Explorations into the membrane hypothesis of schizophrenia

Wybe van der Kemp

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1. Introduction

Symptoms and diagnosis of schizophrenia

Schizophrenia is a neuropsychiatric disorder that usually has a late adolescent- or early adulthood onset. Symptoms of the disease are usually categorized in: positive, negative, disorganised and cognitive, as shown in Table 1.I.

Table 1.I. Categorisation of possible symptoms in schizophrenia (Beers *et al.*, 2006)

Category	Symptoms
Positive	Delusions and hallucinations.
Negative	Blunted affect, poverty of speech, anhedonia, and asociality.
Disorganised	Disorganised thinking and speaking, bizarre behaviour.
Cognitive	Impairment in: attention, processing speed, working memory, abstract thinking, problem solving, and understanding of social interactions.

These symptoms can manifest themselves in patients with varying degrees throughout life. The DSM IV diagnosis of schizophrenia can be briefly summarized as: The patient shows for at least a month (or less if effectively treated) at least one of the positive symptoms, or symptoms from two of the other categories. The patient shows for at least 6 continuous months some evidence of the disorder and for much of this time the patient's ability to work, study, socialize or provide self-care is impaired.

Epidemiology and etiology

The epidemiology and etiology of schizophrenia have recently been reviewed by Tandon *et al.* (2008). An overview of genetic and environmental risk factors of developing schizophrenia from their review is shown in Table 1.II. The overall risk of developing schizophrenia over one's lifetime averages approximately 0.7%. Apparent from the data in Table 1.II is the large genetic component as a risk factor for developing schizophrenia. This ranges from a factor 2 for a 3rd degree relative, to a factor 50 to 70 for monozygotic twins.

Relatively large risk factors that are missing in Table 1.II are maternal iron deficiency and maternal diabetes mellitus. Maternal iron deficiency expressed as a haemoglobin concentration lower than 10 g/dl increases the risk of developing schizophrenia in offspring with almost a factor 4 as compared to a haemoglobin level of 12 g/dl or higher (Insel *et al.* 2008). Children of mothers who experienced diabetes mellitus during their pregnancy are 8 times more likely to develop schizophrenia is one of the findings of a meta-analytic review on obstetric complications and schizophrenia by Cannon *et al.* (2002). However, the confidence interval of this number is rather large (CI 1,37-43.9, p<0.03) and more studies are needed to obtain a more robust estimate of the relative risk. Recently van Lieshout *et al.* (2008) elaborated on the possible underlying pathophysiology of maternal diabetes being a risk factor in developing schizophrenia in the offspring. An overview of a high level pathophysiological mechanism adopted from their review is shown in Figure 1.1. Interesting to note from Figure 1.1 is that iron deficiency, a risk factor in itself, also plays a role in the pathophysiology of maternal diabetes related schizophrenia.

Table 1.II. Estimates of approximate relative risk RR for schizophrenia due to various genetic and environmental risk factors adopted from Tandon *et al.* (2008).

Risk factor	RR
Family history of schizophrenia (see specification below)	2-70
Monozygotic twin	50–70
Both parents affected	40–60
Dizygotic twin or 1st degree relative	9–18
2nd degree relative (e.g., grandparent)	3–6
3rd degree relative (e.g., 1st. cousin)	2–3
Any specific single gene variant	1.1–1.5
Urbanicity	2–3
Migration	2–3
1st or 2nd trimester maternal infection or malnutrition	2–3
Winter birth	1.1
Obstetric and perinatal complications	2–3
Cannabis or stimulant use	2–3
Paternal age >35 years	1.5–3
Male gender	1.4

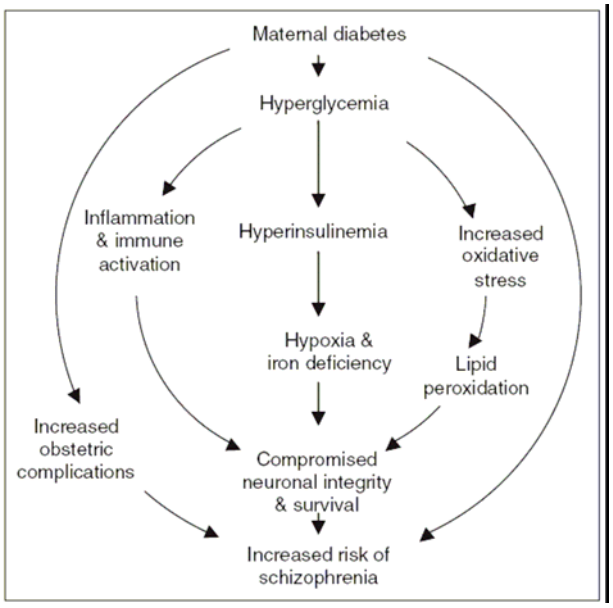

Fig. 1.1. Pathophysiology of gestational diabetes and schizophrenia in the offspring (Van Lieshout *et al.* 2008).

Considering the environmental factors, of which quite a few are maternal and perinatal, one would suspect a neurodevelopmental process which doesn´t give rise to overt clinical features in early childhood but leads to the onset of schizophrenia later in life. Evidence for the neurodevelopmental hypothesis has been reviewed by Marenco and Weinberger (2000).

Medical treatment

Unfortunately there is no cure for schizophrenia and medical treatment is directed towards reducing the positive symptoms with anti-psychotics (anti-dopaminergic medication), family support, education and rehabilitation of the patient.

The first anti-psychotics discovered in the 1950s were the so called *typical* or conventional anti-psychotics. The most common substances in this group are haloperidol and chlorpromazine. Extra-pyramidal adverse effects such as restlessness, muscle spasms and Parkinsonism are often seen in patients treated with typical anti-psychotics.

Clozapine, a so called *atypical* (or second generation) anti-psychotic, was introduced in clinical practice in 1970. A number of atypical anti-psychotics followed in the 1990s and 2000s. Well known examples are risperidone, olanzapine, and paliperidone. Less extra-pyramidal side effects and less cognitive blunting are major benefits of the atypical anti-psychotics.

The dopamine hypothesis of Schizophrenia

The first (classical) dopamine hypothesis of schizophrenia formulated in the 1960s was that schizophrenia is caused by a hyperactivity of the dopaminergic transmission. This was primarily based on the fact that all antipsychotics are dopamine D_2 receptor antagonists and that the substance amphetamine, which stimulates dopamine release, is able to induce symptoms of psychosis quite similar to the positive symptoms seen in schizophrenia. This first hypothesis, which only explains the positive (psychotic) symptoms, has been more refined later to include the negative symptoms as well. Evidence from functional imaging studies, post-mortem investigation of schizophrenic brains and studies in non-human primates indicated that an impairment of the dopaminic transmission in the prefrontal cortex gives rise to the negative symptoms in schizophrenia. A more refined dopamine hypothesis is that there exist a *hypo*activity in dopamine transmission in the prefrontal cortex, giving rise to the negative symptoms, and because cortical dopamine systems generally show an inhibitory effect on subcortical dopamine systems, the *hyper*activity in the subcortical mesolimbic pathway, giving rise to the positive symptoms, is explained as well (Weinberger and Laruelle, 2002).

Recently a substance with antipsychotic activity that not directly targets the dopamine pathway, but the glutamate pathway, has been discovered (Patil *et al.*, 2007). However a link with the dopamine system still exists because dopamine tunes the excitability of glutamate and GABA neurons (Weinberger, 2007).

Lipids as a possible biochemical substrate for schizophrenia

Lipids are important constituents of the human brain. They comprise approximately 1/3 of the dry weight composition of gray matter, 2/3 of white matter and almost 4/5 of myelin in the human brain (O'Brien and E. L. Sampson, 1965).

Neuronal membranes are particularly rich in phospholipids. The growth of axons and dendrites, and the making of new- and pruning of old synaptic connections all require phospholipids anabolism and catabolism. In fact, phospholipids form the physicochemical framework in which neurotransmitter receptors, ion channels and other proteins involved in

signal transduction are embedded. The tertiary structure of these proteins is influenced by the phospholipid environment. Neurotransmitters and calcium, involved in neuronal signal transduction, are released from synaptic phospholipid vesicles. This requires the realignment of phospholipid molecules. Moreover, phospholipids and their fatty acid constituents also form part of many cell-signalling systems. (Horrobin, 1998). This makes phospholipids and fatty acids a possible substrate for a number of brain diseases. In this thesis the focus will be on schizophrenia. The 'membrane hypothesis of schizophrenia' and the evidence for it will be discussed.

Overview of this thesis

Lipids, fatty acids and the membrane hypothesis of schizophrenia are dicussed in chapter 2. The evidence for an altered fatty acid metabolism in schizophrenic patients based on erythrocyte membranes and postmortem brain studies are reviewed in chapters 3 and 4 respectively. This thesis concludes with chapter 5, in which a possible link between excessive gray matter density loss, as observed with MRI, in schizophrenic patients and the membrane hypothesis of schizophrenia is discussed.

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2. Lipids, fatty acids and the membrane hypothesis of schizophrenia

Introduction

Cell membranes are composed of lipid bilayers in which other structural elements such as for instance receptors, ion channels and carrier proteins are embedded. The lipid bilayers are mainly composed of phospholipids and cholesterol, which have a polar head group and an aliphatic tail, or in case of cholesterol a combination of an alicyclic and aliphatic tail. Submersed in a polar medium such as water, lipids can form micelles, liposomes and bilayers, in which the polar head groups are directed towards the polar medium and the apolar tails are directed to each other. A schematic drawing of a cell membrane (bilayer) is shown in Figure 2.1.

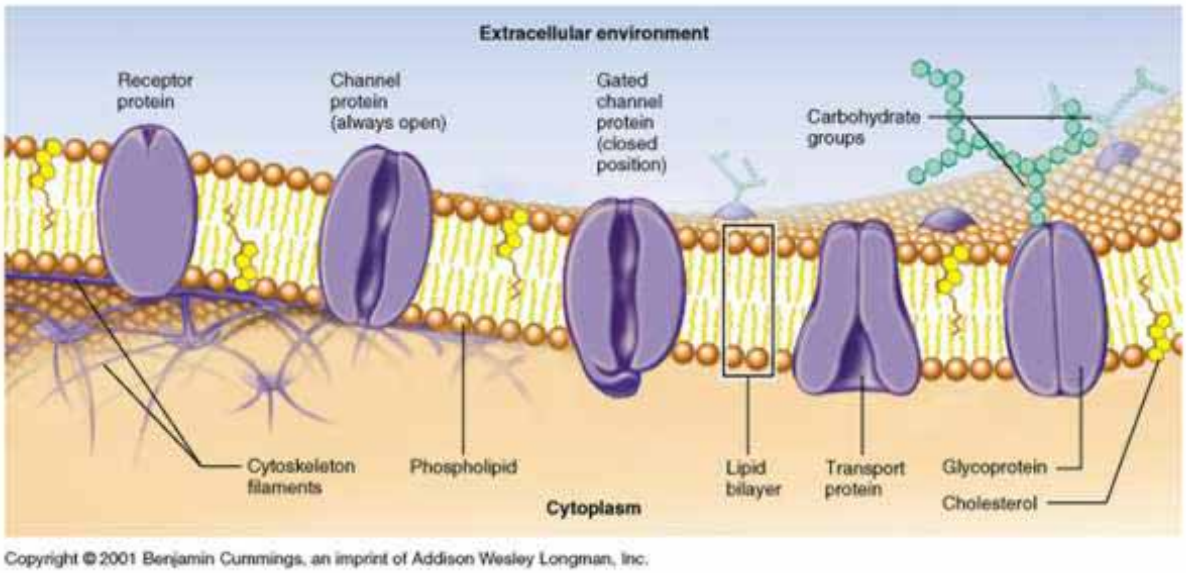
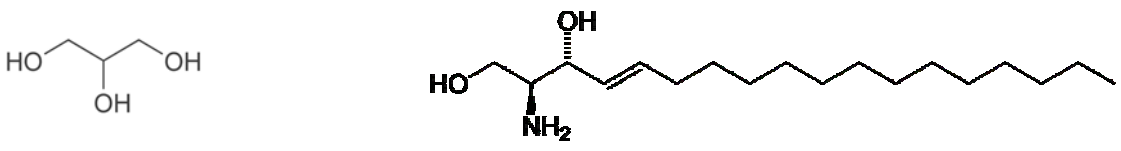


Figure 2.1. Schematic drawing of a cell membrane, adopted from S.S. Mader (2004).

Phospholipids

Phospholipds can be subdivided in two main classes: the glycerophospolipids and the sphingolipids, although not all sphingolipids have to be phospholipids, see Figure 2.3. The glycerophospolipids are composed of a glycerol (Figure 2.2) backbone in which two –OH groups (the sn1 and sn2 positions) have been esterified with fatty acids and one –OH group (the sn3 position) has been esterified with a phospate group. Usually a saturated fatty acid is attached to the sn1 position and an unsaturated one to the sn2 position. The characteristic group, which gives the phospolipid the last part of its name, is attached to the phospate group. These groups are choline, ethanolamine, inositol and serine. So the glycerolphospholipds are (glycero-) phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidylserine. The 'glycero' part of the name is usually omitted.



glycerol sphingosine

Figure 2.2. Chemical structure of glycerol and sphingosine, important precursors of glycerophospholipids and

sphingolipids.

The sphingolipids do not have a glycerol backbone but a sphingosine (Figure 2.2) backbone, which is an aminoalcohol.

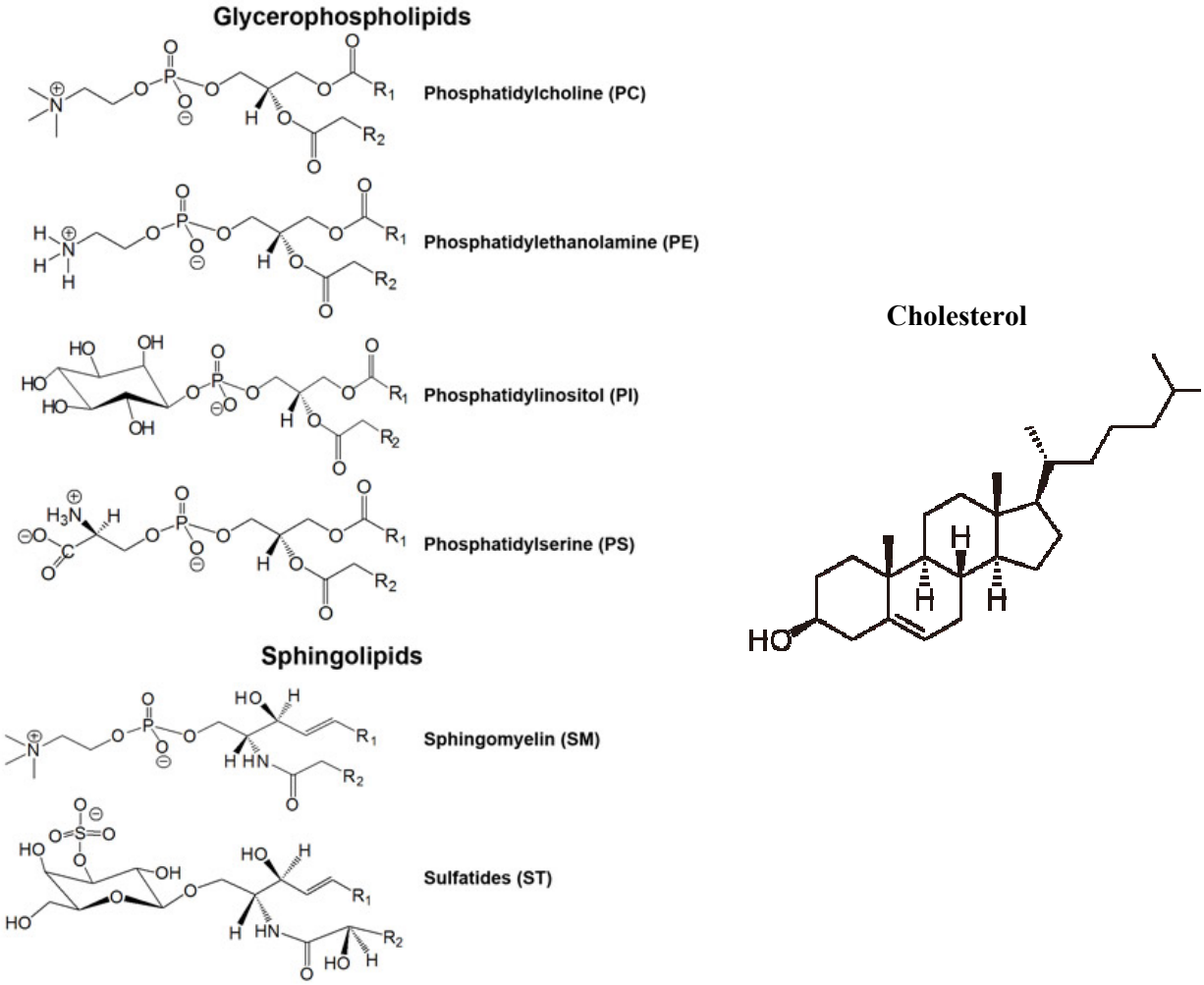


Figure 2.3. Chemical structure of glycerophospholipids, sphingolipids and cholesterol. (C-atoms and H-atoms attached to carbon not shown explicitly). Note that not all sphingolipids are phospholipids, and that only one type of sphingophospolipid (sphingomyelin) is shown.

Phospholipid metabolism involves many enzymatic steps. A schematic overview for the production and breakdown of a (glycero-) phosphatidylcholine, adopted from Glunde *et al.* (2006) is shown in Figure 2.4. Reaction intermediates, shown in Figure 2.4, that often occur in the ^{31}P MRS literature are the so called phosphodiesters and phosphomonoesters, which in this case are glycerophosphocholine and phosphocholine, respectively. As is evident from Figure 2.4 increased concentrations of PDE indicate phospholipid breakdown and increased PME indicate phospholipid synthesis. However, the ^{31}P peaks of PDE and PME at low spectral resolution include also other molecular phosphor species, so there can be some ambiguity in interpreting these signals.

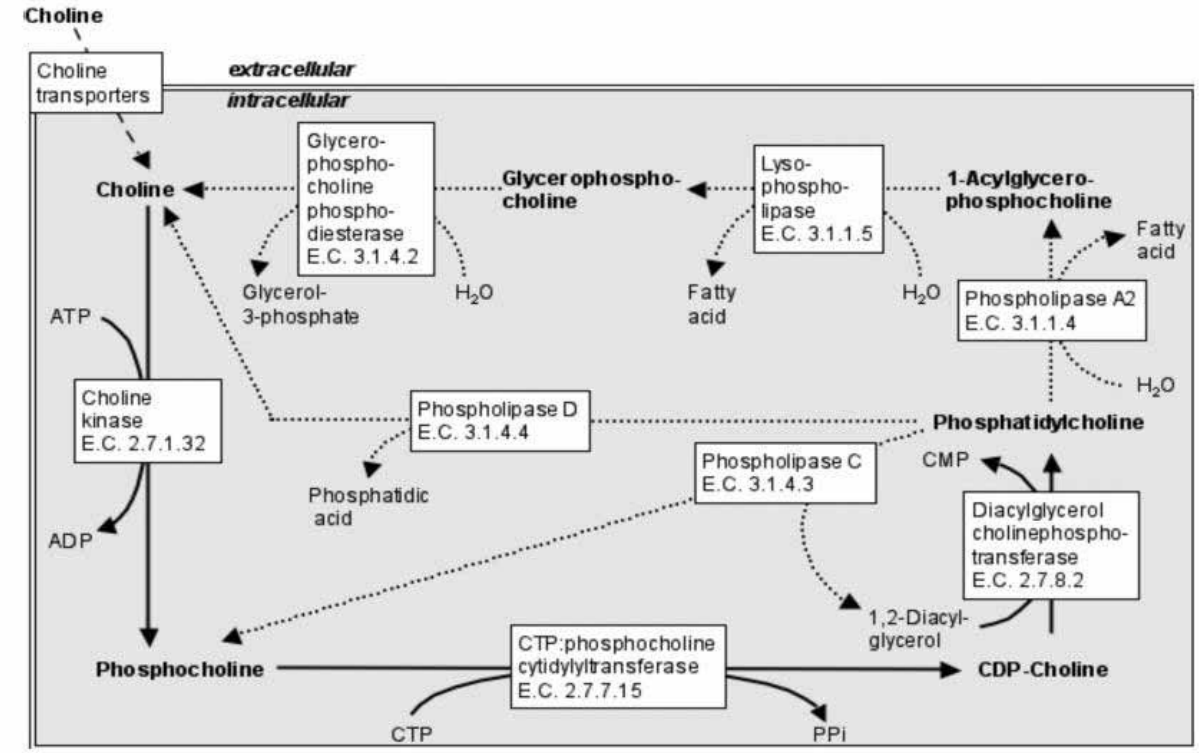


Figure 2.4. Schematic overview of phospholipid metabolism with phosphatidylcholine as example, Glunde *et al.* (2006).

Fatty acids, eicosanoids and docosanoids

Fatty acids esterified in biological cell membranes consist of a long (usually between 14 and 24) unbranched carbon chain of which one carbon (the head group COOH) is attached to glycerol or sphinghosine. Some 3D examples of free fatty acids (not esterified) are shown in Figure 2.5. Fatty acids can be saturated, monounsaturated or polyunsaturated. The saturated fatty acids have a straight acyl chain whereas unsaturated fatty acids have an acyl chain that is curved, due to the one (mono) or more (poly) double carbon bonds in the acyl chain. Unsaturated and polyunsaturated fatty acids make the membrane bilayer more fluid, while saturated fatty acids and cholesterol make it more rigid. An overview of the nomenclature of the fatty acids is shown in Table 2.I.

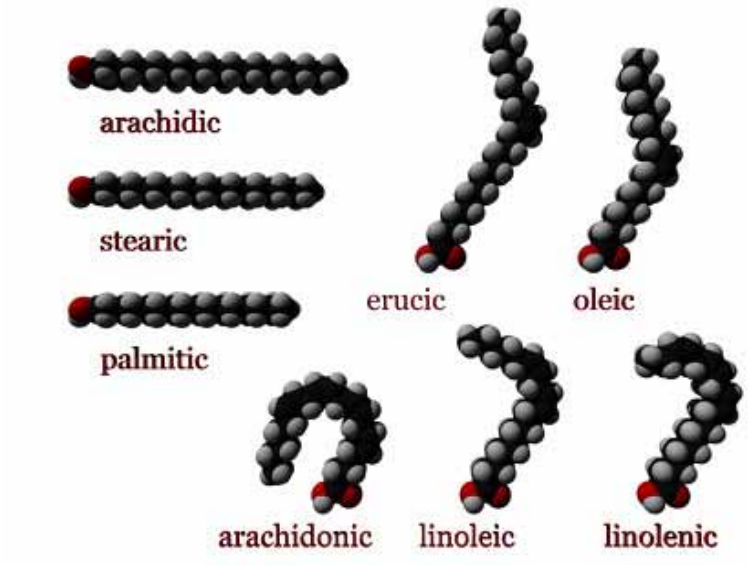


Figure 2.5. Some examples of the 3D structure of fatty acids.

Table 2.I. Overview of some fatty acids and their nomenclature. The first number in the chemical short hand notation is the total number of carbon atoms, the number after the colon (:) is the number of double (unsaturated) bonds, the number after the n (or sometimes written omega ω) represents the location of the double carbon bond counted from the methyl (CH_3) tail. The systematic chemical names of saturated fatty acids end with 'anoic' and the names of the unsaturated fatty acids end with 'enoic'. In the systematic nomenclature the location of the unsaturated bonds is counted from the carboxyl group. The trivial names and their abrevations are most often used in the literature.

short hand chemical notation	abrevation & trivial name	systematic chemical name
saturated		
14:0	MA myristic acid	tetradecanoic acid
16:0	PA palmitic acid	hexadecanoic acid
18:0	SA stearic acid	octadecanoic acid
20:0	-- arichidic acid	eicosanoic acid
monounsaturated		
18:1n9	OA oleic acid	*cis*-9-octadecenoic acid
24:1n9	NA nervonic acid	*cis*-15-tetracosenoic acid
polyunsaturated omega-3		
18:3n3	ALA (α-Linolenic acid)	*all-cis*-9,12,15-octadecatrienoic acid
20:3n3	ETE (eicosatrienoic acid)	*all-cis*-11,14,17-eicosatrienoic acid
20:4n3	ETA (eicosatetraenoic acid)	*all-cis*-8,11,14,17-eicosatetraenoic acid
20:5n3	EPA (eicosapentaenoic acid)	*all-cis*-5,8,11,14,17-eicosapentaenoic acid
22:5n3	DPA (docosapentaenoic acid)	*all-cis*-7,10,13,16,19-docosapentaenoic acid
22:6n3	DHA (docosahexaenoic acid)	*all-cis*-4,7,10,13,16,19-docosahexaenoic acid
polyunsaturated omega-6		
18:2n6	LA linoleic acid	*all-cis*-9,12- octadecadienoic acid
20:3n6	DGLA dihomo-γ-linolenic acid	*all-cis*-8,11,14-eicosatrienoic
20:4n6	AA arachidonic acid	*all-cis*-5,8,11,14-eicosatetraenoic acid
22:4n6	DTA adrenic acid	*all-cis*-7,10,13,16-docosatetraenoic acid

Fatty acids in biological tissues usually have an even number of carbon atoms because their biosynthesis involves acetyl-coenzym A, which provides two carbon atoms at a time.

The polyunsaturated fatty acids constitute an important class in humans and other mammals, they are called *essential fatty acids*, because they can not be syntesized *de novo* and have to be included in the diet. Strictly speaking only the 18-carbon fatty acids α-linolenic and linoleic acid are essential. The others can be synthesized from them, by *desaturase* enzymes (making more unsaturated carbon bonds) and *elongase* enzymes (making longer chains), although the desaturase process is slow and the efficiency decreases with aging. Essential fatty acids that are most abundant in the brain are AA, DTA and DHA, these comprise approximately 20 % of the dry weight of the brain. Phospatidylethanolamine is the most rich in polyunsaturated fatty acids.

The twenty-carbon essential fatty acids (ω-3 and ω-6) can be oxygenated to form *eicosanoids*, a very important class of substances which control inflammatory responses and act as intra- and extracellular messengers. The eicosanoids comprise four different classes: prostaglandins, prostacyclins, thromboxanes and leukotrienes. In the central nervous system eicosanoids are involved in: the modulation of neuroreceptor activity, thermoregulation, synaptic plasticity and long term potentiation which are important in learning and memory, and the regulation of inflammatory processes (Glen and Ross, 2004).

Another important class of substances, the *docosanoids*, is derived from DHA. These substances are anti-inflammatory and have neuroprotectins and resolvins as well known examples.

Thus cell membranes are a store of polyunsaturated fatty acids that can be used for the production of eicosanoids and docosanoids. The polyunsaturated fatty acids can be released from the membrane phospholipids by the action of *phospholipases*, for instance by phospholipase A2, which removes fatty acids from the sn2 position of the glycerol backbone.

The membrane hypothesis of schizophrenia

Suggestions that ultimately led to the membrane hypothesis of schizophrenia, by Horrobin, were posed by Feldberg in 1976 and Horrobin in 1977. Both pointed to a link between prostaglandins and schizophrenia. Horrobins article was entitled: "Schizophrenia as a prostaglandin deficiency disease". (Prostaglandins are family of the eicosanoids discussed in the previous section). At that time in 1977, it was based on three types of observations:

1. All anti-schizophrenic drugs stimulate prolactin secretion and prolactin is a precursor of prostaglandin.
2. Schizophrenic patients are often resistant to pain and inflammation and are free of rheumatoid arthritis. Prostaglandins play an important role in pain, inflammation and rheumatoid arthritis.
3. High dosis of drugs that are anti-prostaglandinic, such as anti-malarials give rise to schizophrenia like syndromes.

The term 'the membrane hypothesis of schizophrenia' was introduced by Horrobin *et al.* in 1994, in a paper with that same title, although a description of it was already given by Horrobin *et al.* in 1991, but then it still refered to the essential fatty acid / prostaglandin hypothesis of schizophrenia. Here the work by Horrobin (*et al.*) dedicated to the membrane hypothesis will be briefly summarized from the papers by Horrobin (*et al.*) (1994), (1996), (1998). Entries to the literature can be found in the above mentioned papers and some more recent entries are added below.

The membrane hypothesis of schizophrenia ".. suggests that schizophrenia is a disorder in which the metabolism and structure of membrane phospholipids are abnormal, not just in the brain, but in other tissues also". And more specific: "The abnormality of membrane biochemistry relates to the way in which 20 and 22 carbon essential fatty acids (EFAs) are incorporated into and removed from membranes, and in which these fatty acids are converted to further metabolites, including cyclo-oxygenase and lipoxygenase derivatives" (Horrobin *et al.*, 1994). *Cyclo-oxygenases* and *lipoxygenases* are enzymes that convert polyunsaturated fatty acids (e.g. arachidonic acid) into prostaglandins and leukotrienes, repectively.

Other observations that support the membrane hypothesis are:

4. Essential fatty acid levels in periferal - and central nervous tissue membranes of schizophrenic patients are often reduced. However, not all findings reported in the literature are consistent. The evidence from erythrocyte membranes and brain tissue for lowered levels of essential fatty acids is reviewed in chapters 3 and 4.

5. Increased levels of phospholipase A2 in the blood of schizophrenic patients. Phospholipase A2 cleaves fatty acids from the sn2 position of the glycerol backbone of glycerophospholipids. The literature on PLA2 in schizophrenic patients has been reviewed by Law *et al.* (2006) and it appears that schizophrenic patients show an overall increase in calcium-independent PLA2 activity.

6. Some magnetic resonance spectroscopy (^{31}P) studies on schizophrenic patients have shown increased phosphodiesters (PDE, e.g. glycerophosphocholine) and reduced phosphomonoesters (PME, e.g. phosphocholine) which suggest that membrane breakdown is dominant. However, a lot of contradictory findings have been reported in the literature and the PME and PDE peaks measured with MRS are thought to be not (exclusively) specific for membrane turnover (Weinberger and Laruelle, 2002). MRS studies on ^{1}H show a more consistent picture and have been reviewed by Steen *et al.* (2005). Schizophrenic patients show a decreased n-acetylaspartate (NAA) signal in a

broad range of brain tissues. Unfortunately, NAA is not a very specific marker and a decreased signal is seen in many illnesses.

7. Schizophrenic patients show a reduced flushing response to oral or topical administered niacin. Niacine provokes the arachidonic acid - prostaglandin cascade in the skin and causes small blood vessels to dilate, giving rise to flushing of the skin. A reduced flushing response, as is often seen in schizophrenic patients, could indicate that AA levels are reduced. A study of first episode patients (FEP) and chronically ill patients by Smesny *et al.* (2005), with a review of the literature, showed a decreased flushing response in FEP but not in chronically ill patients. However, the chronically ill patients in this study were mostly on atypical antipsychotics, which may be related to this different flushing behaviour. Evidence for the different action of atypical and typical antipsychotics on phospholipid metabolism is discussed in chapters 3 and 4. In the light of these chapters, and because there are quite a few conflicting reports on niacin flushing in the literature, it would be interesting to review the literature on niacin flushing response in schizophrenic patients with an emphasis on medication type and on whether the age and sex of the control groups are carefully matched with the patient groups. A study by Smesny *et al.* (2004) revealed that males are less sensitive to niacin and that the flushing response (male and female) is strongly inverse correlated with age; so age and sex matching patients and controls is very important.

8. Schizophrenic patients show a reduced amplitude of the flash electroretinogram. The amplitude of the electroretinogram depends on the availability of DHA for cell signalling. A recent study on the subject, with some entries to the literature is by Balogh *et al.* 2008. These authors found a reduced amplitude in schizophrenic patients that were approximately 4 weeks on atypical anti-psychotics, however the significance of the effect disappeared after 8 weeks on atypical medication. The authors conclude that the decreased amplitude is only observed in patients with acute symptoms and that medication is not of influence because the patient groups measured at 4 weeks and after 8 weeks (follow up) were both on (atypical) neuroleptics. It is argued here that a medication effect can still be the case because it may take several weeks before the retinal membrane DHA concentration is restored to normal levels. A study on DHA deficient rhesus monkeys, by Connor *et al.* (1990) has shown that repletion of plasma, red blood cells and brain phospolipids to normal DHA-levels, by a diet high in fish oil (containing EPA and DHA), takes 6 to 12 weeks.

9. Clozapine appears to enhance AA and DHA levels in red blood cell phospholipids of schizophrenic patients, as has been found in a preliminary study by Glen *et al.* (1994) in four patients. This finding is confirmed in a later study by Waldo *et al.* (2003) comparing the effect of clozapine and prolizin on AA and DHA levels. In chapters 3 and 4 additional evidence is discussed.

The membrane hypothesis as the biochemical basis for the neurodevelopmental hypothesis of schizophrenia

In his 1998 paper Horrobin poses the membrane hypothesis as the biochemical basis for the neurodevelopmental hypothesis of schizophrenia. The neurodevelopmental hypothesis of schizophrenia states that schizophrenia is caused by an interplay of genetic and environmental factors that influence brain development from conception to early adulthood. This involves neuron formation, migration, synaptogenesis, pruning and apoptosis. The formation of neurons and their connections is dependent on phospholipid synthesis. Modelling, remodelling and apoptosis of neurons all depend on *eicosanoids* for which

essential fatty acids are precursors. An abnormality in essential fatty acid metabolism will be of influence on these processes. Such an abnormality has been found in the overactivity of the PLA2 enzyme in schizophrenic patients which has a genetic origin, see Law *et al.* (2006) for a review on this topic. The onset after puberty fits in this concept, because a surge in synaptic remodelling, under the influence of gonadotrophins and gonadal hormones, takes place around puberty. A disturbed essential fatty acid metabolism will have its impact on this process.

However, from longitudinal imaging studies we know today that volume loss of gray matter is progressive in adult chronically ill schizophrenic patients, see for instance Hulshoff Pol and Kahn (2008). This does not falsify the neurodevelopmental hypothesis but indicates that a neurodegenerative process, in which focal gray matter density decreases, continues after onset of the first psychotic episode.

The (often circumstantial) evidence for the membrane hypothesis as a biochemical basis for the neurodevelopmental hypothesis will be discussed below in separate points. Entries to the literature prior to 1998 can be found in Horrobin (1998). One should keep in mind that the points below are not causative in itself, but are factors that increase the risk of developing schizophrenia when a genetic predisposition is present.

1. Gender

Men are more susceptible to develop schizophrenia than women, see Table 1.II, and a difference in age at onset between men and women exist. Most men develop schizophrenia in the age range 15 to 25 years, while the age range for females is 15 to 30 years. On average schizophrenia tends to strike women 3 to 4 years later than men. The probability age curve of women also shows a small peak just after the menopause between 45 and 50 years (Hafner, 2003). In general women tend to have a milder form of schizophrenia than men. These gender differences in developing schizophrenia and the severity of the disease parallels a difference in phospholipid synthesis and bioavailability of AA and DHA between men and women. It appears that these fatty acids are more easily synthesized and incorporated in phospholipids in women than in men, which is partially dependent on oestrogen levels. Schizophrenic women with higher oestrogen levels tend to have milder psychiatric symptoms than those with lower oestrogen levels. Also the menopause effect, mentioned above, is probably related to oestrogen.

2. Age

In both sexes a schizophrenia-like syndrome that can emerge at old age exists. After the age of approximately 65 years the incidence rate of psychosis increases with age, Van Os *et al*. (1995). Phospholipids and essential fatty acids of brain tissue decrease with age, see chapter 4. The production rate of DHA and AA from dietary precursors declines with increasing age and a marginal problem in for instance the acyltransferase pathway can aggravate the incorporation rate of these fatty acids into phospholipids.

3. Pregnancy and perinatal events

A number of environmental influences during pregnancy or around birth are known to increase the risk for the occurrence of schizophrenia later in life. Most of these influences can be shown to affect the supply or incorporation of essential fatty acids into brain phospholipids as well.

Breast feeding has been associated with a decreased risk for developing schizophrenia later in life. Human milk contains substantially more omega-3 and 6 polyunsaturated fatty acids than most infant formulas.

Maternal food deprivation increases the risk for schizophrenia in the offspring. The link with reduced bioavailability of essential fatty acids for the foetus seems plausible.

Brain growth of the foetus is dependent on arachidonic acid supply. Small head circumference at birth is a marker of AA deprivation and a risk factor for schizophrenia.

Obstetric complications, resulting in hypoxia or premature birth are a risk factor for schizophrenia. Hypoxia is known to increase phospholipase A2 activity and mobilises polyunsaturated fatty acids from cell membranes in the brain. Children with hypoxia at birth, or who were premature at birth, are also less likely to be breast fed, thus enhancing the risk for schizophrenia.

Maternal stress increases the risk for schizophrenia in the offspring. Stress is mediated by cortisol and catecholamines which reduces the rate of formation of brain specific polyunsaturated acids (e.g. AA, DHA, DPA) from their dietary precursors, with possibly a subsequent reduction in availability of these fatty acids for the foetus.

Maternal viral infections such as influenza enhance the risk for schizophrenia in the offspring. It is known that viral infections can inhibit the δ-6 desaturation of precursor fatty acids in the production of brain PUFAs, thus possibly reducing the availability of these PUFAs for a short time in the foetus.

Winterbirth increases the risk for developing schizophrenia later in life. From studies on ectodermal lipids it is known that there is a seasonal influence on the PUFA content of these lipids, leading to a decrease in PUFA content in winter. It is suggested here that this effect may be mediated by vitamin D3, which production decreases when there is less sun exposure of the skin. Moreover, studies in rats have shown that vitamin D3 stimulates phospholipid synthesis in fetal lung membranes, Marin *et al.* (1990), and vitamin D3 deficient rats show a decrease in phosphor content of brain cortex tissue. A tritium and radiocarbon labeling study by Matsumoto *et al.* (1981) revealed that vitamin D3 enhances the synthesis of phosphatidylcholine and enhances the incorporation of unsaturated fatty acids in phosphatidylcholine in duodenal mucosal cells of chicks. In the light of the above it might not be a coincidence that the list of deficiency diseases of vitamin D3 and of polyunsaturated fatty acids are similar (depression, hypertension, heart disease, various cancers, impaired immune function, osteoporosis, etc.). A recent review on vitamin D3 deficiency and brain dysfunction, discussing the evidence for the important role of vitamin D3 in brain development and function is written by McCann and Ames (2008).

Maternal and perinatal vitamin D3 deficiency in itself appears to be a risk modifying factor for developing schizophrenia, see McGrath (1999) and Burne *et al.* (2006). The link proposed here between vitamin D3 and phospholipid synthesis (including the incorporation of polyunsaturated fatty acids) in relation to schizophrenia could be an interesting field of research.

Other pregnancy related events, that were not mentioned by Horrobin but, that are known to increase the risk of schizophrenia in the offspring are: maternal diabetes, maternal iron deficiency and preeclampsia. All of these are related to polyunsaturated fatty acids. A possible mechanism for the effect of maternal diabetes, involving increased oxidation of polyunsaturated fatty acids, is discussed by Van Lieshout *et al.* (2008). Maternal iron deficiency, as well as preeclampsia may cause a state of chronic hypoxia in the foetus and this enhances phospholipase A2 activity, leading to a loss of PUFA from cell membranes. Iron also plays an important role in neurodevelopment and is required in the biosynthesis of lipids and the neurotransmitter dopamine, *Insel et al.* (2008).

Recent progress: the n-3 polyunsaturated fatty acid dopamine hypothesis of schizophrenia
The evidence for the membrane hypothesis of schizophrenia is often a bit circumstantial. It is beyond reasonable doubt that there is something peculiar in the fatty acid metabolism of schizophrenic patients, although conflicting data exists. As shown above, the concept of the membrane hypothesis can explain quite a lot of observations, and in that sense it fits the shoe, but it lacks more specific experimental evidence. The statement: polyunsaturated fatty acids influence membrane fluidity and the physicochemical surroundings of structural elements such as neuroreceptors, ion channels et cetera, so they are co-responsible for the tertiary structure and correct functioning of these elements, is probably true, but it lacks precise observation. The same holds for the statement: neurotransmitters and calcium, which are necessary for signal transduction, are released from phospholipid vesicles, this requires the re-alignment of phospholipids and PUFA are influencing this process. Intuitively this is evident but direct experimental evidence is not given.
An attempt to be more specific, although not completely elucidative, has been made by Ohara (2007) in a paper entitled: "The n-3 polyunsaturated fatty acid/dopamine hypothesis of schizophrenia". Based on experimental evidence from animal studies Ohara combined the dopamine hypothesis and the membrane hypothesis as schematically shown in Figure 2.6.

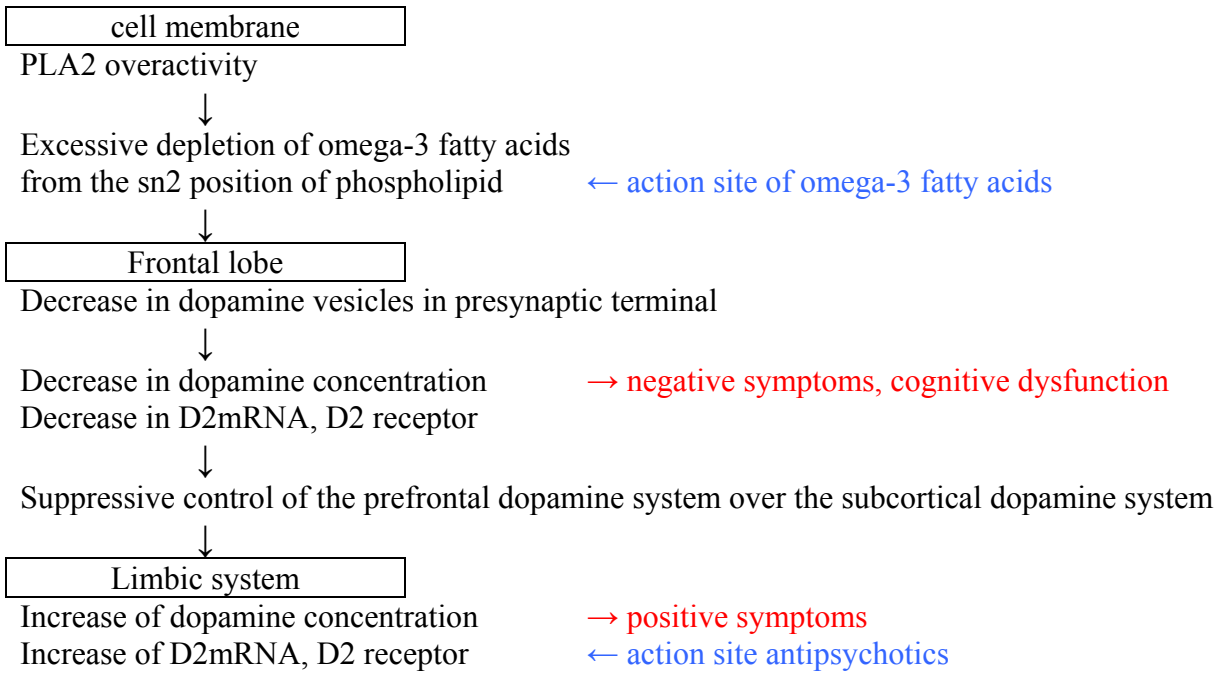


Figure 2.6. Schematic representation of the omega-3 fatty acid/dopamine hypothesis of schizophrenia. Modified from Ohara (2007).

Overactivity of phospholipase A2, and possibly other enzymes, cause excessive depletion of polyunsaturated fatty acids at the sn2 position of phospholipids in cell membranes. Animal studies, see Ohara (2007) for entries to the literature, have shown that n-3 deficient animals have a decreased number of dopamine vesicles, decreased dopamine concentration and a decreased number of D2 receptors in prefrontal presynaptic terminals. This hypofunction of the prefrontal dopamine system causes negative symptoms and cognitive disorders. The prefrontal cortex has a suppressive influence on the subcortical dopamine system, this influence declines due to the hypofunction of it, and as a result the subcortical dopamine system gets upregulated and the dopamine concentration and the number of D2-receptors in the striate body increase. This hyperactivity of the subcortical and limbic dopamine systems induces the positive symptoms of schizophrenia. Although more specific and a leap forwards, something that still has to be clarified is: why depletion of

polyunsaturated fatty acids has a different action on the prefrontal cortex than it has on the subcortical and limbic systems? Are there focal regions of polyunsaturated fatty acid depletion? If so, why?

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3. The fatty acid composition of erythrocyte membranes of schizophrenic patients compared to normal controls: a meta-analysis.

Introduction

The literature on essential fatty acid abnormalities in (peripheral) tissues in schizophrenia has been reviewed / summarized by Fenton *et al.* (2000). Their review comprises 8 studies on erythrocyte (red blood cell) membranes, 4 studies on plasma, 2 studies on platelets, 1 study on cultured fibroblasts, and 1 study on post-mortem brain tissue. Essential fatty acids that are most frequently assessed in studies prior to the year 2000 are linoleic acid (LA, 18:2n6), arachidonic acid (AA, 20:4n6) and docosahexaenoic acid (DHA, 22:6n3). Fenton *et al.* (2000) evaluated qualitatively whether an increase or decrease was reported in fatty acids and made no comment on the cases where data are lacking or were non-significant. However, to obtain an clear view one would like to know whether a fatty acid has not been measured or that it was measured but that the change in concentration was found to be non-significant. Moreover details of the studies (number of subjects, males and females, medication status, average age) were not reported. Fatty acid data on erythrocyte membranes are the most abundant in the literature. Erythrocyte membrane fatty acid composition shows not too much short term fluctuations caused by diet influence. This makes erythrocyte membranes of schizophrenic patients a good target for a meta-analysis, which at present does not exist in the literature. Literature data are summarized here and a meta-analysis is done in the sections below for: neuroleptic naïve first episode patients, chronically ill patients (mostly) on typical antipsychotics and for a mixed group of first episode and chronically ill patients (mostly) on atypical antipsychotics. This subdivision in groups, based on medication type, may well be the key to resolve a lot of conflicting reports on erythrocyte membrane fatty acid composition. (See also chapter 2, point 9 of the observations that support the membrane hypothesis).

Method

All fatty acid values reported in the sections below are the percentage difference compared to normal controls (relative fatty acid difference *RFAD*), equation (1), with a standard error of mean, *SEM,* given by Fiellers theorem, equation (2), see for instance Motulski (1995). Reporting the relative difference makes a comparison between different studies easier.

$$RFAD = \frac{[FA]_{SZ}}{[FA]_{NC}} - 1 \qquad (1)$$

$$SEM_{RFAD} = \frac{RFAD + 1}{(1-g)} \sqrt{(1-g)\left(\frac{SEM_{SZ}}{[FA]_{SZ}}\right) + \left(\frac{SEM_{NC}}{[FA]_{NC}}\right)^2} \qquad (2)$$

The square brackets denote concentrations, NC are normal controls, SZ schizophrenic patients and *g* is an intermediate variable given by

$$g = \left(t^* . \frac{SEM_{NC}}{[FA]_{NC}}\right)^2, \qquad (3)$$

where *t** is the *t*-value of the Students *t*-distribution in which the number ν for the degrees of freedom is calculated from

$$\nu = \frac{\left(SEM_{NC}^2 + SEM_{SZ}^2\right)^2}{\frac{SEM_{NC}^4}{n_{NC}+1} + \frac{SEM_{SZ}^4}{n_{SZ}+1}} - 2 . \qquad (4)$$

In equation (4) the assumption of equal variances is omitted. In case of equal variances it leads to the well known

$$\nu = n_{NC} + n_{SZ} - 2. \quad (5)$$

The 95% confidence interval of the *RFAD* can subsequently be calculated from

$$\frac{RFAD}{(1-g)} - t^* \cdot SEM_{RFAD} \quad \text{to} \quad \frac{RFAD}{(1-g)} + t^* \cdot SEM_{RFAD}. \quad (6)$$

For the meta-analysis each study i is given a statistical weight w_i equal to the reciprocal $SEM_{\mathrm{RFAD},i}$ of the study

$$w_i = \frac{1}{SEM_{RFAD,i}}. \quad (7)$$

The $RFAD_{\mathrm{meta}}$ obtained from meta-analysis is then calculated from

$$RFAD_{meta} = \frac{\sum_i RFAD_i \cdot w_i}{\sum_i w} \quad (8)$$

and the 95% confidence interval for $\mathrm{RFAD_{meta}}$ can be calculated from equation (6), when substituting the meta-values for RFAD and $\mathrm{SEM_{RFAD}}$ and using the appropriate t^* value.

Neuroleptic naïve first episode schizophrenic patients.

Literature data on neuroleptic naïve first episode schizophrenic patients compared to normal controls are summarized in Table 3.I. Most striking in Table 3.I are the large decreases in polyunsaturated (essential) fatty acids and the high increases in TBARS in the study by Kahn *et al.* (2002) and to a lesser extent the study by Arvandakshan *et al.* (2003). [TBARS stand for thiobarbituric acid reactive substances which a measure for the amount of lipid peroxidation or oxidative stress]. It seems likely that these extreme numbers represent some kind of artefact. Although inter-laboratory differences regarding experimental procedures exist, all studies are based on a comparison with normal controls so basically one would expect only variations in error margins between laboratories and of course the possible influence of the usual suspects (differences in: diet, smoking and alcohol habits, numbers of males & females, medication, age, of patient and control groups). However, it is known from storage studies (Otto *et al.*, 1997) on erythrocytes that polyunsaturated fatty acids are susceptible to degradation. Moreover the degradation rate (during storage at –20 °C) of polyunsaturated fatty acids in RBC from schizophrenic patients appears to be significantly higher than that of normal subjects, Fox *et al.* (2003). Enhanced phospholipase A2 activity (Fox *et al.* 2003) as well as enhanced lipid peroxidation (Otto *et al.* 1997) are possible mechanisms that can alter fatty acid composition of erythrocytes after sampling. Although Khan *et al.* (2002) reported a storage temperature of -70 °C for the RBC, addition of an antioxidant to the RBC was not mentioned. Oxidative stress in the blood samples (TBARS) was assessed by Kahn *et al.* (2002) and Arvandakshan *et al.* (2003) and very high values for their patient groups (increases of 713% and 72%) were reported, see Table 3.I. It seems unlikely that the change of polyunsaturated fatty acid concentrations reported by Khan *et al.* (2002) represent in vivo patient conditions. In addition, the increases in saturated fatty acids reported by Khan *et al.* (2002) for the schizophrenic groups (NN FEP and chronically ill, Tables 3.I and 3.II) are out of line with the other studies. For palmitic acid this is caused by the very low concentration measured for the normal control group (15% of total fatty acid vs 20-29% of total fatty acid for control groups of other studies, although statistically not an outlier). The palmitic acid concentrations for the patient groups of Khan *et al.* (2002) are in agreement with other studies. For stearic acid the situation is reversed *i.e.* the values

reported for the patient groups of Khan *et al.* 2002 are higher than for the patient groups of other authors (24% vs 15-17 % of total fatty acids) whereas the values for the control groups are in good agreement.

Table 3.I. Fatty acid composition of erythrocyte membranes in neuroleptic naïve first episode schizophrenic patients. Fatty acid data are reported as a percentage change compared to the normal control group with ± SEM. NC: normal controls; SZ schizophrenic patients; na: not available; ns: non significant; ↓ shows a down trend at significance level: $0.05 \leq p \leq 0.1$; All fatty acid changes expressed in numbers are significant at $p < 0.05$ (reported p levels for a significant change between SZ and NC, vary between 0.001 and 0.05). TBARS: thiobarbituric acid reactive substances change in %; TBARS are a measure for lipid peroxidation and oxidative stress. LPO: lipid peroxides

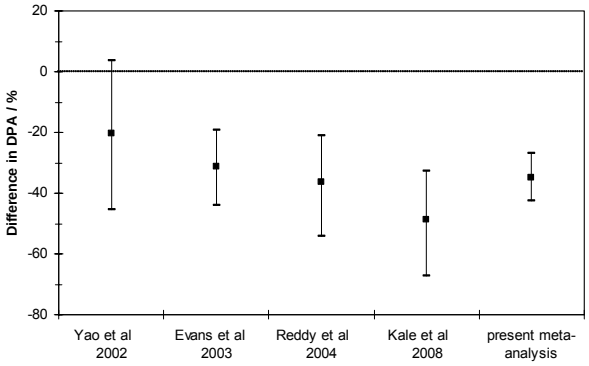
	Khan *et al.* (2002)	Yao *et al.* (2002)	Arvan-dakshan *et al.* (2003)	Evans *et al.* (2003)	Reddy *et al.* (2004)	Yao *et al.* (2005)	Kale *et al.* (2008)
NC Male:Female	14:2	6:5	25:20	na : na (25)	20:11	26:10	25:21
Average age	24 ± 6	26 ± na (19-39)	29 ± 9	25 ± 4	27 ± 8	28 ± 9	34 ± 9
SZ Male:Female	18:4	6:5	12:8	14:2	17:7	23:10	14:17
Average age	22 ± 4	26 ± na (17-44)	29 ± 10	19 ± 5	28 ± 8	24 ± 8	33 ± 8
Medication A:T:DF	0:0:22	0:0:11	0:0:20	0:0:16	0:0:24	0:0:33	0:0:31
illness duration (m)	0.2	na	30±24	< 1	na ~ 18	na ~ 11	6 ± 7
MA 14:0	na	ns	na	na	na	na	na
PA 16:0	66 ±11	ns	na	na	na	ns	ns
SA 18:0	52 ± 7	ns	na	na	na	ns	ns
Σsaturated	na	ns	4 ±3	na	ns	ns	na
OA 18:1n9	28 ± 9	ns	na	na	na	ns	ns
NV 24:1n9	ns	ns	na	↓ ns	na	ns	ns
Σmonounsat.	na	ns	na	na	ns	ns	na
EPA 20:5n3	na	ns	na	na	na	na	ns
DPA 22:5n3	-92 ± 6	ns	na	-31 ± 6	-36 ± 8	ns	-49 ± 9
DHA 22:6n3	-92 ± 5	ns	-75 ± 6	-30 ± 8	-26 ± 8	↓ ns	-20 ± 6
LA 18:2n6	-39 ± 10	ns	na	ns	ns	ns	ns
DGLA 20:3n6	na	ns	na	na	na	ns	na
AA 20:4n6	-79 ± 4	-19 ± 6	-52 ± 5	ns	-18 ± 5	-12 ± 5	ns
DTA 22:4n6	na	ns	na	na	ns	-21 ± 9	na
Σpolyunsat.	na	↓ ns	na*	na	-13 ± 5	na	na
TBARS/LPO	713 ± 87	na	72 ± 20	139 ± 32	na	na	na

* Total polunsaturated fatty acids reported by Arvandakshan *et al.* (2003) erroneously also include mono-unsaturated fatty acids.

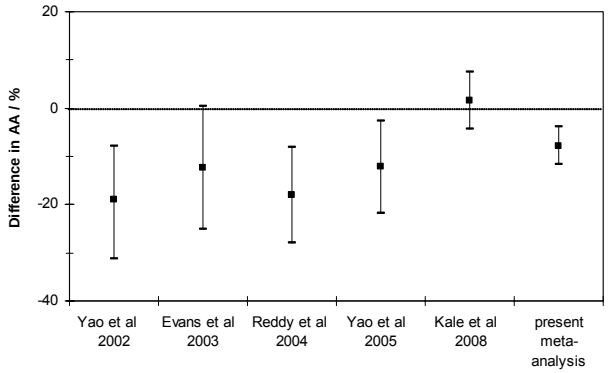
Arvandakshan *et al.* (2003) reported the amount of total fatty acids for neuroleptic naïve first episode patients to be 84 % (Σ[saturated + (mono + poly)unsaturated fatty acids]), *i.e.* during the experimental procedure 16 % of total fatty acids were not recovered. Unfortunately no comments were made on this by the authors. The large and significant decrease in AA and DHA for this group of patients could be caused by incomplete recovery of the fatty acids, due to oxidation (as polyunsaturated fatty acids are the most reactive, they will disappear first). The results reported by Khan *et al.* (2002) and the NN FEP results

reported by Arvandakshan *et al.* (2003) are suspect, in the light of the above, and the values have been grayed out in Table 3.I.

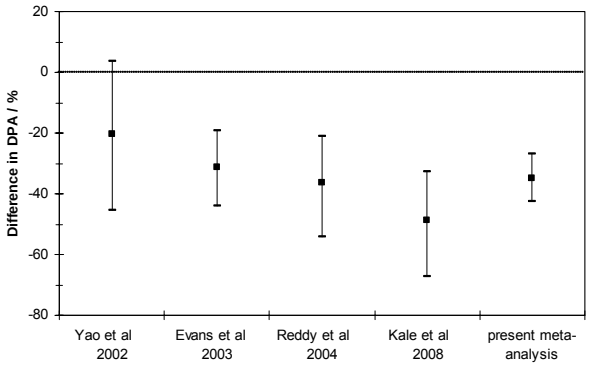
A meta-analysis was done for four of the polyunsaturated fatty acids: AA, DHA, DPA, and LA and 95% confidence intervals of the relative fatty acid difference were calculated with equation (6). The studies by Khan *et al.* (2002) and Arvandakshan et al (2003) were discarded for the reasons discussed above. Figures 3.1 A to D show the results of the meta-analysis.



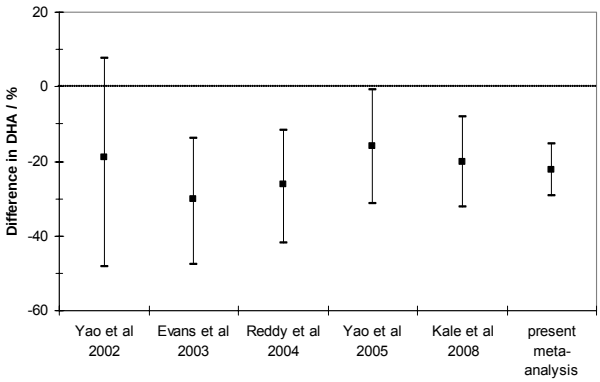
A. Linoleic acid (LA, 18:2n6).



B. Arachidonic Acid (AA, 20:4n6).



C. Docosapentaenoic acid (DPA, 22:5n3).



D. Docosahexaenoic acid (DHA, 22:6n3).

Figure 3.1. Relative difference in fatty acid composition of erythrocyte membranes of *neuroleptic naïve* first episode schizophrenic patients as compared to normal controls. The figures show the 95 % confidence intervals of the relative difference in fatty acid composition of the various studies and the present meta-analysis.

For linoleic acid the difference in fatty acid concentration is not very convincing as the confidence interval of the meta-analysis contains zero. For the other poly-unsaturated fatty acids AA, DPA and DHA there is convincing evidence that neuroleptic naïve first episode patients show a pronounced decline in concentration in erythrocyte membranes.

Chronically ill schizophrenic patients mostly on typical antipsychotics

The literature data on chronically ill schizophrenic patients (mostly) on typical antipsychotics are summarized in Table 3.II. The study by Doris *et al.* (1998), has been grayed out in Table 3.II because of the large number of patients in this group that were receiving *atypical* antipsychotics. This study was excluded from the meta-analysis because there is a remarkable difference in influence of atypical and typical antipsychotics on fatty acid concentrations of erythrocyte membranes as will be evident in the next section.

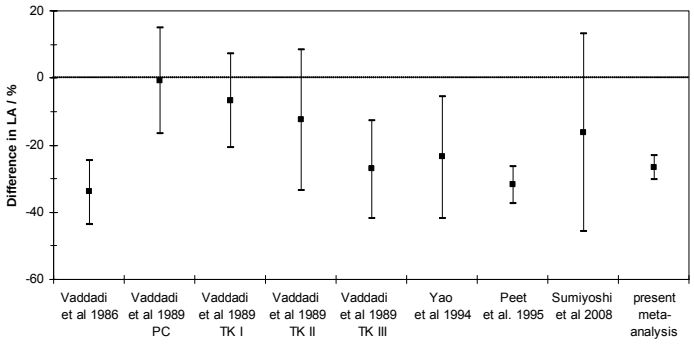
Table 3.II. Summary of literature data on chronically ill patients mostly on *typical* antipsychotics. Fatty acid data are reported as a percentage change compared to the normal control group with ± SEM. NC: normal controls; SZ schizophrenic patients; na: not available; ns: non significant; ↓ shows a down trend at significance level: $0.05 \leq p \leq 0.1$; All fatty acid changes expressed in numbers are significant at $p < 0.05$ (reported p levels for a significant change between SZ and NC, vary between 0.001 and 0.05). Medication: A atypical; T typical; DF drug free; Some patients receive more than one type of medication so the sum of medication is often larger than the number of patients. TBARS: thiobarbituric acid reactive substances change in %; LPO: lipid peroxides.

	Vaddadi *et.al.* (1986)	Vaddadi *et.al.* (1989)				Yao *et al.* (1994)	Peet *et al.* (1995)	Doris *et al.* (1998)	Sumi-yoshi *et al.* (2008)
NC Male:Female	na : na (14)	11:9				22:0	na:na (16) matched	34:6	18:14
Average age	na matched	na matched				32 ± 10	na matched	35 (22-63)	33 ± 8
			tardive kinesia groups						
SZ patients		PC*)	mild	mode-rate	severe	**)			
Male:Female	na:na (16)	10:7	31:17			24:0	16:7	34:6	14:11 ***)
			(15)	(11)	(13)				
Average age	na (20-55)		52.7			37 ± 8	55 (28-75)	37 (21-63)	35 ± 12
Medication A:T:DF	0:16:0	0:≥13:≤4	0:~15:~0	0:~11:~0	0:~13:~0	0:24:0	0:24:0	16:25:1	0:16:0 ***)
PA 16:0	ns	na	na	na	na	ns	32 ± 10	na	ns
SA 18:0	ns	na	na	na	na	ns	ns	na	ns
Σsaturated	na	na	na	na	na	ns	na	na	ns
OA 18:1n9	ns	na	na	na	na	ns	29 ± 7	na	ns
NV 24:1n9	na	na	na	na	na	ns	na		
Σmonounsat.	na	na	na	na	na		na	na	ns
EPA 20:5n3	na	ns	ns	ns	-31 ± 17	na	-38 ± 7	↓ ns	ns
DPA 22:5n3	-24 ± 8	-29 ± 4	28 ± 11	-28 ± 15	-34 ± 14	-42 ± 9	ns	na	ns
DHA 22:6n3	32 ± 15	-25 ± 13	ns	ns	-30 ± 11	-45 ± 10	-49 ± 7	ns	ns
LA 18:2n6	-34 ± 5	ns	ns	ns	ns	-23 ± 9	-32 ± 3	na	ns
DGLA 20:3n6	ns	ns	-30 ± 12	-30 ± 14	-32 ± 12		ns	22 ± 7	
AA 20:4n6	-23 ± 8	-24 ± 10	↓ ns	↓ ns	-32 ± 9	-37 ± 9	-31 ± 8	ns	ns
DTA 22:4n6	-26 ± 9	-31 ± 10	-39 ± 10	-39 ± 11	-55 ± 10	-34 ± 10	↓ ns	na	
Σpolyunsat.	na	na	na	na	na	-33 ± 9	na	na	-16 ± 6
TBARS/LPO	na	na	na	na	na	na	ns	na	na

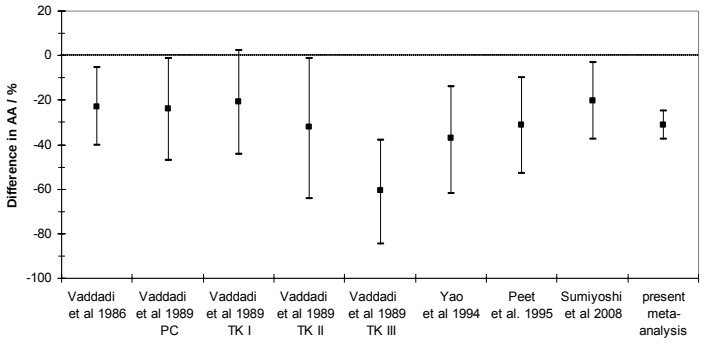
*) The study by Vaddadi *et al.* (1989) also includes a psychiatric control group consisting of 14 SZ patients and 3 patients with a personality disorder, which is treated here as all SZ patients.

**) The study by Yao *et al.* (1994) consists of three groups of patients. One group treated with haloperidol (typical agent) and two drug free groups. The drug free groups are classified as < 5 weeks drug free and > 5 weeks drug free.

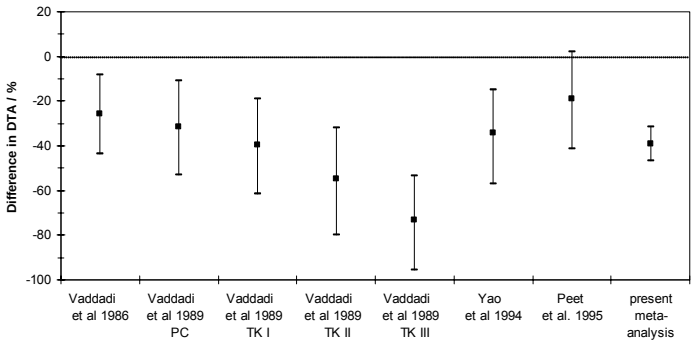
***) The study by Sumiyoshi *et al.* (2008) contains one group of 16 SZ patients on typical antipsychotics and one group of 9 patients which were at the moment of studying drug free. Only the patients on typical antipsychotics are taken into account here.



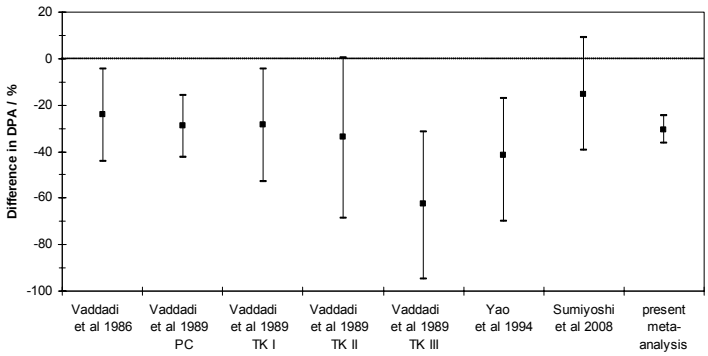
A. Linoleic acid (LA, 18:2n6).



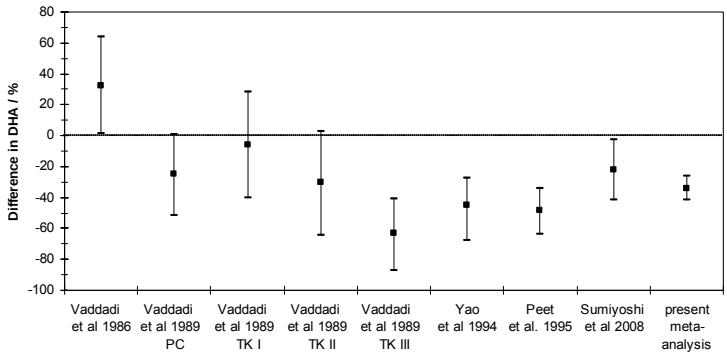
B. Arachidonic Acid (AA, 20:4n6).



C. Docosatetraenoic acid (DTA, 22:4n6).



D. Docosapentaenoic acid (DPA, 22:5n3).



E. Docosahexaenoic acid (DHA, 22:6n3).

Figure 3.2. Relative difference in fatty acid composition of erythrocyte membranes of chronically ill schizophrenic patients (mostly) on *typical* antipsychotics as compared to normal controls, reported by various authors. The figures show the 95 % confidence intervals of the relative change in fatty acid composition of the different studies and the present meta-analysis.

Figure 3.2 A-E shows convincing evidence that there exists a pronounced decline in LA, AA, DTA, DPA and DHA concentrations of erythrocyte membranes in schizophrenic patients on typical antipsychotics as compared to normal controls.

Chronically ill schizophrenic patients mostly on <u>atypical</u> antipsychotics

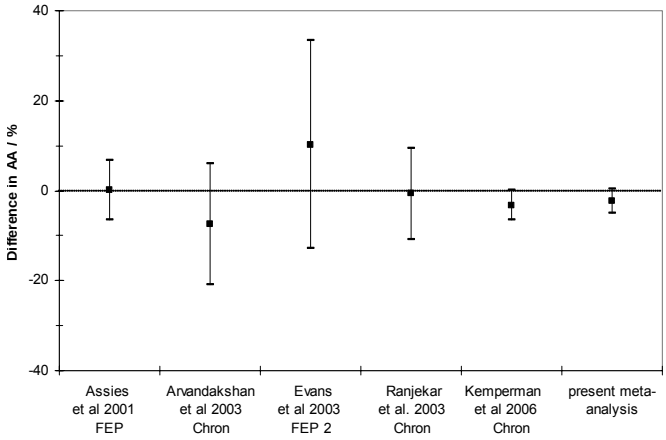
Data from the literature for first episode and chronically ill patients that were mostly on atypical antipsychotics are gathered in Table 3.III.

Table 3.III.Summary of literature data on first episode and chronically ill schizophrenic patients mostly on *atypical* antipsychotics. Fatty acid data are reported as a percentage change compared to the normal control group with ± SEM. NC: normal controls; SZ schizophrenic patients; na: not available; ns: non significant; ↓ shows a down trend at significance level: $0.05 \leq p \leq 0.1$; All fatty acid changes expressed in numbers are significant at $p < 0.05$ (reported p levels for a significant change between SZ and NC, vary between 0.001 and 0.05). Medication: A atypical; T typical; DF drug free; Some patients receive more than one type of medication so the sum of medication is often larger than the number of patients. TBARS: thiobarbituric acid reactive substances change in %; LPO: lipid peroxides.

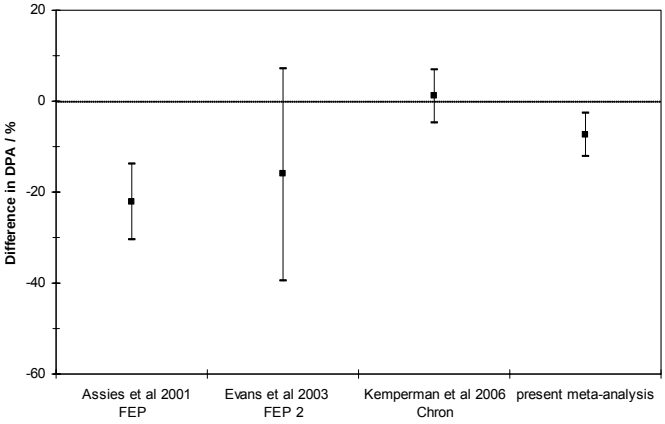
	Assies *et al.* (2001)	Khan *et al.* (2002)	Arvan-dakshan *et al.* (2003)	Evans et al (2003)	Ranjekar et al. (2003)	Kemper-man *et al.* (2006)
NC Male:Female	12:2	14:2	25:20	na : na (25)	na:na (27)	na:na (67)
Average age	21 ± 2	24 ± 6	29 ± 9	25 ± 4	matched	35 ±na
SZ patients	FEP	Chron	Chron	FEP	Chron	Chron
Male:Female	17:2	30:0	21:11	na : na (10)	31:0	37:24
Average age	21 ± 2	46 ± 6	31± 10	20 ± 5	37 ± 7	32 ± 9
Medication A:T:DF	15:4:2	20:10:0	24:8:0	10:0:0	mostly A	≥47:na:2
PA 16:0	ns	76 ± 12	na	na	na	2 ± 1
SA 18:0	ns	64 ± 8	na	na	na	2 ± 1
Σsaturated	ns	na	5 ± 3	na	ns	2 ± 1
OA 18:1n9	ns	55 ± 8	na	na	na	8 ± 1
NV 24:1n9	-8 ± 5	-94 ± 2		-16 ± 5		11 ± 3
Σmonounsat.	ns	na	na	na	na	5 ± 1
ALA 18:2n3					-63 ± 44	
EPA 20:5n3	ns	na	na	na	-24 ± 24	na
DPA 22:5n3	-22 ± 4	-76 ± 5	na	ns	na	ns
DHA 22:6n3	-22 ± 7	-64 ± 6	ns	ns	-29 ± 16	-17 ± 4
LA 18:2n6	ns	-20 ± 6	na	ns	na	↓ ns
DGLA 20:3n6	ns			na		13 ± 4
AA 20:4n6	ns	-54 ± 5	ns	ns	ns	-3 ± 2
DTA 22:4n6	ns			na	na	
Σpolyunsat.	ns	na	na	na		-5 ± 1
TBARS	na	585 ± 75	↑ ns	126 ± 36	26 ± 13	na

[1)] Fatty acid data by Kemperman was reported as median, here taken as mean, and the SD was estimated from the reported range.

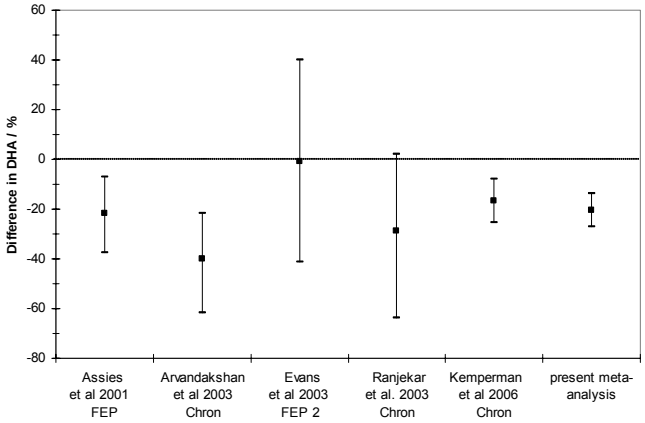
The study by Khan *et al.* (2002) was excluded for the reasons outlined in section 2.1.1 and because the average age of normal controls and patients are not matched at all, see Table 3.III. Unfortunately most groups contain also some patients who received typical antipsychotics. However patients that received atypical antipsychotics outnumber the ones that received typical antipsychotics by at least a factor three, so if there is a difference in influence of medication on fatty acid concentration one expects to detect it. Note that error margins in the study by Ranjekar *et al.* (2003) are very large. The authors claim that the measured difference between patients and controls are significant but a two tailed *t*-test, and a more strict two tailed Welch test (*t*-test with unequal variances) do not show any significant difference for ALA, EPA and DHA (p>0.1), not even a trend.



A. Arachidonic Acid (AA, 20:4n6).



B. Docosapentaenoic acid (DPA, 22:5n3).



C. Docosahexaenoic acid (DHA, 22:6n3).

Figure 3.3. Relative difference in fatty acid composition of erythrocyte membranes of chronically ill schizophrenic patients (mostly) on *atypical* antipsychotics as compared to normal controls, reported by various authors. The figures show the 95 % confidence intervals of the relative change in fatty acid composition of the different studies and the meta-analysis.

Apparent from Figure 3.3 is that AA and DPA concentrations have almost normalised and DHA still shows a decline as compared to normal controls.

Comparison between neuroleptic naïve patients and patients on typical and atypical anti-psychotics.

From the previous sections it is evident that there exists a pronounced difference in poly-unsaturated fatty acids concentration of erythrocyte membranes among the different patient groups as far as antipsychotic medication is concerned. Figure 3.4 shows an overview of the fatty acids that have been meta-analysed in all three different medication groups.

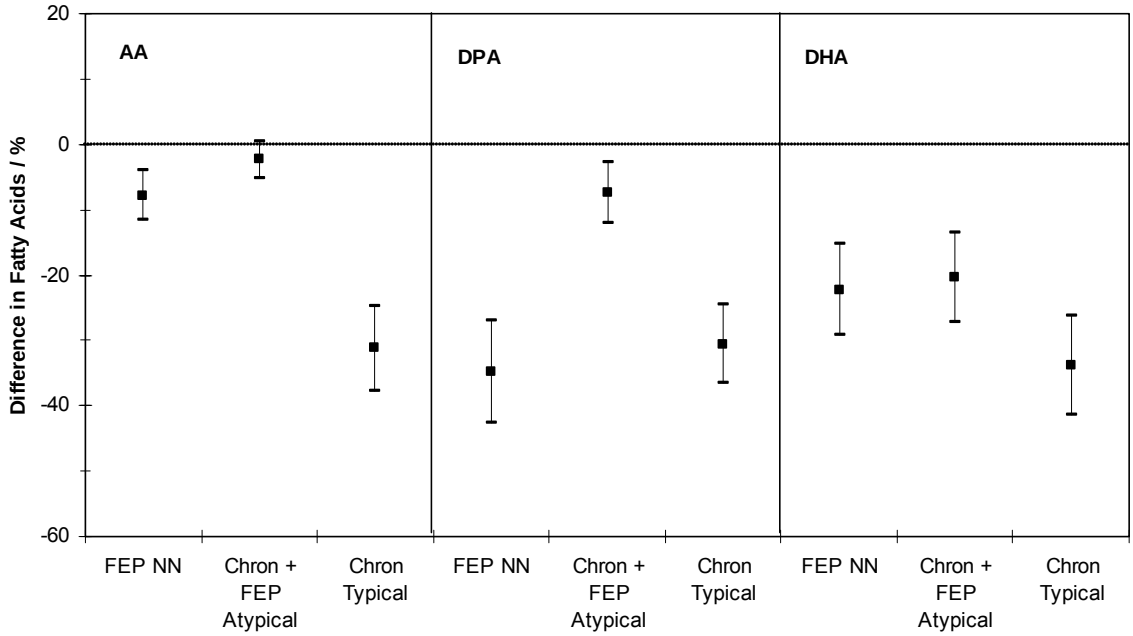


Figure 3.4. Comparison of AA, DPA and DHA 95 % confidence intervals, derived from meta-analysis, for patient groups on different medication regimes (neuroleptic naive, atypical antipsychotics, typical antipsychotics).

As is evident from Figure 3.4 *atypical* antipsychotics tend to normalise the erythrocyte membrane polyunsaturated fatty acids. For AA and DPA this effect is almost complete *i.e.* the difference with normal controls almost disappears. For DHA there is only a tendency to less difference with normal controls. It should be noted however, see Table 3.III, that the patients from the atypical antipsychotics group were not all on atypical antipsychotics. This group also contains patients on typical medication and drug free patients. Only the majority of these patients was on atypical antipsychotics, which implies that the true normalizing effect of the atypical antipsychotics is larger than shown in Figure 3.4. The present data do not allow for a further subdivision in medication. Some atypical agents may have a more pronounced normalising effect than others and some typical agents may be more de-normalising than others. Additional evidence for the normalising effect of clozapine (an atypical agent) on AA concentrations in RBC comes from Waldo *et al.* (2003). They found that patients on clozapine showed no significant difference in AA concentration, but patients on typical antipsychotics did show a decrease in AA. Unfortunately, the data reported by these authors in a conference abstract was not detailed enough to summarize in Table 3.II and 3.III or to take into account in the meta-analysis. Other studies reported in the literature that were excluded are summarized below.

Glen *et al.* (1994).
The data by these authors are not compared in tabular form to a normal control group but a comparison is made between chronically ill patients (all on neuroleptic medication) with predominantly positive vs predominantly negative symptoms. The authors found a bimodal distribution in AA and DHA. Patients with predominantly negative symptoms showing low concentrations of AA and DHA whereas the patients with positive symptoms showing virtually normal AA and DHA concentrations. Because medication is not specified in typical or atypical, the results cannot be interpreted unambiguously.

Peet *et al.* (1996).
These authors found a similar effect as Glen *et al.* (1994), unfortunately the data are not tabulated but only depicted in figures.

Hibbeln *et al.* (2003)
These authors studied the influence of smoking, gender and dietary influences on fatty acid composition of erythrocyte membranes of schizophrenic patients that were all on atypical antipsychotics. No tabular comparison with normal controls is reported. However their frequency distribution figures on AA, DPA and DHA include smooth lines showing normal comparison curves. These figures show AA concentrations which are normalised and DPA and DHA that are in line with Figure 2.3 B and C, *i.e.* an average relative concentration difference in the range -10 to -20 % (estimated) with large error bars. Comparing smoking to non-smoking schizophrenic patients led to a statistically significant ($p<0.01$) relative DHA difference of -17 %. Suggesting increased lipid peroxidation in smokers, however it appeared that the non-smokers were on a healthier diet as far as unsaturated fatty acids is concerned. The authors did not find a bimodal distribution for AA or DHA as in *Glen et al.* (1994) and Peet *et al.* (1996) nor did they found a decrease in AA and they argue that this may be a smoking artefact. However in the light of this chapter it seems more likely that it is related to antipsychotic medication.

Peet *et al.* (2004)
This paper was published in a journal that is not in the collection of the UU library and unfortunately the authors did not respond to an email request for a reprint. Two groups of Asian unmedicated schizophrenic patients were investigated. No significant difference in AA, a significantly decreased concentration of DTA and a significantly increased concentration of DHA in erythrocyte membranes of the patients were found. The abstract was not detailed enough to interpret these results.

All studies that measured TBARS (lipid peroxidation) found higher values in the patient groups. Enhanced lipid peroxidation may be one of the mechanisms that causes reduced polyunsaturated fatty acids levels in erythrocyte membranes. The other mechanism is increased phospholipase A2 activity in schizophrenic patients that has been observed by many authors. Entries to the literature on increased PLA2 activity can be found in Law *et al.* (2006).

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4. Lipid and fatty acid composition of the normal and schizophrenic brain: post-mortem studies

Lipid composition changes in the healthy aging human brain

The major lipid constituents of human brain are phospholipids, cholesterol and galacto-cerebroside. Of the phospholipids, phosphatidylcholine (PC) and phosphatidylethanolamine (PE) are the most abundant in the brain. An overview of the lipid composition in gray and white matter of normal adult human brain is shown in Table 4.I. These values are only indicative because the lipid composition of the brain changes with age.

Table 4.I. Lipid composition (weight %) of normal adult human brain in gray mater (GM) and white matter (WM). Recalculated from dry weights for individual components as reported by G.J. Siegel *et al.* (1998).

constituent	GM (%)	WM (%)
Total phospholipids	69.6	51.2
PE	24.7	22.1
PC	28.8	14.1
Sphingomyelin	6.2	7.0
Phosphoinositides	2.4	0.8
PS	7.5	7.2
Cholesterol	19.4	25.3
Galactocerebroside	4.8	18.2
Galactocerebroside sulfate	1.6	5.0
Ganglioside	4.6	0.3

PE, phosphatidylethanolamine; PC, phosphatidylcholine; PS, phosphatidylserine

A study on 118 post-mortem brains of healthy subjects, who died suddenly and unexpectedly, in the age range 20 to 100 years, by Svennerholm *et al.* (1994), showed for the frontal and temporal cortex and white matter, that dry solids and lipids decrease dramatically with age. Results for whole brain (101 male, 83 female) where reported in a later study by Svennerholm *et al.* (1997). A summary of the most important findings from these two studies, calculated from regression equations fitted to the experimental data, is shown in Table 4.II and Figures 4.1 to 4.6.

There appears to be a pronounced difference in brain mass loss between men and women during aging as shown in Figure 4.1. The mass of the female brain decreases slower than the mass of the male brain.

The average decrease in brain constituents from age 20 to 100 year for the frontal and temporal cortex and white matter are shown in Table 4.II. Data is tabulated as a decrease in concentration and as decrease per brain. The values of decrease in concentration are smaller than the values of decrease per brain, which implies that aging increases the hydration of the brain.

The decrease in constituents per brain for frontal cortex and white matter with age are shown in Figures 4.2 and 4.3. The decrease in cortical tissue is larger than in white matter from 30 to 40 years of age, but after 40 years frontal white matter decreases more rapidly. Temporal cortex and white matter show a similar pattern and figures are not shown here. Note that the values depicted in Figures 4.2 and 4.3 and Table 4.II are calculated from regression equations and are average values (*i.e.* no distinction between males and females has been made). Changes in *whole* brain composition, calculated from the data reported in Svennerholm *et al.* (1997), as a function of age for *men* and *women* are shown in Figures 4.4 to 4.6.

Table 4.II. Calculated average percentage *decrease* of brain constituents from 20 to 100 year in frontal and temporal cortex and white matter. Percentage decrease is calculated as a decrease in concentration mg/g for dry solids and μmol/g for specific components in cortex tissue (Svennerholm *et al.* 1994) and for the cortex of an average human brain as reported by Svennerholm *et al.* (1997). The (hypothetical) average human standard brain, adapted here, weighs 1500 g at age 20 and 1183 g at age 100.

	frontal cortex		temporal cortex	
constituents	decrease in concentration %	decrease per brain %	decrease in concentration %	decrease per brain %
dry solids	14	32	17	35
phospholipids	18	35	21	38
cholesterol	18	35	19	36
ganglioside	11	30	18	35

	frontal white matter		temporal white matter	
constituents	decrease in concentration %	decrease per brain %	decrease in concentration %	decrease per brain %
dry solids	29	44	24	40
phospholipids	31	45	29	44
cholesterol	34	48	33	46
ganglioside	22	39	15	33
cerebroside	35	48	31	46
sulfatide	35	49	34	48

Total phospholipids, phosphatidylcholine and phosphatidylethanolamine have been determined in frontal gray matter, white matter, hippocampus and pons as a function of age by Soderberg *et al.* (1991). A similar trend: a dramatic decrease in total phospholipids with age is observed. The ratio of PC to PE in the different tissues appears to be fairly constant with age. Pons and white matter show a PC to PE ratio (weight percent) of 0.66, frontal gray matter and hippocampus a ratio of 1.2 and 1.05 respectively.

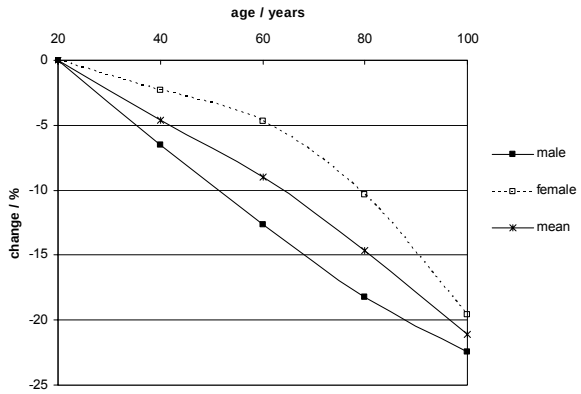

Fig.4.1. Change in brain mass as a function of age calculated from regression equations, Svennerholm *et al.* (1997). Estimated errors ±2% to ±3 % on change scale.

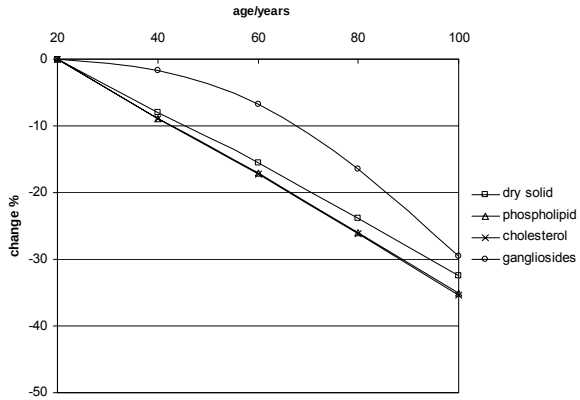

Fig. 4.2. Change in frontal cortex constituents per brain as a function of age, calculated from regression equations published by Svennerholm *et al.* (1994, 1997). Estimated errors ±3 to ±5 % on change scale.

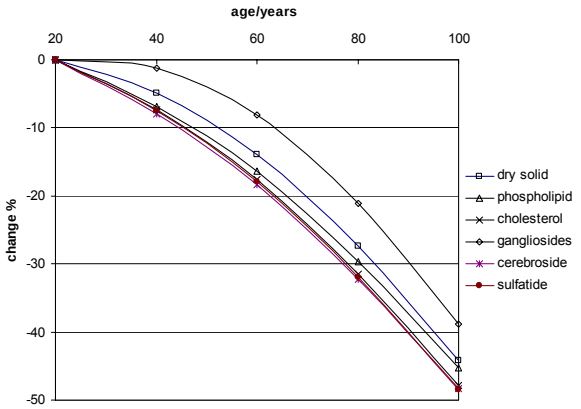

Fig. 4.3. Change in frontal white matter constituents per brain as a function of age, calculated from regression equations published by Svennerholm *et al.* (1994, 1997). Estimated errors ±3 to ±5 % on change scale.

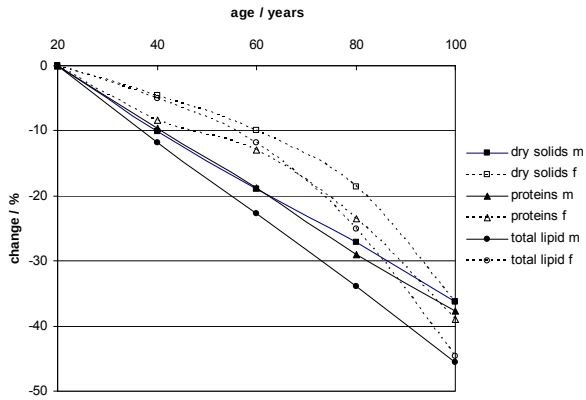

Fig. 4.4. Change in whole brain dry solids, proteins and total lipids for males (m) and females (f) calculated from data reported by Svennerholm *et al.* (1997). Estimated errors ±3 to ±5 % on change scale.

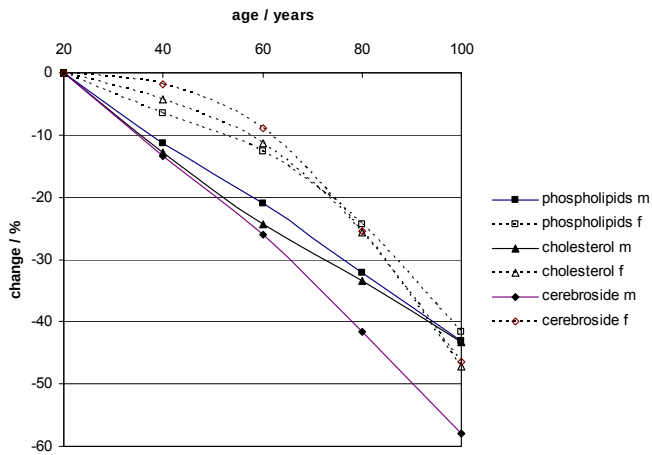

Fig. 4.5. Change in whole brain phospholipids, cholesterol and cerebroside for males (m) and females (f) as a function of age, calculated from data reported by Svennerholm *et al.* (1997). Estimated errors ±3% to ±5 % on change scale.

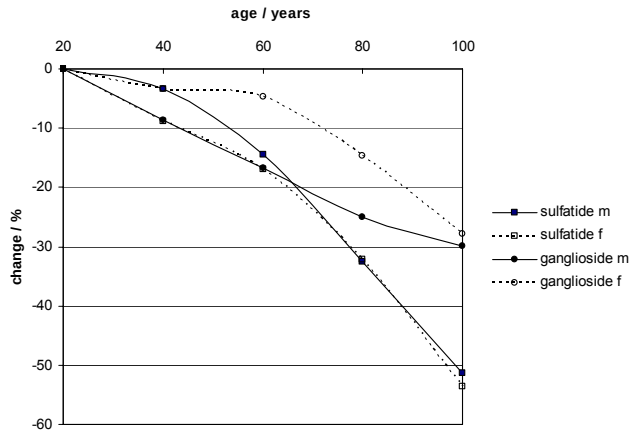

Fig. 4.6. Change in whole brain sulfatide and ganglioside for males (m) and females (f) as a function of age, calculated from data reported by Svennerholm *et al.* (1997). Estimated errors ±3% to ±5 % on change scale.

Fatty acid composition of the (healthy) aging human (frontal) cerebral cortex.
The most comprehensive post-mortem study (58 non-schizophrenic subjects) on fatty acid composition of frontal cerebral cortex is by Carver *et al.* (2001). Fatty acid composition of cerebral cortex appears to be correlated with age. A linear correlation can be obtained by dividing the age of the subjects in two groups. One group of age 18 or younger and one group older than 18 years. In addition, a correlation between fatty acid composition of cerebral cortex and the fatty acid composition of red blood cells exist for subjects older than 18 years. A selection of fatty acids and their correlation with subjects age (age > 18 years) is shown in Table 4.III. The last column of this Table shows the results that were reported in a more recent study by McNamara *et al.* (2008). Apparent from the data by Carver *et al.* (2001) is that saturated fatty acids increase and polyunsaturated fatty acids decrease in cerebral cortex with age. Considering the significance levels there is reasonable agreement between the datasets by Carver *et al.* (2001) and McNamara *et al.* (2008).

Table 4.III. Linear correlations for a selection of fatty acids in frontal cerebral cortex and age for subjects older than 18 years as reported by Carver *et al.* (2001). Numbers in the regression equations refer to weight percentage of total fatty acid. Last column: change in fatty acid composition of the orbito frontal cortex of subjects of average age 34 and 70 year as reported by McNamara *et al.* (2008).

fatty acids	regression equation weight % of total fatty acid	signify-cance regression equation	calculated change from 20 to 100 year %	change for average age groups 34 to 70 year by McNamara *et al.* (2008) %
saturated				
myristic acid (MA) C14:0	0.398 + 0.002X	$p < 0.001$	37	21 (NS)
palmitic acid (PA) C16:0	21.533 + 0.022X	$p < 0.05$	8	-9 (p=0.042)
stearic acid (SA) C18:0	23.927 - 0.0001X	NS	0	-10 (p=0.001)
monounsaturated				
oleic acid (OA) 18:1n-9	18.314 + 0.007X	NS	3	28 (p=0.003)
vaccenic acid (VA) 18:1n-7	3.64* + ???X	NS	?	19 (p=0.003)
omega-6 polyunsaturated				
arachidonic acid (AA) 20:4n-6	10.140 - 0.019X	$p < 0.001$	-16	-15 (p=0.008)
docosatetraenoic acid (DTA) 22:4n-6	5.386 - 0.012X	$p < 0.01$	-19	1 (NS)
docosapentaenoic acid (DPA) 22:5n-6	1.893 – 0.002X	NS	-9	-37 (p=0.007)
omega-3 polyunsaturated				
docosahexaenoic acid (DHA) 22:6n-3	13.620 – 0.006X	NS	-4	-22 (p=0.024)

* Data (read from a figure) reported by McNamara *et al.* (2007) for normal control group (n=26) with an average age of 47 years. Correlation: r=+0.40, p = 0.04, slope not reported. NS: not significant.

Fatty acid compositions of the major phospholipids PC and PE in frontal gray matter, hippocampus, white matter and pons, for subjects in the age range of 33 to 92, have been measured by Soderberg *et al.* (1991). The experimental uncertainties do not permit to calculate age regressions, so their results are summarized here, in Figure 4.7, as averages for the whole age range covered. The PE fraction is the most rich in polyunsaturated fatty acids such as GA, AA, DTA and DHA. The PC fraction is the richest in saturated fatty acids such as MA, PA and SA. Pons and white matter show a large similarity in fatty acid composition and so do frontal gray matter and hippocampus. Oleic acid content shows little difference between PE and PC fractions for pons and white matter.

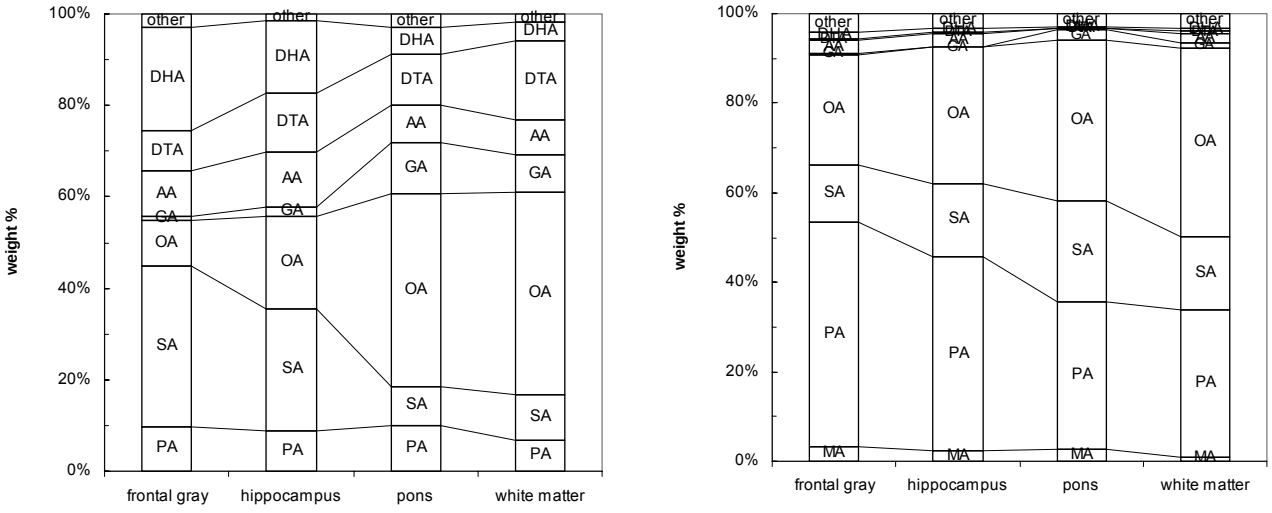

Fig. 4.7. Fatty acid composition of PE and PC fraction, calculated from Soderberg *et al.* (1991). MA: myristic acid; PA palmitic acid; SA stearic acid; OA oleic acid; GA gondoic acid; AA arachidonic acid; DTA docosatetraenoic acid; DHA docosahexaenoic acid.

Fatty acid composition of the frontal cerebral cortex in patients suffering from schizophrenia.

To date, four studies have been published that report on the fatty acid composition of post-mortem brain tissue of patients suffering from schizophrenia. The most comprehensive and recent study is by McNamara *et al.* (2007) and their results are such that the previous reports have to be seen in a different light. An overview of characteristics of patient and normal control groups of all four studies is shown in Table 4.IV.

The most important findings by McNamara *et al.* (2007) will be summarized below. The fatty acid composition of orbitofrontal cortex (OFC) changes with age in normal controls and schizophrenia patients on typical medication, in line with the findings on normal subjects by Carver *et al.* (2001), see above. The polyunsaturated acids DHA and AA decrease and the monounsaturated OA and VA increase with age. However, schizophrenic patients who were treated with *atypical* antipsychotics did *not* show these composition age relations.

Normal females showed a 10 % higher AA (p=0.04) and 4 % higher DTA (p=0.02) concentration. In a more recent and larger study on the OFC of normal subjects by McNamara *et al.* (2008) no differences in fatty acid composition between men and women were found. Carver *et al.* (2001) did not discriminate between male and female subjects, male and female group sizes were not reported, moreover only figures with experimental data were published, the data itself is not reported. Another study on *whole* brain fatty acid composition by Gershbein *et al.* (1985) showed some minor differences in fatty acid composition between men and women.

Unfortunately, experimental data by McNamara *et al.* (2007) are not reported on subject level but only on heterogeneous group level, making true quantitative analysis (and future meta-analysis) impossible. The different heterogeneous groups and their fatty acid characteristics are shown in table 4.V.

Male schizophrenic subjects showed marked (and significant) differences in OFC fatty acid composition as compared to normal male subjects, but OFC fatty acid composition in female schizophrenic subjects were not significantly different from normal control female subjects. Males (and patients on average) showed an increase in OFC monounsaturated fatty acids and a decrease in saturated and polyunsaturated fatty acids compared to normal controls.

Table IV. Postmortem studies on fatty acid composition in schizophrenic patients and normal control subjects.

characteristic	Horrobin et al. 1991	Yao et al. 2000	Landen et al. 2002	McNamara et al. 2007
patients	7	11	11	21
female	1	3	6	7
male	6	8	5	14
age	75 (8)	52(16)	80 (10)	44 (13)
on antipsychotics	>=6	11	9	18
typical	>=6	Not reported*	Not reported**	9
atypical	none	Not reported	Not reported	9
controls	7	14	13	26
female	2	3	5	8
male	5	11	8	18
age	74 (8)	60 (17)	75 (14)	47 (11)
tissue	frontal cortex, cerebellar cortex	caudate nucleus	anterior left gyrus cinguli of prefrontal cortex	orbito frontal cortex
Major findings	no significant changes in cerrebellar cortex. PE fractions of frontal cortex show significant decrease in C18:3n-6 & C20:3n-6***; cholesterolesters lower.	AA & DHA lower, only AA significant; Significantly lower PC and PE fractions; total fatty acids lower	no significant changes	AA, DHA, PA, SA significantly lower; OA & VA significantly higher

* Haloperidol (a typical antipsychotic) is mentioned a few times in the paper but not in direct relation to subjects in the present study.
** Drug usage is expressed in equivalents of chlorpromazine, but medication is not further specified, can be typical and atypical.
*** These are polyunsaturated fatty acids that represent less than 2% of total fatty acid composition.

Drug free schizophrenic subjects and those on typical antipsychotics showed significant differences in OFC fatty acid composition (*i.e.* an increase in monounsaturated and decrease in saturated and polyunsaturated fatty acids) as compared to normal controls, whereas OFC of patients treated with atypical antipsychotics showed no significant differences in fatty acid composition as compared to normal controls.

The numbers in Table 4.V are just to give an impression of the effects involved. Quantitatively they have no meaning whatsoever because of the heterogeneity of the patient groups. Data should be reported on six homogenous groups, *i.e.* a subdivision should be made on gender *and* medication (drug free, typical atypical) to be meaningful. For the study by McNamara *et al.* (2007) this would (probably) lead to a lack of statistical power, because of too small group sizes.

The only study that found no significant change in fatty acid composition was by Landen *et al.* (2002), with more than half of the patients female and possibly some subjects on atypical antipsychotics, which could be responsible for this outcome. The study by Horrobin (1991) showed only a significant decrease in gamma linoleic acid (C18:3n-6) (-83 %, $p<0.05$) and dihomogammalinoleic acid (C20:3n6) (-29 %, $p<0.05$) of the phospatidylethanolamine fraction of the frontal cortex, but these fatty acids do not contribute much to the overall fatty acid composition (less than 2%). Horrobin *et al.* (1991) did not find significant differences for AA or DHA, although AA tended to be lower. The

common factor with the study by Landen *et al.* (2002) is the high average age of the subjects (and the small subject groups), see Table 4.IV. This could lead to the hypothesis that the fatty acid composition of brain tissue of schizophrenic patients normalizes with age, although subject groups that were too small to find significant differences is another plausible explanation.

Table 4.V. Fatty acid composition and ratios in orbitofrontal cortex in heterogeneous patient groups extracted from the paper by McNamara *et al.* (2007). Grayed values are not significant (*i.e* p>0.05). Significance level between brackets. Values are percentage change as compared to the corresponding heterogeneous normal control groups.

		Gender		medication		
	all patients	males	females	drug free	typical	atypical
Σsaturated	-6 (0.01)					
PA	-9 (0.01)			-11 (0.003)	-17 (0.0003)	-1 (1.00)
SA	-4 (0.04)					
Σmonounsaturated	11 (0.02)					
OA	11 (0.03)	15 (0.01)	0.5 (0.95)	24 (0.0003)	13 (0.003)	3 (0.99)
VA	12.5 (0.003)			21 (0.049)	14 (0.003)	5 (0.7)
Σpolyunsaturated	-11 (0.01)					
AA	-10 (0.012)	-11 (0.03)	-7 (0.31)	-26 (0.006)	-12 (0.002)	-3 (0.92)
DHA	-20 (0.0051)	-27 (0.001)	-2 (0.91)	−33 (0.008)	-26 (0.003)	−8 (0.84)
DPA	~0	~0	~0	~0	~0	~0
AA:DHA	17 (0.01)	25(0.003)	-4 (0.57)	29 (0.01)	21(0.003)	7 (0.14)
OA:DHA	41 (0.01)	52 (0.01)	5 (0.83)			
DPA:DHA	28 (0.09)	39 (0.04)	16 (0.19)			
PA:VA	-19 (0.003)					
Σsaturate:Σmonounsat.	-15 (0.01)					
Σsaturate:Σpolyunsat.	7 (0.04)					
Σmonunsat.:Σpolyunsat.	24 (0.01)					

All patients (male and female, drug free, on typical and atypical antipsychotics); males (drug free, on typical and atypical antipsychotics); females (drug free, on typical and atypical antipsychotics); drug free (males and females); typical antipsychotics (male and female), atypical antipsychotics (male and female).

In conclusion: there is evidence from post-mortem studies that brain tissue of schizophrenic patients shows difference in fatty acid composition as compared to normal controls. However the evidence is thin, in the sense that it draws heavily on the study by McNamara *et al.* (2007), because the other studies were hampered by one or more of the following shortcomings: statistically underpowered, no distinction between patients on typical or atypical antipsychotics or drug free, no differentiation for gender, subject groups of very high average age. In addition to this, fatty acid composition in the *normal* aging human brain is dependent on brain tissue type, differences in fatty acid composition between schizophrenic patients and normal controls could also be dependent on brain tissue type.

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5. Excessive gray matter volume loss in schizophrenic patients observed with MRI and the membrane hypothesis: is there a link?

Introduction

From the erythrocyte and postmortem brain studies discussed in the previous chapters it seems plausible that there is a (missing) link between polyunsaturated fatty acid (PUFA) depleted membranes and progressive gray matter volume loss in schizophrenic patients as observed with MRI. It appears that this link between PUFA and gray matter volume is not restricted to schizophrenic patients but is also involved in gray matter increase and decrease in healthy subjects. A recent imaging study on healthy adult subjects, by Conklin *et al.* (2007), revealed a positive correlation between reported DHA and EPA intake and corticolimbic gray matter volume (anterior cingulate cortex and right amygdala). A PUFA study of the aging orbitofrontal cortex by McNamara *et al.* (2008) in which these authors found decreasing levels of PUFA as a function of age made them hypothesize that gray matter volume loss during aging and decreased PUFA status are somehow related to each other.

Evidence for a link between depleted PUFA status and gray matter volume loss in schizophrenic patients

Neuroleptic naïve schizophrenic patients and patients on typical antipsychotics show a significant reduction in polyunsaturated fatty acids of the lipid cell membranes (previous chapters) as compared to normal controls. This reduction in PUFA is probably caused by increased phospholipase A2 activity (Law *et al.*, 2006) and by enhanced lipid peroxidation (Arvandakshan *et al.,* 2003; Evans *et al.*, 2003; Ranjekar *et al.*, 2003). It appears that atypical antipsychotics almost completely restore arachidonic acid and docosapentaenoic acid concentrations in the cell membranes of erythrocytes to normal levels and slightly increases the docasahexaenoic acid concentration above levels of neuroleptic naïve patients (previous chapters). A similar effect has been found in the orbitofrontal cortex of schizophrenic patients by McNamara *et al.* (2007). Voxel based morphometry MRI studies (Lieberman *et al.,* 2005; Hulshoff Pol and Kahn, 2008; Van Haren *et al.*, 2008) indicate that atypical antipsychotics tend to reduce the enhanced brain volume loss rate that is characteristic for schizophrenic patients. The above implies that a link *does* exist.

Membrane fluidity, PUFA and temperature

It is a well known fact that polyunsaturated fatty acids increase cell membrane fluidity. Loss of PUFA leads to more rigidity of membranes and can alter the conformation and functioning of proteins, receptors and ion channels. This is the standard membrane hypothesis which has been elaborated on by Horrobin *et al.* (1994) and Horrobin (1998). Although intuitively evident, additional proof for the influence of PUFA on membrane fluidity comes from brain membranes of teleost species (fish). These show an inverse relationship between unsaturated fatty acid membrane content and the average temperature in which the animals reside (Logue *et al.*, 2000). Lower temperatures would lead to less membrane fluidity and the adaptation mechanism of these species is increasing PUFA concentration of the cell membranes.

It is evident that at a fixed fatty acid composition, the membrane fluidity will increase with temperature. The observation that a psychotic episode in a schizophrenic patient often subsides when, due to an infectious disease, the patient suffers from a fever may be related to this. Although, the temperature effect might be too small and Horrobin *et al.* (1994) suggest that this observation may be caused by a change in prostaglandin levels synthesized from AA.

Influence of fatty acids on cell volume regulation as the missing link.

Arachidonic acid is directly involved in cell volume regulation (Hamill *et al.,* 2001; Hoffmann *et al.*, 2009). Addition of free AA to cells that are swollen in a hypotonic solution inhibits regulatory volume decrease (Lambert, 1987; Sánchez-Olea *et al.*, 1995). It is suggested here that cells depleted in arachidonic acid may show an enhanced opposite effect.

Incubation of ventricular myocytes with palmitic acid, a saturated fatty acid, causes cell shrinkage and apoptosis (De Vries *et al.*, 1997). Incubation of the cells with the unsaturated oleic acid alone or in combination with saturated fatty acid leaves the cells intact. De Vries *et al.* (1997) observed that the free fatty acids exchange rapidly with the esterified fatty acids of the cell membranes, thus enhancing the concentration of the added species in the membrane. For β-pancreatic cells the same phenomenon has recently been observed by Morgan *et al.*, 2008. In the light of the above, PUFA depletion in schizophrenic patients causes a relative increase of saturated fatty acids in cell membranes of brain cells, this may cause cell shrinkage. Notably: there is some evidence that monounsaturated fatty acids show a tendency to increase in schizophrenic patients (McNamara *et al.,* 2007), which may be a compensatory effect to counteract the toxicity of the saturated fatty acids.

A postmortem morphometric study (Rajkowska *et al.*, 1998) on neuronal cells of schizophrenic patients showed reduced neuronal cell size. A recent review by Glantz *et al.* (2006) also points in the direction of altered neuronal apoptosis in schizophrenia. The authors suggest that sub-lethal apoptotic activity may lead to a limited form of apoptosis in for instance terminal neurites and individual synapses to cause synaptic elimination without cell death.

Thus it is hypothesized here that gray matter volume loss observed in schizophrenic patients is caused by the influence of the altered membrane fatty acid composition on cell volume regulation and this leads to sub-lethal apoptotic activity.

Possible future directions in research to test the link between PUFA and brain volume loss and the membrane hypothesis of schizophrenia in general

With high-field MRI microscopy specific brain regions, which are notorious for gray matter volume loss in schizophrenic patients, can be studied. This may lead to identifying in vivo neuronal cell shrinkage in SZ patients as compared to normal controls. Additionally the influence of atypical and typical antipsychotic medication on neuronal cell size can be investigated by means of MRI microscopy.

In vivo measurement of fatty acids in biological membranes with MRS is hampered by the relative immobility of the phospholipids in the bi-layer, however ^{13}C studies in which patients and normal controls are receiving ^{13}C fatty acid tracers might reveal some broad peaks of the spiked fatty acids. When spiked, it is to be expected that gray matter of normal controls show larger ^{13}C peaks in MRS than schizophrenic patients. Moreover one might be able to identify whether polyunsaturated fatty acid depletion is a focal phenomenon, possibly at the same focal points identified by MRI studies on gray matter volume loss, or that it is non-localised. The influence of atypical and typical antipsychotics on fatty acid distribution can also be investigated by means of this technique. Carbon-13 is a stable (non-radioactive) isotope without detrimental health effects so a trial with patients and normal controls is quite feasible. In addition the possible influence of vitamin D3 status on phospholipid synthesis and the incorporation of (^{13}C labeled) PUFA, as discussed in chapter 2, can be investigated.

Brain tissue temperature can be measured and also heated with MRI, so principally a direct influence of tissue temperature and psychotic status of the patient can be revealed. However, for the obvious reasons, such an experiment is not feasible.

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