than for the schizotypal probands (Chen, Liu, Chang, Lien, Chang and Hwu 1998). In this view, sustained attention deficits measured by CPT have been considered as potential vulnerability indicator for schizophrenia (Keri and Janka, 2004).

3.4 Executive functions

“Executive functions” refers to a set of cognitive operations on a higher level that are essential for the production of goal-oriented behaviour. These skills are necessary to generate and execute goal-directed behaviour, especially in novel situations. This involves working memory and being able to change actions in response to feedback. Executive functions are presumed to be mediated by the prefrontal cortical system (Gazzangia, Ivry and Mangun, 2002). The prefrontal cortical system is connected in numerous ways to the rest of the brain, which means that executive functions rely on a set of less demanding processes. Wisconsin Card Sorting Task (WCST) is one of the most commonly used test in this paradigm and tap functions like cognitive flexibility, problem solving, rule-learning and maintaining information “on-line”. A great number of studies confirm that patients with schizophrenia perform poorer on this test (Badcock, Michiel and Rock, 2005).

4.0 Aims of study

Our main objective was to investigate the association between supplements and cognition. We expect a graduation of efficacy on cognitive functions in the following

order: 1) combined EPA and antioxidant treatment, 2) a choice of EPA or active antioxidants and 3) placebo.

5.0 Methods

5.1 Subjects and design

This study is part of a larger multicentere, double-blind, placebo-controlled trial of Ethyl-Eicosapentaenoic Acid (EPA) and antioxidant (vitamins E and C) supplementation in the treatment of younger patients with schizophrenia and related disorders. The aim of the larger study was to elucidate the effect of adding a polyunsaturated fatty acid (eicosapentaenoic acid) and/or antioxidant supplementation (vitamin E and C) to neuroleptic treatment of patients.

Inclusion criteria were: 1. diagnoses of schizophrenia, schizophreniform or schizoaffective disorder (DSM-IV). 2. Admitted to a hospital/department within the previous seven days before screening. 3. Less than six years, in retrospect, since first psychotic symptoms (DSM-IV 295, criteria A, 1-4). 4. Age 18-40 years. 5. Speaks fluently a Scandinavian language. 6. A written informed consent form must be obtained before any trial-related activities. 7. At baseline, significant psychiatric symptomatology as shown by either a score of at least 15 on the positive or negative PANSS subscales or a total PANSS score (P, N and G subscales) of at least 60. Exclusion criteria were: 1. A diagnose of substance dependence (DSM-IV). 2. Known allergy to study medication. 3. Currently taking warfarin or having anamnestic indicators of impaired haemostasis (profuse bleeding, except epistaxis).

The patients are selected from hospitals in southern Norway, during an enrolment period of one year. The experimental intervention lasted four months. The sample was tested at baseline and at week 16. Demographic details are given in Table 1.

5.2 Neuropsychological assessment

Both patients and controls completed a broad battery of neuropsychological tests. The selected tests assessed attention, working memory, verbal short-term memory and executive functions. The battery consisted of the following tests: the Continuiuos Performance Test IP Version (CPT), the Letter-Number span test (LN-span), the Stroop test, the Johns Hopkins Verbal Learning Test (HVLT), the Paced Auditory Serial

Addition Test (PASAT), the Letter Fluency Test (phonological association), the Category Fluency Test (semantic association) and the Kimura Reccuring Recognition Figures Test (Kimura). The Picture Completion test and the Similarities task from the WAIS battery were conducted at occasion one as an estimate of IQ.

The tests were administered in a fixed order, though if the administrator observed signs of fatigue they were free to administrate them in another order, but as close to the original order as possible. The HVLT however, were fixed in all settings because of the nature of the tests: HVLT includes a test of recall and recognition 20 minutes after reading the list and the test of immediate recall. The CPT was also fixed in all settings and was administered in between the two HVLT-tests to maintain a fixed time-ratio for all. The administration of the test-battery was completed by five trained research assistants. All retest were administered within 17 weeks of the first testing.

2.2.1 Attention

Tests of sustained attention typically involve the sequential presentation of stimuli over a period of time with instructions to the patient to indicate in some way when a given target stimulus is perceived. These tasks are generally performed well by persons whose capacity for sustained attention is intact (Lezak, Howieson and Loring, 2004). The Continious Performance Test IP Version (CPT-IP) (Cornblatt et al., 1989) was selected to test sustained attention in this study. Here the subjects had to remove their finger off a mouse button when two successively presented stimuli were identical. In the present study test number 4 was used consisting of trials with four digit numbers and trails with figures. In line with common practice, the performance index reported is the signal detection parameter d' (dprime) and β (beta). The sensitivity index, dprime, measures an individual's ability to discriminate target stimuli from non-target stimuli, while the response criterion index, beta, is considered an indicator of a patient's task-taking strategy (Liu, Hsieh, Hwang, Hwu, Liao and Lin et al., 2006). Variables irrelevant to the intended comparison of sensitivity, such as differences in level of motivation or cooperativeness, would yield differences in beta rather than in dprime (Cornblatt and Keilp, 1994). Typically, schizophrenia patients and subjects in populations at risk for schizophrenia are characterized by lower dprime than controls. On the other hand, these populations do not show consistent difference in beta (Cornblatt and Keilp, 1994). The dprime and beta were calculated across all trials and used in the analysis.

Stroop tests are based on the findings that it takes longer time to call out the colour name of coloured patches than to read words, and even longer to name the colour of the ink in which a colour name is printed when the print ink is different than the colour name (Dyer, 1973 cited in Lezak, 2004). The latter phenomena have raised a debate to explain this effect. Some argue that the slower response is due to a response conflict, while others postulate a failure in selective attention (cited in Lezak, 2004, p.366).

In this study a Norwegian version of the Stroop test (Spreen and Strauss, 1998) was used. It includes three subtests: naming colour, reading words and colour and colour-word. In the colour-word subtest the participants are asked to name the colour of the ink the words are written in. Each stimulus in the conflict condition consists of two sorts of information; colour words (e.g. green) are written in an incongruent ink colour (e.g. blue). The test requires the participants to selectively focus on the ink-information, and to ignore the written word. In the analysis, the time used on the conflict condition was calculated.

5.2.2 Working memory

As a measure of the capacity of working memory the Letter-Number span (LN-span) (Gold, Arndt, Napoulos, O'Leary, and Andreasen, 1997)³ was chosen. Here the subjects are presented with a line of alternating letters and numbers. The subjects are instructed to respond by first saying the numbers in order from 1-9, followed by saying the letters in alphabetic order. It consists of 24 trials with increasing difficulty (from 2 to 7 items). This assess the capacity of working memory because the subjects are given increasingly difficult lines of letters and numbers and are then asked to do standard Working Memory operation by sorting the information given to them. The capacity increases with longer correctly completed strings of items. In the analysis a total of correctly completed trails were used.

To assess the attention control of the working memory, a Norwegian version of the Paced Auditory Serial Addition Test (PASAT)³ (Spreen and Strauss, 1998) was used. This test consists of sixty recorded digits presented to the subject on tape. The digits are

³ Norwegian version by Nils Inge Landrø, University in Oslo.

presented in a two second ratio, and are asked to sum the two latest read digits. The subject has to respond to the two first digits read, and then taking attention off from their own answer, listen to the new digit read, remembering the digit which was read prior to their responding, sum them up and give a new response. The total of the correct responses was used in the analysis.

5.2.3 Episodic memory

As a measure of Verbal episodic memory the John's Hopkins Verbal Learning test (HVLT) (Lacritz and Cullum, 1998) was conducted. This is a verbal memory test where recall, recognition and learning potential can be measured. The participants are asked to remember a list of twelve words. The list is read for the participants three times registration of the immediate recall in between. The list items were changed from occasion one to occasion two. The selected target variable was overall learning. This is a sum score of the three registrations of immediate recall.

5.2.4 Non-verbal memory

To assess non-verbal memory the Kimura Recurring Figures Test was used. The material consists of cards containing either geometric or nonsense figures (Lezak et al., 2004, pp. 450). The subject's task is to indicate what item were seen previously. We use a Kimura total sum score where false positives are subtracted from the total score to correct for guessing.

5.2.5 Executive functions (fluency tasks)

The verbal fluency test is used to assess initiation processes and strategies for guiding the search for words (Lezak et al., 2004, pp.518). This test comprises two subtests: The Letter Fluency Test (phonological association) and the Category Fluency Test (semantic association). For the activation task the subjects were instructed to say aloud as many words as they could think of beginning with a specified target letter (letter fluency; phonological association) or a specified target category (animals, jobs, fruit and vegetables). The subjects were asked to produce as many items they could think of within the time limit of one minute for each letter/category. A sum score of all words generated was used in the analysis.

5.3 Study drugs

1. Generic name: ethyl eicosapentaenoate

Trade name: Ethyl-EPA

Presentation: capsules containing 500 mg ethyl eicosapentaenoate.

2. Generic names: RRR- α -tocopherol, ascorbic acid

Trade name: CellaVie (Ferrosan A/S)

Presentation: tablets containing 91 mg RRR- α -tocopherol and 250 mg slow release ascorbic acid

5.3.2 Placebo

1. Placebo EPA

Presentation: capsules providing 500 mg mineral oil

2. Placebo antioxidant

Presentation: tablets providing dicalciumphosphate (cfr. Ferrosan A/S)

5.1.3 Dosage and administration

The four treatment arms are:

Active EPA: 2 g/day delivered as 2 capsules with EPA x 2 (with food), and

Active antioxidants: RRR- α -tocopherol 364 mg and ascorbic acid 1000 mg a day, delivered as 2 tablets x 2 (with food).

Active EPA: 2 capsules x 2 ; *placebo antioxidant*: 2 tablets x 2.

Active antioxidant: 2 tablets x 2; *placebo EPA*: 2 capsules x 2

Placebo EPA: 2 capsules x 2; placebo antioxidant: 2 tablets x 2

5.5 Statistical analyses

The statistical tests were carried out using the Statistical Package for the Social Sciences (SPSS, version 12.0). The significance level was set at $p \leq 0.05$ for all analyses.

To analyse differences between patients and healthy controls in demographical data, a Pearson's Chi-Square or Fisher's exact test were used for categorical variables. For the remaining variables independent two-sample *t*-tests were used. To analyse group differences on cognitive test scores, a *t*-test was used (Table 2).

Missing data is an important issue for analyzing and interpreting results, especially in clinical groups. Patients with schizophrenia have a high drop-out rate in follow-up studies, and this entails a high proportion of missing data. The appropriate model for analyzing will be a Linear Mix Model that takes in consideration the unbalanced nature of longitudinal data and which explores both fixed and random effects. The pattern of missing data is not known on forehand, and these data will therefore be treated as randomly distributed (Tu, Zhang, Kowalski, Shults, Feng and Sun et al., 2006).

We used the Linear Mixed Model (LMM) programme to analyse longitudinal effects. We examined the effect on neurocognitive tests of time, treatment groups and baseline PUFA. Time was coded (0,1), and treatment categories were EPA (0,1) and antioxidants (0,1). PUFA was a continuous variable (referred to as "omegasum" in the tables), (≥ 0 $\mu\text{g/g}$ Red Blood Cells).

"Subject" (intercept) was treated as the only random effect. The fixed effects were intercept, time, time x EPA, time x antioxidants and time x EPA x antioxidants in the model. Placebo and active treatment groups were assumed to be equal by design as for the outcome variable at baseline, because allocation to treatment groups was random. Thus the main effects of EPA and antioxidants were not included in the statistical model. In the model adjusted for a moderator (baseline PUFA), fixed effects of PUFA, time x EPA x PUFA, time x antioxidants x PUFA and time x EPA x antioxidants x PUFA were added to the raw model.

Data imputed in General Linear Model (GLM) (figures) from Linear Mixed Model analyses (SPSS 12.0). *p-values*: LMM moderator (PUFA at baseline) has been centered on typical patients from whole group, low and high groups, respectively; whole sample (n=49) used. In order to display the results graphically, the GLM programme (SPSS 12.0) had to be used. Values for the dependent variables predicted by LMM replaced missing values in GLM analyses. In GLM analyses, adjustment was done for baseline dependent variables in order to have the same point departure for the treatment options in each figure.

6.0 Results

6.1 Demographical and psychometric characteristics

There were significant differences between the patients and controls in age, sex and education (Table 1). Both groups completed the Similarities and the Picture Completion test, which is part of the WAIS battery and from which an IQ-score can be roughly estimated, as the first test is highly correlated with verbal IQ and the second with non-verbal IQ. The patients scored significantly below the controls on both tests, as can also be seen in Table 1.

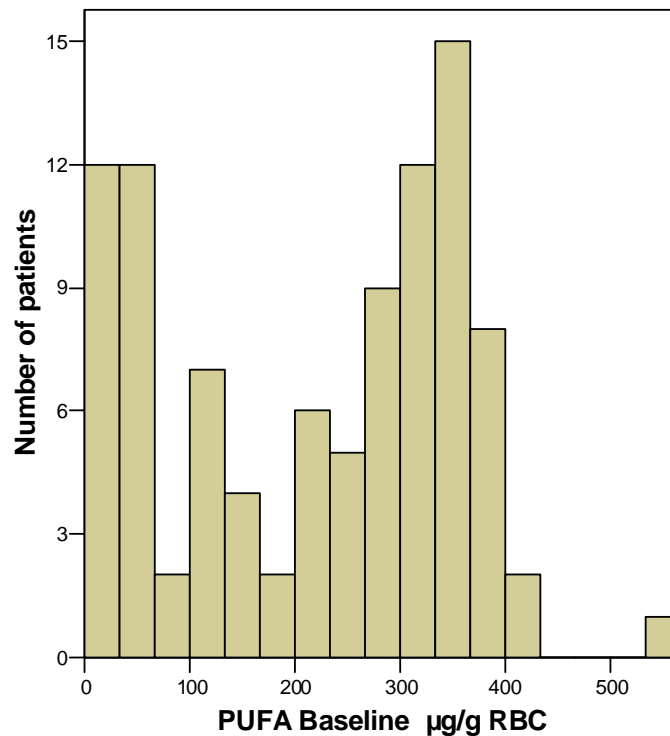
Table 1

Mean and S.D of demographic variables for the patients and the controls and t-test of the difference between the groups

	Patients (n=53)	Controls (n=20)
Age (years)	26.2 (5.0)	31.1 (5.3) *
Education (years)	11.7 (1.9)	14.6 (1.5) *
Gender (m/f)	37/16	11/9
Doi (years)	3.9 (3.7)	----
Similarities ¹	9.0 (2.8)	11.7 (2.5) *
Picture completion ¹	7.8 (3.0)	11.8 (2.9) *
Doi =Duration of illness	¹ Both tests are part of WAIS battery	* p<0.05

Retrospective analysis of the whole trial sample (n=99) showed that the concentration of polyunsaturated fatty acids in red blood cells at baseline was bimodally distributed (Bentsen, Solberg and Lingjærde et al., in prep 2007). It has been shown statistically that the sample can be divided in two groups according to the patients PUFA levels: a high PUFA group ($> 137\mu\text{g/g RBC}$) and a low PUFA group ($\leq 137\mu\text{g/g RBC}$) as seen in figure 2.

Figure 2 Distribution of RBC polyunsaturated fatty acids in all patients at baseline (n=99)



6.2 Cognition

a) Comparison between patients and healthy controls

The mean neuropsychological test scores for the patients and the controls and a t-test of the group differences at baseline are presented in Table 2. As expected, the overall

performances of the clinical groups were below the control groups. The controls perform significantly better on all tests than the patients, except for the CPT beta and Kimura.

Table 2

Mean and S.D. of the neuropsychological test scores for the patients and the controls and t-test of the difference between the two groups at baseline.

	Patients (n=53)	Controls (n=20)	Significance
<i>Attention</i>			
CPT dprime	1.2 (0.5)	2.0 (0.6)	<.001
CPT beta	2.1 (1.5)	1.7 (0.8)	n.s.
Stroop conflict	59.3 (19.7)	43.2 (7.2)	<.001
<i>Working memory</i>			
LN span	11.6 (3.5)	14.6 (3.6)	.008
PASAT	37.9 (14.2)	50.7 (8.5)	<.001
<i>Episodic memory</i>			
HVLT	20.3 (6.7)	27.0 (3.8)	<.001
<i>Non-verbal memory</i>			
Kimura	22.3 (7.3)	24.7 (6.5)	n.s.
<i>Executive functions (fluency tasks)</i>			
Phonological fluency	18.2 (8.1)	24.1 (8.7)	.008
Semantic fluency	31.6 (9.5)	45.3 (8.5)	<.001

b) Effects of treatment in the patient group

The effect of trial drugs was analyzed by the standard adjusted LMM on the following cognitive functions: verbal episodic memory (Hopkins), attentional control of working memory (PASAT), capacity of working memory (Letter-number span), phonological fluency (The Letter Fluency Test), semantic fluency (The Category Fluency Test),

sustained attention (CPT, dprime), selective attention (Stroop, conflict; time) and non-verbal memory (Kimura). Attention turned out to be the only domain affected (CPT dprime and Stroop) by trial drugs. The standard adjusted LMM analyses showed only significant effects of antioxidants on Stroop conflict time and of EPA on CPT dprime. Both were moderated by the level of baseline PUFA, thus the effect was less beneficial or more harmful the lower the PUFA. We selected these attention variables for further LMM analyses of the effect of healthy control-patient status and time (Table 3 and 4).

Table 3 shows that EPA impaired the course of CPT dprime over the trial ($p=0.02$), but this effect was positively modified by the PUFA level at baseline ($p=0.02$). Thus, the lower the PUFA, the less beneficial was the effect of EPA on the course of CPT. There was no effect of antioxidants on the course of CPT. However, the effect of EPA was positively modified by antioxidants ($p=0.03$). Thus, combining EPA and antioxidants entailed a beneficial effect on CPT compared to placebo or one supplement alone.

Table 3

Estimates of Fixed Effects

Parameter	Estimate	95% Confidence Interval	Sig.
Intercept	1.10	.76 -1.45	.000
Time	.33	.10 - .56	.006
EPAgroup x Time	-.56	-1.03 - -.10	.019
Antiox.group x Time	-.07	-.71 - .58	.832
EPAgroup x Antiox.group x Time	1.2	.12 - 2.29	.031
Omeegasum*	.000	-.000 - .002	.481
EPAgroup x omeegasum* x Time	.002	.000 - .003	.021
Antigroup x omeegasum* x Time	.000	-.003 - .004	.904
EPAgroup x Antiox.group x Omeegasum* x Time	-.004	-.008 - .001	.143

Dependent Variable: CPT dprime (attentional sensitivity). *Omeegasum = PUFA level at baseline.

Table 4 shows that antioxidants impaired the course of the Stroop test over the trial ($p=0.01$), but that this effect was negatively modified by the PUFA level at baseline

(p=0.008). Thus, the lower the PUFA, the less beneficial was the effect of antioxidants on the course of the Stroop test. Antioxidants or combining the trial drugs did not have any effect on the course of the Stroop test.

Table 4

Estimates of Fixed Effects

Parameter	Estimate	95% Confidence Interval	Sig.
Intercept	21.34	12.18 – 30.50	,000
Time	2.66	-8.55 – 13.87	.64
EPAgroup x Time	-6.65	-29.81 – 16.50	.57
Antiox.group x Time	35.22	8.09 – 62.36	.01
EPAgroup x Antiox.group x Time	-26.64	-76.03 – 22.74	.29
Omegasum*	.009	-.024 - .04	.59
EPAgroup x Omegasum* x Time	-.002	-.076 - .07	.95
Antiox.group x Omegasum* x Time	-.15	-.26 - -.04	.008
EPAgroup x Antiox.group x Omegasum* x Time	.15	-.04 - .33	.12

Dependent variable: Stroop Conflict.

*Omegasum = PUFA level at baseline.

The effects of trial drugs in the low versus high PUFA groups are illustrated in figures 3-4. The significant levels refer to the statistical models shown in table 3-4, but the “omegasum” (= PUFA at baseline) have been centred on the median PUFA of the whole sample and the mean PUFA levels in the high and low PUFA groups respectively. Thus, p-values correspond to treatment effects according to LMM for the typical patient (as for PUFA level) in these samples.

Figure 3 illustrates the whole group and the two PUFA groups performance on CPT. Significant results is observable for the low PUFA group receiving only EPA (p=0.03). This shows that the single EPA group decrease performance on CPT. Significant (p=0.03) result were also found in the low PUFA group receiving the combination intervention (EPA and vitamin E + C). Here, on the other hand, performance on CPT

increased. In comparison to the placebo group there was a non-significant beneficial effect on CPT dprime of the combined treatment in the low PUFA group ($p=0.15$).

*Figure 3: Continuous Performance Test dprime
Effects of EPA alone or EPA and Vitamins E+C combined*

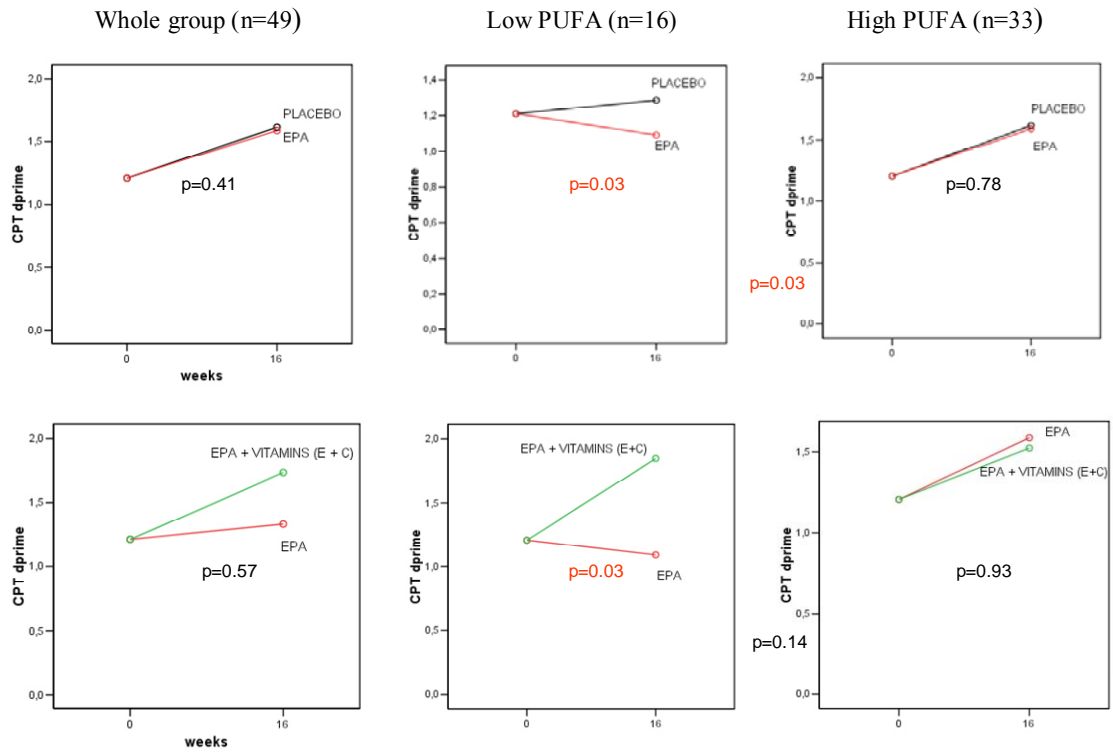
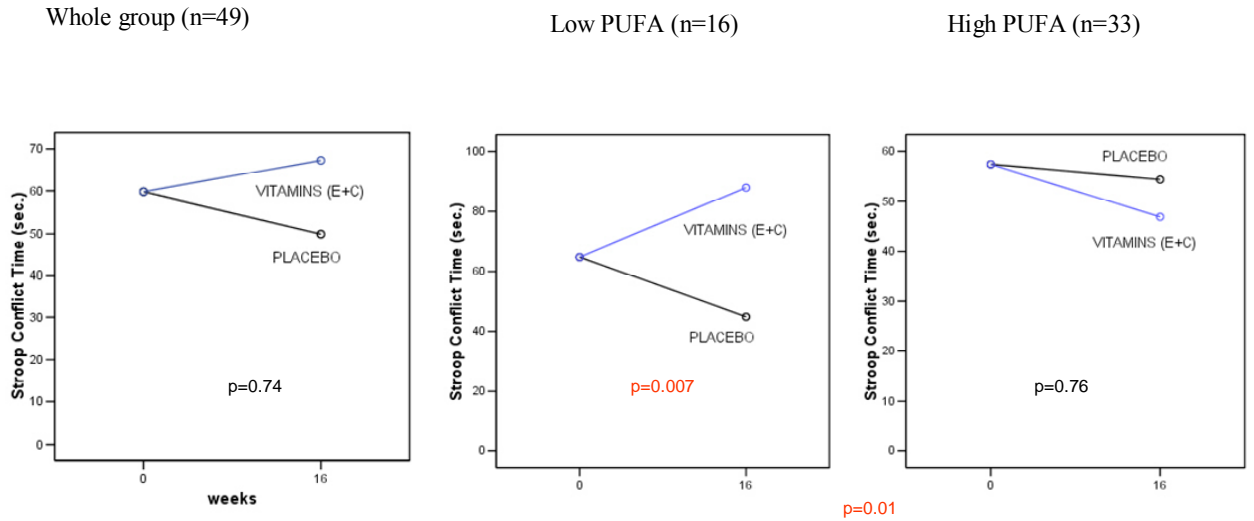


Figure 4 illustrates the whole group and the two PUFA groups performance on Stroop. Significant results ($p=0.007$) were obtained in the low PUFA group receiving vitamin E and C. This shows that the low PUFA group receiving only antioxidants reduces their performance on the Stroop.

Figure 4: Stroop conflict time, effects of vitamins E+C alone



7.0 Discussion

The purpose of this study was to investigate the effect of an omega-3 fatty acid (EPA) and antioxidants (vitamin E and C) on cognitive functions in people with schizophrenia. To our knowledge, this randomized, double-blind, placebo-controlled study is the first to be executed on the effects of EPA and antioxidant supplementation as treatment for cognitive deficits in schizophrenia. The only way to verify whether EPA and antioxidants are potential therapeutic options in the management of cognitive functions in schizophrenia is to conduct large randomized controlled trials similar to those required for the licensing of any new pharmacological treatment, as we have done here.

We expected that the group that received double supplements would perform better on the cognitive tests after 16 weeks, than the groups who received single supplements or placebo. That is, we expected a graduation of efficacy in the following order: 1) combined EPA and antioxidant treatment, 2) EPA in combination with placebo 3) antioxidants in combination with placebo and 4) double placebo. We assumed that the

addition of antioxidants to EPA would reduce oxidative stress and reinforce the effect of EPA. Oxidative stress is considered to have a negative effect on the neuronal membranes and furthermore affect the CNS through maladaptive neurotransmitter communication and neurodevelopment. In turn, this is associated with lower cognitive performance (Mahadik and Yao, 2006). We anticipated this outcome in individuals with schizophrenia because they are thought to have an altered cell- metabolism, and an impaired antioxidant defence system (AODS) which makes them more vulnerable to oxidative stress (Mahadik, 1994).

Overall, the results did not confirm our expectations concerning the graduation of efficacy. Hence, we did not find a positive link between cognitive performances and the adjunctive use of fatty acids and antioxidants. The significant results we obtained were negative in that performance on attention variables was decreased and this effect was restricted to the groups receiving single supplement groups. The EPA-group performed poorer on CPT, while the antioxidant-group performed worse on the Stroop-test. The performances on the rest of the cognitive tests were not influenced by the trial drugs or placebo.

We also looked at retrospective analyzes from the larger study (Bentsen et al. in prep, 2007). These analyzes reveals a division of patients in a high and low PUFA group as measured by PUFA levels in red blood cells (Bentsen et al. in prep. 2007). These levels show a bimodal distribution, where one of three patients had very low levels of PUFA's compared to healthy controls (Bentsen et al. in prep. 2007). In line with this, earlier studies have shown PUFA levels to be bimodally distributed among patients with schizophrenia (Glen, Glen, Horrobin, Vaddadi, Spellman and Morse-Fisher et al., 1994; Peet, Laugharne, Rangarajan, Horrobin and Reynolds, 1995). This is suggested to reflect a metabolic abnormality of aetiological importance (Peet et al., 1995).

The interesting aspect of the bimodal distribution in PUFA levels is that these are related to cognitive performance. Stroop was only affected in the low PUFA group when the antioxidants were given alone, while CPT was only affected in the low PUFA group when EPA was given. Antioxidants increased the time on Stroop and the unfavorable effect was also significantly higher in the low- than in the high PUFA group. Similar results were obtained for the CPT when receiving EPA. EPA reduced the

CPT dprime, and the effect was also restricted to the low PUFA group. The higher the PUFA, the less was this disadvantageous effect. Hence, low PUFA levels were linked to poor attentional performance. The low PUFA group receiving both interventions demonstrated the only combination effect (increased performance on CPT), that we on forehand expected for all groups and tests, although this effect was non-significant to placebo treatment. The combination effect was not observable in the first analyses, probably because it was diminished when the patients (and hence the difference in PUFA-levels) were mixed.

The failure of EPA to produce cognitive improvement in patients with chronic schizophrenia in the study by Fenton et al. (2001, op.cit.pp.12) has several possible explanations. To begin with, the dose of EPA is not optimal. Peet and Horrobin (2002) conducted a study of patients with schizophrenia in where placebo was compared with different doses of EPA. The best results were achieved at the 2g/day dose, which produced an increase in red cell EPA without any decrease in red cell AA, which would be unfavourable. Fenton et al. (2001) may have used too high EPA (3g/ day) doses that depleted AA fatty acids. Based on these findings 2g/day dose was used in the current study. Additionally, Fenton et al. (2001) based their study on the exploratory interest of improving cognitive functions since fatty acids have shown to relief symptoms, and did not ground the trial on the theory of oxidative stress. However, this study was developed with respect to explore the mechanism of oxidative stress and therefore prompted use of high-dose antioxidants, in contrast to Fenton et al. (2001). This study also used a combination of vitamin E and C for optimal reduction of oxidative stress. This is believed to provide complete antioxidant defence because the potency of vitamin E is maintained by vitamin C. Besides this, age and illness duration have implications for the effectiveness of the interventions. Fenton et al's (2001) subjects had a higher mean age and duration of illness than our participants. Fenton et al. (2001) also had an insensitive design, lacking a control group.

Taken together, the analyses show that 1) single antioxidant- or EPA supplementation impaired attention variables in the low PUFA groups and only in these groups, 2) it reveals a bimodal distribution of PUFA levels and 3) the combination effect was only evident in the CPT test in the low PUFA group when the patients were divided in groups according to PUFA-levels, but not significantly superior to placebo.

7.2 Attention as a vulnerability marker in schizophrenia

Abnormalities in attention have long been viewed as one of the fundamental underlying cognitive deficits in schizophrenia and are likely to contribute both to formation of some types of symptoms and particularly to the substantial work and social impairments that often follow schizophrenia (Nuechterlein, Pashler and Subotnik 2006). Yet, the precise nature of the attentional deficits in schizophrenia remains poorly understood. Attention is generally regarded as a complex and comprehensive concept and there might only be specific aspects of the attention-construct that are deficient in schizophrenia. The network assumed to underlie the distribution of attention in the brain involves structures in all lobes, including subcortical regions (Posner, 1994). This network is also very specific to certain aspects of attention, something that can be demonstrated through variation in attention deficits that are seen in cases of brain damage.

The CPT-IP is by many researchers regarded as a vulnerability-marker of schizophrenia because studies demonstrate that it consistently discriminates affected patients from controls (Francey, Jackson, Phillips, Wood, Yung and McGorry, 2005). Simultaneously the impairment of sustained attention, as measured by the CPT, is seen in the healthy first-degree relatives of patients with schizophrenia (Chen et al., 2004) and the deficit appears independent of clinical symptoms (Wang, Chan, Sun, Yao, Deng and Sun et al., 2006). Also, the reaction-time of the CPT is suggested as an endophenotypic marker for schizophrenia (Wang et al., 2006). These findings support the notion that sustained attention is a susceptibility indicator for schizophrenia. It is especially genetic researchers that advocate this point of view, highlighting the link between biology and cognition (Mahadik and Yao, 2006). The CPT being such a sensitive test might detect subtle biological alterations in a larger degree than other tests are able to register, which contribute to explain our findings. Generally, the CPT-IP is a difficult task and the EPA given could have changed the biological machinery in means that have enlarged the already existing deficit. This means that the EPA might have influenced neurobiological variables essential for certain attentional capabilities (e.g. processing capacity), which are traced by the CPT-IP. Moreover, the observed attentional deficit appears to represent a true impairment in processing capacity rather than a group difference in response style or motivation, as our participants did not differ from the control group on

the beta variable. Attributing lower performance to motivational factors is therefore ruled out (for a detailed review of the signal detection paradigm, see Appendix, 10.2).

The CPT is also regarded as a measure of working memory because stimuli must be held “online” in order to determine when to respond (Thaden, Rhinewine, Lencz, Kester, Cervellione and Henderson et al., 2006). Still, tests of working memory used in this study (PASAT and LN-span) were not affected in any of the groups. Attention, as measured by CPT, requires less maintenance of internal representations and consequently have smaller load on working memory. Hence, the CPT is conceptualised as a more basic measure of attention. This illustrates the nuances captured by the different tests and support specific attentional deficiencies in schizophrenia. We propose that abnormalities in the development of circuits that subserves specific aspects of attention may be central to the pathophysiology of schizophrenia and that these deficits are perceptible via the CPT-IP.

The Stroop test is the second cognitive tests affected negatively in our study. The phenomenon of a markedly slow naming response when a colour name is printed in ink of a different colour has received a variety of interpretations (Lezak et al., 2004, pp. 366). Some have attributed the slowing to response conflict, some to failure of response inhibition, and some to a failure of selective attention. This is one example of why it is difficult to make inferences about what specific effects the antioxidants have. Like in the CPT we suggest that the supplements affect specific, perhaps more vulnerable parts, of the attentional network. As in the CPT-IP, decreased performance may be caused by unfavourable changes in the biological environment and therefore reflects a slowed rate of information processing. However, since this reduction in performance only concerns the low PUFA group, the results may be best understood in the light of vulnerabilities following this group. For instance can their distractibility threshold be lower than in the high PUFA group.

A shared characteristic of CPT and Stroop is that they require inhibition of a pre-potent response (Lezak et al., 2004). This is a process that demands more capacity than automatic processes, and therefore requests more cognitive flexibility. Anterior cingulate cortex (ACC) is indicated in the process of controlling and correcting responses, and is considered to be a vital contributor to the inhibition of pre-potent

responses (Gazzagnia et al., 2002). Attentional deficits in schizophrenia have in part been ascribed to impairment of the ACC (Yücel, Pantelis, Stuart, Wood, Maruff and Velakoulis et al., 2002). The anterior cingulate in patients with schizophrenia is for instance less activated during Stroop task performance (Carter, Nichols and Cohen, 1997). The decreased performance on CPT and Stroop can be a way of adjusting to a saccade of biological events that is initiated by the adjunctive use of fatty acids or antioxidants. Still, why ACC should be particularly influenced is unclear.

In summary, we found that impairments in attentional performance using the CPT-IP and Stroop become more pronounced when receiving one of the supplements, but only in the low PUFA groups. We also found an increase in performance on the CPT in the group receiving double supplements, also limited to the low PUFA group. This combination effect was not observable in the Stroop test. In sum, these tests seem to be linked to particular attentional constitutional aptitudes, whose relations with the supplements are yet to be explained. Furthermore, CPT-IP is confirmed to be a sensitive measure in detecting subtle cognitive alterations.

7.3 Interpretations of results

We argue that the beneficial combination effect on CPT in the low PUFA group can be explained by the characteristics tied to the low PUFA levels and the sensitivity of CPT. The combination intervention can exert most effect in the low PUFA group and may first be noticed by CPT. Our hypothesis is therefore that the low PUFA group has greater benefit from reduction of oxidative stress and the adding of both PUFA and antioxidants. The non-significant beneficial effect of the combined treatment on CPT compared to placebo can be due to low statistical power. There is also reason to believe that this effect may prove superior to placebo in a larger sample (e.g. 100), as we observe a beneficial tendency.

Why our aims in general were not supported might be due to a variety of factors: Firstly, the heterogeneity of schizophrenia might confound data and make it difficult to reveal larger effects (Zielasek, Ehli, Herrmann and Fallgatter, 2004). Secondly, it might take longer time than 16 weeks to have an effect on the cognitive stage. Correcting abnormal cerebral PUFA levels can take more time than correcting PUFA in red blood cells because they have a high turn over. Thirdly, the link between cell

function and cognitive function is multifaceted and not linear (Mahadik et al, 2001). It might be other steps “on the way” between the cell-level and cognitive level that is maladaptive or aberrant (e.g. brain-structures or functional connectivity) that the reduction of oxidative stress can not modify. Fourthly, the lack of positive results might be because fatty acids and antioxidants have a primary preventive role of developing schizophrenia and not in the treatment of manifested cognitive deficits. It is possible that when a disease is clinically apparent, the neuropathological involvement is too advanced to be substantially attenuated by reduction of oxidative stress. It may also be a more fundamental abnormality in the cell membrane and -metabolism in schizophrenia which is more difficult to correct than by nutritional intervention. Fifthly, it is possible that the use of adjunctive supplements is ineffective; either we were not able to reduce the oxidative stress, or this process is not as closely tied to cognitive functions in schizophrenia like we assumed. Also, due to the lack of guidelines from past research in shaping our hypothesis, it is possible that the formulation of expectations prevented us from observing a relationship. For instance, findings regarding reduction of symptoms when patients receive fatty acids (Mahadik, et al., 2006; Laugharne, et al., 1996,) or antioxidants (Dakhale, et al., 2005) do not have to be transferable to cognitive improvement. Alternatively, the fact that we do not get a positive effect of EPA might indicate that the type of PUFA (i.e. the different therapeutic effects of DHA EPA or AA) is not the crucial part to raise change in cognition. EPA is not a major membrane fatty acid and levels of its metabolites, such as eicosanoids, may increase at high doses. These metabolites have shown to have some unwanted effects (Mahadik et al., 2006) and the lack of knowledge about EPAs side effects might contribute to why we get reduced performance on attention in the EPA group with low PUFA. Finally, the statistical power is low, entailing high risk of type II error.

There are several ways to conceptualise the findings of a bimodal distribution of PUFA levels in our patient group. It indicates that the sample is not homogenous and may reflect two distinct disorders. This result accompany the debate concerning the validity of the schizophrenia diagnose (Malt et al., 2003; Green, 2004). That is, although the international diagnostic systems are valid for clinical diagnostic purposes, it might be insufficient for the aetiological research of schizophrenia (Reddy et al.,). Moreover the distribution can give support to the theoretical view of conceptualising schizophrenia in a dimensional perspective (e.g. schizotaxia). We suggest that the PUFA-level can

represent an *aspect* of a biological susceptibility for developing variations of illnesses in the schizophrenia spectrum. Hence, the liability can be expressed in a larger or lesser degree and end up as diagnosis with differences in course and outcome. This way we also see how the diathesis-stress framework (op.cit. pp. 4) of schizophrenia is significant (Jones and Fernyhough, 2006). There might be tied different characteristics to the differences in PUFA-levels that are specific to the subgroups; this could involve maladaptive absorption of nutrients and/or a difference in cell metabolism, or reflect variations in stress-tolerance Furthermore, the expression of this diathesis can depend on e.g. stress exposure, diet and pro-oxidant behaviour. Since the negative effect only concerns the low PUFA groups in our study, it may be certain vulnerabilities related to a low level of PUFA, or protective factors tied to the high PUFA group. PUFA levels might therefore predict different prognosis in terms of aetiology, symptoms and course.

Another concern regarding the low and high PUFA levels is to ascertain whether the PUFA level is transient or a stable trait (Mahadik et al., 1994; Reddy et al., 2004). If differences in PUFA levels characterize stable traits of schizophrenia, and not just transient states associated with for example psychosis, they would be valuable in clinical practise and as a biological marker for the disorder. Reddy et al., (2004) who investigated 24 neuroleptic-naive patients with first episode schizophrenia or schizoaffective disorder, found that relative to normal subjects, patients had significant reductions in total PUFA. These reductions were not related to age, gender, smoking status, or nicotine levels. They interpret this as supports to that significant PUFA reductions occur early in the illness, prior to initiation of treatment, and raise the possibility that these deficits are trait related.

These findings points to a biological abnormality manifested as low PUFA levels that seem specific to a subgroup in schizophrenia. The differences can elucidate information about variations in pathophysiology. Identifying a biological marker within the clinical group of schizophrenia may yield information about endophenotypes and guide research on good prognostic variables further. We do not claim that the PUFA level is the cause of cognitive dysfunction in schizophrenia, rather that the wide range of genetic and environmental influences contributing to the disorder may operate through different pathways. What biological abnormality that precedes the division in PUFA-levels is yet to be elucidated. We have simply revealed that the group with this “trait” do not profit

on the adjunctive use of fatty acid and antioxidants taken alone. It can also reflect a trait impairment of schizophrenia independent from symptom profile, but we have not focused on symptoms here.

It is vital to identify the best timing to start the adjunctive use of supplements. The schizophrenia pathology can occur at all stages and might influence each other in the process of developing the illness. Therefore it is a complex question to answer when the supplements will exert most effect. Timing may be one of the reasons why we do not obtain positive results in our study. It can exist critical periods for remodelling the neuronal membrane pathology. If the pathology occur at an early stage, it is reasonable to think that it is reinforced later in life, but can profit on supplements. Further, the growth of the pubertal brain increases the process of oxidative stress for individuals having an abnormal cell metabolism. Adolescence may be an especially sensitive period where supplement can make a difference. For example it is proposed that there is increased “pruning” (excessive removal of nerve endings and processes during pubertal maturation) as a result of oxidative injury (Keshavan, Anderson and Pettegrew, 1994). The results hold up that little is known about the pathologic progression from an at-risk state to clinical disease, and which set of processes takes over at some point to cause progression of clinical disease. We believe that oxidative stress is of importance here, but the complexity of our results demonstrates the intricacy of this field.

7.4 Limitations and suggestions for further studies

One of the objections to our study concerns the uptake of supplement. Some studies show that the effect of antioxidants is better obtained from in natural forms, than from capsules. It has for example been demonstrated that vitamin E in its natural forms protects better against lipid peroxidation than E-vitamin pills (Wolf, 1997). Therefore the power of supplements might be weaker when administered as a capsule. Moreover, to find out if oxidative stress is the mechanism behind the altered cell metabolism in schizophrenia, the field is in need for better measures. Methods to date can not ensure the validity of measures we use to assess oxidative stress.

Another limitation is the lifestyle characteristics typical for patients with schizophrenia, involving much pro-oxidant behaviour, like fast –food consumption, alcohol- and marihuana intake and cigarette smoking (Hibbeln, Makino, Martin, Dickerson and

Fenton, 2003; Mahadik and Yao, 2006). Besides this, the pro-oxidant qualities of the atypical antipsychotic medications can additionally increase the oxidative cell injury. Antipsychotics can alter oxidative stress by changing antioxidant defence systems and generating reactive oxygen species (Mahadik and Yaho, 2006). Some anti-cholinergic drugs are prone to cognitive deterioration, and older neuroleptics (e.g. Haloperidol) has a toxic effect on the neuronal membranes and act as a pro-oxidant. Some newer neuroleptics on the other hand, avoids this effect (e.g. Olanzapine, Clozapine). These factors will influence the results when investigating supplement interventions.

Moreover, the stability of patients might be a factor to control for in further studies. We could have obtained different results if we started the intervention at a more stable phase than when acutely hospitalised. Differences in symptoms, length of prodromal phases and illness-onset vary considerably and may contaminate the data. Further studies should aim to select samples that are as similar as possible on these variables. Although schizophrenia is hard to study because of high drop out, further studies should aspire larger samples to increase the statistical power. In relation to the missing data caused by drop outs, LMM analysis should be used.

Subsequent studies must also consider the use of first-degree relatives of schizophrenia patients. This is of relevance because they are one type of at-risk population for developing the illness. Such subjects are optimal for studying the biological mechanisms involved in schizophrenia, since they are free of the confounding variables that are common in research with schizophrenia patients (like drugs and psychosis). Investigating PUFA levels in these relatives will clarify how cognition and oxidative stress is involved in the pathogenesis of the illness.

Overall, our findings cannot serve as basis for general recommendation for cognitive treatment of dietary supplementation of fatty acids and antioxidants in patients with schizophrenia. But it is important to emphasize that the negative effect on dprime and Stroop in the single- supplement groups are eliminated when the supplements are given together in the low PUFA group. This underscores the importance of defining patients PUFA level. The low PUFA group experiences a negative effect of single supplements and we also believe that they are more sensitive to other interventions. For this reason, ethical aspects should be considered in future studies.

Taken together, further studies should conduct larger, randomized, placebo-controlled trials. In these trials varieties of fatty acids, antioxidants and different and daily doses should be compared. They should select younger patients at a stable phase and divide them initially by measuring the PUFA levels and follow the different effects on cognitive variables closely. Repeating studies on the PUFA subgroups adding genetic information is of major interest. This way one can better come around the heterogeneity of schizophrenia and investigate the effects of supplements on different subgroups that react very differently to the adjunctive use of EPA and antioxidants.

8. Conclusion

This study validates earlier findings of cognitive deficits in schizophrenia and that they cover a broad range of functional areas. However, the current study does not reveal a consistent relationship between supplements and cognitive functions in younger patients with schizophrenia spectrum disorders. The study holds up attention as a vulnerable psychological construct, and CPT and Stroop as sensitive measures that are able to detect subtle cognitive changes. Furthermore, a bimodal distribution of PUFA levels was disclosed and linked to performance on these attentional parameters. The combination effect of the supplement improved attentional function in patients with low PUFA levels, although this beneficial effect was not significant compared to the placebo group.

The bimodal distribution of PUFA levels give support to the heterogeneity of schizophrenia. This might contribute to explain why research in this field has been inconsistent and delayed; one may actually overlook important biological parameters by putting together patients from different subgroups. The finding also has implication for clinical practice as initial measure of PUFA levels in patients with schizophrenia could contribute to therapeutic improvement. The study also illustrates the complexity of drawing a line from biological structures, like neuronal membranes, to cognition and behaviour. Our result does not establish that oxidative stress is the crucial aspect of cognitive deficits in schizophrenia, but provides a valuable theoretical standpoint for further exploration of diet supplementation as a new therapeutic strategy.

8.0 References

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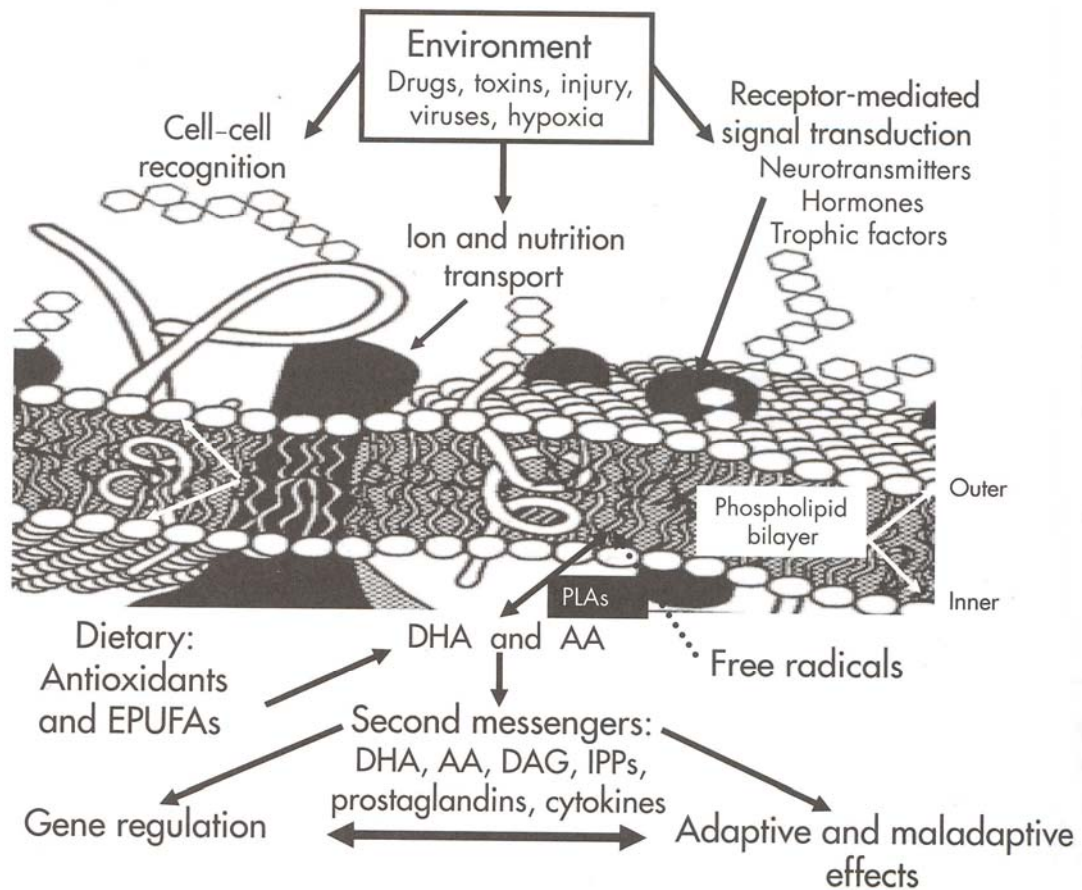
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10.0 Appendix

10.1 The neuronal membrane

Figure 1



Source: Adapted from Mahadik and Yao, 2006.

Figure 1 illustrates the membrane key structures and their function in cell-cell interaction, interaction with environmental factors and receptor-mediated signal transduction (Mahadik and Yao, 2006). The representation gives a picture of the phospholipid bilayer where receptors for e.g. neurotransmitters, growth factors, ions and nutrition are embedded. The phospholipid bilayer constitutes four major phospholipids that are asymmetrically localized. Some of the phospholipids are highly enriched in arachidonic acid (AA) and docosahexaenoic acid (DHA) which are released by receptor-mediated phospholipases (PLAs). AA and DHA and their metabolic products work as second messengers and

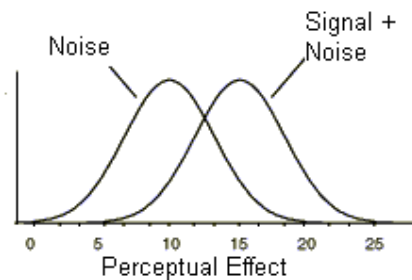
physiological mediators, including gene modulation and hence lead to adaptive and maladaptive cellular changes.

10.2 Signal detection theory

RESPONSE CRITERION

Response criterion refers to an operator's set of rules that they develop when involved in a signal detection task. As we will see later this can be manipulated either by motivation or expectancy.

Before discussing the types of criterion and their implications, let's take a look at the relationship of probability, possible responses, and stimulus in the environment.



- The curve on the left represents the probability that the detection of the signal is due to noise alone.
- The curve on the right represents the probability that the detection of the signal is due to the presence of the signal.
- The point in the center of the intersection represents the point where the detection of the signal is equally probable both to the presence of noise or signal plus noise.
- The area of overlap is where an error occurs, either miss or false alarm.

If the sensation level is above a particular level called the response bias and symbolized with the Greek letter (β) or beta, the observer says "yes"; if it is below the response bias level the observer says "no."

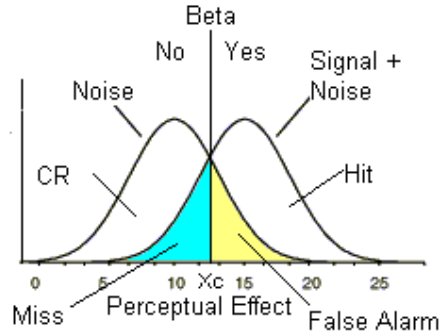
- Signal detection theory assumes that an optimal strategy is used on every trial of an experiment.

There are three types of response criteria in which an operator may adopt each with their own implications:

- **Liberal**--More likely to say signal present most of the time; produces high # hits and false alarms.
- **Neutral**--Equal probability of hits and false alarms
- **Conservative**--more likely to say no signal present most of the time; produces low # hits and false alarms.

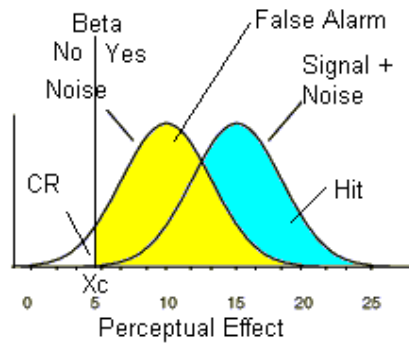
In terms of graphic representation below X_c represents the decision criterion. Beta (β) represents the response bias (β) which is a ratio measure of neural activity. Both beta and X_c define the response criterion

Neutral



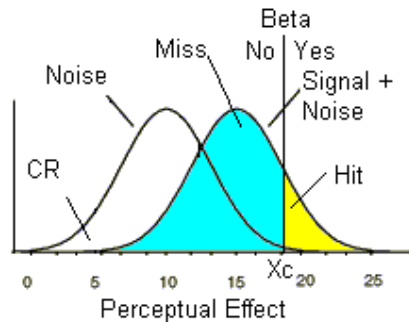
As you can see, there is an equal probability of getting either a miss or a false alarm. Beta=1.0

Liberal



Here the operator says "yes, signal present" all the time leading to high rate of false alarms and a high rate of hits. Beta < 1.0

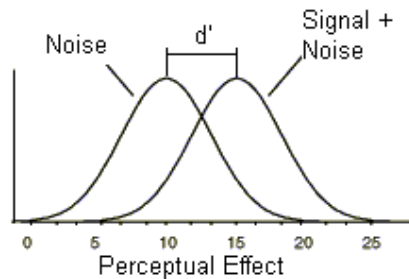
Conservative



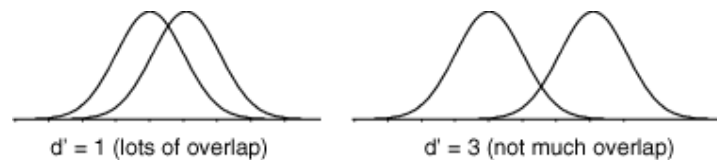
Here the operator says "no, there is no signal present" most leading to a low number of hits & high number of misses yet few false alarms. $\beta > 1.0$

Sensitivity

Sensitivity refers to the keenness or resolution of the detection mechanisms in an operator. Theoretically, sensitivity refers to the separation of the means of the two distributions for noise and signal + noise along the X axis (See Below). This is represented by d' . On the next page it is discussed later how to calculate d' from a single outcome matrix.



The greater the distance between these two points, the greater the sensitivity, and the higher the value of d' .



The greater the sensitivity of the operator, the less probability there are for errors. Remember that the area of the overlap represents the chances for probability of an error. Therefore the less overlap there is between the two, the less chance for error.

Like biases, sensitivity also has an optimal level. The setting of this level is quite complex and can really only be done in a carefully controlled environment. It requires defining precisely the statistical properties of the physical energy in the signal and no signal trials. Operators sensitivity often drifts from optimal sensitivity because the characteristics of the signal are often forgotten.

Calculating Sensitivity

There are two methods of calculating sensitivity. One is based on theoretical assumptions and the other is based on empirical data.

d'-Theoretical Approach

Data required for each point on an ROC curve requires hundreds of trials (to get accurate probabilities for Hits and False Alarms). With a few theoretical assumptions, d' can be calculated from a single outcome matrix.

This method assumes that:

- Noise is normally distributed. Presenting a signal on top of that noise, will therefore shift the amount of sensory activity to the right (higher), by an amount equal to that sensory systems sensitivity to that signal.
 - The difference between the mean amount of sensory activity generated by the noise alone trials and the signal+noise trials will equal sensitivity (d') measured in z-score (standard deviation) units.
- Participants adopt a criterion for dealing with those values of sensory activity that could result from either noise alone or signal plus noise (the area where the noise and signal+noise distributions overlap).
 - If the amount of sensory activity exceeds that amount, the participant will say they detected the signal, any amount less than that and they will say they did not detect the signal.

With these assumptions, the four cells of an outcome matrix can be represented as areas under the two normal distributions .

$$d' = Z_{FA} - Z_{Hit}$$

Tables for the z-score distribution or percent area under the normal curve typically present the z-score distances between the mean and the criterion value beta.

- If you are using such a table, Z_{FA} can be found by looking up the z-score associated with (50 - False Alarm %).
 - If this number is positive, then the z-score to be put into the above formula will also be positive,
 - If it is negative, the z-score value for the formula will also be negative.
 - It is essential that the proper signs be used. A good way of checking would be to draw the distributions and the criterion and see the relationship between d' and the two z-scores.

- Similarly, to find Z_{Hit} , look up (50 - Hit %), again, the resulting sign will be the same as is used for the z-score in the formula.

Signal	Proportion of Responses	
	Yes	No
Present	.60	.40
Absent	.20	.80

$$d' = Z_{FA} - Z_{Hit}$$

$$d' = Z_{(50-20)} - Z_{(50-60)}$$

Looking up the z-score associated with 50-20= 30% of the area under the normal curve, it is .842; for 50-60= -10% it is .253. Since 50 - 60 is a negative, -.253 is put into the formula to get:

$$d' = .842 - (-.253) = .842 + .253 = 1.095$$

Empirical Approach

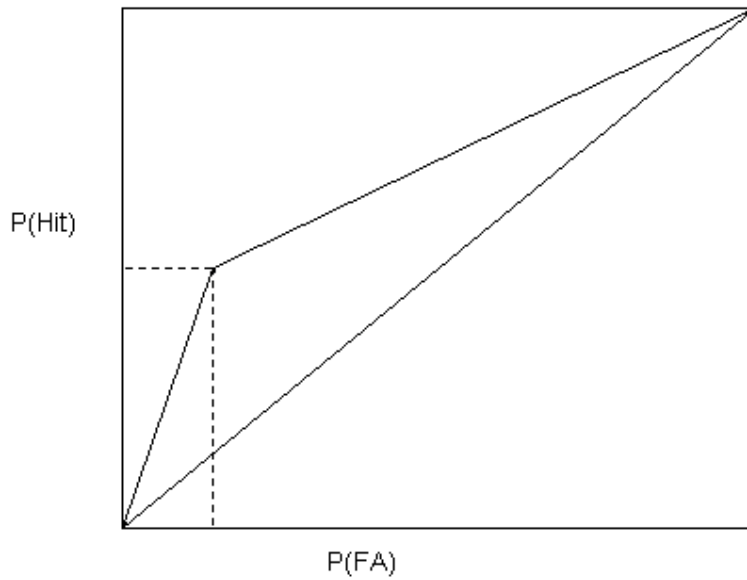
A distinction should be made between actual empirical data collected in a signal detection experiment and the theoretical method discussed above.

- Distributions of noise and signal plus noise are not precisely normal and of equal variance, especially if there is variability in the signal.
- Data collected would consist of a set of discrete points rather than a continuous smooth curve.
- Empirical results in which data are collected from a subject as the criterion is varied provide points that do not fall precisely along a line of constant bowedness as shown in the ROC curve.
- Although it is desirable to generate two or more points on the ROC curve, this may be impossible to do.
 - The experimenter often has neither the luxury nor feasibility of manipulating beta or using rating scales and must use the data available from a single response matrix.

Under these circumstances there is an alternative measure of sensitivity.

This measure is known as P(A)--the area under the ROC curve.

- This represents the area to the right and below the line segments connecting the lower left and upper right corners of the ROC space to the measured data point (See Below).



P(A)-area under ROC curve derived from one point.

This measure does not depend on any assumptions concerning the shape or form of the underlying signal and noise distributions.

P(A) may be calculated by the following formula:

$$P(A) = \frac{P(H) + [1 - P(FA)]}{2}$$

Adapted from: <http://www.csulb.edu/org/hfes/responsecriterion.htm>