THE ROLE OF MEMBRANE ESSENTIAL POLYUNSATURATED FATTY ACIDS IN SCHIZOPHRENIA OUTCOME

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IN

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BY

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CERTIFICATE

Certified that the work in this Ph. D. Thesis entitled **"THE ROLE OF MEMBRANE ESSENTIAL POLYUNSATURATED FATTY ACIDS IN SCHIZOPHRENIA OUTCOME"** submitted by **Ms. MEENA ARJUN MULCHANDANI** was carried out by the candidate under my supervision. The material obtained from other sources has been duly acknowledged in the thesis.

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Research Guide

DECLARATION

I hereby declare that the thesis entitled **"THE ROLE OF MEMBRANE ESSENTIAL POLYUNSATURATED FATTY ACIDS IN SCHIZOPHRENIA OUTCOME"** submitted for Ph. D degree at the University of Pune has not been submitted by me for a degree at any other University.

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dedicated to my beloved parents

INTER-INSTITUTIONAL COLLABORATIVE RESEARCH EFFORT

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(MEENA MULCHANDANI)

LIST OF ABBREVIATIONS

AA	Arachidonic acid
ALA	Alpha linolenic Acid
ANOVA	Analysis of variance
BPRS	Brief Psychiatric Rating Scale
BS-SZ	FES studied at baseline, at the start of the four-year follow-up study
CAT	Catalase
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
CT	Computerized Tomography
DA	Dopamine
DHA	Docosahexaenoic Acid
DNA	Deoxyribonucleic Acid
DPA	Docosapentaenoic Acid
DSM	Diagnostics and Statistical Manual
EDTA	Ethylene diamine tetra acetic acid
EFA	Essential fatty acid
EPA	Eicosapentaenoic acid
EPUFA	Essential polyunsaturated fatty acid
FEP	First episode psychotics
FES	First episode schizophrenics
fMRI	functional Magnetic Resonance Imaging
FU-SZ	FES studied after four years of Follow-Up
G TOT	PANSS General psychopathology cluster score
GABA	Gamma amino butyric acid
GIT	Gastrointestinal tract
GPx	Glutathione peroxidase
HLA	Human leukocyte antigen
HPTLC	High performance thin layer chromatography
IL	Interleukin
LA	Linoleic acid

MRI	Magnetic Resonance Imaging
M-SZ	Medicated schizophrenics
N TOT	PANSS Negative symptom factor score
NC	Normal Controls
NE	Norepinephrine
NMDA	N-methyl D-aspartate
NM-SZ	Never-medicated schizophrenics
O TOT	Outcome scale - tolal score
P TOT	PANSS Positive symptom factor score
PANSS	Positive and Negative Symptom Scale
PC	Phosphatidyl choline
PDE	Phosphodiesters
PE	Phosphatidyl ethanolamine
PI	Phosphatidyl Inositol
PL	Phospholipid
PME	Phosphomonoesters
PS	Phosphatidyl serine
PUFA	Polyunsaturated fatty acids
QOL	Quality of Life scale
RBC	Red blood cell
ROS	Reactive oxygen species
SFA	Saturated fatty acids
SM	Sphingomyelin
SOD	Superoxide dismutase
SPECT	Single Photon Emission Computerized Tomography
SZ	Schizophrenics
SZ-Post	Schizophrenics after supplementation
SZ-Pre	Schizophrenics before supplementation
SZ-WO	Schizophrenics after supplementation wash-out
TBARS	Thiobarbituric acid reactive substances
TLC	Thin layer chromatography
WHO	World Health Organization

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PREFACE

Schizophrenia is a devastating neuropsychiatric disorder. Prevalence of the disorder in the general population is 1%. Its onset is generally early in life; the illness has developmental origins. It generally has a chronic course. Costs involved in its management are enormous and human suffering involved is fathomless.

The etiology of the disorder remains highly speculative. Its neuropathophysiology is only recently beginning to be understood; is heterogeneous, as is its phenomenology. This has been unfavorable to drug development for treatment. The available drugs (antipsychotics) are targeted to antagonize possibly altered receptor functions of several neurotransmitters, which may be secondary to the structural neuropathophysiology. The drugs do not effectively treat the psychopathology but treat the different symptoms in varying degrees. Some of the early antipsychotics (typical or conventional antipsychotics - typicals) effectively treat psychosis but not the recently considered core psychopathology, negative symptoms and cognitive impairment that are critical in illness outcome improvement. Also, these drugs often cause serious side effects. The recently introduced atypical antipsychotics (atypicals) seem to be effective in managing the negative symptoms, improving cognitive performance and causing only mild side effects. The long-term use of typicals, but not the atypicals, is found to impair cognitive performance and CNS cholinergic function in animals (Mahadik et al, 1998; 2001). However, stands to be proved whether the atypicals are effective against the core negative symptoms. Also, as with the typicals, the atypicals also function to alter neurotransmitter receptor functions and not the core neurostructural pathophysiology of schizophrenia. Thus, with a view to achieve development of effective as well as safe drugs for treatment of schizophrenia, there is a pressing need to understand the etiopathophysiology of the disorder.

Various pathophysiological hypotheses have been put forward to explain schizophrenia. The membrane hypothesis may be able to explain the observed neuropathophysiology and also provide a basis for evolving effective and safe treatment for schizophrenia. The "membrane hypothesis" suggests that abnormal membrane structure, that is, altered quantity and quality of various phospholipids [PLs], is prevalent in schizophrenia. Recently these changes have been considered

to be a result of altered metabolism (reduced synthesis fom essential fatty acids [EFAs], and/or increased breakdown) of essential polyunsaturated fatty acids [EPUFAs]. EPUFAs are the ω-3 and ω-6 series of fatty acids that cannot be synthesized by the body and hence are essential in the diet. EFA / EPUFA intake and metabolism govern the availability of EPUFAs for PL synthesis and thus the quantity and quality of membrane PLs in both periphery and the brain (Thompson, 1992). Also, it is the EPUFA component of the PLs that determines fluidity for optimal functioning of membrane proteins and EPUFAs are second messengers for a number of neurotransmitter mediated signal transduction processes, which may account for the multi-transmitter dysfunction observed in schizophrenia. Membrane PL profile in schizophrenia is suggestive of reduced levels of EPUFAs. Accordingly, reduced membrane levels of EPUFAs are consistently reported in red blood cells and brain of chronic medicated schizophrenic patients (Vaddadi et al, 1990; Horrobin et al, 1991; Yao et al, 1994; Glen et al, 1994; Peet et al, 1995; 1996; Assies et al, 2001) and cultured skin fibroblasts from never-medicated patients at the onset of psychosis (Mahadik et al, 1994). This is known to be associated with psychopathology (Glen et al, 1994; Peet et al, 1995).

Other observations with EPUFAs and schizophrenia are also noteworthy. Better illness outcome observed in the developing countries as compared to the developed countries by the WHO (1979) during its multi-national, multi-center, epidemiological and clinical study was reported to correlate with relatively high EPUFA intake in diet (Christensen and Christensen, 1988). Peet et al (1996) have also reported a correlation (negative) between EPUFA intake and the severity of predominant symptoms. These observations strongly implicate a role for membrane EPUFAs in outcome and if proved, can have applications in management of the disorder.

It is generally accepted that schizophrenia is a neurodevelopmental disorder that may lead to dysfunction of several neurotransmitters. There is substantial evidence for the role of EPUFAs (particularly docosahexaenoic acid [DHA] and arachidonic acid [AA]) in brain development, and function as second messengers in

neurotransmitter mediated signal transduction processes. Thus the role of reduced membrane EPUFAs in etiology of schizophrenia may also be considered.

This thesis is an account of the study of the association of membrane EPUFAs with psychopathology, and thereby the clinical outcome of schizophrenia, the mechanisms responsible for the reduced membrane EPUFAs and the application of this finding. Reduced membrane EPUFAs observed in never-medicated patients in early illness, as well as the medicated schizophrenics and its association with psychopathology indicate that it may constitute or contribute to pathophysiology of the illness. Improvement in membrane EPUFA levels with concomitant improvement in psychopathology, observed with a follow-up of first-episode schizophrenic patients, add support to its role in pathophysiology and outcome of schizophrenia. When the mechanism of reduction of membrane EPUFAs, namely intake of EFAs / EPUFAs and antioxidants, and oxidative stress mediated lipid peroxidation was studied, both these factors were found to affect membrane EPUFA levels. Accordingly, supplementation with ω–3 EPUFAs and antioxidants have been considered beneficial to alleviate the psychopathology. ω–3 EPUFA supplementation regimen using low dose of the EPUFAs along with antioxidants was found to be effective in alleviating psychopathology in young patients.

CHAPTER I

INTRODUCTION

ESSENTIAL POLYUNSATURATED FATTY ACIDS AND SCHIZOPHRENIA

I) SCHIZOPHRENIA

Schizophrenia is a devastating neuropsychiatric disorder. It has been labeled the worst disorder that affects mankind. The following describes a schizophrenic; “People with the disorder lose touch with the real world. They hear voices that are not there, speak a language that does not exist, laugh for no reason, or sit motionless for hours on end. The entire human personality is laid waste, and the psychological and social building blocks of everyday life are crushed beyond recognition” (US Department of Health and Human Services: A National Plan for Schizophrenia Research, 1988).

Early onset, high levels of morbidity, low levels of mortality, together contribute to the tragic nature of the disorder. Following is a detailed description of the disorder.

A) PSYCHOPATHOLOGY

Psychopathology is the study of disturbances in various psychological domains caused by the disorder. Schizophrenia involves abnormalities of perception, inferential thinking, speech and language, fluency of thought and speech, social interactions and behavior, attention, volition, hedonic capacity, emotional expression and responsiveness, intellectual creativity, and the ability to abstract.

CLINICAL PHENOMENA

The clinical phenomena associated with schizophrenia can be grouped into positive and negative symptom clusters. Studies of correlation between the various symptoms suggest that they fall into several dimensions. The most popular model involves three dimensions (Fig. 1).

POSITIVE SYMPTOMS

Positive symptoms are defined as behavior or functions not present in a normal individual.

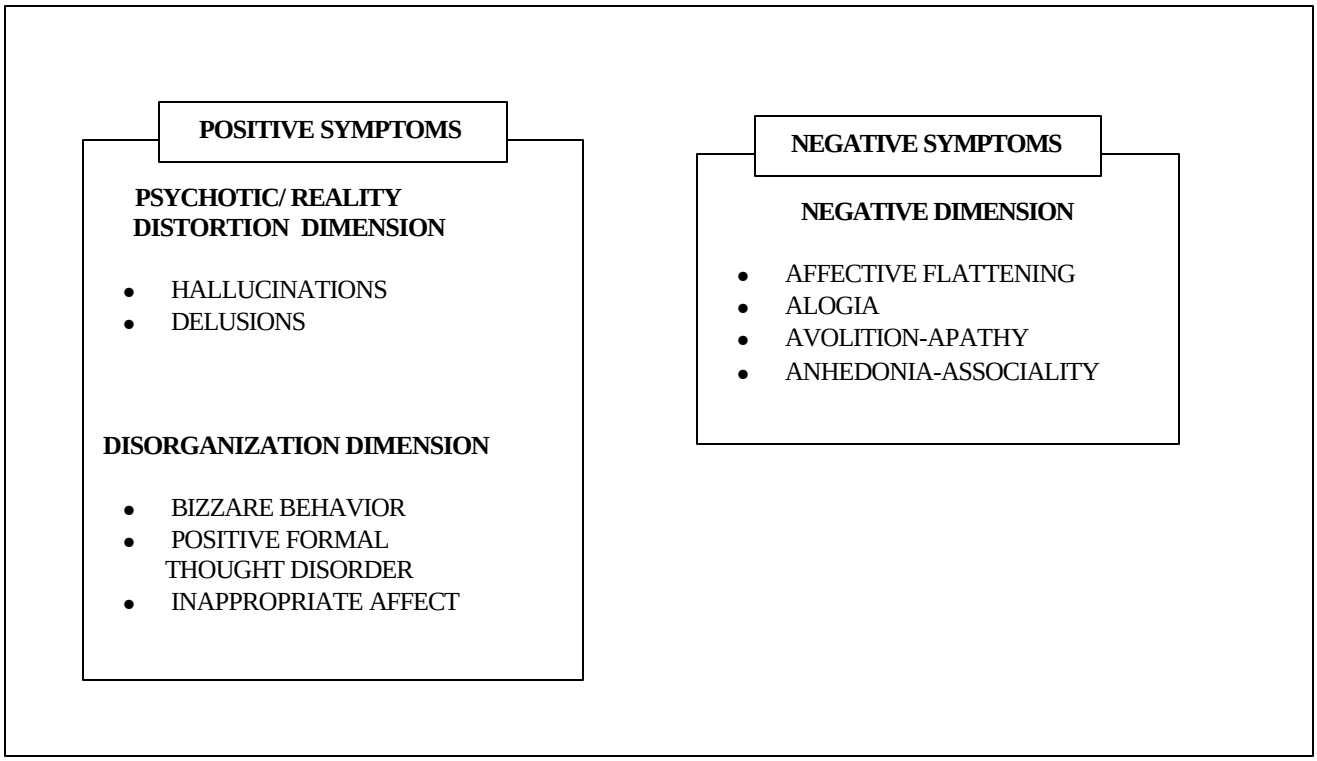


Fig. 1 Symptoms of schizophrenia

PSYCHOTIC / REALITY DISTORTION DIMENSION

HALLUCINATIONS

False perceptions (perceptions in the absence of some identifiable external stimulus); may be experienced in any of the sensory modalities, including hearing, touch, taste, smell and vision

DELUSIONS

False beliefs (beliefs that cannot be expressed on the basis of patient's cultural background)

DISORGANIZATION DIMENSION

BIZZARE BEHAVIOR

Unusual, bizarre or fantastic behavior e.g. patient may paint parts of his body different colors, may dress in a fantastic costume

POSITIVE FORMAL THOUGHT DISORDER

Fluent speech that tends to communicate poorly

INAPPROPRIATE AFFECT

Incongruous affect e.g. patient may laugh or assume a silly facial expression while talking about a sad subject

NEGATIVE SYMPTOMS

Negative symptoms are defined as absence of behavior or functions that are present in a normal individual.

NEGATIVE DIMENSION

AFFECTIVE FLATTENING OR BLUNTING

Impoverished emotional expression, reactivity and feeling

ALOGIA

Thinking processes that seem empty, turgid or slow

AVOLITION-APATHY

Lack of energy, drive and interest

ANHEDONIA-ASSOCIALITY

Difficulty in experiencing interest or pleasure

POOR ATTENTION

No symptom is pathognomonic – each is present in some patients, but none is present in all. Schizophrenia demonstrates a heterogeneous clinical presentation. Patients may range from having fiercely angry to extremely blunt affect. Due to the complex nature of the disorder, attempts are made to subdivide it into various categories.

SHOULD SCHIZOPHRENIA BE SUBTYPED OR IS IT A PSYCHOPATHOLOGICAL CONTINUUM?

Schizophrenia subtyping has a long history. Earlier, distinctions were made on the basis of either phenomenology, etiology or prognosis, namely,

Phenomenology - hallucinatory, hebephrenic, catatonic and simplex forms

Etiology - reactive (psychogenic) / process, organic/endogenous forms

Prognosis - benign/non-benign, process/non-process, deficit/non-deficit, good prognosis/ poor prognosis.

Later, distinctions involved a combination of several disease characteristics - onset, symptoms, course, response to treatment and biological parameters (ventricular enlargement). These were-

TYPE I AND TYPE II SCHIZOPHRENIA (Crow, 1980)

Type I was characterized by acute onset, normal brain structure, normal intellectual function, good response to antipsychotic drugs, possibly increased dopamine D_2 receptors, and absence of negative symptoms.

Type II was characterized by insidious onset, enlarged cerebral ventricles, intellectual deterioration, poor response to antipsychotic drugs and prominent negative symptoms.

POSITIVE AND NEGATIVE SCHIZOPHRENIA (Andreasen, 1982)

Positive schizophrenia was characterized by the prominence of positive symptoms with minimum negative symptoms or none at all.

Negative schizophrenia was defined by the prominence of negative symptoms with minimum positive symptoms or none at all.

POSITIVE, NEGATIVE AND MIXED TYPES (Andreasen, 1982)

Patients who failed to meet the criteria for both positive and negative schizophrenia or had a substantial number of both sets of features were classified as mixed.

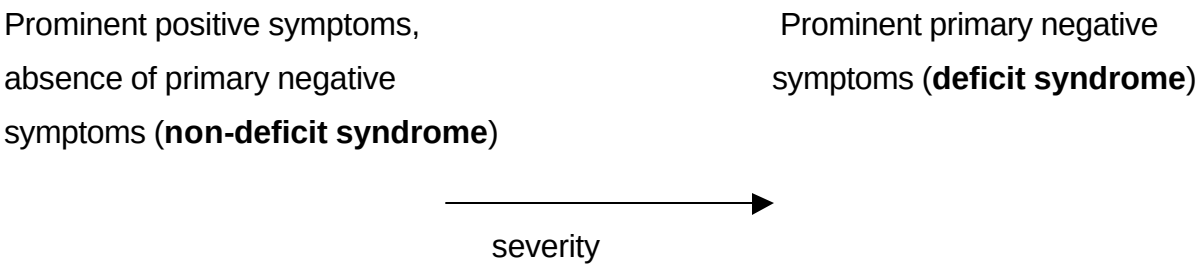
DEFICIT AND NON-DEFICIT SCHIZOPHRENIA (Carpenter, 1988)

Patients with primary, enduring negative (deficit) symptoms, carefully distinguished from transient negative symptoms secondary to other factors, were labeled as the deficit group, and those without the same were the non-deficit group.

There have been problems with subtyping. Pure positive or negative phenomena, on which most of the distinctions were made, are rarely observed. These are not longitudinally independent phenomena, but are seen interchangeably

during the course of schizophrenia. It is thought that deficit symptoms exist only in a subgroup of schizophrenic patients and thus may provide the basis for categorization (Kirkpatrick, 2001). However, this needs to be proved.

Schizophrenic patients with deficit symptoms exhibit poorer social and occupational functioning as compared to patients without these, and thus may indicate more severe forms of the disease. Hence, it seems appropriate to conceptualize schizophrenia as characterized by a psychopathological continuum, with patients with predominantly positive symptoms and the absence of primary negative symptoms (non-deficit syndrome) at one end of the continuum representing a milder form of the disease, and those with predominantly primary negative symptoms (deficit syndrome) at the other end representing the severe form.



The concept of a psychopathological continuum suggests the existence of a core pathology. This may be present in the various brain regions to varying extents and thus account for the heterogeneity of clinical presentation due to diversity of symptoms. It is being increasingly recognized that the phenotype of schizophrenia involves abnormalities in fundamental cognitive processes, which manifest as the positive and negative symptoms.

CORE PSYCHOPATHOLOGY: COGNITIVE IMPAIRMENT

Emil Kraepelin, a pioneer in the study of psychopathology of schizophrenia, had indicated that the core feature of schizophrenia is impaired cognitive performance. It is now being recognized that cognitive defects are intrinsic to schizophrenia, are not due to preoccupation, but may be further exacerbated with hallucinations and delusions or lack of attention, and are not the result of institutionalization or

medication. Evidence for abnormalities in a variety of cognitive domains (viz. executive function, attention and memory) is now amounting.

The 'seemingly' diverse symptoms of schizophrenia may be explained as abnormality in cognition. Andreasen et al (1998) have suggested the use of the term cognition in a broad sense and simply explained the entire array of symptoms as "…abnormality in receiving and processing information from the external world, relating it to information that has already been processed and acting on that information to produce reaction or response."

Cognitive deficits characterize the schizophrenic population as a whole (are not restricted to a class of schizophrenic patients), are persistent, and can theoretically form the basis of the positive, negative and disorganized symptoms, and thus classify as core psychopathological substrates.

The presence of cognitive defects in early childhood, much before the onset of the psychotic symptoms which bring the disease to light, is in keeping with the generally accepted view that schizophrenia is a neurodevelopmental disorder.

ONSET

The psychotic symptoms (hallucinations and delusions) and related aggressive and violent behavior bring the disease to light. Accordingly, the average age of onset is 15-20 years for males and 20-25 years for females. However, neurobiological studies (indicative of neurodevelopmental brain abnormalities - discussed later) and evidence for behavioral abnormalities in early childhood point to neurodevelopmental origins of schizophrenia. Mild disturbance of perception (e.g. illusions), beliefs (e.g. ideas of reference, over-valued ideas), cognition, attention, affect (e.g. depression, hostility, affective blunting) and behavior (e.g. decline in function, social withdrawal) are seen before the onset of frank psychotic symptoms. These are called prodromal symptoms. The average time from onset of prodromal symptoms to the onset of psychotic symptoms is 1-5 years.

Decline in school and social performance (indicative of abnormalities in attention and cognition) may predate the onset of other prodromal symptoms. These

may be of higher predictive value if validity and reliability are established. These can thus be used for early treatment intervention proved to be associated with better outcome.

Schizophrenics occupy 50 % of the beds in psychiatric institutions. The incidence and prevalence rates, course and outcome of schizophrenia are as follows.

B) EPIDEMIOLOGY

Epidemiology is the study of distribution (incidence and prevalence), course and outcome of a disorder/ disease in a population. Epidemiological differences between geographical areas may provide a clue to the etiology or the factor/s affecting course and outcome.

INCIDENCE AND PREVALENCE

Incidence refers to the number of new cases that appear in a population during a specified period. According to the WHO sponsored - Determinants of Outcome of Severe Mental Disorders study (1978-84), the most comprehensive international, multi-site effort to date, the annual incidence rates for 'nuclear schizophrenic symptom profile cases' are similar across the various geographical areas studied, that is 0.7-1.4 cases per 10,000 population.

Prevalence refers to the number of cases present at a particular point in time, or a period of time – the week, month, or year prior to evaluation. According to the National Institute of Mental Health sponsored Epidemiologic Catchment Area study; the annual and lifetime prevalence rates are 1.0% and 1.4% respectively.

Although the incidence and prevalence rates of schizophrenia are similar across countries, the course and outcome are known to be variable.

COURSE AND OUTCOME

Course is prognosis of the disorder, usually studied at various time intervals while outcome is the result of the course studied at a particular time point.

Kraepelin (1896) conceived 'dementia praecox' (later named 'schizophrenia' by Eugene Bleuler) as an illness always leading to deterioration in psychological and behavioral functions over time. Hence the concept of a homogenous long-term course had prevailed then. Bleuler (1911), however, observed that the course of schizophrenia is variable and chronicity, characterized by an unfavorable course and bad outcome, is not a rule.

Heterogeneity in course and outcome of schizophrenia can be said to be prevalent at two levels -

HETEROGENEITY ACROSS INDIVIDUALS

Patients may -

1) remit completely after a single psychotic episode
2) exhibit florid pathology (usually in the beginning) with stable residual psychopathology later
3) exhibit florid pathology and suffer continuously deteriorating psychopathology later

HETEROGENEITY ACROSS POPULATIONS

A marked difference in course and outcome is observed between populations of the developing and developed countries. This was indicated by the WHO ten-country, multi-center, clinical and epidemiological study. Interestingly, the developing countries were found to have a milder course and better outcome as compared to the developed countries.

The conclusions drawn from the WHO studies have been questioned. Inclusion of cases of acute reactive, brief or schizophreniform psychosis, which are high in developing countries and have a favorable course and better outcome as compared to schizophrenia, could have accounted for similar incidence across nations and better outcome in the developing countries (Stevens, 1987; Torrey, 1987). *This then leads to the conclusion that the incidence of schizophrenia is low in the developing countries.*

The study of possible "protective elements" in the developing countries that account for good outcome and / or low incidence would prove useful in the prevention and management of the disorder.

The course and outcome of schizophrenia have been studied with the aim of improvement of the same. These studies have yielded a number of factors that may affect the course and hence decide the outcome-

Obstetric complications: The presence of obstetric complications at birth of schizophrenics is associated with bad outcome

Onset: An insidious onset of the illness, as opposed to an acute onset, is associated with a bad outcome of illness

Premorbid functioning: Poor premorbid functioning is associated with bad outcome

Duration of untreated psychosis: A longer duration of untreated illness is known to be associated with bad outcome

Ventricular enlargement: Schizophrenics with ventricular enlargement are known to have a bad outcome

Gender: Females, as opposed to males, have a benign course and good outcome

Family history: A family history of the illness is associated with bad outcome

Seasonality of birth: Schizophrenics with winter births are known to have a bad outcome

Diet: A high calorie, high fat diet which is poor in essential fatty acids and rich in saturated fatty acids, is associated with bad outcome of schizophrenia

An understanding of the neuropathophysiology of schizophrenia should aid in delineating possible candidate "protective elements". An account of the same follows.

C) NEUROPATHOPHYSIOLOGY

NEUROANATOMICAL ABNORMALITIES

MORPHOMETRIC NEUROIMAGING STUDIES

Schizophrenia, for a long time, was thought to be a functional psychosis despite Kraepelin's belief in the organic basis of the disorder. Computerized tomography (CT)

scans of brains of schizophrenic patients provided the first definite proof of brain pathology (Johnstone et al, 1976). Dilated lateral ventricles were reported in these patients. Later studies using CT and magnetic resonance imaging (MRI) confirmed these findings.

FINDINGS OF NEUROIMAGING STUDIES

Enlarged ventricles

Enlarged ventricles, particularly the lateral and third, is the most robust observation of neuroimaging studies. The abnormality is characteristic of the entire schizophrenic population, rather than a subgroup (Daniel et al, 1991). It is observed in never-medicated patients, at the onset of psychosis (Degreef et al, 1992; Lim et al, 1996; Gur et al, 1998; Whitworth et al, 1998; Zipursky et al, 1998) and thus is not a result of medication or illness chronicity. In fact, the pathology predates the onset of psychosis since it is observed in adolescents and young adults at risk of developing schizophrenia (Cannon et al, 1993).

Reduced cortical volume

Reduced cortical volume, wherein gray matter reduction is more than that observed with white matter (Laurie and Abukmeil, 1998; Zipursky, 1998) is reported. Evidence indicates that the pathology predates the onset of psychosis (Cannon et al, 1993).

Reduction in volume of a number of brain regions is observed. Reduction in temporal lobe volume (Barta et al, 1990; Schlaepfer et al, 1994; Zipursky et al, 1994; Flaum et al, 1995; Menon et al, 1995; Tune et al, 1996; Hajek et al, 1997; Marsh et al, 1997; Reite et al, 1997; Hirayasu et al, 1998; Sullivan et al, 1998) is most prominent. Frontal, parietal and occipital lobes are also reported to be affected (Andreasen et al, 1994; Bilder et al, 1994; Schlaepfer et al, 1994; Zipursky et al, 1994). In addition to cortical regions, a number of subcortical structures, namely, thalamus, corpus callosum, basal ganglia, cerebellum, cavum septum pellucidi are found to be affected.

POST-MORTEM STUDIES

In keeping with the above studies, post-mortem studies of brains of schizophrenic patients have reported a decrease in brain weight (Brown et al, 1986; Pakkenberg et al, 1987; Bruton et al, 1990), brain length (Bruton et al, 1990) and volume of cerebral hemispheres (Pakkenberg et al, 1987).

The findings indicate that neuropathology of schizophrenia is not localized but is widespread.

NEUROCYTOARCHITECTURAL ABNORMALITIES

POST-MORTEM STUDIES

Neurohistological abnormalities such as neuronal atrophy, focal demyelination and metachromatic bodies were reported in schizophrenic patients as early as late nineteenth - early twentieth century (Alzheimer, 1897; Wernicke, 1900; Alzheimer, 1913). However, negative results reported by some studies (Lewis, 1923; Dunlap, 1924; Peters, 1937) and lack of confidence with identification of artifacts with the newly developed histopathological techniques discouraged search along these lines for a considerable period of time. Extensive data have now accumulated that indicate cytoarchitectural abnormalities in schizophrenia.

FINDINGS OF NEUROHISTOPATHOLOGICAL STUDIES

Absence of gliosis

Absence of gliosis was reported in the histopathological studies. This rules out the possibility of neurodegeneration, and supports, if not confirm, abnormal neurodevelopment, as being responsible for the observed neuropathophysiology.

Alteration in cortical cytoarchitecture

Neurons are observed to be mis-placed, mis-sized and disorganized (Kovelman and Scheibel, 1984; Jakob and Beckmann, 1986; Benes et al, 1986; Benes and Bird, 1987; Arnold et al, 1991; Benes et al, 1991; Conrad et al, 1991; Arnold et al, 1995; Zaidel et al, 1997; Rajowska et al, 1998). Reduced cell numbers and density (Bogerts

et al, 1983; Falkai and Bogerts, 1986; Falkai et al, 1988b; Jeste and Lohr, 1989; Pakkenberg, 1990; Akbarian et al, 1993; Jonsson et al, 1997; Benes et al 1998; Beasley and Reynolds, 1997; Danos et al, 1998; Bernstein et al, 1998; Tran, 1998) reported in certain brain regions were thought to account for the reduced volumes of the brain regions seen with the imaging studies. Reduction in neuropils (Arnold et al, 1991b; Browning et al, 1993; Eastwood and Harrison, 1995a; Eastwood et al, 1995b; Goldsmith and Joyce, 1995; Blennow et al, 1996; Perrone-Bizzozero, 1996; Glantz and Lewis, 1997; Garey et al, 1998; Thompson et al, 1998; Woo et al, 1998; Harrison and Eastwood, 1998; Young et al, 1998; Higley et al, 1999; Selemon and Goldman-Rackic, 1999) and cell size and associated increased cell density (Daviss and Lewis, 1995; Selemon et al, 1995; Anderson et al, 1996) were also reported. Reduced cell size and neuropils may cause the cells to pack together closely (Harrison, 1997) and these, rather than cell number, are now thought to be responsible for the reduced volumes of brain regions.

WHAT ARE THE BRAIN ABNORMALITIES IN SCHIZOPHRENIA ATTRIBUTED TO, GENES OR ENVIRONMENT?

Structural imaging studies of monozygotic twins discordant for schizophrenia have shown that the affected twins have larger ventricles (Reveley et al, 1982; Suddath et al, 1990), and more clearly, smaller temporal lobes and hippocampus (Noga et al, 1996), as compared to the unaffected twins. Monozygotic twins have almost identical genetic material and it seems most probable that environmental conditions, such as unequal availability of building material or factors necessary for neurodevelopment could have been responsible for the pathology. Family studies have shown that schizophrenics have larger ventricles and smaller brains as compared to the unaffected relatives (Honer et al, 1994; Sharma et al, 1998; Silverman et al, 1998). It has also been shown that relatives of schizophrenics have larger ventricles as compared to relatives of normal controls (Sharma et al, 1998; Lawrie et al, 1999). Certain habitual practices run in families and may account for the above observation. Thus, although genes can be implicated in the etiopathology, the effect of environment, which has not received the deserved attention, cannot be ruled out.

IS THE NEUROPATHOLOGY STATIC OR PROGRESSIVE?

Studies on progression of the neuroanatomical pathology in schizophrenia by measuring the increase in ventricular size with the duration of illness have yielded contradictory findings indicating that the issue of a static or progressive nature of this pathology is controversial. Differences with respect to cohort composition, stage of the illness studied, duration of follow-up interval, and brain regions may be responsible for the variations between studies. It is possible that changes are progressive during early stages of illness and may plateau in later stages. Thus absence of change in ventricular size may be due to the fact that these studies involve chronic patients (DeLisi, 1999). Also, it is possible that the disease may be progressive in a subpopulation of schizophrenics with poor prognosis (Garver et al, 1997).

NEUROCHEMICAL ABNORMALITIES

ABNORMAL NEURAL/ NEUROTRANSMITTER CIRCUITRY

FUNCTIONAL NEUROIMAGING STUDIES

Regional abnormalities in brain activity have been observed, at rest and with cognitive activation techniques, in schizophrenics as compared to controls, with positron emission tomography (PET) (Ingvar and Franzen, 1974a; Ingvar and Franzen, 1974b; Buchsbaum, 1982; Little, 1987; 1991; Frith, 1991a; Frith, 1991b; Allen, 1993; Frith, 1995; Dolan, 1995), single photon emission computerized tomography (SPECT) (Mathew et al, 1982; Gur et al, 1983; Devous et al, 1985; Weinberger et al, 1986; Berman et al, 1987; Weinberger et al, 1988; Woods and Flowers, 1990; Andreasen et al; 1992) and functional MRI (fMRI). In addition to abnormalities in regional connectivities, disconnectivities between different brain regions, that is, abnormal long-range connectivity is also implicated in schizophrenia. Fronto-temporal connectivities (Friston et al, 1992) and cortico-cerebellar thalamic cortical circuitry (Andreasen, 1999) are known to be aberrant.

POST-MORTEM STUDIES

The abnormalities in anatomical circuitry can cause disruptions of the chemical anatomy of the brain. Loss of non-pyramidal GABAergic neurons in the hippocampal circuitry (Benes et al, 1999) has been observed. Similar changes may underlie the observations of hyper- and hypo- dopaminergic activities (O'Donnell and Grace, 1998), increased cholinergic tone (Tandon, 1999), decreased glutamatergic tone (Tamminga, 1999) and resultant monoaminergic tone (Carlson, 1999).

ABNORMAL NEUROTRANSMISSION

Neurotransmitter synthesis and degradative enzymes, release and reuptake, and receptors are implicated to be abnormal in schizophrenia. Extensive studies have been carried out with the dopaminergic system due to the prevalence of dopaminergic hypothesis of schizophrenia. The finding of increased dopamine (D_2) receptors in the striatum is controversial, since both positive and negative results have been observed. A small number of studies indicate excessive synthesis and release of dopamine in schizophrenia. Additionally, abnormalities of other neurotransmitter systems that is, decreased 5-HT_{2A} and increased 5-HT_{1A} receptors in cortical regions, and increased $GABA_A$ receptor density in the cingulate cortex, and finally decreased glutamatergic activity have been reported in schizophrenic patients.

The neurochemical studies indicate abnormalities of several neurotransmitter systems implicating multitransmitter dysfunction in schizophrenia.

NEURODEVELOPMENTAL PATHOLOGY

Absence of gliosis indicates, though does not prove, that the neuropathology of schizophrenia has developmental origins. Also observed are pathological and neuroimaging evidences consistent with early developmental brain (disturbed brain asymmetries and abnormalities of sulcogyral patterns) and developmental defects in associated structures (e.g., craniofacial) of ectodermal origin.

Additional proof to neurodevelopmental pathology of schizophrenia was provided by the fact that certain observations in schizophrenic patients are common-

1) Behavioral disturbances, namely, delay in attainment of early milestones, excess neuromotor integrative disturbance in early childhood (Walker and Lewine, 1990; Jones et al, 1994; Waddington et al, 1998)
2) Altered neuronal migration and misalignment (Jacob and Beckmann, 1986; Falkai et al, 1988a; Arnold et al, 1991a; Akbarian et al, 1993).
3) Increased frequency of perinatal / obstetric complications
4) Nutritional deficiencies at the time of prenatal neurodevelopment

It is now generally accepted that schizophrenia has neurodevelopmental origins and is probably characterized by progressive neuropathology, atleast in a subgroup of patients. A number of hypotheses have been proposed to explain the neuropathology of schizophrenia -

D) ETIOPATHOPHYSIOLOGICAL HYPOTHESES

GENETIC HYPOTHESIS

Schizophrenia is known to run in families giving rise to the hypothesis regarding the genetic basis of schizophrenia. Higher rates of schizophrenia are seen in offsprings (9%) with affected parents, second (6%) and third (2%) degree relatives as compared to the general population (1%). Thus, increase in the risk of schizophrenia with the degree of biological relatedness to the patient is observed. Twin studies indicate higher monozygotic (48%) than dizygotic (16%) concordance. Monozygotic twins raised apart seemed to have only slightly higher concordance rates than those raised together. Also, higher rate of schizophrenia was observed in biological and not adoptive relatives of patients.

"Familiality" does not necessarily indicate the effect of genes and can also be due to environmental (including cultural) factors. Monozygotic twins share 100% of their genes and hence should show 100% concordance. Dizygotic twins share 50% of their genes and hence should show half the concordance of that observed in monozygotic twins. However, the fact that the above does not hold true suggests the important contribution of environment in the etiology of schizophrenia. The other observations also do not implicate the sole effect of genes in causing schizophrenia

since environmental factors, especially prenatal and intra-uterine, may also have been responsible for the above effects.

Schizophrenia does not exhibit the Mendelian pattern of inheritance. Results of genetic studies rule out the involvement of a single or a single major gene (Gottesman, 1991; Tsuang et al, 1999). In fact, studies have confirmed a polygenic model of inheritance of susceptibility to schizophrenia. Linkage and candidate gene approaches have been used to identify the abnormal chromosomal regions and genes respectively.

LINKAGE STUDIES

Abnormalities of chromosomal regions 6p and 8p (Moises et al, 1995a; Pulver et al, 1995; Schwab et al, 1995; Wang et al, 1995; Schizophrenia Linkage Collaborative Group for Chromosomes 3, 6 and 8, 1996), 10p (Straub, 1994; Faraone et al, 1998; Schwab et al, 1998), 13q (Blouin et al, 1998), 15q (Freedman et al, 1997; Kaufmann et al, 1998) and 22q (Polymeropoulos et al, 1994; Pulver et al, 1994; Moises et al, 1995b; Schizophrenia Linkage Collaborative Group [Chromosomes 22], 1996) have been implicated in schizophrenia. However, these results have not been consistently replicated.

CANDIDATE GENE STUDIES

A number of genes speculated to be involved in the pathophysiology of schizophrenia have been studied with respect to polymorphism, namely,

1. dopamine receptors (Moldin, 1997)
2. serotonin receptor, 5-HT_{2A} (Inayama et al, 1996)
3. dopamine transporter (Byerley et al, 1993; Li T et al, 1994)
4. GABA receptors (Asherson et al, 1991; Coon et al, 1994)
5. corticosteriod receptor (Gershon and Rieder, 1992)
6. tyrosine hydroxylase (Byerley, 1993)
7. interleukin receptor (Pulver et al, 1994)
8. HLA gene complex (Nimgaonkar et al, 1995)

Although studies have reported association of polymorphism in some of the candidate genes with schizophrenia, negative results have also been prominent.

Extensive linkage and association studies carried out to date have not been able to discover 'schizophrenia genes'. Moreover, over 60% of the schizophrenic patients have no family history of schizophrenia or schizophrenia in first and second degree relatives.

DOPAMINE HYPOTHESIS

According to the dopamine (DA) hypothesis, which was based on indirect evidence, schizophrenic illness is a manifestation of hyperdopaminergic state (Matthysse, 1973). The neuroleptics, which were effective against the symptoms of schizophrenia and were found to bind to dopamine (D_2) receptors in the brain, served as dopamine antagonists, and the potency of blockade correlated with the clinical efficacy of the neuroleptic. However, the discovery that atypical neuroleptics are most effective for schizophrenia and display the weakest D_2 receptor binding affinity as compared to the typical neuroleptics used to treat schizophrenia, weakens the hypothesis. Massive evidence has accumulated against the hyperdopaminergic hypothesis over the years.

A number of neurotransmitters are found to be abnormal in schizophrenia. GABAergic predominance or glutamatergic deficit is implicated in the pathology of schizophrenia. The glutamate hypothesis of schizophrenia states that reduced glutamatergic transmission causes relative dopaminergic excess in the basal ganglia and limbic systems (NA). Loss of glutamatergic neurons from selective brain regions is implicated in the pathology of schizophrenia (Squires, 1993). Glutamate receptor dysfunction in schizophrenia has also been suggested (Olney and Farber, 1995). Abnormalities of sigma receptors that are thought to regulate the activity of glutamatergic system via NMDA receptors are also suggested to underlie the pathophysiology of schizophrenia.

The cholinergic hypothesis states that schizophrenia involves increased cholinergic tone in the pons (NA). Noradrenergic dysfunction has been implicated in

schizophrenia (Glazer, 1987; Yamamoto, 1994; Friedman, 1999). Impaired homeostasis of norepinephrine (NE) and DA, which causes instability in NE and DA firing, is also suggested in the pathology of schizophrenia. Glycine binding sites are observed to be increased in the cerebral cortex of schizophrenic patients as compared to the controls.

Thus, there has been a shift from the dopamine hypothesis to a multitransmitter dysfunction hypothesis in schizophrenia.

VIRAL HYPOTHESIS

Schizophrenic symptoms are observed in some viral illness which led to the belief that schizophrenia may be due to a gene-virus interaction. It was found that 35% of influenza associated psychiatric admissions to Boston Psychopathic hospital during the 1918-influenza pandemic were schizophrenics (Menninger, 1919; 1926). It was suggested that latent virus when activated precipitated psychosis (Raskin and Frank, 1974) or that viral attack may alter cell function (without killing it) and the alterations can cause schizophrenia (Oldstone et al, 1989; de la Torre J et al, 1991). Another notion that was put forth was that the virus or the mother or fetuses reaction to the virus damages the CNS and may cause schizophrenia. A number of viruses have been implicated in the pathophysiology of schizophrenia, namely cytomegalovirus (Torrey et al, 1983), reovirus (Averback, 1982), influenza (Menninger, 1926; 1928), and retroviruses (Crow 1984; 1986). A number of studies have been carried out to search for viruses or viral genes in the CSF of schizophrenic patients and the results of such studies are varied.

To date, no virus is definitely implicated in the pathology of schizophrenia.

AUTOIMMUNE HYPOTHESIS

The autoimmune hypothesis proposes that schizophrenia is one of the spectrum of neuropsychiatric disorders, which is caused by an immune attack on the brain. Increased prevalence of autoimmune diseases, elevated serum immunoglobulin

levels, decreased mitogen responses, morphologically abnormal lymphocytes, increased antibodies to nuclear factor, increased anticytoplasmic antibodies, decreased lymphocyte interleukin-2 (IL-2) production, increased serum IL-2 receptor concentration, increased serum IL-6 concentration, decreased $CD4^+$ T cells in schizophrenia, all observed with other autoimmune diseases, formed the basis of this hypothesis. These findings have been reviewed and it appears that these abnormalities may be due to antipsychotic medication, duration of illness, the clinical state (psychotic or remitted), nutritional status, substance abuse, and concurrent medical illness of patients (Ganguli, 1994).

Thus autoimmunity is not established as an etiological mechanism in schizophrenia.

MEMBRANE HYPOTHESIS

The "membrane hypothesis" states that schizophrenia is a manifestation of abnormal membrane phospholipid (PL) metabolism. Observations indicative of decreased synthesis (decreased phosphomonoesters - PME) and increased breakdown (increased phosphodiesters - PDE) of PLs in several brain regions have been made. A significant correlation between this pathology and psychopathology was reported. In addition, PL analyses of cell membranes revealed differences in chronic schizophrenics and normal controls. Significantly decreased levels of PLs rich in essential polyunsaturated fatty acids (EPUFA) were reported. Increased levels of the PLs rich in saturated fatty acids compensated for these decreases. This increase correlated with psychopathology. The abnormal membrane phospholipid metabolism was stated to be related to abnormal essential polyunsaturated fatty acid (EPUFA) metabolism. Accordingly, reduced membrane EPUFAs have been reported in chronic medicated schizophrenic patients. Lower levels of ω-6 EPUFA, arachidonic acid (AA) (Yao et al, 1994); or ω-3 EPUFAs, docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA) (Mahadik et al, 1996b; Assies et al, 2001)**;** or both ω-6 and ω-3 EPUFAs (Vaddadi et al, 1990; Glen et al, 1994; Peet et al, 1995; Peet et al, 1996) have been found in cell membranes from chronic medicated schizophrenic patients and from first-episode psychotic patients. This pathology has been found to

correlate with psychopathology. The membrane hypothesis may be able to explain the neuropathophysiology and also provide a basis for evolving effective and safe treatment for schizophrenia. This is discussed in detail in the latter section.

To summarize -

THE STATE OF AFFAIRS

It seems that the core psychopathology of schizophrenia is beginning to get unraveled. It is now generally accepted that cognitive impairments underlie the obvious positive, disorganized and negative symptoms of schizophrenia. Also, the neuropathophysiology of schizophrenia is beginning to be understood. Structural brain abnormalities, enlarged ventricles and reduced volumes of several brain regions, have been observed. Reduced cell size and reduced neuropils are the cytoarchitectural abnormalities that probably underlie the anatomical abnormalities. Neural circuits are hypothesized to be abnormal. A number of neurotransmitter systems are affected in schizophrenia. However, there is no obvious association between the neurostructural and neurochemical findings. It has been suggested that hypoactivity of neurotransmitter glutamate may lead to the loss of neurons (Olney and Farber, 1995) observed in certain brain regions, and also affect other neurotransmitter systems. It is likely that the neurodevelopmental insults, reported in schizophrenia, may result in reduced cell size and neuropils and thus affect synaptic connectivity. The defect in the development and/or maintenance of synaptic connectivity may cause abnormal or reduced development of neural circuitry (Bullmore et al, 1997) and may account for the neurochemical abnormalities observed in schizophrenia. Thus there seems to be a conceptual link between the neuroanatomical and neurochemical abnormalities. Abnormal neural circuitry, due to reduced synaptic connectivity, can also explain impairment of cognitive functions observed in schizophrenia (McGlashan and Hoffman, 2000; Silva et al, 2000) and thus the association between the psychopathology and neuropathophysiology of schizophrenia. The heterogeneity of the neuropathological findings with respect to brain regions may account for the clinical heterogeneity of schizophrenia. However,

these associations remain to be experimentally proved and the cause of the observed neuropathology has not yet come to light. The etiology of schizophrenia thus remains highly speculative though genes, viruses and nutritional deficiencies are implicated. Thus the lacuna is the unknown etiology. Do we have any clues?

It is now generally accepted that schizophrenia is a neurodevelopmental disorder. There is substantial evidence for the role of EPUFAs, particularly docosahexaenoic acid (DHA) and arachidonic acid (AA) in brain development; their role in formation of synapses and synaptic function is particularly relevant to schizophrenia. Also, the EPUFA component of the membrane PLs determines fluidity for optimal functioning of membrane ion channels and receptors and acts as second messengers for a number of neurotransmitter mediated signal transduction, which may account for the multitransmitter dysfunction observed in schizophrenia. With this in mind, it is tempting to make a proposition implicating reduced EPUFAs in the etiology of schizophrenia.

The available drugs (antipsychotics) are targeted to antagonize possibly altered functions of several neurotransmitters, which may only be a result a consequence of the core neuropathophysiology. The drugs do not effectively treat the psychopathology and treat the different symptoms in varying degrees. Some of the early antipsychotics (typical or conventional antipsychotics - typicals) effectively treat psychosis but not the recently considered core psychopathology, negative symptoms and cognitive impairment that are critical in illness outcome improvement. Also, these drugs often cause serious side effects. The recently introduced atypical antipsychotics (atypicals) seem to be effective in managing the negative symptoms, improving cognitive performance and also causing none or few side effects, the latter exemplified by the fact that the long-term use of typicals, but not the atypicals, is found to cause impaired cognitive performance and CNS cholinergic function in animals (Mahadik et al, 1998; 2001). Based on these animal studies and the clinical effects, the atypicals are considered to alter neurotransmitter receptor functions and probably also the altered neuroanatomy of schizophrenia. However, it stands to be proved whether these are effective against the core negative symptoms.

If abnormal neural circuitry due to reduced synaptic connectivity indeed reflects the neuropathology of schizophrenia, it only seems logical that effective drugs for treatment would be those that correct this aberration. EPUFAs theoretically can achieve this (discussed later). Moreover, treatment with atypicals which seems to be effective against negative symptoms and cognitive impairments, is found to increase membrane EPUFA levels (Horrobin et al, 1999; Khan et al, in press). Safety of the drugs of treatment is an aspect to be borne in mind. EPUFAs will prove safer than currently available drugs. Thus it seems important with the current state of affairs to formulate a hypothesis to implicate reduced EPUFAs in the etiopathophysiology of schizophrenia.

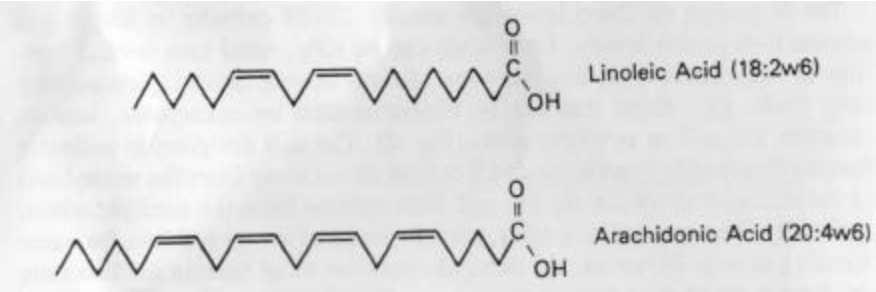
A comprehensive description of essential polyunsaturated fatty acids and a discussion of their relevance to schizophrenia seems pertinent at this stage.

II) ESSENTIAL POLYUNSATURATED FATTY ACIDS (EPUFAs)

Fatty acids of the ω-6 and ω-3 series, although indispensable, cannot be synthesized by the body and hence are "essential" in the diet. Linoleic acid [LA, 18:2 ω-6] and α-linolenic acid [ALA, 18:3 ω-3] are the essential fatty acids [EFAs]. LA, identified as the first essential fatty acid, is the precursor of arachidonic acid [AA, 20:4 ω-6] and other long chain polyunsaturated fatty acids [LCPUFA] of the ω-6 series. ALA is the precursor of eicosapentaenoic acid [EPA, 20:5 ω-3], docosahexaenoic acid [DHA, 22:6 ω-3] and other LCPUFA of the ω-3 series. AA, EPA and DHA, since they are made from the EFAs, and contain 20-22 carbon atoms and three or more double bonds, are called essential polyunsaturated fatty acids [EPUFAs] (Fig. 2).

It was recognized as early as 1930s (Burr and Burr 1929; 1930) that the EFAs are necessary for development of animals and possibly humans, but it became apparent only later when clinical deficiency was observed in infants fed skim-milk based formulas (Hansen et al, 1963) and in patients given lipid-free parental nutrition (Collins et al, 1971; Paulsrud et al, 1972). ω-3 and ω-6 deficiency syndrome together include poor growth; skin lesions; decreased skin pigmentation; loss of muscle tone; degenerative changes in the kidney, lung and liver; increased metabolic rate;

A) ω-6 fatty acids



B) ω-3 fatty acids

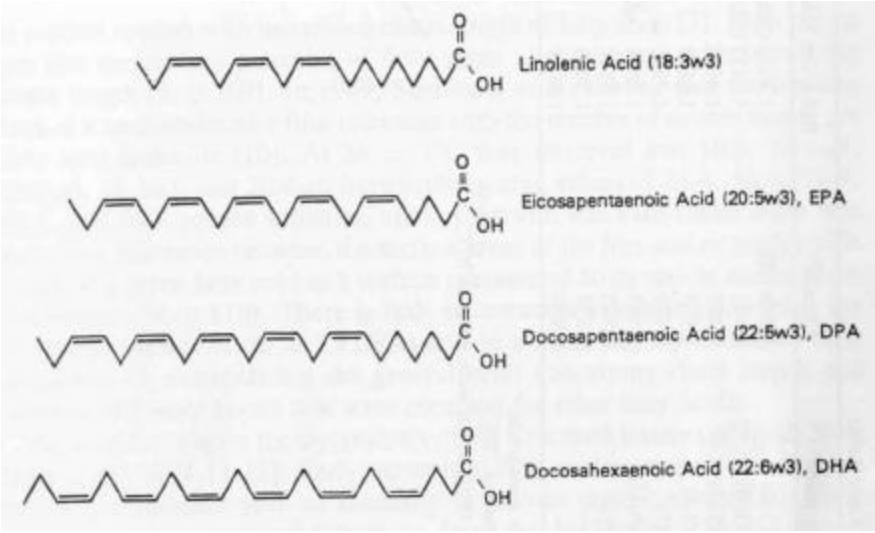


Fig. 2 Structures of EFAs and important EPUFAs

impaired water balance; increased fragility and permeability of cell membranes and increased susceptibility to infection. These abnormalities are by and large overcome by ω-6 fatty acid supplementation. ω-3 EFA deficiency was observed to be associated with skin changes (unresponsive to LA supplementation), and more importantly, with abnormal visual function and peripheral neuropathy.

A) METABOLISM

The EFAs, LA and ALA, are present in green leafy vegetables, some fruits, cereals and pulses and EPUFAs are obtained from fresh fish and fish oils.

As shown in Fig. 3, dietary EFAs are taken up by the gastrointestinal tract (GIT) and are largely transported to the liver for conversion to EPUFAs (discussed

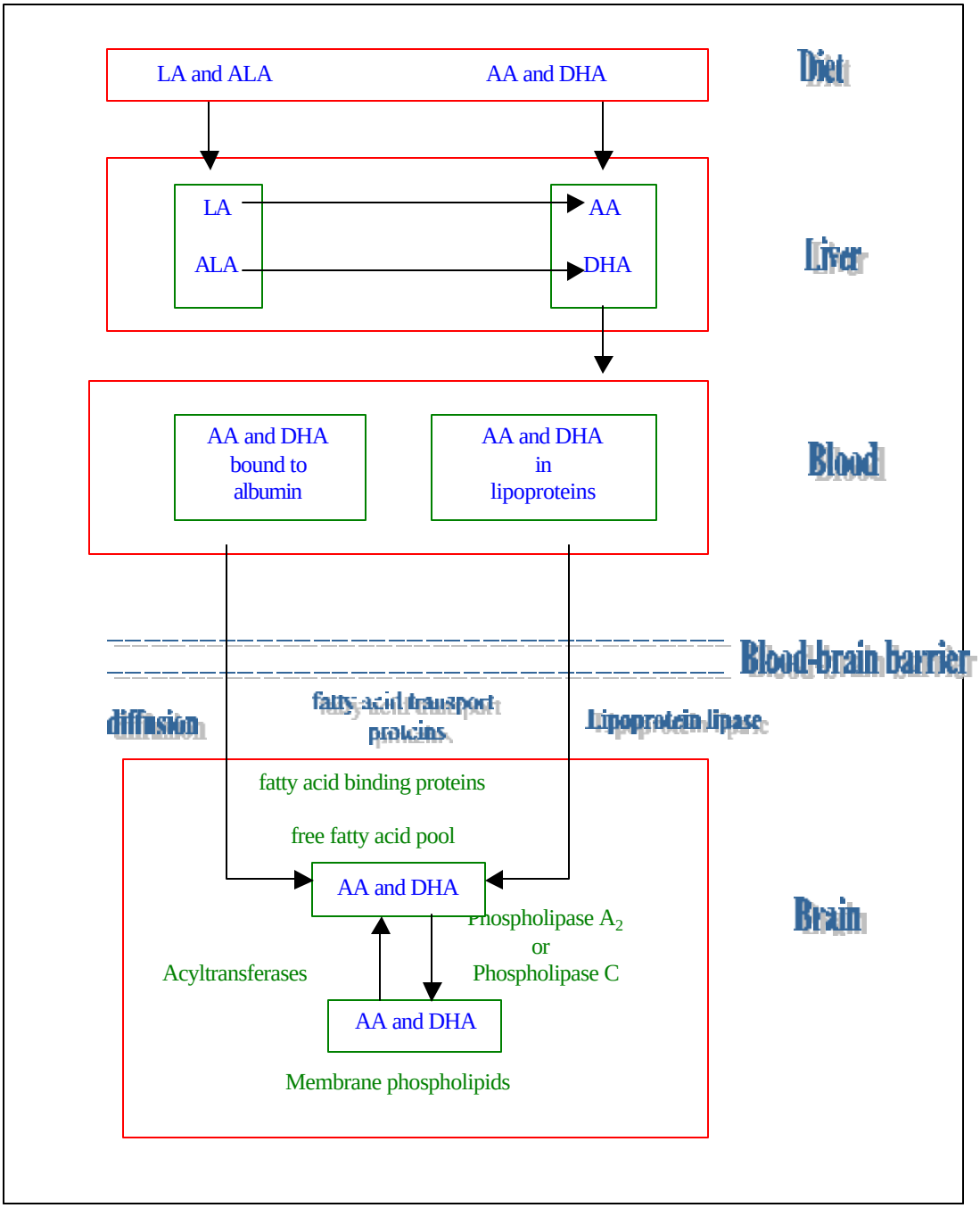


Fig. 3 Supply of EPUFAs to brain

later). The EFAs/EPUFAs from the GIT and the liver enter blood circulation to reach the target organs. In the blood, they are either bound to albumin or are in the form of triglycerides associated with lipoproteins and these then diffuse across the blood-brain barrier. In some cases, transport mechanisms may be involved. The blood brain barrier is also one of the possible sites for elongation and desaturation of LA and ALA (Dopeshwarker, 1983). These are released from albumin by diffusion. Release from triglycerides requires lipoprotein lipase of the endothelial cells. From the extracellular fluid, these are taken up into cells mainly by simple diffusion through the membranes and partly with the aid of fatty acid transport proteins.

The EFAs are taken up by the glial cells for conversion to EPUFAs and are supplied to the neurons with the help of at least three fatty acid binding proteins. Neurons are thought to have limited capacity for making EPUFAs.

ANABOLISM OF EPUFAs

The metabolic pathways of EFAs are now delineated in considerable detail and are more complicated than previously envisaged (Fig. 4). LA and ALA undergo alternating desaturation and elongation, to make their distinct cascades of metabolic products upto 22 or more carbons long which is carried out in the microsomes. The chain shortening and saturation of double bonds during retroconversion may occur in the inner mitochondrial membrane or peroxisomes.

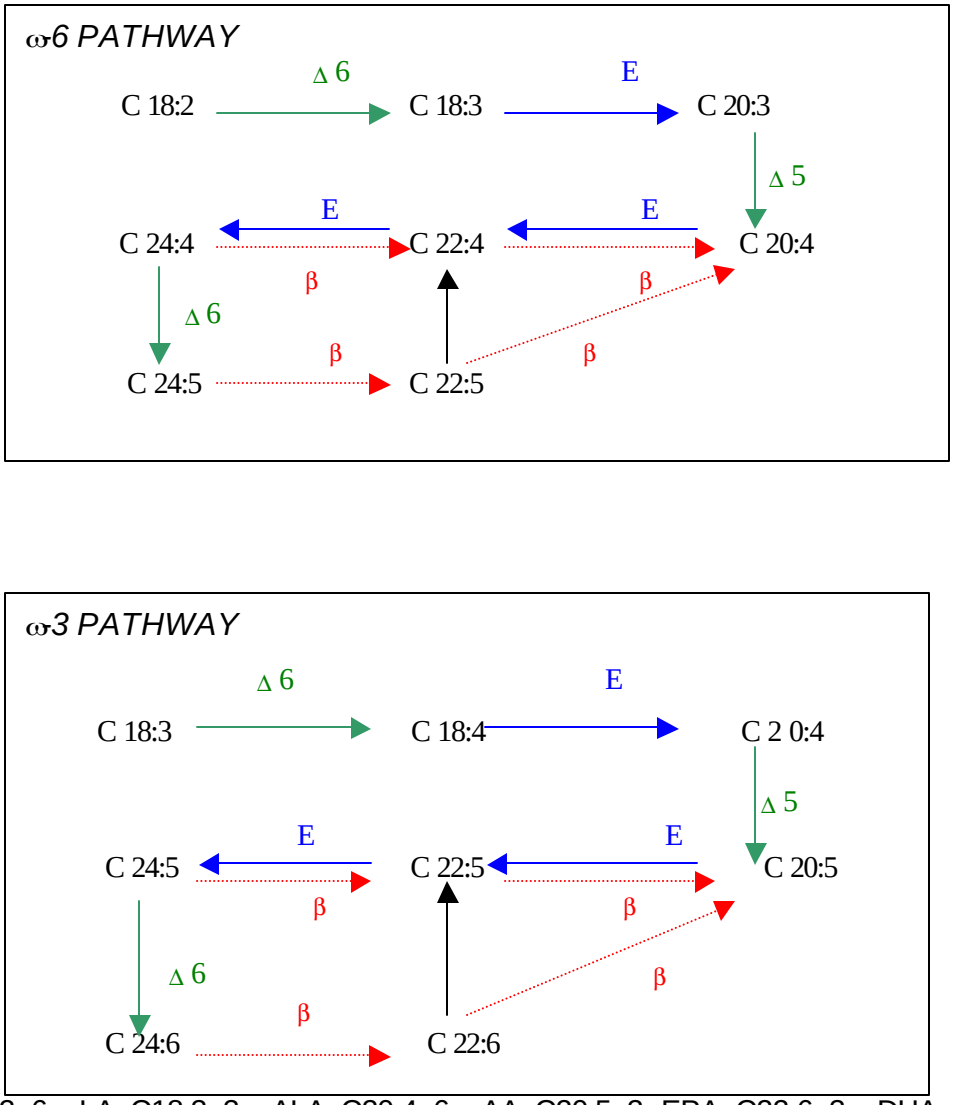
ω-6 and ω-3 fatty acids compete with each other for the same enzymes for elongation and desaturation. However, the ω-6 and ω-3 fatty acids constitute discrete series and there is no interconversion between them.

CATABOLISM OF EPUFAs

DAMAGE BY REACTIVE OXYGEN SPECIES

EPUFAs are the most unsaturated of the polyunsaturated fatty acids species and are highly prone to peroxidation by reactive oxygen species (ROS). ROS, namely, $O_2^{\cdot -}$, $OH^{\cdot}$, OH^{-}, $NO^{\cdot}$ and $ONOO^{-}$ are generated during aerobic metabolism, that is, mitochondrial oxidations and monoamine oxidations. They can affect a number of molecules of the cell including lipids, proteins and DNA. Under normal

circumstances, ROS are eliminated by cellular enzymatic and non-enzymatic antioxidant defenses. Oxidative stress is defined as a state of higher cellular levels of ROS, either due to excessive ROS production or inadequate antioxidant defense or both.



C18:2ω6 – LA, C18:3ω3 – ALA, C20:4ω6 – AA, C20:5ω3- EPA, C22:6ω3 – DHA

Fig. 4 Metabolic cascades of LA and ALA

Preferential vulnerability of brain to oxidative injury

Brain has high aerobic metabolism and blood perfusion. It also has relatively poor enzymatic antioxidant defense. It is enriched in essential polyunsaturated fatty acids that are preferentially vulnerable to oxidative damage. Also, neurons in the adult

brain do not multiply and the DNA damage by ROS proves cumulative making the brain preferentially vulnerable to oxidative injury as compared to other organs.

Lipid peroxidation

Oxidative damage to the unsaturated lipids is termed as lipid peroxidation. It is initiated by the attack of the free radical (or a species capable of abstracting hydrogen) on a fatty acid molecule. The greater the number of double bonds in a

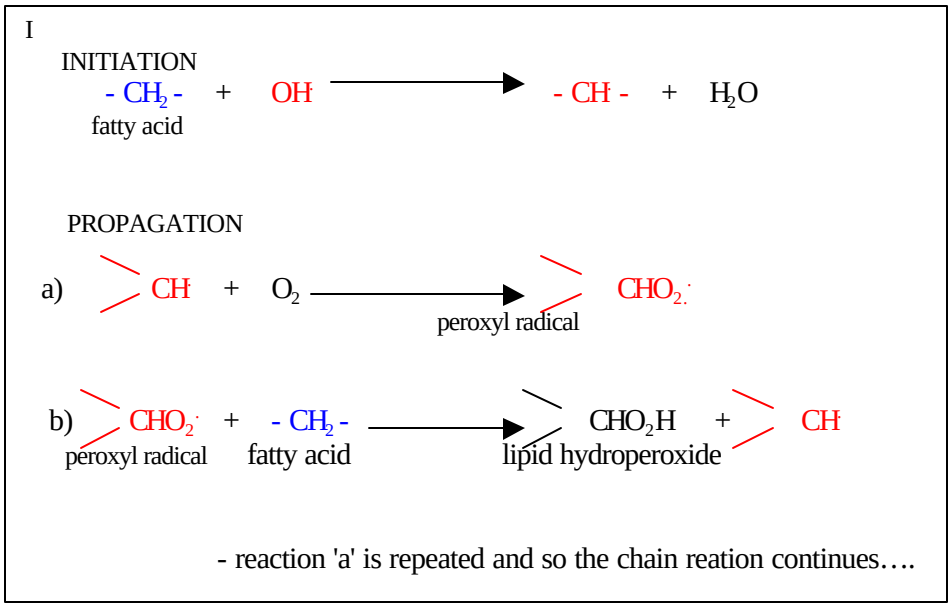


Fig. 5 Lipid peroxidation : a radical chain reaction

molecule, the easier it is to remove hydrogen atom leaving behind an unpaired electron on the carbon atom to which it was originally attached. The resulting carbon centered lipid radical can react with O_2 to form a peroxyl radical which can abstract hydrogen from adjacent fatty acid chains thus initiating a chain reaction of lipid peroxidation. Hence, a single event can result in destruction of hundreds of fatty acid side chains into lipid peroxides. (Fig. 5).

The lipid peroxidation chain reaction can be terminated by providing easily donatable hydrogen for abstraction by the peroxyl radical which is provided by antioxidants. The most important chain breaking antioxidant in human lipids is α-

tocopherol (Burton and Ingold, 1989). α-Tocopherol radical can be converted back to tocopherol by reduction with ascorbic acid at the surface of biological membranes (Tappel, 1968; Esterbauer et al, 1989). β-Carotene, urate, sulphydryl containing proteins and peptides such as glutathione (GSH) (Burton and Ingold, 1990; Halliwell and Gutteridge, 1990) represent the nonenzymatic antioxidant molecules.

The human body also has a complex antioxidant defense system the antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT). SOD converts superoxide to hydrogen peroxide which is decomposed to water and oxygen by the heme protein CAT thereby preventing the formation of hydroxyl radicals. GPx converts hydrogen peroxide to water or more critically converts toxic hydroperoxides to less toxic alcohols (Fig. 6). SOD, CAT and GPx are critical to different stages of free radical metabolism and altered activity of one enzyme without compensatory changes in other enzymes may leave membranes vulnerable to damage.

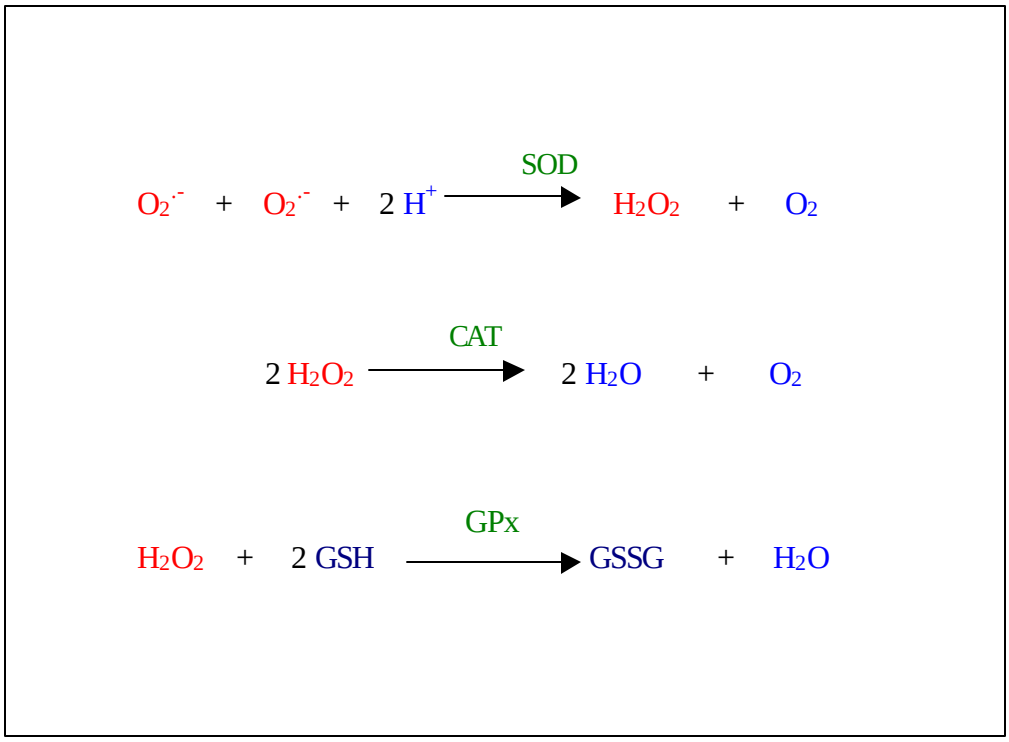
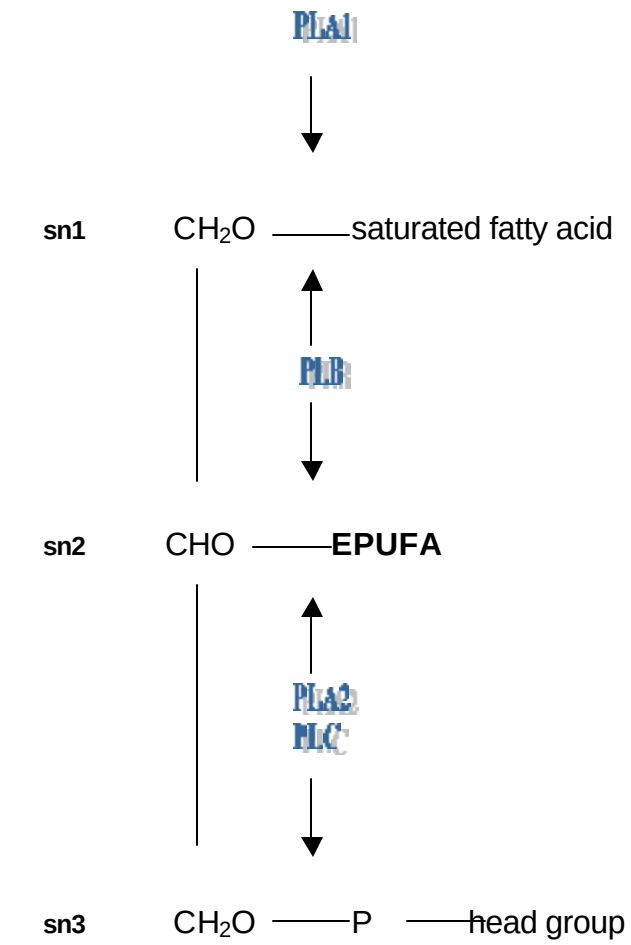


Fig. 6 Reactions catalysed by antioxidant enzymes

RELEASE FROM MEMBRANE PHOSPHOLIPIDS

EPUFAs generally occupy the sn2 position of membrane phospholipids. Phospholipase A_2 group of enzymes, when activated, hydrolyze the unsaturated fatty acids from their sn2 position in the phospholipids (Fig. 7).

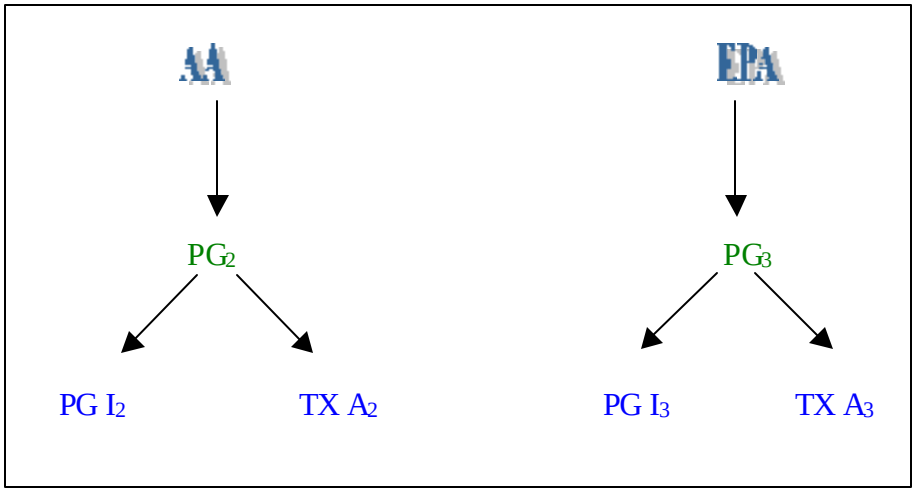


PLA_2 – Phospholipase A_2

Fig. 7 Phospholipases : sites of action

The released fatty acids regulate various cell functions, for example protein kinases; the nature of the regulation depends on the particular fatty acid molecule and on its geographical location within the cell (Nunez, 1993; Goodfriend and Elliot, 1995).

The fatty acids may also be converted to an array of signaling molecules known as eicosanoids (Fig. 8) which include prostaglandins, leukotrienes and hydroxyacids, among many others. They have a huge array of direct as well as indirect actions mediated via cyclic nucleotides, calcium and protein kinases. The range of effects produced depends on the chemical nature of the specific fatty acids, as well as upon the geographical location in the neuron where the fatty acid is released (Nunez, 1993; Goodfriend and Elliot, 1995).



PG – Prostaglandins; TX – Thromboxanes

Fig. 8 The Eicosanoids

B) IMPORTANCE IN BRAIN STRUCTURE AND FUNCTION

Lipids constitute 50 % of the brain tissue dry weight. The central nervous system (CNS) is second only to the adipose tissue in containing the highest lipid concentration. However, in the brain, lipids largely serve as structural and functional components of cell membranes. The amount of storage lipid namely, triglyceride is negligible (Eichenberg, 1969; Rissiter, 1970; Sastry, 1985).

Essential fatty acids and brain

EFAs constitute about 20 % of the brain tissue dry weight and about one third of these fatty acids belongs to the ω-3 family (Bourre and Dumont, 1991). DHA, the biologically functional ω-3 EPUFA, is a major component of the brain as well as the retinal tissue. Accordingly, low LNA and no ω-3 EPUFAs in the diet are observed to be associated with altered learning behavior in rats (Walker, 1976; Lamptey and Yamamoto et al, 1988; Bourre et al, 1989a), lower visual acuity thresholds and reduced amplitudes of electroretinograms in rhesus monkeys (Connor et al, 1984; Neuringer et al, 1984; Neuringer et al, 1986; Anderson and Connor, 1989), rats (Futterman et al, 1971; Benolken, 1973; Wheeler et al, 1975; Lamptey and Walker, 1976; Bourre et al, 1989a) and in premature infants (Uauy et al, 1990).

ω-3 fatty acids are important to the brain

It was found that when dietary LA was held constant, increasing the levels of dietary ALA suppressed the content of the ω-6 products. Similarly it was found that increasing the level of LA suppressed the ω-3 products. Also, it was noted that strong suppression of ω-6 metabolism was accomplished by < 2% of calories of ALA, whereas an equal suppression of ω-3 metabolism required nearly 10 times as much dietary LA. This implies that ω-3 EPUFAs are more strongly conserved than ω-6 EPUFAs. Also, the elongation and desaturation processes at the blood brain barrier are more extensive for ω–3 fatty acids than for ω–6 fatty acids (Moore et al, 1990). Additionally, Bourre et al (1992) found that in rats which were fed LNA deficient diets for 7 months, brain DHA levels did not decline although significant reductions were observed in other organs such as the liver and heart. Thus ω-3 fatty acids, especially DHA, are importance to the brain.

Cellular location of essential polyunsaturated fatty acids

EFAs taken up by tissues are recovered in relatively high proportions as components of phospholipids (Carlier et al, 1991). This implies that the EFAs are largely localized

in the membranes. Here, these are present as their long-chain derivatives, the EPUFAs. Saturated fatty acids are attached to sn1 position and EPUFAs are attached to sn2 position of PLs (Fig. 9). In the brain, unlike other tissues, usually the sn-2 position is occupied by EPUFAs (O'Brien et al, 1964; Crawford et al, 1976). DHA is the primary ω-3 EPUFA of the cell membranes whereas AA is the primary ω-6 EPUFA. DHA is primarily found in ethanolamine and serine phosphoglycerides whereas AA is more prominent in the inositol phosphoglycerides (Fliesler and Anderson, 1983; Sastry et al, 1985).

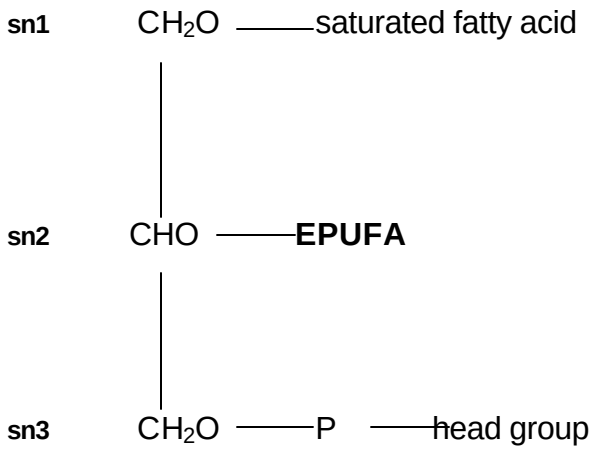


Fig. 9 Phospholipid structure

The EPUFAs as constituents of cell membrane phospholipids (PLs) (Fig. 10), form the hydrophobic interiors of all biological membranes. They contribute structurally and functionally to the membranes by modulating fluidity, permeability and activity of membrane bound enzymes (Clandinin et al, 1991). EPUFAs, AA, EPA and DHA largely affect membrane fluidity on the basis of their high degree of unsaturation. The replacement of even a single double bond in these EPUFAs is sufficient to exert a profound effect on the physical properties of membranes (Cohen and Zubenko, 1987; Mason et al, 1992). Viani (1991) reported that age related declines in the polyunsaturated fatty acids resulted in a decrease in Na^+,K^+ ATPase activity. The exact mechanism by which membrane lipids influence the properties of

enzymes and transporters is not understood. It is possible that these affect membrane fluidity, which affects the protein orientation in the membrane, thereby, their function. However, the mechanisms by which EPUFAs, especially DHA, affect cell membrane functions are not investigated. The fact that a high concentration of EPUFAs is present in the synaptic terminals and visual terminals of the retina may implicate that these are important determinants for normal functioning of excitable tissue, possibly playing a role beyond that of maintaining the high fluid properties of membranes (Innis, 1991).

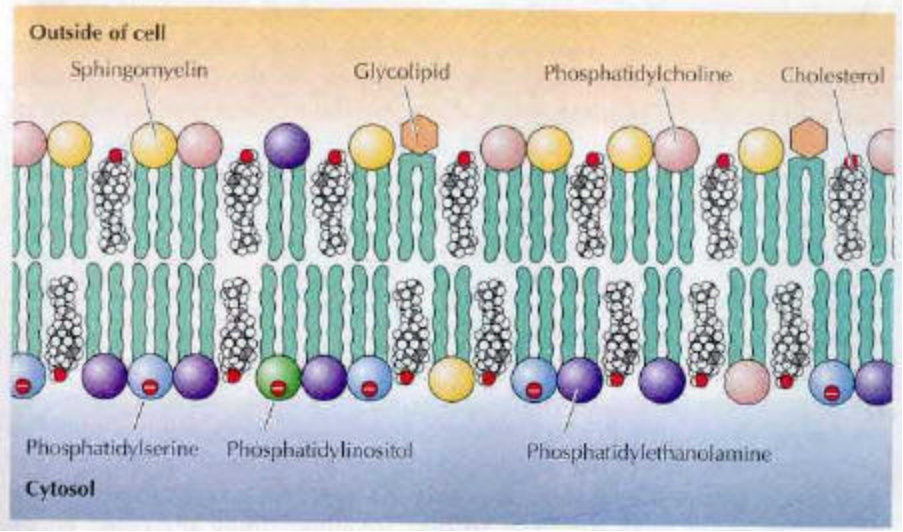


Fig. 10 Membrane structure

High levels of DHA have been reported in subcellular fractions in synaptosomes (Viani, 1991; McGee, 1994), synaptic vesicles (Weisinger, 1995), mitochondria (Willumsen, 1996), microsomes (Srinivasarao, 1997) and nerve growth cones (Martin, 1992).

There is evidence to implicate the importance of EPUFAs, especially that of the ω3 series, in neurodevelopment and behavior (Sinclair, 1990; Simopoulos, 1991; Crawford, 1992; Wainwright, 1992). Although, the exact mechanisms underlying the

role of DHA in neural development are not yet fully understood, DHA deficiency predominantly affects brain development (Neuringer, 1986).

ROLE IN NEURODEVELOPMENT

The effects of EPUFA deficiency are widely documented in experimental animals whereas information from humans is scarce. Lampety and Walker (1976), Walker (1967), Wheeler (1975), Crawford et al (1981) and Clandinin et al (1981) pioneered the investigation of the role of EPUFAs in neurodevelopment. This was followed by significant contributions by Martinez et al (1974, 1987, 1988, 1989), Innis (1989), Carlson (1989), Neuringer (1984, 1986), and Bourre et al (1989b). The studies on EPUFAs in brain development have yielded important information on the patterns of EPUFA accumulation during the course of development and the functional effects of deficiency of EPUFA on brain development.

The human fetus obtains its source of EPUFAs by placental transfer (Portman et al, 1969) and additionally during the third intrauterine trimester by elongation and desaturation of the transferred 18 carbon fatty acids (Friedman et al, 1978). The ω-3 and ω-6 fatty acids are present in low concentrations in immature brain and accumulate rapidly during brain growth spurt (Alling, 1974; Galli, 1975; Hitzmann, 1981; Sinclair,1972). During early growth, AA and DHA increase together while the deposition of DHA becomes quantitatively more prominent later (Galli, 1975; Sinclair, 1972). In fact, the increase in brain lipid DHA/AA, which occurs at about 23 days of age in rats, has been proposed to be a possible useful indicator of normal maturation. As in rodents (Hitzmann, 1981; Samulski, 1982; Walker, 1967), human brain contains a relatively high amount of 22:5 ω-6 (DPA ω-6) in the early stages of development, which is later followed by a quantitative predominance of DHA (White, 1971). Martinez (1989) observed that after 30 weeks of gestation there is a preferential desaturation of the long-chain ω–3 fatty acids in the brain.

Accordingly, a large number of studies have shown that essential fatty acid deficiency results in pronounced alterations in the fatty acyl composition of the developing brain and its cell and subcellular fractions (Mohrhauer, 1963; Walker, 1967; Galli, 1970; Galli, 1971; White, 1971; Galli, 1972; Alling, 1972; Alling, 1974;

Sun, 1974a; 1974b; Karlson, 1975; Lampety, 1978; Matheson, 1980; Samulski, 1982). There is an increase in the ω-9 and decrease in ω-3 and ω-6 EPUFA, predominantly DHA and AA (Innis, 1991). A deficiency of ω-3 and/or ω-6 fatty acids during development is known to result in altered activity of a variety of CNS membrane associated enzymes, receptors and transport systems, cognitive behaviors, visual function and prostaglandin synthesis (Calwell, 1966; Paoletti, 1972; Bernsohn, 1974; Sun, 1974a; 1974b; Borgman, 1975; Galli, 1975b; Lampety, 1976; 1978; Brenneman and Rutledge, 1979; Morgan, 1981; Ruthrich, 1984; Coscina, 1986; Crane, 1987; Hannah, 1987; Yamamoto, 1987; 1988). Thus although the consequences of EPUFA deficiency at the anatomical, cellular and biochemical level are not known, it is only logical that the deficiency of molecules required for the various events of brain development can affect the structural and biochemical maturity of the brain and can have lasting effects. For example, a restricted diet (creating a protein and EFA deficiency) during the period of brain growth spurt results in a reduction in growth, that is reduced brain weight and cell numbers and also behavioral impairments (Dobbing, 1972).

On the basis of available information a role for the EPUFAs in various stages of brain development can be envisaged. It can be conceived that DHA is required in all the stages of neurodevelopment -

Proliferation: It has been suggested that DHA is required to maintain the rapid rate of neuronal plasma membrane synthesis (O'Brien and Samson, 1985).

Migration: DHA is also critical for maintaining high membrane fluidity that may help promote growth and membrane growth factor receptor function (Stubbs and Smith, 1990) which are important at this stage.

Synaptogenesis: Green and coworkers (1999) found that at the 17^{th} embryonic day, the increase in rat brain PUFA concentration plateaued, except that of DHA, which accumulated further. This accumulation was found to occur just before synaptogenesis, which is in keeping with the fact that the synaptosomal membranes are rich in DHA.

The timing of this sequence differs from one cell type to another and from one region to another. Thus the process of brain development at a temporal cross section

can be perceived as a complex pattern of events and deficiency of EPUFA may have differential effects on the various brain regions.

ROLE IN NEUROTRANSMISSION

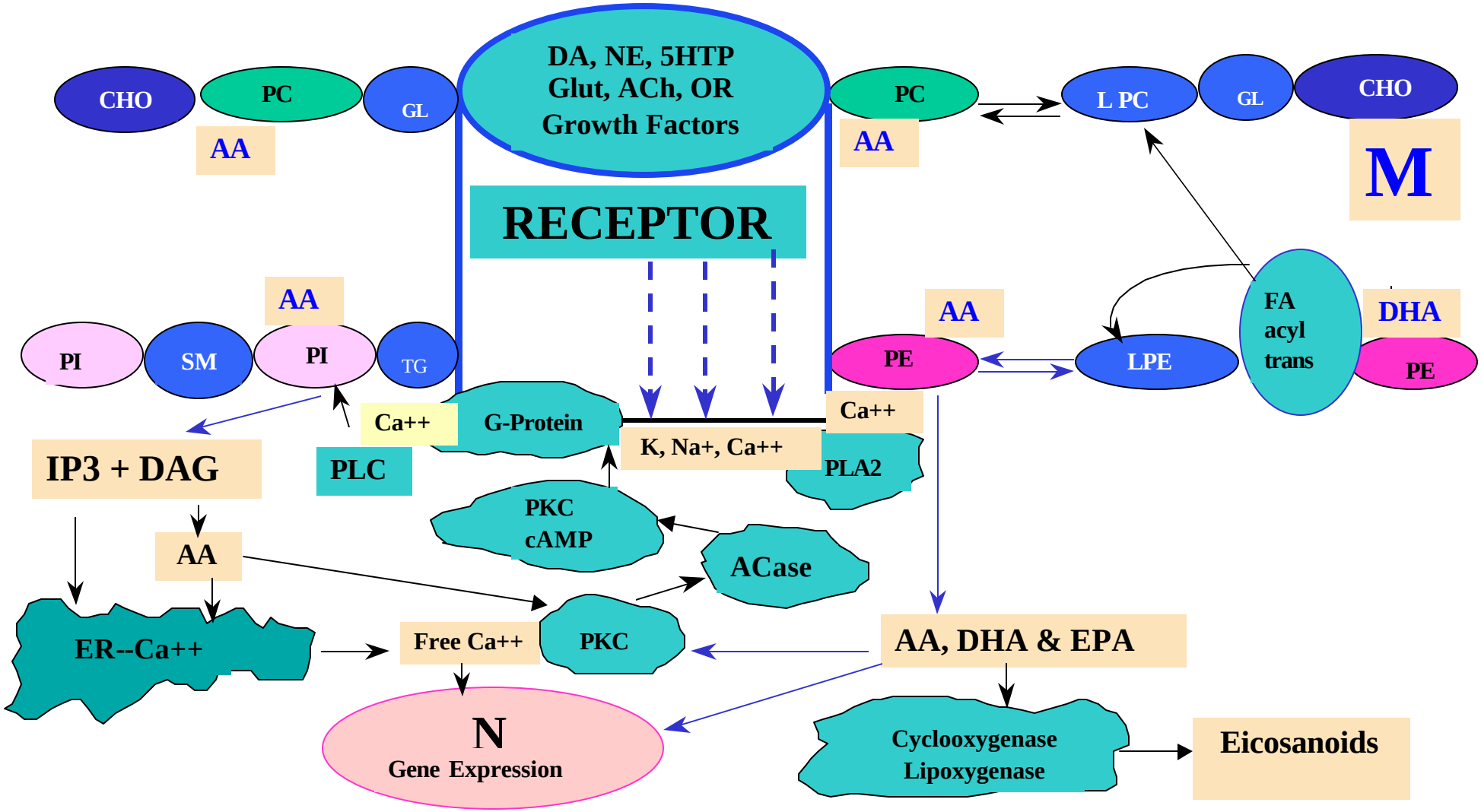
EPUFAs are crucial in determining the quantity and quality of neuronal phospholipids (PLs) (Horrocks et al, 1982; Thompson et al, 1992) and maintain membrane fluidity appropriate for membrane receptors. Some of the PLs are hydrolyzed by receptor mediated processes generating second messengers, namely, diacylglycerol, inositol polyphosphates, AA, prostaglandins and leukotrienes (Berridge, 1981; Axelrod, 1990; Rana and Hokin, 1990; Horrobin et al, 1994)

AA functions as a second messenger in signal transduction pathway (Fig. 11). DHA is the most unsaturated fatty acid species of the cell membranes. The highest levels of DHA are found in the brain (cerebral cortex) and retina, especially in the phospholipids of the synaptosomal membranes and photoreceptor outer segment membranes. Reduced DHA is expected to alter the physical and functional properties of membranes. Also, Jones and co-workers (1997) demonstrated that following intravenous infusion of [4,5 ^{3}H] DHA, plasma [^{3}H] DHA incorporation into synaptic membrane phospholipids of rat brain in response to cholinergic activation is selectively increased indicating a role for DHA in phospholipid mediated signal transduction at the synapse.

C) RELATIONSHIP TO SCHIZOPHRENIA

FAT CONSUMPTION AND SCHIZOPHRENIA

Christensen and Christensen (1988) reported that quality and quantity of fat in the diet was associated with the outcome of schizophrenia. A highly significant correlation between favourable ratings of course and outcome of schizophrenia in patients from different countries (cultures) and low percentage of dietary total fat (r =



IP3-inositol triphosphate; DAG-diacylglycerol; PKC-protein kinase C; cAMP-cyclic AMP;Acase-Adenylate cyclase

Fig. 11 AA in signal transduction

0.8 - 0.9, $p < 0.05$) and saturated fat (fat from land animals and birds) ($r = 0.91$-0.95, $p < 0.01$) was reported. They observed that 98% of the variation in the course of schizophrenia could be explained by variations in fat intake (Food and Agricultural Organization, 1980); patients in the developed countries where the major dietary fat is obtained from vegetables and sea-food (enriched in ω-3 and ω-6 EPUFAs) had a better course of the illness than seen in patients in the developing countries where a high fat diet, with majority of fat from land animals and birds (poor in the EPUFAs) is common.

Thus higher intake of EPUFAs in the developing countries was implicated with better outcome observed.

ω-3 FATTY ACIDS: THE LIMITING FATTY ACIDS

ω-6 EPUFAs are abundantly available in the diet, which implies that the limiting EPUFAs associated with the outcome of schizophrenia, are the ω-3 EPUFAs. Also, as stated earlier, ω-3 fatty acids are pertinent to normal brain anatomy and function and accordingly appear to be better conserved than the ω-6 EPUFAs, confirms their relative importance to the brain.

D) ω-3 FATTY ACIDS AND SCHIZOPHRENIA: THE DHA HYPOTHESIS OF SCHIZOPHRENIA

DHA HYPOTHESIS : The hypothesis states that reduced DHA is involved in the etiology and neuropathophysiology of schizophrenia

REDUCED DHA AND NEUROSTRUCTURAL PATHOLOGY

In schizophrenia, various abnormalities have been reported namely, enlarged ventricles and reduced volumes of several brain regions, reduced cell numbers and cell size, mis-placed and mis-aligned cells and reduced neuropils. This indicates that the processes of neurodevelopment, namely, proliferation, migration and forming of synaptic connections are affected. As mentioned earlier, DHA may be an important requirement for all these processes. Dietary DHA deficiency per se or conditions

responsible for oxidative damage of DHA may thus be responsible for the neuroanatomical abnormalities of schizophrenia.

REDUCED MEMBRANE DHA AND NEUROCHEMICAL ABNORMALITIES

DHA is very important for maintaining the appropriate membrane fluidity for the optimal functioning of membrane proteins, which may thus affect a number of cell functions including signal transduction. AA is abundantly available in the diet, however, reduced membrane AA is concomitantly observed with reduced DHA. DHA may play a role in regulation of membrane AA levels. Dopamine receptor activates the AA cascade, which is the basis of D_1/D_2 synergism. D_1/D_2 synergism is found to be abnormal in schizophrenia. Moreover, multitransmitter dysfunction is now considered to contribute to the pathophysiology of schizophrenia. AA is a second messenger for a number of neurotransmitter mediated signal transduction processes. DHA as stated earlier also plays a role in the signal transduction mechanisms. Thus, reduced membrane DHA may be responsible for the neurochemical abnormalities of schizophrenia.

CHAPTER II

MEMBRANE EPUFA IN SCHIZOPHRENIA OUTCOME

A PART OF THE CONTENT OF THIS CHAPTER HAS BEEN COMMUNICATED TO BIOLOGICAL PSYCHIATRY

INTRODUCTION

Phospholipids (PLs) play an important role in membrane structure and function. PL derived second messengers namely, arachidonic acid (AA), diacylglycerol, inositol polyphosphates and prostaglandins are involved in signal transduction. PLs are also important for the function of membrane proteins e.g. phosphatidyl choline (PC) is required for Na^+-K^+ ATPase activity. Hence alterations in membrane PLs can affect cell membrane functions.

Altered membrane PL metabolism has been observed in schizophrenia. ^{31}P magnetic resonance spectroscopy studies have indicated decreased synthesis (decreased phosphomonoesters - PME) and increased breakdown (increased phosphodiesters - PDE) of PLs in several brain regions. These observations have been made in early (first-episode schizophrenics - Pettegrew et al, 1991; Keshavan et al, 1993a) as well as chronic stages of the illness (chronic schizophrenic patients - Fujimoto et al, 1992; Stanley et al, 1994; Fukuzako et al, 1996). The abnormality has been associated with severity of psychopathology, for example, a significant positive correlation was found between levels of PDE and scores of positive symptoms on Brief Psychiatric rating scale (BPRS) (Fukuzako et al, 1996).

In addition to abnormal metabolism, membrane PL composition analyses have revealed differences in schizophrenics and normal controls. Although, the results of such studies have been varied, the consensus is in favor of increased phosphatidyl serine (PS) and decreased phosphatidyl ethanolamine (PE) (Rotrosen and Wolkin, 1987) in schizophrenics as compared to normal controls. Later studies with unmedicated patients have indicated significantly decreased PE and a trend towards decreased phosphatidyl inositol (PI) in erythrocytes from chronic schizophrenic patients (Keshavan et al, 1993b) and additionally, decreased PS in cultured skin fibroblasts from patients at the onset of psychosis (Mahadik et al, 1994). These decreases were found to be compensated by increased sphingomyelin (SM) and cholesterol esters. Total BPRS scores showed a significant positive correlationship to SM levels (Keshavan et al, 1993b).

Abnormal membrane PL composition and metabolism may be explained by reduced EPUFA intake and increased breakdown observed in schizophrenia. Essential polyunsaturated fatty acids (EPUFAs) at the sn2 position of glycerol are characteristic of the particular PL species. These determine the content of membrane PLs, especially in the brain (Thompson, 1992), where AA and docosahexaenoic acid (DHA) make up over 90% of the EPUFAs. Increased oxidative stress has been suggested in schizophrenia (Mahadik and Mukherjee, 1996; Reddy and Yao, 1996). Increased EPUFA peroxidation due to oxidative stress may be one of the causes of increased breakdown of membrane PLs. In addition, low dietary availability of EPUFAs (Christensen and Christensen, 1988) may probably contribute to decreased synthesis of membrane PLs. Moreover, EPUFAs largely contribute to fluidity of the membranes and thereby affect membrane protein function. It is established that AA is a second messenger and that DHA is involved (mechanism unknown) in cell signal transduction processes used by several neurotransmitters. Thus reduced membrane EPUFAs may affect neurotransmitter mediated signal transduction processes accounting for the multitransmitter dysfunction observed in schizophrenia. Hence, reduced membrane EPUFAs may be the basic membrane abnormality in schizophrenia. However, despite the strong implications, the association between altered PL and EPUFA composition in schizophrenia remains to be confirmed. Simultaneous estimations of membrane PL and EPUFA compositions of membranes can confirm the same.

Membrane PL profile in schizophrenia is suggestive of decreased EPUFAs and increased saturated fatty acids. Low levels of ω-6 EPUFA, AA (Yao et al, 1994); or ω-3 EPUFAs, DHA and docosapentaenoic acid (DPA) (Assies et al, 2001)**;** or both ω-6 and ω-3 EPUFAs (Vaddadi et al, 1990; Glen et al, 1994; Peet et al, 1995; Peet et al, 1996) have been found in RBC membranes from chronic medicated schizophrenic patients. Low levels of membrane EPUFAs in cultured skin fibroblasts from first-episode psychotic patients have been reported (Mahadik et al, 1996). Low levels of EPUFAs, especially AA, are found in a subgroup of patients with negative symptoms (Glen et al, 1994; Peet et al, 1995). Persistent negative symptoms and cognitive impairments are considered to indicate poor outcome of schizophrenia (Carpenter et

al, 1988; Andreasen et al, 1999). Hence membrane EPUFA status may be the pertinent index of membrane pathology in schizophrenia and may correlate with outcome. However, the role of EPUFAs in psychopathology is not clear. Most of the studies have been carried out on chronic medicated schizophrenic patients and both EPUFAs and psychopathology, are altered with years of illness and treatment with antipsychotics. Investigations in never-medicated schizophrenic patients with short duration of illness will help fill this lacuna.

Erythrocyte / red blood cell (RBC) membranes have been found to be suitable to study neuronal membrane PL (Keshavan et al, 1993a) and EPUFA (Bourre et al, 1992; Carlson et al, 1986; Connor et al, 1993) status and can be used in studies to answer the above questions. This, however, requires that diet of subjects not be very variable over time. We have met the criteria of dietary homogeneity with the selection of subjects from Indian population.

OBJECTIVES

The objectives were to study membrane abnormalities in schizophrenia in terms of -

A) association of membrane PL and EPUFA composition

In order to determine whether membrane PL composition reflects a pattern of fatty acid composition, particularly with respect to EPUFAs, RBC membrane PL and fatty acid composition of never-medicated - first episode psychotic and schizophrenic patients with a short duration of illness, were compared with that of normal controls. Patients with the above characteristics were chosen to eliminate confounds due to drug treatment and chronicity of illness.

B) association of membrane EPUFA levels to psychopathology

Two designs were used to study the relationship of membrane EPUFAs and psychopathology -

***cross sectional*:** involved comparisons of membrane EPUFA levels and psychopathology of patients in various stages of illness and, with and without treatment, namely, never medicated first-episode schizophrenics (FES), never-

medicated schizophrenics with short duration of illness (NM-SZ) and medicated schizophrenics (M-SZ), and

***longitudinal*:** involved comparisons of membrane EPUFA levels of first-episode schizophrenics at baseline (BS-SZ) and after a four-year follow up (FU-SZ).

METHODS

SUBJECTS

The patients enrolled were consecutive admissions to outpatient treatment units of Kripamayee Institute of Mental Health, Miraj, India, and private hospitals in Pune, India. Diagnosis of schizophrenia was according to DSM IV (American Psychiatric Association, 1994). The first episode psychotics (FEP) were patients with less than one month of duration of illness. The FES patients were those who met the diagnosis of schizophrenia after six months of follow-up. The controls consisted of healthy volunteers, who did not have history of psychosis and major mood disorder and did not use any medication. These were enrolled from the general population and academic community via advertisement. They were matched for age and gender. The patients and controls were excluded for seizure disorder, head injury with loss of consciousness, alcohol and substance abuse and dependence, and for diabetes, cardiovascular disease, hypertension or a family history of the same. Informed consent was obtained from all the subjects. The demographic characteristics of the subjects are considered in the respective sections.

BIOCHEMICAL ANALYSES

The analyses were carried out by investigators blind to subject status.

Fasting venous blood was collected on the day of enrolment in tubes containing 100 µl of 0.5 M EDTA. RBCs were separated by centrifugation of the whole blood at 800 *g* for 15 min. 1 ml of packed RBCs was used for quantitation of PL classes, and remaining stored at -70^{o}C until sent to Laxdale Ltd. for fatty acid analysis or required for future use.

PL ANALYSIS

RBC MEMBRANE ISOLATION

RBC membranes were isolated using a protocol that was modified from the method described by Dodge et al (1967). 1 ml of packed RBCs was lysed with 9 ml of chilled distilled water. This was then centrifuged at 8000 *g* for 15 min. at 4^{o}C. The supernatant was discarded and pellet washed twice with 10 ml of normal saline (0.9%). The pellet was resuspended in 1 ml normal saline. This was used for lipid extraction.

RBC MEMBRANE LIPID EXTRACTION

Membrane lipids were extracted by the method described by Folch et al (1957). 20 volumes of 2:1 chloroform : methanol (containing 0.005% butylated hydroxy toluene) was added to 1 ml of RBC membrane suspension. This was vortexed, allowed to stand for 15 min. and centrifuged at 600 *g* for 10 min. Supernatant (lipid extract) was collected and dried completely under argon. RBC membrane lipids were redissolved in 1 ml of 2:1 chloroform: methanol and stored at -70^{o}C until used for separation and quantitation of the PL classes.

SEPARATION AND QUANTIFICATION OF MEMBRANE PL CLASSES

Separation and quantification of the various PL classes was carried out by the method described by Skipski et al (1964). The PL classes were separated using high performance thin layer chromatography (HPTLC) and quantitated using scanning laser densitometry. 50 µl of the lipid extract was applied to HPTLC plates (20 $\times$ 20 cm, precoated with 0.2 mm silica gel 60 obtained from E. Merck, catalogue no.1.05641) at a distance of 1 cm above the edge of the plate, as 6 mm streaks with a space of 5 mm between adjacent streaks, with an automated sample applicator (LINOMAT IV, CAMAG) equipped with a 100 µl syringe. Plates were activated prior to sample application by heating at 110^{o} C for 10 min. The composition of the mobile phase used for separation was chloroform: methanol: glacial acetic acid: water: 60: 50: 1: 4. The development chamber was preconditioned with the mobile phase for one hour. The plate was developed till the mobile phase reached 7 cm from the

bottom edge. The plate was air dried and then dipped in a tank containing molybdenum blue reagent (Sigma Chemical Co. Ltd., Cat. No. M3389) and heated at 120^{o}C for 5 min. Dark blue bands were observed against a blue background. The background was decolorized by exposing the plate to ammonia vapors. The bands were quantitated with a TLC scanner II (CAMAG). Intensities were measured with a light beam slit of 4 $\times$ 5 mm in the reflectance-absorbance mode at 720 nm. Relative absorbance percentages were computed using Camag TLC software (CATS, version 3.1).

FATTY ACID ANALYSIS

The RBCs were air freighted to Laxdale Ltd, Scotland, UK, under dry ice for fatty acid analysis. The procedure used was revised from the original method of Manku et al (1983). The method is detailed below.

LIPID EXTRACTION

On thawing, RBCs were suspended in NaCl/H_2SO_4 aq.(17mmol/L NaCl, 1mmol/L H_2SO_4, 1.8 ml), 3 ml of methanol was added and was thoroughly mixed. 6 ml of chloroform was added and the sample was stirred vigorously using a vortex mixer. After centrifugation at 2000 *g* for 10 min, the lower layer containing the lipid extract was carefully removed and filtered through sodium sulfate before evaporation to dryness.

PREPARATION OF METHYL ESTERS

The lipid extract was transesterified using H_2SO_4/methanol. These were purified by loading onto a isohexane-washed silica column prior to elution with isohexane: diethyl ether (95:5).

ANALYSIS BY GAS CHROMATOGRAPHY

The resulting methyl esters of the fatty acids were separated and measured using a Hewlett Packard HP 5890 Series II Plus Gas chromatograph (Cp-wax 52CB 25m capillary column, Chrompack UK). The carrier gas was hydrogen (1 ml/min.). Oven temperature was programmed to rise from 170^{o}C to 220^{o}C at 4^{o}C/min. Detector

temperature was 300^{o}C and injector temperature 230^{o}C. Retention times and peak areas were automatically computed by Hewlett-Packard HP 3365 Chem Station software (Revision A.06.01). Peaks were identified by comparison with standard methyl fatty acid ester (Sigma UK, Poole, Dorset).

CLINICAL ASSESSMENTS

Patients were rated for psychopathology using Positive and Negative Symptom Scale (PANSS) (Kay et al, 1987), Brief Psychiatric Rating Scale (BPRS) (Overal and Gorham, 1962), Heinrichs' Quality of Life (QOL) (Heinrichs et al, 1984) and Strauss and Carpenter Outcome Scale (O). The assessments were carried out within the week of enrolment by trained psychologists, who were tested at intervals for inter-rater reliability with one of the co-investigators (Dr. Madhav Ghate). Dr. Ghate has established inter-rater reliability (>0.89) with investigators in the USA (Drs. Denise Evans, Veterans Affairs Medical Center, Augusta, GA; and Dr. Matchery Keshavan, University of Pittsburgh, Pittsburgh, PA) by assessing the patients in the USA as well as in India. Drs. Evans and Keshavan have been consultants on this project for the last 5 years. Assessments were based on the information from the patient, a relevant informant accompanying the patient and from hospital records.

STATISTICAL ANALYSES

Nonparametric statistics was employed to analyze the data. Kruskal-Wallis analysis of variance (ANOVA) was used for comparisons between the groups and Mann-Whitney tests for bivariate comparisons. Fisher Exact test was used to compare group differences for categorical variables. Correlation between variables was studied using Pearson's correlation analysis.

A) ASSOCIATION OF MEMBRANE PL AND EPUFA COMPOSITION

RESULTS

Table IIa shows the demographic characteristics of the patient groups, first episode psychotics (FEP) and never-medicated schizophrenics (SZ), and normal controls (NC).

These groups do not differ significantly from each other with respect to age and ratio of males to females. The age of onset of illness between the patient groups, FEP and SZ, was not significantly different.

TABLE IIa: Demographic characteristics of subjects

GRP	AGE	M:F	AGE-OF-ONSET (y)	DURATION-ILLNESS
FEP (n=15)	32.47 ± 9.69	9:6	32.47 ± 9.69	9.0 (d) ± 6.32
SZ (n=11)	29.64 ± 10.13	8:3	25.91 ± 8.01	3.91 (y) ± 6.23
NC (n=4)	34.0 ± 10.54	3:1	NA	NA

FEP - first episode psychotics; SZ - never-medicated schizophrenics; NC - normal controls; M:F - Male:Female Ratio; d - days; y - years.Values are expressed as Mean ±SD.

There were differences with respect to membrane PL composition between the patient groups and normals (Table IIb) (Fig. 12 and 13).

TABLE IIb: Distribution of various membrane PL classes of first episode psychotics, chronic schizophrenics and normal controls

GRP	SM	PC	PE	PI	PS	PC+PE+PI+PS
FEP (n=15)	22.02 [a] ± 5.99 p=0.01	34.73 ± 3.45	27.43 ± 4.55	1.22 ± 0.78	14.32 ± 4.1	77.62 [a] ± 7.09 p=0.01
SZ (n=11)	22.05 [a] ± 4.23 p=0.02	36.55 ± 4.76	28.67 ± 3.91	0.83 ± 0.49	11.91 ± 3.57	77.96 [a] ± 4.25 p=0.02
NC (n=4)	14.64 ± 1.58	38.09 ± 4.64	31.27 ± 1.88	1.7 ± 1.7	14.26 ± 1.58	85.31 ± 1.73

FEP - first episode psychotics; SZ - never-medicated schizophrenics; NC - normal controls; SM - Sphingomyelin; PC - Phosphatidyl choline; PE - Phosphatidyl ethanolamine; PI - Phosphatidyl inositol; PS - Phosphatidyl serine;
Values are expressed as Mean ±SD. The phospholipids (PL) are expressed as % of total PL.
a - statistically significant differences between FEP or SZ groups and NC.

PC, PE, PI, PS and the sum of these - (PC+PE+PI+PS) were lower in patients (exception: PS in FEP was higher) than in normals. SM was higher in patients as compared to normals.

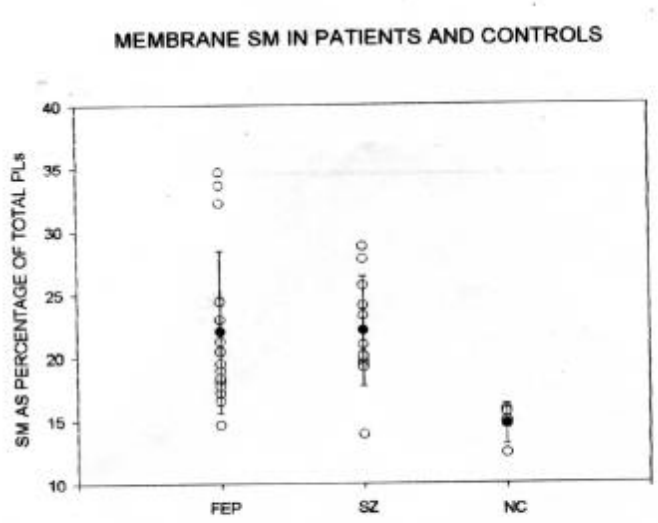


Fig . 12

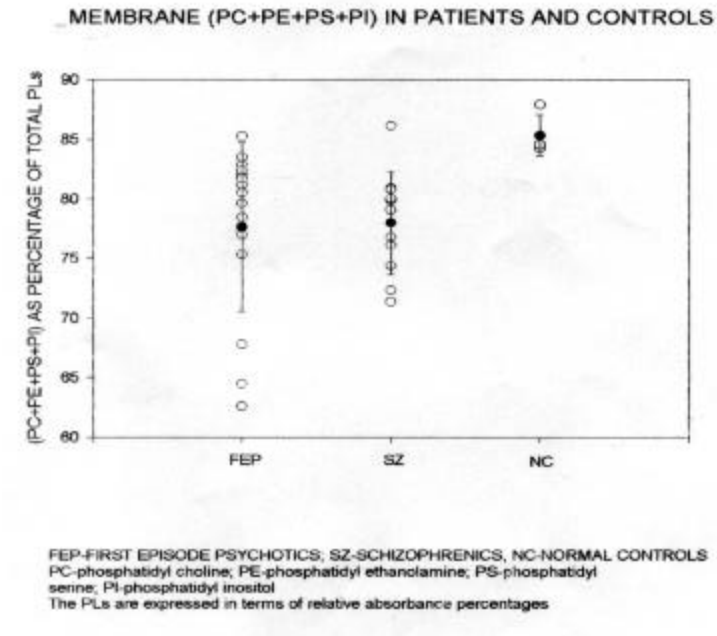


Fig. 13

ANOVA showed a significant effect of group on the distribution of SM (H=8.07, df=2, P=0.02) and the sum of PC, PE, PI and PS (H=7.95, df=2, P=0.02). Post hoc Mann-Whitney tests revealed that the differences with respect to SM and the sum of PC, PE, PI and PS were statistically significant. ANOVA did not show a significant effect of group on the distribution of individual PLs, PC, PE, PI and PS.

There were differences with respect to membrane fatty acid composition between the patient groups and normals (Table IIc). AA, total ω-6 fatty acids, DHA and total ω-3 fatty acids were lower in patients as compared to normals. Compensatory increases in saturated, total ω-7 and ω-9 fatty acids were observed.

TABLE IIc: Distribution of membrane fatty acids of first episode psychotics, chronic schizophrenics and normal controls

GRP	AA	T ω6	DHA	T ω3	T SFA	T ω7	T ω9
FEP (n=15)	5.71 ± 2.62	16.76 [a] ± 5.68 *p=0.08*	0.48 ± 0.38	0.98 [a] ± 0.74 p=0.05	45.51 ± 8.45	2.36 ± 0.35	18.47 ± 3.34
SZ (n=11)	4.9 ± 1.48	16.11 [a] ± 2.55 p=0.01	0.38 ± 0.2	0.68 [a] ± 0.4 p=0.02	44.19 ± 4.04	2.58 ± 0.36	17.42 ± 2.18
NC (n=4)	7.58 ± 3.98	23.46 ± 5.37	1.14 ± 0.93	2.23 ± 1.28	44.02 ± 8.03	2.23 ± 0.44	18.58 ± 4.8

FEP - first episode psychotics; SZ - never-medicated schizophrenics; NC - normal controls
AA-arachidonic acid; DHA - docosahexaenoic acid;
T SFA - total saturated fatty acids = C14:0, C16:0, C18:0, C20:0, C22:0, C24:0;
T ω7 - total ω7 fatty acids = C16:1ω7, C18:1ω7;
T ω9 - total ω9 fatty acids = C18:1ω9, C20:1ω9, C20:3ω9, C22:1ω9, C24:1ω9;
T ω6 - total ω6 fatty acids = C18:2ω6, C18:3ω6, C20:2ω6, C20:3ω6, C20:4ω6, C22:4ù-6, C22:5ω6;
T ω3 - total ω3 fatty acids = C18:3ω3, C18:4ω3, C20:3ω3, C20:5ω3, C22:5ω3, C22:6ω 3;
Values are expressed as Mean ± SD. The fatty acids (FA) are expressed as % of total FA.
a - statistically significant differences between the SZ groups and NC.

ANOVA showed a significant effect of group for total ω-6 (H=6.4, df=2, P=0.04) and total ω-3 (H=6.84, df=2, P=0.03) fatty acids. Post hoc Mann-Whitney tests revealed significant differences between the patient groups (FEP and SZ) and normal controls (NC).

ANOVA did not show a significant effect of group on the distribution of AA, DHA, and total saturated, ω-7 and ω-9 fatty acids.

DISCUSSION

This is the first report on the association of altered EPUFA distribution with the abnormal membrane PL composition in first episode psychotics and schizophrenics. We found significantly lower levels of the sum of the PLs, PC, PE, PI and PS in the patients as compared to normals. In the same patients, we also found significantly lower levels of membrane total ω-6 and ω-3 fatty acids as compared to normal controls. This is in keeping with the fact that these are the phospholipids, which attach the EFAs at the sn-2 position. Our results are consistent with the observation of others. Keshavan et al (1993a) have reported significantly low levels of PE and a trend towards decreased PI in RBCs from unmedicated chronic schizophrenic patients. This was attributed to decreased PL synthesis and increased breakdown observed with ^{31}P magnetic resonance spectroscopy studies. PE is especially enriched with EPUFAs, and hence prone to breakdown due to increased peroxidation attributed to oxidative stress in schizophrenia. We did not observe significantly reduced PE levels in our sample population probably because the EPUFA reduction in our population is much lower as compared to the population (from USA) used by Keshavan et al (1993a). In fact we found that the sum of all the PLs, which contain EPUFAs, namely, PE, PC, PS and PI were significantly lower in the patient groups as compared to controls. Mahadik et al (1994) have also reported significantly decreased PE, PI and PS in cultured skin fibroblasts from unmedicated first break psychotics.

The decreased levels of the PLs, PE, PC, PS and PI have been found to be compensated for by increased SM. We found significantly increased SM in both the first episode psychotics and schizophrenics as compared to normals. Although lower

levels of saturated, ω-7 and ω-9 fatty acids, (which are likely to be attached to SM at the sn2 position) were found in the patients, the differences were not statistically significant.

Earlier studies, reviewed by Rotrosen and Wolkin (1987), also found altered levels of membrane PLs in schizophrenic patients. Although, the results of these studies have been varied, reduced PE has been a common finding. Increased PS and increased or decreased PC and PI have also been reported. We have found varying fatty acid profiles, particularly with respect to saturated, ω-7 and ω-9 fatty acids which are proportionally higher in SM, PC, PS, PI as compared to PE, in schizophrenic patients in early illness and in chronic stages, and in never-medicated and those treated with antipsychotics (reported in the latter section). Thus the variations in the membrane PL profiles may only be a reflection of the fatty acid profiles in these studies which have used different types of patients. Some of these studies (Kelsoe et al, 1982; Tolbert et al, 1983) have found reduced activity of the enzymes responsible of the synthesis of PLs (PC). However, this situation may also be the result of low availability of substrate, namely, EPUFAs required for the synthesis of the PLs. The increase in SM explains the operation of compensatory mechanisms since it utilizes endogenously synthesized fatty acids for its synthesis.

EPUFAs are especially enriched in the brain, where they play a prominent role in determining the PL composition of cell membranes (Thompson, 1992). In view of this, low EPUFA availability may cause drastic changes in neuronal PL composition, and alter cell membrane structure and affect function.

B) ASSOCIATION OF EPUFA WITH PSYCHOPATHOLOGY

a) CROSS SECTIONAL DESIGN

RESULTS

Table IId shows the demographic characteristics of the patient groups, first episode schizophrenics (FES), never-medicated schizophrenics (NM-SZ) and medicated schizophrenics (M-SZ), and the normal controls (NC). These groups do not differ significantly from each other with respect to age. The male to female ratio was higher in patient groups, similar to that generally reported for schizophrenia. The age of

onset of psychosis was significantly higher in the never-medicated groups (FES and NM-SZ) as compared to M-SZ. The mean duration of illness of NM-SZ and M-SZ was statistically significant.

TABLE IId: Demographic characteristics of subjects

GRP	AGE	M:F	AGE-OF-ONSET (y)	DURATION-ILLNESS
FES (n=7)	28.28 ± 10.17	3:4	28.57 ± 10.19	9.43 ± 6.43 (d)
NM-SZ (n=13)	30.00 ± 9.25	9:4	26.38 [c] ± 8.26 p=0.04	3.78 [c] ± 5.7 (y) p<0.001
M-SZ (n=32)	31.31 ± 10.31	21:11	21.19 ± 7.33	10.12 ± 6.92 (y)
NC (n=45)	29.24 ± 8.87	25:20	NA	NA

FES - first episode schizophrenics; NM-SZ - never-medicated schizophrenics;
M-SZ - medicated schizophrenics; M: F is Male:Female Ratio; d - days; y - years
Values are expressed as Mean ±SD.
c - statistically significant differences between the NM-SZ and M-SZ

The percent distribution of RBC membrane AA and DHA, as well as total ω-3 and ω-6 fatty acids was lower in all the patient groups (FES, NM-SZ, and M-SZ) compared to NC. Compensatory increases were observed in percent distribution of saturated, ω-7 and ω-9 fatty acid (Table IIe) (Fig. 14 and 15).

ANOVA showed a significant effect of group on the percent distribution of AA (H=30.4, df=3, P=< 0.001), total ω-6 fatty acids (H=36.22, df=3, P=<0.001), DHA (H=26.69, df=3, P=<0.001) and total ω-3 fatty acids (H=22.15, df=3, P=<0.001). Post hoc Mann-Whitney tests showed that differences with respect to the EPUFAs for FES as well as NM-SZ, as compared to M-SZ and NC group were statistically significant. The M-SZ, although seemed to have lower percent distribution of membrane EPUFAs as compared to NC, the decrease was not statistically significant.

TABLE IIe: Distribution of RBC membrane fatty acids of never-medicated and medicated schizophrenics and normal controls

GRP	AA	T ω6	DHA	T ω3	T SFA	T ω7	T ω9
FES (n=7)	6.44 [ac] ± 3.58 p=0.002 p=0.008	18.56 [ac] ± 7.05 p<0.001 p<0.001	0.6 [ac] ± 0.63 p=0.005 p=0.03	1.26 [ac] ± 1.26 p=0.02 p=0.02	44.79 [abc] ± 4.07 p<0.001 p<0.001 p<0.001	2.33 [bc] ± 0.28 p<0.001 p=0.007	17.31 ± 0.28
NM-SZ (n=13)	5.43 [ac] ± 1.97 p<0.001 p<0.001	17.22 [ac] ± 4.14 p<0.001 p<0.001	0.46 [ac] ± 0.30 p<0.001 p<0.001	0.92 [ac] ± 0.82 p<0.001 p<0.001	45.03 [a] ± 4.45 p<0.001	2.72 [ac] ± 0.54 p<0.001 p<0.001	17.99 ± 2.59
M-SZ (n=32)	11.23 ± 3.78	31.67 ± 7.07	1.2 ± 0.89	2.53 ± 1.25	45.68 [a] ± 5.59 p<0.001	2.16 [a] ± 0.36 p=0.01	18.4 [a] ± 3.55 p=0.03
NC (n=45)	12.12 ± 3.33	32.22 ± 6.45	2.02 ± 1.28	3.60 ± 1.70	43.34 ± 3.97	1.96 ± 0.29	16.97 ± 2.42

FES - first episode schizophrenics; NM-SZ - never-medicated schizophrenics;
M-SZ - medicated schizophrenics; NC - normal controls
AA-arachidonic acid; DHA - docosahexaenoic acid;
T SFA - total saturated fatty acids = C14:0, C16:0, C18:0, C20:0, C22:0, C24:0;
T ω7 - total ω7 fatty acids = C16:1ω7, C18:1ω7;
T ω9 - total ω9 fatty acids = C18:1ω9, C20:1ω9, C20:3ω9, C22:1ω9, C24:1ω9;
T ω6 - total ω6 fatty acids = C18:2ω6, C18:3ω6, C20:2ω6, C20:3ω6, C20:4ω6, C22:4ω6, C22:5ω6; T ω3 - total ω3 fatty acids = C18:3ω3, C18:4ω3, C20:3ω3, C20:5ω3, C22:5ω3, C22:6ω3
Values are expressed as Mean ± SD. The fatty acids (FA) are expressed as % of total FA.
a - statistically significant differences between the SZ groups and NC
b - statistically significant differences between the FES and NM-SZ
c - statistically significant differences between the FES or NM-SZ and M-SZ

ANOVA also showed a significant effect of group on the percent distribution of total saturated (H=47.69, df=3, P=<0.001) and total ω-7 (H=29.86, df=3, P=<0.001) fatty acids. Post hoc Mann-Whitney tests showed that differences with respect to percent distribution of total saturated fatty acids for FES, NM-SZ and M-SZ, as compared to NC were statistically significant. FES also had significantly lower percent distribution of saturated fatty acids as compared to the chronic SZ groups.

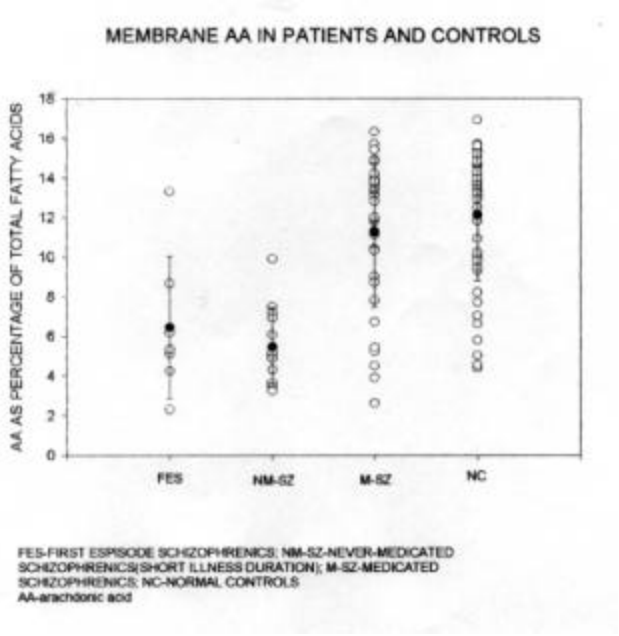


Fig. 14

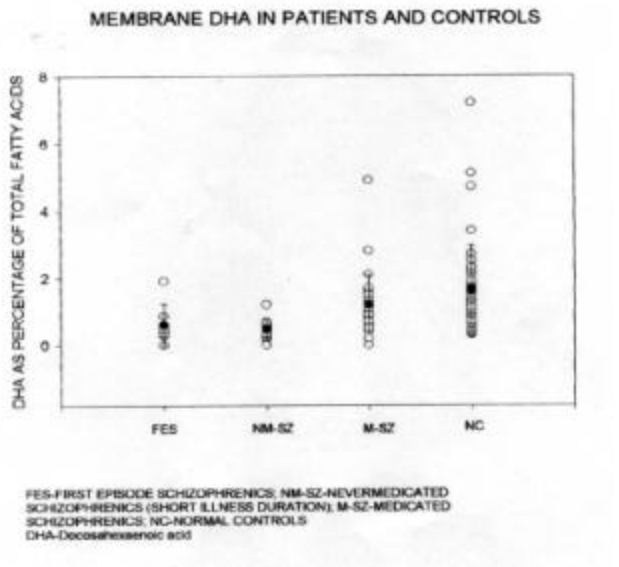


Fig. 15

Percent distribution of total ω-7 fatty acids was significantly lower in the FES as compared to NM-SZ, however both were significantly higher with respect to the M-SZ. ANOVA did not show a significant effect of group on the percent distribution of

total ω-9 fatty acids. However, post hoc Mann-Whitney tests revealed a significant difference between the M-SZ and NC.

FES showed higher positive symptom scores than both NM-SZ and M-SZ, whereas negative symptom scores were higher in the chronic SZ group (Table IIf). However, PANSS total scores were highest for NM-SZ group that also had the lowest membrane, EPUFA levels, and the lowest for the M-SZ that had the highest membrane EPUFA levels among the three groups (Fig. 16).

TABLE IIf: Psychopathology scores of never-medicated and medicated schizophrenics

GRP	P TOT	N TOT	G TOT	PANSS T	BPRS T
FES (n=7)	21 [c] ± 7.1 p=0.02	11 [bc] ± 5.3 p=0.008 p<0.004	43 [bc] ± 4.5 p=0.03 p=0.03	75 ± 11.5	44 ± 7.9
NM-SZ (n=13)	17 [c] ± 4.4 p=0.03	22 [c] ± 7.0 p=0.09	43 [c] ± 5.4 p=0.006	85 [c] ± 10.5 p=0.003	41 ± 10.2
M-SZ (n=32)	14 ± 6.3	18 ± 6.3	36 ± 8.2	68 ± 14.0	36 ± 7.8

FES - first episode schizophrenics; NM-SZ - never-medicated schizophrenics;
M-SZ - medicated schizophrenics;
P TOT - PANSS - positive symptom factor score; N TOT - PANSS - negative symptom factor score; G TOT - PANSS - general psychopathology cluster score;
PANSS T - Positive and Negative Symptom Scale - Total score= P TOT + N TOT + G TOT;
BPRS T - Brief Psychiatric Rating Scale - Total;
Values are expressed as Mean ± SD.
b - statistically significant differences between the schizophrenic groups
c - statistically significant differences between the FES or NM-SZ and M-SZ

ANOVA did not show a significant effect of group on BPRS scores of the patients. However, it did show a significant effect of group on PANSS total score (H=9.2, df=2, P=0.01), PANSS-positive symptom factor (H=8.8, df=2, P=0.012), PANSS-negative symptom factor (H=11.5, df=2, P=0.003) and PANSS-general psychopathology cluster scores (H=10.3, df=2, P=0.006). Post hoc Mann-Whitney

tests revealed significant differences with respect to total PANSS, positive, negative and general psychopathology scores of both the FES and NM-SZ, as compared to M-SZ group.

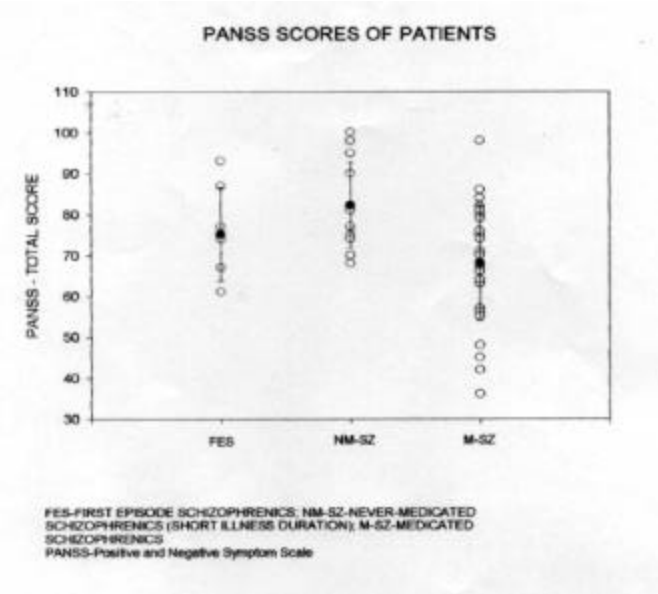


Fig. 16

In the case of M-SZ, the EPUFAs showed a trend towards a negative correlationship with total PANSS scores and that of a positive correlationship with the saturated, total ω-7 and ω-9 fatty acids (Table IIg).

TABLE IIg: Correlation between various fatty acids and psychopathology of chronic, medicated schizophrenics

	T SFA	T ω7	T ω9	AA	T ω6	DHA	T ω3
N TOT	r= 0.28 p=0.13	0.34 p=0.07	0.09 p=0.65	-0.27 p=0.16	-0.25 p=0.18	-0.25 p=0.19	-0.27 p=0.14

AA-arachidonic acid; DHA - docosahexaenoic acid; T SFA - total saturated fatty acids = C14:0, C16:0, C18:0, C20:0, C22:0, C24:0; T ω7 - total ω7 fatty acids = C16:1ω7, C18:1ω7; T ω9 - total ω9 fatty acids = C18:1ω9, C20:1ω9, C20:3ω9, C22:1ω9, C24:1ω9; T ω6 - total ω6 fatty acids = C18:2ω6, C18:3ω6, C20:2ω6, C20:3ω6, C20:4ω6, C22:4ω6, C22:5ω6; T ω3 - total ω3 fatty acids = C18:3ω3, C18:4ω3, C20:3ω3, C20:5ω3, C22:5ω3, C22:6ω3 .

DISCUSSION

EPUFA LEVELS IN THE SCHIZOPHRENIC PATIENT GROUPS AND NORMAL CONTROLS

This is the first detailed report of reduced RBC membrane EPUFAs in never-medicated patients. We found low membrane EPUFAs, AA and DHA, in never-medicated, both first episode schizophrenics (FES) and schizophrenics with a short duration of illness (NM-SZ) as well as in chronic patients treated with antipsychotics (M-SZ), as compared to normal controls. Reduced RBC membrane EPUFAs in FES indicates the presence of the abnormality in early stages of the illness, and thus, negates its attribution to disease chronicity and drug treatment. Low membrane ω-3 fatty acids in cultured skin fibroblasts of never-medicated first episode as well as chronic schizophrenics have also been reported by Mahadik et al (1996). The decrease in only DHA, and not AA, as reported in this study is probably because low levels of AA are due to lipid peroxidation occurring under disease state (Peet et al, 1995; Ramchand et al, 1996), and skin fibroblasts in culture are spared from the factors responsible for lipid peroxidation in schizophrenics. Decrease in Δ4 desaturation was also cited as a possibility for decreased DHA (since decreased C22:5 ω-6 was also observed); this would not affect AA. In medicated patients, EPUFA levels, except DHA, were comparable to levels in normals. This indicates that treatment response is probably related to improved DHA status of the membranes.

There are several reports of reduced levels of RBC membrane EPUFAs in chronic medicated patients (Vaddadi et al, 1989; Yao et al, 1994; Glen et al, 1994; Peet et al, 1995; Assies et al, 2001). However, our data clearly indicate that reduced EPUFA levels are associated with the onset of psychopathology of schizophrenia, and that treatment with antipsychotics (primarily atypicals - see later) may improve the EPUFA levels and thereby psychopathology. The reductions in the levels of EPUFAs in our study are smaller as compared to that in some studies that are done in chronic and older patients (Yao et al, 1994; Glen et al, 1994; Peet et al, 1995). Our data is similar to the study of patients in early psychosis by Assies et al (2001). Assies et al (2001) found significant reductions in levels of ω-3 EPUFAs, particularly DHA, but not AA.

EPUFA LEVELS IN NEVER-MEDICATED SCHIZOPHRENIC GROUPS (FIRST EPISODE SCHIZOPHRENICS AND SCHIZOPHRENICS WITH A SHORT DURATION OF ILLNESS)

The fact that EPUFAs are higher, on an average, in never-medicated FES than in NM-SZ (though this difference is not statistically significant) indicates that the low membrane EPUFAs are present in early stages of the disease and that further decrease with disease progression in the absence of treatment is possible. The total PANSS scores are also lower in the FES as compared to NM-SZ. However, characteristically, the positive symptom scores are higher in the FES and negative symptom scores in the NM-SZ. Thus our results indicate that the membrane abnormality, low AA and DHA, and total ω-6 and ω-3 fatty acids are associated with the prominent positive symptoms in the early stages of the disease. A probable further decrease of these with disease progression may be responsible for the manifestation of the negative symptoms. However, the small number of patients in the two groups, due to the difficulty in obtaining such patients, make these conclusions highly speculative. It is noteworthy, though, that Glen et al (1994) have also found higher AA and DHA in patients with prominent positive symptoms and low levels of the same in patients with negative symptoms.

EPUFA LEVELS IN NEVER-MEDICATED AND MEDICATED SCHIZOPHRENIC GROUPS

We found low membrane EPUFA levels and severe psychopathology in never-medicated as compared to medicated schizophrenic patients. Never-medicated patients had a significantly later onset of psychosis and also longer duration of untreated illness compared to medicated patients. These patients had a rural domicile and were enrolled at a rural Mental Health Center. Traditionally, the rural Indian population has a very high tolerance for illness. Patients may have been suffering with mild symptoms for a significantly long period of time; mild course of illness has been reported in patients from rural India (WHO, 1973; Jablensky et al, 1991). They may seek care only after significant deterioration. In addition, inaccessible mental care may have contributed to the delayed observed/reported illness by patient and family and hence the later onset and the longer duration of untreated illness. These characteristics are similar to that generally found in patients

from community mental health centers. Medicated patients, on the other hand, were from urban community where patients generally have less tolerance for the illness, more awareness of treatment, affordability of the treatment costs, and easily accessible mental care. In addition, they may have had severe symptoms at onset. These differences provide important insights into the role of EPUFAs in psychopathology and outcome.

The EPUFA levels suggest that in never-medicated patients, illness may have had an initial mild course, but if left untreated for a long period of time it may lead to further reductions in levels of EPUFAs and probably some progressive neurostructural changes contributing to the severe psychopathology as reported by Wyatt et al (1997) which has been suggested as an important rationale for the early intervention (Wyatt and Henter, 2001). In patients with early onset and severe symptomatology, early itreatment intervention may have prevented the progressive deterioration and hence the observed mild psychopathology.

Majority of the patients in the M-SZ group was treated with atypical antipsychotics. Thus the data indicates that treatment with atypical antipsychotics is associated with higher membrane EPUFA levels. Although the medicated patients are different from never-medicated patients in terms of the age of onset and chronicity of illness, all the patients have same racial makeup and similar dietary patterns and life style. Horrobin (1999) has reported a significant increase in RBC membrane AA and DHA in schizophrenic patients after 10-12 weeks of treatment with atypical antipsychotic, clozapine.

There was no significant correlation between individual fatty acids and any of the clinical scores in NM-SZ or M-SZ patients. Glen et al (1994) have reported higher membrane AA and DHA in patients with prominent positive symptoms as compared to those with prominent negative symptoms. We only found a trend towards a negative correlation of the EPUFAs with the negative symptom scores in the M-SZ. However, in medicated patients, the elevated levels of EPUFAs, particularly DHA and AA were significantly associated with improved positive, negative and general psychopathology clusters of PANSS scores.

Thus, among the patient groups, lowest membrane EPUFAs were found in NM-SZ with the highest PANSS scores and highest membrane EPUFAs in M-SZ with the lowest PANSS scores suggesting that membrane EPUFA levels may correlate with psychopathology and thereby outcome.

ASSOCIATION EPUFA WITH PSYCHOPATHOLOGY

b) LONGITUDINAL ANALYSIS

RESULTS

Table IIh shows the demographic characteristics of the patient group and normal controls (NC).

TABLE IIh: Demographic characteristics of subjects

GRP	AGE	M:F	AGE-OF-ONSET (y)	DURATION-ILLNESS
FES (n=7)	28.28 ± 10.17	3:4	28.57 ± 10.19	9.43 (d) ± 6.43
NC (n=45)	29.24 ± 8.87	25:20	NA	NA

FES - first episode schizophrenics; NC - normal controls;
M:F is Male:Female Ratio; d - days; y - years
Values are expressed as Mean ±SD.

These groups do not differ significantly from each other with respect to age and the ratio of males to females.

The percent distribution of RBC membrane AA and DHA, as well as total ω-3 and ω-6 (except for the FU group) fatty acids was lower in the patient groups (BS-SZ and FU-SZ) as compared to NC (Table IIi) (Fig. 17 and 18). Compensatory increases were observed in percent distribution of saturated, ω-7 and ω-9 fatty acid.

ANOVA showed a significant effect of group on the percent distribution of AA ($H=15.31$, $df=2$, $P=< 0.001$), total ω-6 fatty acids ($H=12.63$, $df=2$, $P=0.002$) and DHA ($H=8.67$, $df=2$, $P=0.013$). Post hoc Mann-Whitney tests showed that the differences with respect to the EPUFA for the BS-SZ and normals were statistically significant;

total ω-6 fatty acids were also significantly different between the schizophrenics at baseline, BS-SZ and at four year follow-up, in FU-SZ.

TABLE IIi: Distribution of membrane fatty acids of schizophrenics at baseline and after a four year follow-up

GRP	AA	T ω6	DHA	T ω3	T SFA	T ω7	T ω9
BS-SZ (n=7)	6.44 [a] ± 3.58 p=0.002	18.56 [ab] ± 7.05 p<0.001 p=0.004	0.60 [a] ± 0.63 p=0.005	1.26 [ab] ± 1.26 p=0.02 p=0.04	44.79 [ab] ± 4.07 p<0.001 p<0.001	2.33 [b] ± 0.28 p=0.004	17.31 ± 3.12
FU-SZ (n=7)	8.66 [a] ± 2.09 p=0.009	32.76 ± 4.93	1.11 ± 0.35	2.71 ± 0.43	42.83 [a] ± 2.92 p=0.02	3.43 [a] ± 0.8 p<0.001	19.01 [a] ± 2.43 p=0.04
NC (n=45)	12.12 ± 3.33	32.22 ± 6.45	2.02 ± 1.28	3.60 ± 1.70	43.34 ± 3.97	1.96 ± 0.29	16.97 ± 2.42

BS-SZ - FES studied at baseline, at the start of the four-year follow-up study
FU-SZ - FES studied after four years of follow-up; NC - normal controls
AA-arachidonic acid; DHA - docosahexaenoic acid;
T SFA - total saturated fatty acids = C14:0, C16:0, C18:0, C20:0, C22:0, C24:0;
T ω7 - total ω7 fatty acids = C16:1ω7, C18:1ω7;
T ω9 - total ω9 fatty acids = C18:1ω9, C20:1ω9, C20:3ω9, C22:1ω9, C24:1ω9;
T ω6 - total ω6 fatty acids = C18:2ω6, C18:3ω6, C20:2ω6, C20:3ω6, C20:4ω6, C22:4ω6, C22:5ω6; T ω3 - total ω3 fatty acids = C18:3ω3, C18:4ω3, C20:3ω3, C20:5ω3, C22:5ω3, C22:6ω3; Values are expressed as Mean ± SD. The FA are expressed as % of total FA.
a - statistically significant differences between the SZ groups and NC
b - statistically significant differences between the BS-SZ and FU-SZ

ANOVA did not show a significant effect of group on the percent distribution of total ω-3 fatty acids. However, post hoc Mann-Whitney tests revealed significant differences with respect to the BS-SZ and normals and between the schizophrenics at baseline, BS-SZ and at four-year follow-up, in FU-SZ.

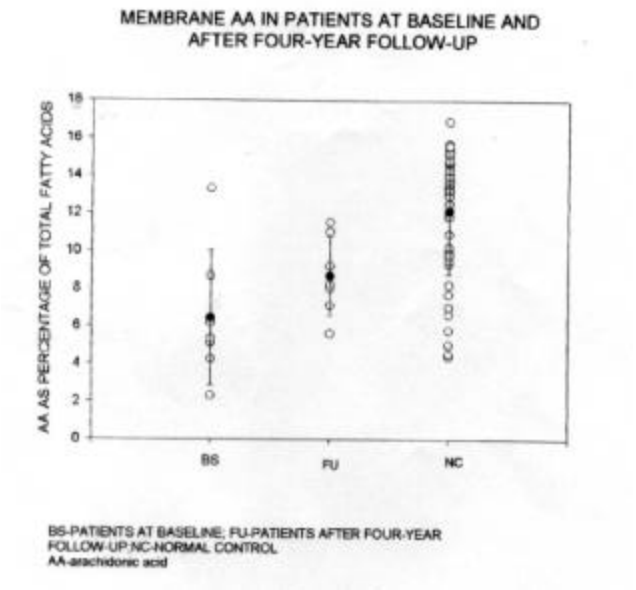


Fig. 17

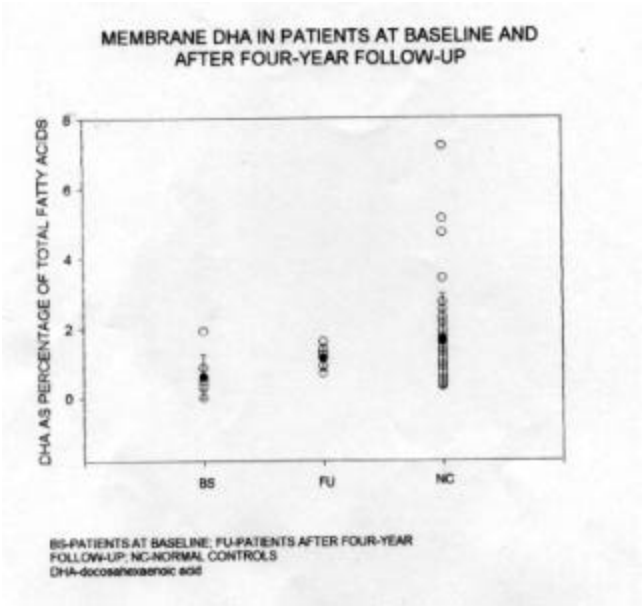


Fig. 18

ANOVA showed a significant effect of group on the percent distribution of total saturated (H=22.45, df=2, P=< 0.001) and total ω-7 fatty acids (H=14.76, df=2, P=<0.001). The differences were statistically significant between the BS-SZ and FU-SZ as well as between FU-SZ and normals as revealed by post hoc Mann Whitney tests. Distribution of saturated fatty acids was significantly different between the BS-

SZ and normals. ANOVA did not show a significant effect of group on the percent distribution of total ω-9 fatty acids. However, Mann-Whitney tests showed that the differences between the FU-SZ and NC were statistically significant.

The psychopathology scores were lower in the schizophrenics at follow up as compared to that at baseline (Table IIj) (Fig. 19). ANOVA did not show a significant effect of group on total BPRS and PANSS, or the positive, negative, general psychopathology clusters of PANSS scores. However, post hoc Mann-Whitney revealed significant differences between the schizophrenics at base line and follow-up with respect to total BPRS, and the positive, negative and general psychopathology subset of PANSS scores.

TABLE IIj: Psychopathology scores of schizophrenics at baseline and after a four year follow-up

GRP	P TOT	N TOT	G TOT	PANSS T	BPRS T
BS-SZ (n=7)	21 **b** ± 7.1 p=0.007	11 **b** ± 5.3 p=0.03	43 **b** ± 4.5 p=0.001	75 ***b*** ± 11.5 *p=0.07*	44 **b** ± 7.9 p=0.007
FU-SZ (n=7)	12 ± 4.2	16 ± 5.8	32 ± 6.0	60 ± 14.4	33 ± 5.4

BS-SZ - FES studied at baseline, at the start of the four-year follow-up study;
FU-SZ - FES studied after four years of follow-up; P TOT - PANSS - positive symptom factor score; N TOT - PANSS - negative symptom factor score; G TOT - PANSS - general psychopathology cluster score;
PANSS T - Positive and Negative Symptom Scale - Total score P TOT + N TOT + G TOT;
BPRS T - Brief Psychiatric Rating Scale - Total; Values are expressed as Mean ± SD.
b- statistically significant differences between the schizophrenic groups

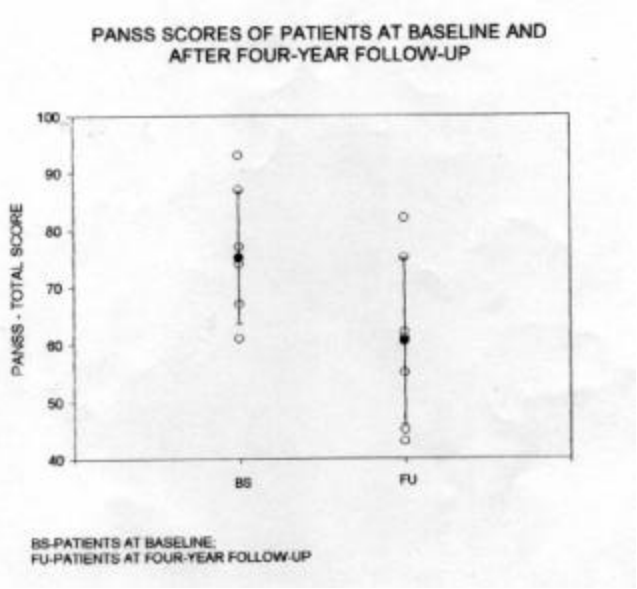


Fig. 19

DISCUSSION

This is the first report of membrane EPUFA levels in first episode schizophrenics at baseline and after a four-year follow-up. We observed significantly lower levels of membrane EPUFAs in the FES at baseline as compared to normal controls. These patients were subsequently treated with antipsychotics. Their EPUFA levels, except for AA, were not significantly different at follow-up as compared to normals. Significant improvement was also observed in their psychopathology scores. This indicates that improvement in membrane EPUFA levels in the schizophrenic patients was associated with improvement in psychopathology. In fact, we found that 3 out of the 7 patients showed marked improvement, as judged by the investigating psychologist, that reflected in the Quality of Life and Outcome scale scores. These patients showed significant improvement in the EPUFA, AA and DHA levels and a significant improvement in the psychopathology scores. The remaining 4 patients who only showed a moderate improvement also did not show significant improvement in the EPUFA or psychopathology scores. Although, these results are dramatic, the small number of patients in each group warrants further investigation on these lines.

These results, although on small number of patients, also suggest that membrane EPUFA levels may correlate with psychopathology and hence outcome.

CONCLUSIONS

The following conclusions can be drawn on the basis of data presented above -

1. Altered membrane PL composition is a result of the altered EPUFA levels
2. Reduced membrane EPUFA levels are present at the onset of psychosis
3. Treatment with antipsychotics, particularly with atypicals, is associated with improved membrane EPUFA levels.
4. Improvement in membrane EPUFA status is associated with improvement in psychopathology

Membrane EPUFA levels is associated with psychopathology and thus the course and outcome of schizophrenia. Oxidative stress observed in schizophrenia could result in low membrane EPUFA levels. Low availability and abnormal utilization may also result or contribute to the same.

CHAPTER III

DIETARY EPUFAs, LIPID PEROXIDATION AND MEMBRANE EPUFA LEVELS

THE CONTENT OF THIS CHAPTER IS UNDER PREPARATION AS A FULL– LENGTH PAPER

INTRODUCTION

Reduced membrane essential polyunsaturated fatty acids (EPUFAs) have been found in brain (Horrobin et al, 1991), erythrocytes (Glen et al, 1994; Yao et al, 1994; Peet et al, 1995; Peet et al, 1996; Assies et al, 2001) and cultured skin fibroblasts (Mahadik et al, 1996) of schizophrenic patients. EPUFAs are the pertinent components of the plasma membrane phospholipids (PLs) that contribute to a large extent to membrane fluidity and thus protein activity, and serve as second messengers in several neurotransmitter mediated signal transduction processes. Thus, low neuronal membrane EPUFAs may account for multitransmitter dysfunction and thereby contribute to the neuropathophysiology of schizophrenia. Data presented in Chapter II indicates that membrane EPUFA levels correlate with psychopathology, and thereby the course and outcome of schizophrenia. If reduced membrane EPUFAs are causative of severe psychopathology, knowledge of the factors affecting membrane EPUFA levels such as their dietary intake, utilization (elongation, desaturation and incorporation in membrane lipids), and breakdown (primarily by free radicals to peroxides) can have important implications in the management of schizophrenia.

Human beings cannot synthesize ω-3 and ω-6 series of fatty acids, the essential fatty acids (EFAs) and these must be obtained from the diet. These are further elongated and desaturated to form EPUFAs to be incorporated in membrane PLs. Alternatively, EPUFAs can be obtained from the diet. Therefore, dietary EPUFAs and their precursors will largely affect membrane EPUFA levels. EFA deficiency is known to be associated with low membrane EPUFA levels and concomitant altered brain development and membrane function (Simopoulos, 1991; Wainwright, 1992), which are also observed in schizophrenia. Moreover, intake of ω-3 fatty acids in schizophrenic patients is found to correlate negatively with the severity of symptoms (Peet et al, 1996). Also, the International Pilot Study of Schizophrenia (IPSS) has concluded that developing countries have a better outcome of the illness as compared to the developed countries (WHO, 1973; Jablensky et al, 1991; Leff et al, 1992) and correlation studies by Christensen and Christensen (1988) have indicated that a low fat, essential fatty acid rich diet of the

developing countries (indicative of better membrane EPUFA status) may have contributed to the better outcome. However, it remains to be directly confirmed that low membrane EPUFA levels in schizophrenia may be due to their low intake.

The ω-3 and ω-6 series EPUFAs are the most unsaturated fatty acid species and thus are highly susceptible to damage by reactive oxygen species [ROS]. Illness, high caloric intake, smoking, alcohol consumption and anti-psychotic drug treatment, conditions that may cause excessive production of ROS, are prevalent in schizophrenia (Mahadik and Gowda, 1996; Mahadik and Mukherjee, 1996). Increased levels of lipid peroxides, that is, thiobarbituric acid reactive substances (TBARS), have been reported in plasma (Peet et al, 1993; McCreadie et al, 1995; Evans et al, 1996) and cerebrospinal fluid [CSF] (Pall et al, 1987; Lohr et al, 1989) from chronic schizophrenic patients and never-medicated patients at the onset of psychosis (Mahadik et al, 1998). Also, there is evidence for impaired enzymatic (Buckman et al, 1990; Reddy et al, 1991; Liday et al, 1995; Mukherjee et al, 1996; Reddy and Yao, 1996) and non-enzymatic (Richardson-Andrews, 1990; Suboticanec et al, 1990; Brown, 1994; Liday et al, 1995) antioxidant defense. Thus it seems likely that lipid peroxidation due to oxidative stress may be responsible for low membrane EPUFA levels in schizophrenia.

We have found low membrane EPUFAs, AA and DHA, and total ω-6 and ω-3 fatty acids, in never-medicated, both first episode (FES) and schizophrenics with short duration of illness (NM-SZ), and in schizophrenics treated with antipsychotics (M-SZ) as compared to normal controls (NC) (Chapter II). In a subset of this sample, we studied the effect of both, intake of foods rich in ω-3 fatty acids (foods with an ω-6:ω-3 ratio < 10) and antioxidant (Vitamin C and β-carotenes), and lipid peroxidation on membrane EPUFA levels and the psychopathology of schizophrenia. We examined the effect of frequency of intake of ω-3 rich foods, since ω-6 fatty acids are abundantly available in diet.

OBJECTIVES

The objectives of this study were to investigate the mechanisms associated with the reduced membrane EPUFAs in schizophrenia by examining the effects of -

A) frequency of dietary intake of foods with ω-6 : ω-3 < 10 and those rich in antioxidants

B) lipid peroxidation

METHODS

SUBJECTS

The patients enrolled were consecutive admissions to out-patient units of Kripamayee Institute of Mental Health, Miraj, India, and private hospitals in Pune, India. Diagnosis of schizophrenia was according to DSM IV (American Psychiatric Association, 1994). The controls consisted of healthy volunteers, who did not have history of psychosis and major mood disorder and did not use any medication. These were enrolled from the general population and academic community via advertisement. They were matched for age and gender. The patients and controls were excluded for seizure disorder, head injury with loss of consciousness, alcohol and substance abuse and dependence, and for diabetes, cardiovascular disease, hypertension or a family history of the same. Informed consent was obtained from the subjects. We combined the never-medicated first episode schizophrenics (FES) and never-medicated schizophrenic patients with a short duration of illness (NM-SZ) since the differences in the percent distribution of EPUFAs between them were not statistically significant.

As shown in Table IIIa, patient groups (NM-SZ and M-SZ) and normal controls (NC) did not differ significantly from each other with respect to age and the ratio of males to females. The age of onset between the patient groups was significantly different. The mean (±SD) duration of illness of FES was 9.43 (± 6.43) days; and that of NM-SZ was 2.12 (± 2.21) and M-SZ was 10.12 (± 6.92) years which was statistically significant P=0.005.

TABLE IIIa: Demographic characteristics of subjects

GRP	AGE (y)	AGE OF ONSET (y)	M:F
NM-SZ (n=12)	27.83 ± 8.18	27.25 [b] ± 8.25 p=0.02	7:5
M-SZ (n=30)	31.31 ± 10.31	21.19 ± 7.33	19:11
NC (n=18)	31.8 ± 8.62	NA	12:6

NM-SZ - never-medicated schizophrenics; M-SZ - medicated schizophrenics;
NC - normal controls
M:F is Male:Female Ratio; y - years.
Values are expressed as Mean ±SD.
b- statistically significant differences between the schizophrenic groups

DIETARY ASSESSMENTS

FFQ developed by Rao et al (2001) was used to estimate the frequency of intake of foods with an ω-6: ω-3 fatty acid ratio < 10 and those rich in vitamin C and β-carotenes. The questionnaire consisted of 17 food groups and a number of foods (approximately 10) were listed under each of the food groups. The frequency of intake of the foods was recorded on an eight-point scale from 'never' to 'thrice daily'. Thus, monthly scores were calculated for each food item. For example, an item consumed once a week will have a score of 4 while that consumed daily will have a score of 30. We did not estimate the absolute intake of ω-3 and ω-6 fatty acids of subjects since the available food tables did not provide the ω-3 and ω-6 content of all foods. Instead, we examined the differences in dietary patterns of patients and normals with respect to EFAs. Selection of food with ω-6: ω-3 < 10 was, therefore, based on reported values by Ghafoorunissa et al (1996). Similarly, foods rich in antioxidants (Vitamin C and β-carotenes) were identified using 'Nutritive Values of Indian Foods' (Gopalan et al, 1994). Thus, monthly scores for foods with ω-6: ω-3 < 10 was calculated and added to get a total score FQ-ω-6/ω-3 and that for antioxidant rich foods was reported as FQ-ANOX.

The assessment was carried out within the week of enrolment by trained investigators blind to subject status. Information was obtained from the subjects and/or a relevant informant who monitored the subjects' intake of food.

BIOCHEMICAL ANALYSES

SAMPLE COLLECTION

Fasting venous blood was collected on the day of enrolment, in tubes containing 100 µl of 0.5 M EDTA. Plasma and RBCs were separated by centrifugation of whole blood at 800 *g for* 15 min. Plasma was stored at -70^{o}C till used for analysis of lipid peroxides (TBARS). The analysis was carried out within a week of sample collection. RBCs were stored at -70^{o}C until sent to Laxdale Ltd. for fatty acid analysis.

ANALYSIS OF PLASMA LIPID PEROXIDES

TBARS were estimated as reported by Konings and Drijver (1979). Briefly, 1 ml of plasma was used. The proteins were precipitated using 30 % trichloroacetic acid. The sample was heated with thiobarbituric acid at 80^{o}C for 30 min and centrifuged at 800 *g* for 10 min. The supernatant was separated and absorbance was read at 532 nm. Tetramethoxypropane was used as standard, and the results were expressed as TBARS (nmoles) per ml of plasma.

FATTY ACID ANALYSIS

The RBCs were air freighted to Laxdale Ltd, Scotland, UK, under dry ice for fatty acid analysis. The procedure used was revised from the original method of Manku et al (1983). The method was detailed in the previous chapter.

CLINICAL ASSESSMENTS

Patients were rated for psychopathology using Positive and Negative Symptom Scale (PANSS) (Kay et al, 1987) and Brief Psychiatric Rating Scale (BPRS) (Overal and Gorham, 1962). The assessments were carried out within the week of enrolment by trained psychologists with inter-rater reliability established at intervals as described in Chapter II. The details of assessments were mentioned in the previous chapter.

STATISTICAL ANALYSES

Nonparametric statistics was employed to analyze the data. Kruskal-Wallis analysis of variance (ANOVA) was used for comparisons between the groups and Mann-Whitney tests for bivariate comparisons. Fisher Exact test was used to compare group differences for categorical variables. Correlation between variables was studied using Pearson's correlation analysis.

RESULTS

Higher frequency of intake of ω-3 fatty acid and of antioxidant rich foods and lower plasma lipid peroxides was associated with higher membrane EPUFAs, DHA and AA, total ω-3 fatty acids, and total ω-6 fatty acids, and vice versa (Table IIIb).

TABLE IIIb: Membrane EPUFA and frequency of intake of ω-3 fatty acid and antioxidant rich foods and lipid peroxidation of never medicated and medicated schizophrenics and normal controls

GRP	FQ-ω-6/ω-3 < 10	FQ-ANTIOX	DHA	MEM-TOT-ω-3	AA	MEM-TOT-ω-6	TBARS
NM-SZ (n=12)	82.50 [ab] ± 50.53 p<0.001 p<0.001	35.42 [a] ± 26.30 p=0.05	0.47 [ab] ± 0.51 p<0.001 p=0.004	0.92 [a b] ± 1.05 p<0.001 *p=0.07*	5.64 [ab] ± 2.99 p<0.001 p<0.001	17.83 [ab] ± 5.77 p<0.001 p<0.001	1.17 [ab] ± 0.32 p<0.001 p<0.001
M-SZ (n=30)	166.50 ± 51.55	47.97 ±28.00	1.16 [a] ± 0.86 p<0.001	2.48 [a] ± 1.24 p<0.001	11.23 [a] ± 3.9 p=0.003	31.70 [a] ± 7.29 p=0.006	0.96 [a] ± 0.66 p<0.001
NC (n=18)	185.83 ± 53.09	65.28 ± 45.2	2.58 ± 1.59	4.2 ± 2.1	13.83 ± 1.91	35.7 ± 4.22	0.5 ± 0.31

NM-SZ - never-medicated schizophrenics; M-SZ - medicated schizophrenics;
NC - normal controls ;
MEM-TOT-ω3 – is the total RBC membrane ω3 fatty acids expressed as percentage of total fatty acids; FQ-INTAKE ω6/ ω3<10 - is total of monthly scores of frequency of intake of foods with a ω6/ ω3 < 10; Values are expressed as Mean ±SD.
a- statistically significant differences between the schizophrenic group and normals
b- statistically significant differences between the schizophrenic groups

ANOVA showed a significant effect of group on the FQ-ω-6/ω-3 < 10 (H=20.12, df=2, P=<0.001), membrane DHA (H=37.8, df=2, P=<0.001), AA (H=26.02, df=2, P=<0.001), MEM-TOT-ω-3 (H=33.86, df=2, P=<0.001), MEM-TOT-ω-6 (H=28.86, df=2, P=<0.001) and plasma TBARS (H=39.28, df=2, P= <0.001). Post hoc Mann Whitney tests revealed significant differences with respect the EPUFAs, DHA and AA, MEM-TOT-ω-3, MEM-TOT-ω-6, and FQ-ω-6/ω-3 < 10, and plasma TBARS between NM-SZ as compared to NC. NM-SZ also differed significantly on the all of the above measures, except MEM-TOT-ω-3 (which was marginally significant), as compared to M-SZ. The M-SZ differed significantly with respect to the membrane EPUFAs and plasma TBARS but not the FQ-ω-6/ ω-3 < 10 as compared to NC.

ANOVA did not show a significant effect of group on FQ-ANOX. However, post hoc Mann Whitney showed that the differences between NM-SZ and NC were statistically significant.

Multiple regression analysis with membrane DHA and AA taken separately as dependant variables, and FQ-ω-6/ω-3 < 10 and FQ-ANOX as independent variables revealed different results in the NM-SZ, M-SZ and NC (Table IIIc). In the NM-SZ, FQ-ω-6/ω-3 < 10 and FQ-ANOX, together predicted membrane DHA (F=5.66, P=0.03) and MEM-TOT-ω-3 (F=5.57, P=0.03), while this was not the case for M-SZ and NC. Correlation (Spearman) between both, membrane DHA and MEM-TOT-ω-3 taken separately, and each of the independent variable was not significant for NM-SZ and NC, but in case of M-SZ was significant for MEM-TOT-ω-3 and FQ-ω-6/ω-3 < 10 and FQ-ANOX, and marginally significant for DHA and FQ-ω-6/ω-3<10. Significant correlation was not found between membrane AA and total ω-6 fatty acids with FQ-ω-6/ω-3 < 10 and FQ-ANOX, for the patients (NM-SZ and M-SZ) or NC, with either multiple regression or Spearman correlation analysis.

TBARS also did not show significant correlation with the membrane EPUFAs.

TABLE IIIc: Multiple regression (MR) and Spearman correlation (SP) analysis to determine further association of dietary intake and membrane EPUFAs among experimental groups.

GRP	MR: DHA v/s FQ-ω6/ω3 <10 and FQ-ANOX	MR: Tω3 v/s FQ-ω6/ω3<10 and FQ-ANOX	SP : DHA - FQ-ω6/ ω3<10 DHA - FQ-ANOX	SP : Tω3 - FQ-ω6/ ω3 <10 T ω3 – FQ-ANOX
NM-SZ (n=12)	F=5.66, **p=0.03**	F=5.57, **p=0.03**	r= -0.25, p=0.42 r= 0.29, p=0.35	r= -0.27, p=0.37 r= 0.26, p=0.40
M-SZ (n=30)	F=2.0, p=0.16	F=1.46, p=0.25	r= 0.32, p=**0.08** r=0.24, p=0.2	r=-0.47, p=**0.008** r= 0.44, p=**0.02**
NC (n=18)	F=1.05, p=0.37	F=0.86, p=0.44	r= -0.03, p=0.9 r= -0.02, p=0.92	r= -0.15, p=0.54 r= -0.12, p=0.63

NM-SZ - never-medicated schizophrenics; M-SZ - medicated schizophrenics; NC - normal controls

Psychopathology was severe in NM-SZ as compared to M-SZ (Table IIId).

TABLE IIId: Psychopathology scores of never-medicated and medicated schizophrenics and normal controls

GRP	PTOT	NTOT	GTOT	PANSS T	BPRS T
NM-SZ (n=12)	19 [b] ± 6.2 p=0.01	14 [b] ± 7.3 p=0.05	42 [b] ± 4.3 p=0.01	75 [b] ± 9.0 *p=0.12*	41 [b] ± 7.2 *p=0.16*
M-SZ (n=30)	14 ± 6.3	18 ± 6.3	36 ± 8.2	68 ± 14.1	36 ± 7.8

NM-SZ - never-medicated schizophrenics; M-SZ - medicated schizophrenics;
P TOT - PANSS - positive symptom factor score; N TOT - PANSS - negative symptom factor score; G TOT - PANSS - general psychopathology cluster score;
PANSS T - Positive and Negative Symptom Scale - Total score= P TOT + N TOT + G TOT;
BPRS T - Brief Psychiatric Rating Scale - Total; Values are expressed as Mean ± SD.
b - statistically significant differences between the schizophrenic groups

Mann Whitney tests revealed significant differences in positive and negative symptom factor and general psychopathology cluster scores and marginally significant differences in total PANSS and BPRS scores between the patient groups.

DISCUSSION

Concomitant effects of intake of ω-3 fatty acid and antioxidant rich foods and lipid peroxidation on membrane EPUFA levels have not been studied earlier, although these have individually been indicated to affect membrane EPUFA levels in schizophrenia. This is, thus, the first report of the same.

We found that the never-medicated schizophrenics had a lower frequency of intake of ω-3 fatty acid rich foods as compared to the medicated schizophrenics. This low frequency of intake was associated with reduced membrane EPUFA levels and severe psychopathology. Negative symptoms, however, were less severe in the never-medicated schizophrenics probably because the majority of patients in the group were first episode schizophrenics and negative symptoms are manifested with disease chronicity. Peet et al (1996) have also reported that higher intake of ω-3 fatty acids is associated with less severe symptoms. Christensen and Christensen (1988) have found that the intake of a low fat, EFA rich diet is associated with better illness outcome. These studies, however, do not report membrane EPUFA measures in the patients and hence our study bridges this gap.

We found significantly increased plasma TBARS in both the never-medicated as well as medicated schizophrenic patients as compared to normal controls. Increased TBARS in plasma (Peet et al, 1993) and CSF (Pall et al, 1987; Lohr et al, 1990; Tsai et al, 1998) in chronic medicated as well as never-medicated (McCreadie, 1995) schizophrenic patients, and in never-medicated patients at the onset of psychosis (Mahadik et al, 1998) have been reported. We found significantly lower plasma TBARS and less severe psychopathology in medicated as compared to the never-medicated patients although the frequency of intake of antioxidant rich foods in both these was not significantly different. Majority of our patients was being treated with atypical antipsychotics. Atypical antipsychotics have been found to have antioxidant properties (Jedding et al, 1995; Joffe et al, 1998). Also, it has been

suggested that atypical antipsychotics may inhibit phospholipase A_2 (that releases EPUFA from membrane phospholipids), reported to be elevated in schizophrenia (Gattaz et al, 1990; Hudson et al, 1996) and thus may be responsible for increased membrane EPUFA levels. However, it is more likely that treatment with atypical antipsychotics ameliorates psychopathology that may result in reduced cellular metabolic activity and thereby reduce oxidative stress mediated lipid peroxidation and account for increased membrane EPUFA levels.

An association of both, intake of ω-3 fatty acid and antioxidant rich foods, and lipid peroxidation, was observed with membrane EPUFA levels and psychopathology in schizophrenia. We studied the possible mechanisms associated with reduced membrane EPUFAs using RBC membranes, which are relevant to study neuronal membrane EPUFA status (Bourre, et al, 1992; Carlson et al, 1986; Connor et al, 1993), and plasma lipid peroxides that reflect global lipid peroxidation including changes in the brain (Kramer et al, 1987; Sinet et al, 1982; Richardson et al, 1993). In this study, never-medicated patients had a significantly later onset of psychosis and longer duration of untreated illness compared to medicated patients. These patients had a rural domicile and were enrolled at a rural Mental Health Center. Traditionally, rural Indian population has a high tolerance for illness, and possibly these patients may have had mild symptoms in the initial period of illness. This may have led them to seek care only after significant deterioration. In addition, inaccessibility to mental care may have contributed to the delayed observed/reported illness onset by patient and family. Also, these characteristics are similar to that generally found in patients from community mental health centers. Medicated patients were from urban community, which have less tolerance for illness, more treatment awareness, affordability of the treatment costs, and easy access to mental care. In addition, they may have had severe symptoms at onset and thus the observed/reported earlier onset.

We found significantly lower levels of membrane AA and total ω-6 fatty acids and higher levels of plasma lipid peroxides in both the patient groups, never-medicated and medicated schizophrenic patients, as compared to normal controls. Membrane AA levels are particularly susceptible to lipid peroxidation (Peet el al,

1995, Ramchand et al, 1996). However, we did not find a significant correlation between membrane AA and lipid peroxide levels as reported by Peet et al (1995). It has been indicated that membrane ω-3 EPUFA levels may regulate ω-6 levels despite adequate dietary availability (Mahadik et al, 1996).

Varying relationships between membrane EPUFA and intake of ω-3 fatty acid and antioxidant rich foods, and lipid peroxidation in the different subject groups was observed.

EFFECT OF INTAKE OF w-3 FATTY ACID AND ANTIOXIDANT RICH FOODS, AND LIPID PEROXIDATION ON MEMBRANE EPUFA LEVELS IN-

Never-medicated schizophrenics

Never-medicated schizophrenics had significantly lower levels of membrane EPUFAs as compared to medicated schizophrenics and normal controls. They also had higher plasma lipid peroxides. The frequency of intake of ω-3 fatty acid rich foods was also significantly lower in these patients as compared to medicated schizophrenics and normal controls. The frequency of consumption of antioxidant rich foods was also lower as compared to normal controls. Multiple regression analysis indicated that the frequency of intake of both ω-3 fatty acid and antioxidant rich food may have contributed to low membrane DHA and total ω-3 fatty acids levels. Thus in these patients, low frequency intake of ω-3 fatty acids and antioxidants, and oxidative stress mediated lipid peroxidation were responsible for reduced membrane EPUFA levels.

Chronic medicated schizophrenics

The chronic-medicated schizophrenics had significantly lower membrane EPUFA levels as compared to normal controls. The frequency of intake of ω-3 fatty acid and antioxidant rich foods was not significantly lower in these patients as compared to normal controls. However, they had higher plasma lipid peroxides. Membrane DHA levels correlated with the frequency of intake of the ω-3 rich foods and membrane total ω-3 fatty acid levels correlated with both, frequency of intake of ω-3 fatty acid and antioxidant rich foods taken individually (Spearman correlation

analysis). Thus, membrane DHA and total ω-3 fatty acid levels did depend on the frequency of intake of ω-3 fatty acid rich foods, although, significantly higher intake of these and lower peroxides (due to antipsychotic action and antioxidant properties of the medicines - atyicals) in these patients, ameliorated the combined effect of both ω-3 fatty acid and antioxidant rich foods seen in the never-medicated schizophrenics. This may mean that the consumption of antioxidant rich foods and treatment with (atypical) antipsychotics can reduce lipid peroxidation and hence increase the incorporation of dietary ω-3 fatty acids into plasma membranes. However, it appears that high lipid peroxidation in these patients (as compared to normal controls) may increase the requirement of ω-3 fatty acids. This implies that supplementation of these patients with antioxidants (to reduced lipid peroxidation) will bring down the increased EPUFA requirement.

Normal controls

The normal controls had significantly higher membrane EPUFA and lower plasma lipid peroxides levels than both the patient groups. Also, the frequency of intake of ω-3 fatty acids and antioxidant rich foods was higher in normal controls as compared to patients (though not statistically significant with respect to the chronic-medicated schizophrenic patients). The frequency of intake of ω-3 fatty acid and antioxidant rich foods taken together (multiple regression analysis) or individually was not responsible for membrane DHA and total ω-3 fatty acids levels. Thus adequate intake of ω-3 fatty acid and antioxidant rich foods and low lipid peroxidation eliminates this relationship which indicates that the intake meets the requirements.

Thus, the EPUFA levels suggest that in never-medicated patients combined with a low frequency of intake of ω-3 fatty acids and antioxidant rich foods and illness related oxidative stress-mediated lipid peroxidation worsened the situation with resultant decreased membrane EPUFA levels. In the medicated schizophrenics, with adequate intake of ω-3 fatty acid and antioxidant rich foods (comparable to normals), the decreased levels of membrane EPUFAs may be attributed to illness related oxidative stress despite treatment with atypical antipsychotics that may ameliorate

some of the oxidative stress as well as loss of EPUFAs. This conclusion highlights the importance of antioxidant supplementation along with that of ω-3 fatty acids in schizophrenia which has been overlooked in supplementation studies with the EFAs despite evidence for increased oxidative stress in schizophrenia.

We did not estimate the absolute intake of ω-3 fatty acids and antioxidants. We found that the caloric intake of the never-medicated and medicated schizophrenics was not significantly different. This only implies that a low frequency of intake of the above foods may be a primary factor in membrane EPUFAs. Future studies, however, should confirm this possibility. Although not explored, it is possible that abnormalities in genes or genetic expression of enzymes responsible for elongation and desaturation of EFAs to EPUFAs and their incorporation in the membrane phospholipids and antioxidants may also be responsible for low membrane EPUFA levels in schizophrenia (Mahadik et al, 1996; 2001).

This study, however, further indicates that intake of ω-3 and antioxidant rich foods and lipid peroxidation contribute to membrane EPUFA levels in schizophrenia which in turn contributes to the pathophysiology and thus psychopathology. Hence, supplementation with ω-3 EPUFAs and antioxidants in schizophrenia should ameliorate psychopathology and possibly improve course and outcome of schizophrenia.

CONCLUSIONS

1) Increased lipid peroxidation and low intake of ω-3 EFAs are both significantly associated with the reduced membrane EPUFA levels in schizophrenia.

2) Treatment with antipsychotics (atypicals), that probably reduces oxidative stress mediated lipid peroxidation, and with an increased dietary intake of ω-3 EFAs and antioxidants may be responsible for improved membrane EPUFA levels, and concomitant improvement in psychopathology.

Both, low intake of ω-3 rich foods and antioxidants, and increased lipid peroxidation significantly contribute to reduced membrane EPUFA levels in

schizophrenia. Although, treatment with antipsychotics (atypicals) may reduce some lipid peroxidation and the dietary availability of EFAs, that may result in improved membrane EPUFA levels and psychopathology, membrane EPUFA levels are still significantly lower and plasma TBARS are significantly higher in medicated patients as compared to normals. Hence adjunctive supplementation of w-3 EPUFAs and antioxidants should further improve EPUFA and psychopathology.

CHAPTER IV

ω-3 EPUFA-ANTIOXIDANT SUPPLEMENTATION, MEMBRANE EPUFA LEVELS AND OUTCOME

THE CONTENT OF THIS CHAPTER HAS BEEN COMMUNICATED TO SCHIZOPHRENIA RESEARCH

INTRODUCTION

Treatment of schizophrenia, particularly the most disturbing symptoms such as psychosis and agitation, is most effectively accomplished with conventional antipsychotics, typical antipsychotics (typicals). Recently, newer antipsychotics, atypical antipsychotics (atypicals), seem to have prophylactic effects against negative symptoms and cognitive deficits that are considered to be difficult to treat. However, treatment with typicals cause serious side effects such as tardive dyskinesia and cognitive deficits and atypicals cause weight gain, insulin resistance, abnormal lipid metabolism and hypertension along with a significant loss of quality of life. It is, therefore, important to develop alternative or adjunctive treatment strategies for schizophrenia.

Altered membrane phospholipid (PL) composition has been studied in schizophrenia for over 30 years (Rotrosen and Wolkin, 1987; Horrobin et al, 1994; 1998). Recently, these changes have been considered to be a result of altered metabolism (reduced synthesis from EFAs, and/or increased breakdown) of EPUFAs. EFA / EPUFA intake and metabolism govern the availability of EPUFAs for PL synthesis and thus the quantity and quality of membrane PLs in both periphery and the brain (Thompson, 1992). Reduced membrane levels of EPUFAs are consistently reported in red blood cells (RBCs), brain, and skin fibroblasts from chronic medicated schizophrenic patients as well as never-medicated patients (Vaddadi et al, 1990; Horrobin et al, 1991, Yao et al, 1994; Glen et al, 1994; Mahadik et al, 1994; Peet et al 1995, 1996; Assies et al, 2001; Arvindakshan et al, in communication). This pathology is found to be associated with psychopathology (Glen et al, 1994; Peet et al, 1995; Arvindakshan et al, in communication). Also, increased oxidative stress has been suggested in schizophrenia (Mahadik et al, 1996a; Reddy and Yao, 1996). Our data also indicate that membrane EPUFA deficits (Chapter II) and peroxidative injury (Chapter III) contribute to the pathophysiology. Thus the pathophysiology may easily be corrected but with careful experimental planning (Mahadik et al, 2001).

Increased breakdown of EPUFAs, predominantly by peroxidation, has been considered to be a critical factor for reduced EPUFA levels and hence, theoretically,

also for the associated pathology. However, a number of studies have reported variable prophylactic effects of supplementation with antioxidants namely, vitamins E primarily on tardive dyskinesia, cognitive performance and even BPRS (Adler et al, 1993; Peet et al, 1993; Mahadik et al, 1999). Supplementation with vitamin C has not been tried although its use in preventing intracellular peroxidative injury by restoration of active vitamin E has been suggested (Mahadik et al, 2001).

Few studies have been published on the beneficial effects of EPUFA supplementation using small number of schizophrenic patients by investigators from only one institution (Peet et al, 1995; 2001; Mellor et al, 1996). One report on a very large number of patients did not find such effects (Fenton et al, 2000). These reports have indicated critical issues that must be considered in designing and carrying out such studies. These are: 1. Age of the patients and years of illness: It is likely that in older patients with long duration of illness, the membrane phospholipid pathology may reach a point of difficult to correct. After 50 years of age, antioxidant defense declines rapidly and majority of elderly patients becomes non-responsive to conventional antipsychotics. Use of preferably younger patients in their early stages of illness may be most preferable. 2. Adjunctive medication: Typical antipsychotics have pro-oxidant properties and also affect the EPUFA metabolism (Jedding et al, 1995) whereas available atypical antipsychotics have antioxidant effects and have been found to improve membrane EPUFA levels (Horrobin, 1999). 3. Type of EPUFA: It has been indicated by earlier studies that supplementation with ω-3 fatty acids (EPA > DHA) is preferable compared to ω-6 EPUFAs (Vaddadi et al, 1989; Peet et al, 2001). Rather, the use of high levels of ω-6 EPUFAs may cause complications. 4. The dose and the quality of ω-3 EPUFAs: It is known that high does of EPUFAs, if not balanced with dietary antioxidants or protected in packing, can form peroxides that are toxic to several plasma membrane functions. Moreover, as indicated earlier, since oxidative cellular injury is observed in schizophrenia, use of a combination of EPUFAs and antioxidants may be the choice of cocktail. 5. Duration of treatment: Earlier studies have shown that supplementation for at least 4 months is required to restore the RBC membrane EPUFA levels since RBCs have a half life of 120 days. Importantly, the same period is required to restore EPUFA levels in the

brain. However, one study has indicated that the supplementation with EPA had prophylactic effects within a few weeks indicating a role for its metabolites.

Although there are several published studies of supplementation with vitamin E alone that have resulted in variable and often marginal effects, primarily on tardive dyskinesia. Supplementation with both, EPUFAs and antioxidants, has not yet been studied. As stated earlier, this is critical since peroxidative cellular injury must be first contained with antioxidants only then can the membrane EPUFA levels be restored.

OBJECTIVES

To determine the effect of supplementation of chronic schizophrenics with a combination of ω-3 EPUFAs (EPA: DHA, 180: 120 mg) and antioxidants (vitamins E: C, 400IU: 500mg) twice a day for 4 months on psychopathology, quality of life and outcome measures.

METHODS

SUBJECTS

The patients enrolled were consecutive admissions to outpatient treatment unit of private hospitals in Pune, India. Diagnosis of schizophrenia was according to DSM IV (American Psychiatric Association, 1994). The controls consisted of healthy volunteers, who did not have history of psychosis and major mood disorder and did not use any medication. These were enrolled from the general population and academic community via advertisement. They were matched for age and gender. The patients and controls were excluded for seizure disorder, head injury with loss of consciousness, alcohol and substance abuse and dependence, and for diabetes, cardiovascular disease, hypertension or a family history of the same. Informed consent was obtained from all the subjects.

SUPPLEMENTATION WITH EPUFA AND ANTIOXIDANTS

33 patients enrolled for the study. All patients were clinically stable for at least a month on a fixed treatment plan. Supplementation was done with a mixture of ω-3 EPUFAs, EPA: DHA (180: 120 mg; Maxiguard, ICI India Ltd., Chennai, India) and a

mixture of antioxidants, vitamins E: C (400IU; Bio E: 500 mg; Celin, from American Remedies Ltd, Chennai, India and Glaxo, India Ltd., Mumbai, India, respectively). This dose was given one in the morning and one in the evening with the meals, for 4 months. One-month-supply was given at the time of doctor's visit. Of the 33 patients that initiated the supplementation, 28 completed the 4 months of supplementation course and of these 21 were followed up further 4 months without supplementation to monitor the effects of supplementation washout. We did not use the placebo group. Instead, the pre-, post- and washout (Sz-Pre, Sz-Post and Sz-WO) measures within the subjects were used to assess the change in the psychopathology. The RBC membrane EPUFAs and plasma peroxides were also determined to assess the effects of supplementation. The clinical coordinator responsible for the assessments was not aware of the treatment schedules and not involved in research interests.

Table IVa shows the demographic characteristics of the patient group (SZ) and the normal controls (NC).

TABLE IVa: Demographic characteristics of subjects

GRP	AGE (y)	M:F	AGE OF ONSET (y)	DURATION OF ILLNESS (y)
NC (n=15)	31.29 ± 9.86	10:5	NA	NA
SZ (n=28)	29.57 ± 7.03	18:10	19.43 ± 4.11	10.14 ± 6.04

NC - normal controls; SZ - chronic schizophrenics;
y : years; M:F : male : female;
Values are expressed as Mean ± SD.

BIOCHEMICAL ANALYSES

FATTY ACID ANALYSIS

Fasting venous blood was collected immediately on enrolment in tubes containing 100 µl of 0.5 M EDTA. RBCs were separated by centrifugation, coded and stored at -70^{o}C until used for analysis. The analyses were carried out blind to the subject status. The procedure used was revised from the original method of Manku et al (1983). Briefly, total lipids were first extracted using 2:1 chloroform: methanol. The

phospholipid fraction was separated. Transesterification of this phospholipid fraction was carried out using methanolic HCl. These were separated and quantitated using a Shimadzu (GC-17A) Gas chromatograph (SP-2330 30m capillary column, Supelco Inc.). Nitrogen was used as carrier gas at 1 ml/min. Oven temperature was held at 175^{o}C for 15 min, programmed to rise from 175^{o}C to 220^{o}C at 10^{o}C/min, held at 220^{o}C for 10 min. Detector temperature was 275^{o}C and injector temperature 240^{o}C. Retention times and peak areas were automatically computed. Peaks were identified by comparison with standard fatty acid methyl esters (Sigma, USA).

ANALYSIS OF PLASMA TBARS

TBARS were estimated as reported by Konings and Drijver (1979). The method was detailed in the previous Chapter.

CLINICAL ASSESSMENTS

Patients were rated for psychopathology using Positive and Negative Symptom Scale (PANSS) (Kay, et al, 1987) and Brief Psychiatric Rating Scale (BPRS) (Overal and Gorham, 1962), Heinrichs' Quality of Life (Heinrichs et al, 1984) and Strauss and Carpenter Outcome Scale. The assessments were carried out within the week of enrolment by trained psychologists with inter-rater reliability established at intervals as described in Chapter II. Assessments were based on the information from the patient, a relevant informant accompanying the patient and from hospital records.

STATISTICAL ANALYSES

Nonparametric statistics was used to analyze the data. Kruskal-Wallis analysis of variance (ANOVA) was used to compare between the groups, Mann-Whitney tests were used for bivariate comparisons. Fisher Exact test was used to compare group differences for categorical variables. Pearson's correlation analysis was used to study correlation between variables.

RESULTS

The percent distribution of RBC membrane EPA and DHA were increased and AA decreased in SZ-Post as compared to SZ-Pre, SZ-WO and NC (Table IVb) (Fig. 20).

TABLE IVb: Effect of supplementation with ω- 3 fatty acids and antioxidants on distribution of membrane fatty acids of chronic schizophrenics

GRP	SFA	Ol	LA	AA	EPA	DHA	TBARS
NC (n=15)	48.25 ± 2.37	12.45 ±1.24	16.58 ±2.09	19.29 ±2.61	0.53 ±0.41	3.28 ±2.27	0.68 ±0.3
SZ-Pre (n=28)	48.20 [c] ± 2.1 p<0.001	13.19 ± 1.34	15.87 ± 1.9	20.3 [b] ± 1.42 p<0.001	0.26 [b] ± 0.36 p<0.001	2.48 [b] ± 1.2 p<0.001	0.51 [a] ± 0.21 p<0.04
SZ-Post (n=28)	49.11 [d] ± 5.03 p<0.001	13.04 ± 1.37	15.77 ± 1.95	16.21[ad] ± 3.62 p=0.003 p<0.001	0.68 [d] ± 0.23 p=0.01	5.25 [ad] ± 1.48 p<0.001 p=0.003	0.60 ± 0.21
SZ-WO (n=21)	46.63 [a] ± 4.37 p<0.001	13.58 ± 1.0	16.62 ± 2.08	19.96 ± 3.04	0.3 ± 0.17	3.16 ± 1.56	0.61 ± 0.14

NC - normal controls; SZ-Pre - schizophrenics before supplementation; SZ-Post - schizophrenics after supplementation; SZ-WO - schizophrenics after supplementation wash-out;
SFA-total saturated fatty acids = C16:0, C18:0; Ol- Oleic acid; LA - linoleic acid; AA-arachidonic acid; EPA- eicosapentaenoic acid; DHA-docosahexaenoic acid;
Total major fatty acids- SFA + Ol + LA + AA + EPA + DHA;
Values are expressed as Mean ± SD. The fatty acids (FA) are expressed as % of total major FA.
a : statistically significant difference between SZ groups and normals
b : statistically significant difference between SZ-Pre and SZ-Post groups
c : statistically significant difference between SZ-Pre and SZ-WO groups
d : statistically significant difference between SZ-Post and SZ-WO groups

ANOVA showed a significant effect of group on the percent distribution of AA (H=31.89, df=3, P=< 0.001), EPA (H=16.47, df=3, P=<0.001) and DHA (H=33.08, df=3, P=<0.001). Post hoc Mann-Whitney tests showed that the differences with respect to the EPUFA for SZ-Post as compared to SZ-Pre, SZ-WO and NC (except for EPA) were statistically significant.

The percent distribution of total saturated fatty acids was lower in the SZ-WO. ANOVA showed a significant effect of group (H=18.86, df=3, P=<0.001). Post hoc

Mann-Whitney tests showed that the differences for SZ-WO as compared to SZ-Pre, SZ-Post and NC were statistically significant.

TBARS were lower in SZ-Pre as compared to SZ-Post and NC (Fig. 21). ANOVA did not show a significant effect of group on TBARS levels. However, post hoc Mann-Whitney tests revealed a significant difference (P=0.04) between the SZ-Pre and NC.

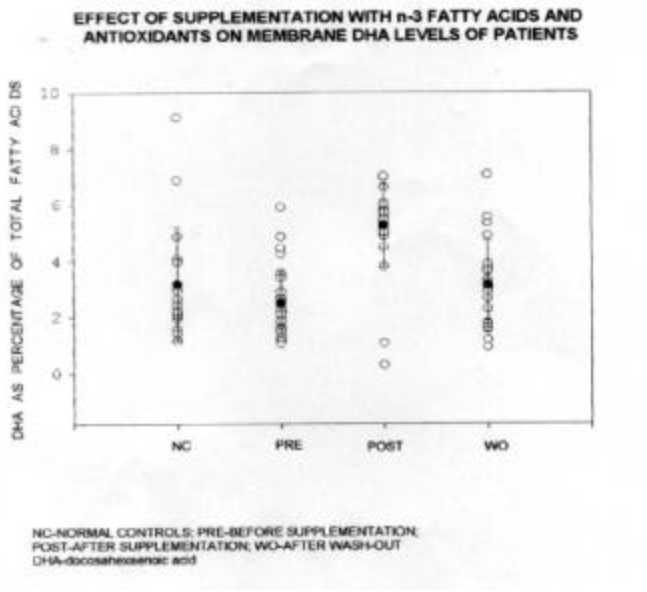


Fig. 20

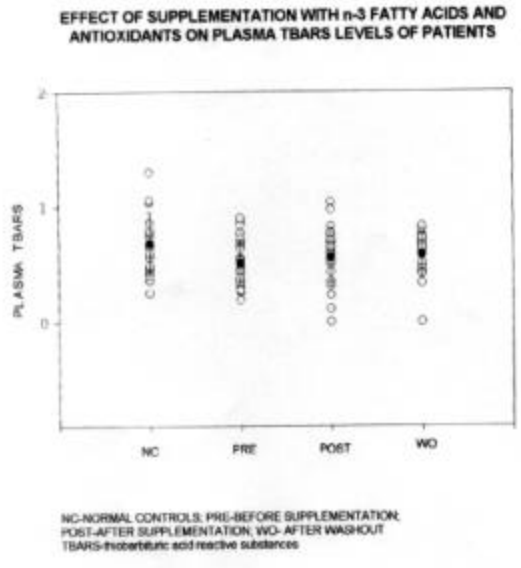


Fig. 21

The psychopathology was improved in both SZ-Post and SZ-WO as compared to SZ-Pre as indicated by reduced scores of BPRS, GTOT, PANSST with increased scores of QOL and OTOT (Table IVc) (Fig. 22). ANOVA showed a significant effect of group on BPRS (H=9.26, df=2, P= 0.01), general psychopathology-PANSS (H=13.95, df=2, P=<0.001) PANSS total (H=10.48, df=2, P=0.005), and Quality of Life (H=8.78, df=2, P=0.012) scores. Post hoc Mann-Whitney tests showed that differences with respect to the scores for SZ-Pre as compared to SZ-Post and SZ-WO were statistically significant.

TABLE IVc: Effect of supplementation with ω-3 Fatty Acids and antioxidants on psychopathological characteristics of chronic schizophrenics

GRP	BPRS T	P TOT	NTOT	GTOT	PANSS T	QOL	O TOT
SZ-Pre (n=28)	45 [bc] ± 16.5 p=0.02 p=0.006	19 [c] ± 9.0 p=0.04	20 [c] ± 8.1 *p=0.09*	42 [bc] ± 13.6 p=0.001 p=0.001	80 [bc] ± 26.9 p=0.005 p=0.005	51 [bc] ± 23.3 p=0.01 p=0.02	9 [b] ± 3.0 p=0.035
SZ-Post (n=28)	35 ± 13.7	14 ± 6.7	15 ± 6.8	31 ± 11.5	61 ± 22.7	65 ± 22.9	11 ± 3.2
SZ-WO (n=21)	32 ± 9.9	13 ± 6.1	16 ± 6.3	30 ± 8.1	59 ± 17.4	67 ± 20.6	11 ± 3.1

SZ-Pre - schizophrenics before supplementation; SZ-Post - schizophrenics after supplementation; SZ-WO - schizophrenics after supplementation wash-out;
P TOT - PANSS - positive symptom factor score; N TOT - PANSS - negative symptom factor score; G TOT - PANSS - general psychopathology cluster score;
PANSS T - Positive and Negative Symptom Scale - Total score = P TOT + N TOT + G TOT;
BPRS T - Brief Psychiatric Rating Scale - Total score; QOL - Quality of life scale - total score; O TOT - Outcome scale - total score; Values are expressed as Mean ±SD.
b : statistically significant difference between SZ-Pre and SZ-Post groups
c : statistically significant difference between SZ-Pre and SZ-WO groups
d : statistically significant difference between SZ-Post and SZ-WO groups

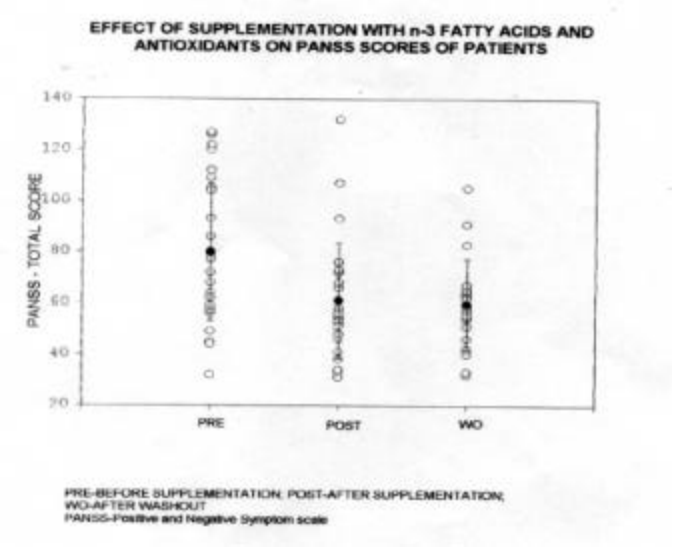


Fig. 22

ANOVA did not show a significant effect of group on positive-PANSS, negative-PANSS and Carpenter's outcome scale scores. However, post hoc Mann-Whitney tests revealed a significant difference with respect to positive-PANSS between SZ-Pre and SZ-WO, negative -PANSS scores showed marginal significance (P=0.09), and Carpenter's Outcome Scale scores were significantly different between SZ-Pre and SZ-Post.

DISCUSSION

This is the first study to show that supplementation with low dose ω-3 EPUFAs in combination with antioxidants is effective in improving psychopathology in chronic medicated schizophrenic patients. Significant improvements were seen on most of the clinical measures. The use of this combination is justified by several studies indicating that schizophrenia pathophysiology involves both reduced EPUFAs and increased oxidative stress-mediated lipid peroxidation. Furthermore, the few studies with supplementation with either EPUFAs or vitamin E alone have reported variable improvements in clinical measures.

Recently, Peet et al (2001) have reported strong prophylactic effects of sole supplementation with EPA. EPA is not a major membrane constituent and its role in the membrane is not known. It is considered a metabolic product formed by

retroconversion from DHA that is released during neuronal activity. EPA is used primarily for prostaglandin (PG) synthesis. PGs are quick acting neuromodulators. There is evidence that PG levels are altered in schizophrenia and supplementation with PGs is associated with clinical improvements (Kaiya et al, 1985). Accordingly, it was indicated that supplementation with EPA had very quick response which may be related to its function as a PG precursor. However, its long-term effect may be related to its conversion to DHA and subsequent incorporation in membrane phospholipids. Improved levels of DHA after EPA supplementation reflect this. The lower beneficial effects of DHA compared to EPA in this study may be because supplemental DHA is not a substrate for PG synthesis. Moreover, it is likely that high supplemental dose and membrane levels of DHA may have proved to be toxic due to increased oxidative stress-mediated lipid peroxidation in schizophrenia; antioxidants were not administered simultaneously. However, this is not confirmed since this and most of the earlier EPUFA supplementation studies have not measured the plasma levels of peroxides. To avoid the possible toxic effects, we used a low dose mixture of EPA and DHA along with antioxidants. With this mixture, we did not find elevated levels of plasma peroxides. Also, it is unlikely that the prophylactic effects we observed are solely due to antioxidants since the primary role of antioxidants is to prevent the cellular membrane EPUFAs that are the functional membrane constituents. Also, several earlier studies with vitamin E supplementation had observed only marginal improvements, primarily on tardive dyskinesia (Adler et al., 1993; Reddy and Yao, 1996; Mahadik and Scheffer, 1996; Mahadik and Gouda, 1996). Therefore, we did not consider the need to have a separate comparison group for supplementation with only antioxidants.

The use of 2 gm (and higher) of EPA in earlier studies (Mellor et al, 1995; Peet et al, 1996; 2001; Shah et al, 1998) is not justified since the daily requirement for total ω-3 fatty acids is < 0.5 gm. However, dose < 2 gm have proved ineffective (Peet et al, personal communication) because it is possible that EPA gets peroxidated in the periphery as well as in the brain. Moreover, oxidative stress is known to be associated with schizophrenia. This is evidenced by the toxic effects of even high doses of EPA or DHA or fish oil used in studies in bipolar disease (Stoll et al, 1999).

However no visible side effects were observed with 2 gm of EPA supplementation (Peet et al, 2001). Our patients were younger (29.57 ± 7.03 yr) as compared to those in studies reported earlier (average 43 yr) (Mellor et al, 1996; Peet et al, 2001). This may also be a reason for observing prophylactic effects with low dose EPUFAs.

It has been suggested that EPA inhibits phospholipase A_2 (PLA_2) that is elevated in schizophrenia, and that this may cause the observed improvement of membrane EPUFAs. However, the findings of elevated levels of PLA_2 are inconsistent. Also, it seems that the increase in PLA_2 is a result of increased plasma lipid peroxides and worsening of clinical symptoms (Scheffer et al, 2000). Several studies have indicated that elevation of PLA_2 is associated with increased peroxidation and is thus a repair response to replace damaged PL EPUFAs. Infact, it is likely that use of antioxidants will reduce the lipid peroxidation, reduce the levels of PLA_2 and thus may contribute to the increase in membrane EPUFAs with supplementation. This also supports the need for low dose EPUFA supplementation if administered along with antioxidants.

Although the EPUFA levels in patients returned to pre-treatment levels after 4 months of washout, the improvement in psychopathology was retained. Rather, we found significant improvement in positive and negative symptom cluster scores at the end of the washout period. This is probably because it is hypothetically possible that the EPUFAs may correct the structural brain changes, namely repair of synaptic connections known to contribute to disrupted neural circuits in schizophrenia. This, however, needs confirmation. The patients were also not continued on the same treatment regimen during the washout period since the doctor was keen on introduction of atypicals, as these are preferred for their low side effects. Atypical antipsychotics, which are more effective than typicals in controlling the psychotic symptoms and improving the core negative symptoms, also have antioxidant properties. Continued protection against oxidative stress offered by atypicals to the repaired brain by supplementation may have led to an improvement in psychopathology.

The low dose of EPUFAs was not associated with any gastro-intestinal problems as reported by similar studies using high dose. Of the 33 patients enrolled

in the study, 28 completed the supplementation course. Remaining patients discontinued due to the fishy odor of the capsules, which could not be tolerated by the patients who consumed vegetarian diet due to religious reasons. Of the 28 patients, only 21 were followed up to study the effects of supplementation washout, since 2 patients continued to take EPUFA supplementation because they personally strongly felt that these were beneficial and 5 patients could not be contacted.

We did not have a placebo group but used pre-, post- and washout measures within subjects. Although the placebo is known to have prophylactic effects in psychiatric disorders, we feel that results with a substantially large number of patients (N=28) and within subjects measures indicate significant and reliable prophylactic effect of supplementation in our study. Also, as indicated earlier, the clinical rater was blind to the treatment protocol, both antipsychotic medication and supplementation. Moreover, placebo effects generally do not last for extended periods of time, such as in our study; the prophylactic effects continued over 4 months after discontinuation of supplementation.

Although the results of these studies are encouraging, further studies need to be done with still larger number of patients and with longer duration of supplementation. The effect of EPUFA - Antioxidant treatment in never-medicated patients, would help determine the use of these as sole treatment agents. However, it is possible that antipsychotics may be required to control the acute psychotic symptoms of the disease.

CONCLUSIONS

Thus, supplementation with a combination of ω-3 EPUFAs and antioxidants does improve psychopathology, quality of life and outcome measures of young, chronic schizophrenic patients.

CHAPTER V

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

A ROLE FOR ω-3 EPUFAs IN THE ETIOPATHOPHYSIOLOGY AND OUTCOME OF SCHIZOPHRENIA

GENERAL DISCUSSION

THE MEMBRANE PATHOLOGY

Data presented in this thesis, for the first time, strongly support the concept that the membrane pathology in schizophrenia is primarily related to reduced membrane essential polyunsaturated fatty acids (EPUFAs). It identifies the ω-3 EPUFAs, particularly docosahexenoic acid (DHA) as the key molecules responsible for this pathology. The above conclusions were based on facts mentioned below.

EPUFAs are essential components of major membrane phospholipids (PLs), namely, phosphatidyl ethanolamine (PE), phosphatidyl choline (PC), phosphatidyl inositol (PI) and phosphatidyl serine (PS). The availability of EPUFAs may limit the synthesis of the PLs. Increased lipid peroxidation of PL-EPUFAs may be responsible for increased breakdown of membrane PLs, since EPUFAs are particularly vulnerable to oxidative damage. Altered membrane PL composition observed in this study and in other studies, reflects the underlying altered EPUFA composition of membranes.

Although the ω-3 EPUFA, DHA and ω-6 EPUFA, AA, are both reduced in cell membranes of schizophrenic patients, reduced intake of DHA (or its precursor) and its vulnerability to peroxidative damage due to high degree of unsaturation may be the cause of the observed pathology. The ω-6 EPUFAs are abundantly available in the diet and it is proposed that DHA may be involved in regulating AA levels. Thus, low membrane DHA levels may cause low AA levels. Low levels of membrane AA may also be due to peroxidative damage.

The useful aspect of the above findings is that it implicates that it may be possible to correct the pathology of schizophrenia, by supplementation with ω-3 EPUFAs and antioxidants.

THE DHA HYPOTHESIS

Reduced membrane EPUFAs, predominantly DHA, in never-medicated schizophrenic patients at the onset of psychosis and their association with the severity of illness indicates their role in pathophysiology of the illness. If the pathology

predates onset of psychosis as early as embryonic development and prevails during later neurodevelopment, it opens up the possibility of their role in the etiopathophysiology of schizophrenia. DHA has been found to be a pertinent requirement for the various stages of neurodevelopment. Low availability of DHA either due to low intake or due to increased lipid peroxidation may thus affect cell proliferation, migration and synaptogenesis and, thereby, alter the cytoarchitecture of the brain and the neurotransmitter circuitry. Reduced cell numbers and size, mis-alignment and mis-placing of cells, and reduced neuropils, which may be responsible for reduction in volume, especially gray matter, in several brain regions have been observed with neuroimaging and post-mortem studies in schizophrenic patients. It seems probable that reduced DHA may be responsible for such changes. In addition to the neurodevelopment pathology, the ongoing pathology, due to oxidative stress mediated lipid peroxidation and low intake of EFAs (observed in this study), may constitute the 'total' pathology observed at any point of time. These together may determine the course and account for the outcome of schizophrenia.

The statement of an etiopathophysiological hypothesis in schizophrenia obligates the need to propose its potential to explain the heterogeneity of the clinical presentation of the illness. The variable timing of the neurodevelopmental insult/s, due to reduced DHA, may be responsible for the differences in the brain regions affected in schizophrenia. The magnitude of the insult may be responsible for the severity of the pathology. The nature and extent of both the neurodevelopmental and ongoing insult may thus account for the qualitative and quantitative differences in the symptoms and thus the heterogeneity of clinical presentation in schizophrenia.

It is also obligatory that the hypothesis explain the observations often reported in schizophrenia.

FAMILY HISTORY

Increased incidence, prevalence and poor outcome of schizophrenia seem to be associated with a family history, suggesting its genetic transmission (Kety, 1980; Gottesman, 1991). However, ω-3 EPUFAs are critical for the expression of several genes involved in adaptive and survival mechanisms (Fernandes et al, 1996; Palmer and Paulson, 1997). Also, genes coding for enzymes for conversion of precursor to

DHA have been suggested to be abnormal. Furthermore, family and siblings share the environment that significantly affects the gene expression.

SEX DIFFERENCES

Females show a later onset, better premorbid functioning and a more benign course of schizophrenia as compared to males (Lewine, 1988; Hambrecht et al, 1992; Castle et al, 1995). Estrogen appears to play a critical role in this sex difference (Haffner et al, 1998). Desaturases, enzymes involved in the synthesis of polyunsaturated fatty acids, are known to be regulated by estrogen (Brenner, 1981). Animal studies have shown that female rat pups maintain brain EPUFA status much better than the male rat pups and contribute to improved cognitive performance (Yamamoto et al, 1987).

PRENATAL AND POSTNATAL FACTORS

Several prenatal and neonatal factors, such as nutrition (e.g. studies during famine), maternal stress, smoking, drugs of abuse, alcohol consumption, obstetric complications such as neonatal hypoxia, and breast feeding, have been associated with schizophrenia (Geddes and Lawrie, 1995; McNeil, 1995; Mahadik and Gowda, 1996; Glover, 1997; Hultman, 1997). These factors have in common the potential to affect cellular membrane EPUFA status. Hypoxia has been found to affect brain development by oxidation of EFAs. Maternal stress, use of alcohols and drugs of abuse and smoking, can affect the intake of EPUFA and antioxidants and increase peroxidative breakdown of EPUFAs. Milk of mammals is enriched in ω-3 fatty acids, which are required for neurodevelopment. It has been reported that fewer schizophrenics than non-schizophrenic patients had been breast-fed (McCreadie, 1997).

SEASONALITY OF BIRTH

Several studies have reported that in both, northern and southern hemispheres, schizophrenic patients are more likely to have been born in winter than in summer (Bradbury and Miller, 1985; Dalen, 1990; O'Callahan et al, 1991; McGrath

et al, 1995). Enlarged ventricles, indicative of abnormal neurodevelopment, was reported in these patients (Revely et al, 1984; Zipursky and Schulz, 1987). It is noteworthy that these patients had low genetic risk (Kinney and Jacobsen, 1978; Shur, 1982; O'Callahan et al, 1991). Populations living in winter zones have been found to suffer from unavailability of fresh fruits and vegetables, which are rich in EPUFAs and antioxidants. Additionally, they are also exposed to winter weather stress that can increase the oxidative stress. However, increasing evidence indicates that this season-of-birth effect is disappearing, probably due to improvement in communication and transport systems as well as storage facilities that help availability of fresh food rich in EPUFAs and antioxidants.

These associations provide support to the involvement of EPUFA, particularly DHA, in the pathophysiology of schizophrenia. The confirmation of the role of DHA in the etiopathophysiology of schizophrenia may provide means to prevent schizophrenia.

w-3 EPUFAs, ANTIOXIDANTS AND ATYPICAL ANTIPSYCHOTICS IN TREATMENT OF SCHIZOPHRENIA

Atypical antipsychotic drugs (atypicals) have revolutionized the management of schizophrenia by improvement of, especially, the core negative symptoms and cognitive impairments, and additionally causing only mild side effects. These may be related to their antioxidant as well as favorable effects on EPUFA synthesis, since treatment with these are associated with improved membrane EPUFAs and psychopathology; typical antipsychotics (typicals) are known to have pro-oxidant effects. However, the results of effect of long term use of atypicals are still awaited, as these have only recently been introduced in psychiatric care. Studies with animals treated with the atypicals have also demonstrated beneficial effects compared to detrimental side-effects, primarily due to oxidative stress mediated pathology, found with typical antipsychotics (Mahadik et al, 1998; 2001). High membrane EPUFAs, low lipid peroxidation and concomitant improved psychopathology observed with patients treated with atypicals (predominantly) as compared to never-medicated patients in this study indicates that improvement in membrane EPUFAs and reduction in lipid

peroxidation may be responsible for the antipsychotic effects. In our study, adjunctive treatment with the ω-3 EPUFAs and antioxidants further improved the psychopathology of patients treated with antipsychotics, predominantly with atypicals. It is also possible that atypicals, by their effects on a number of neurotransmitter receptors, reduce the psychomotor mediated oxidative stress associated with psychotic symptoms and the administration of ω-3 EPUFAs under such conditions may be responsible for repairing some of the structural defects, namely, damaged synaptic connections and thus probably correcting neural circuitry. Accordingly, in our study improvement in psychopathology was retained after withdrawal of supplementation. It remains to be seen whether ω-3 EPUFA and antioxidant treatment alone can manage the symptoms of schizophrenia, although it seems probable that antipsychotics may be required to attenuate the acute psychotic symptoms. It is possible though that the effective antipsychotic dose required may be reduced (as indicated by some studies) thus reducing the harmful side effects of the antipsychotics. In conclusion, adjunctive treatment with ω-3 EPUFAs and antioxidants can open more effective and safer avenues to the treatment and management of schizophrenia.

FUTURE PERSPECTIVES

Studies presented in this thesis have provided strong evidence for the role of EPUFAs in the pathophysiology and outcome and thereby the treatment of schizophrenia. A number of issues however, still remain unresolved (detailed below). Studies addressing these will finally provide alternative means to manage, if not cure, the most serious illness of the mankind.

Data on membrane PL composition and its component fatty acids indicate that future studies of fatty acid changes in the individual PL classes would bring to light the specific compensatory molecular mechanisms that may underlie the pathophysiology and outcome of schizophrenia.

Follow-up study of membrane EPUFA levels at first episode and at regular intervals of six months upto five years to study variations in membrane EPUFA levels

with psychopathology at every cross section may help to establish the role of EPUFAs in the course and outcome.

We have only studied these measures at baseline and at the end of four years, in a small number of patients, as the principal clinician met with a crippling accident, due to which most patients were lost to follow-up

As proposed by the hypothesis, both the neurodevelopmental and the ongoing insults, should determine the course and account for the outcome. The neurodevelopmental insult may be estimated by brain scans using MRI. Quantitative MRI scans and membrane EPUFA levels, and their correlation with psychopathology and outcome measures in never-medicated schizophrenics will help understand their relationship in psychopathology and outcome. The association of altered CNS PL metabolism by ^{31}P MRS with peripheral RBC EPUFA levels may also help to establish better the importance of peripheral EPUFAs to brain changes.

Our efforts in this direction were constrained due to limited availability of equipment and lack of detailed technical know-how required for such studies.

Cognitive impairment, recognized as the core psychopathology in schizophrenia, would be most appropriate psychopathology measure to be correlated with membrane pathology (EPUFA levels). It is important to indicate that ω-3 EPUFA deficiency has been found to lead to brain abnormalities and cognitive deficits in rats and primates.

We did not have suitably adapted versions of cognitive scales at the time of the study.

As indicated by this study never-medicated, relatively chronic patients have low membrane EPUFA levels and higher oxidative stress as compared to the first episode schizophrenics. This, thus, may point to the progressive nature of the pathology and suggested deteriorating psychopathology. To confirm this, MRI scans and membrane EPUFA levels in never-medicated schizophrenic patients at the onset of psychosis with follow-ups at several intervals, will help gain an insight into the static or progressive nature of the pathology.

We found that untreated illness was associated with larger reductions in membrane EPUFAs and severe psychopathology when compared with medicated patients. However using patients with varying duration of untreated illness, which however are difficult to find, MRI scans, membrane EPUFA levels and psychopathology and outcome measures, can be used to establish these findings.

Treatment with atypicals is known to improve membrane EPUFA levels and psychopathology. Membrane EPUFA levels of patients treated with atypicals and typicals may be compared on to determine whether this is the mechanism of effectiveness of the former. This may provide an insight into the further mechanism of antipsychotic action of the drugs, subsequent to their effects at neurotransmitter levels.

We did not have a large enough group of patients treated with typicals, due to growing popularity of use of atypicals, to compare with the patients treated with atypicals on measures of membrane EPUFA and psychopathology.

Placebo controlled studies using larger numbers should be undertaken to confirm the findings of our study with supplementation with ω-3 EPUFA and antioxidants. Longer duration of supplementation may be required to observe improvements with negative symptoms and cognitive impairments as indicated by our study. Furthermore, since duration of untreated illness is known to be associated with bad outcome, treatment initiation early in illness, at onset, during prodromal period, or earlier, in high-risk subjects, would prove beneficial.

The etiological cues for schizophrenia indicate interplay between environment and genes. Although this study does not involve investigation of genes associated with fatty acid metabolism implicated in schizophrenia, because of the strong implications of earlier studies to intake of EPUFA and oxidative stress as being individually responsible for low membrane EPUFA levels in schizophrenia. However, low membrane EPUFA levels may also have genetic causes.

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