BACKGROUND

Nutritional Management of Cognitive Decline (Dementia)

By: Nancy L. Morse B.Sc. (Hons), CNPA, NWS

TABLE OF CONTENTS

1.0 Introduction
  1.1 What is Age-Associated Memory Impairment/Cognitive Decline (AAMI)? What is dementia?
  1.2 What causes dementia?
  1.3 How common is dementia?
  1.4 What are the risk factors for dementia?
  1.5 What are the most important early indications of dementia?
  1.6 What are the symptoms of Age Related Cognitive Decline, Dementia and Alzheimer's disease?
  1.7 How is Age Associated Memory Impairment/Cognitive Decline Diagnosed?
  1.8 How is Dementia Diagnosed?
  1.9 What are the Treatments for Dementia?

2.0 What is the connection between Docosahexaenoic Acid (DHA) and Cognitive Decline?
  2.1 Epidemiological Evidence
    2.1.1 Evidence indicating low DHA levels in patients with Alzheimer's disease.
    2.1.2 Evidence supporting the link between low DHA status and the development of cognitive decline
    2.1.3 Evidence supporting a link between high fish and more specifically high docosahexaenoic acid (DHA) intake and a reduced incidence of developing dementia
  2.2 Preclinical Biochemical Studies
  2.3 Preclinical Intervention Studies
  2.4 Clinical Trials using DHA Supplementation

3.0 What is the connection between Phosphatidylserine (PS) and Cognitive Decline?
  3.1 What is Phosphatidylserine (PS) and how is it involved in brain function?
  3.2 Preclinical Intervention Studies
  3.3 Clinical Trials using PS Supplementation
  3.4 Approved Health Claims pertaining to PS and Cognitive Decline

4.0 What is the connection between the B Vitamins and Cognitive Decline?
  4.1 Epidemiological Evidence
  4.2 Clinical Trials using B Vitamins Supplementation
5.0 What is the connection between Ginkgo biloba and Cognitive Decline?
   5.1 Clinical Trials using Ginkgo biloba supplementation

6.0 Studies on the Efalex Active 50+ Formulation
   6.1 Preclinical

7.0 Suggested Dosages of DHA, PS, B Vitamins and Ginkgo biloba for Cognitive Decline

8.0 Rationale for the Efalex Active 50+ formulation

9.0 Safety

10.0 References

11.0 Package Insert
1.0 Introduction

Many people believe that memory loss and confusion are a normal part of aging. However, scientists now know that most people can remain both alert and able as they age, even though it may take them longer to remember things.

Some memory problems are serious, and others are not. People who have serious changes in their memory, personality, and behavior may suffer from a form of brain disease called dementia or cognitive decline. Alzheimer’s disease is one of many types of dementia. It is one of the most disabling and burdensome health conditions globally because it seriously affects a person’s ability to carry out daily activities. Although the origin of dementia’s many forms can vary, research clearly shows that nutritional management can play a beneficial role to prevent and reduce its effects. The following nutritional supplements are of particular benefit.

**Omega 3 Nutrients, docosahexaenoic acid (DHA)** – are the building blocks of the brain and help with the transmission of messages between nerves.

**Phosphatidylserine (PS)** - plays an important role in healthy nerve function through the central nervous system including the brain.

**Vitamin B12 and folic Acid** – may help maintain brain cell integrity and nerve cell function.

**Ginkgo biloba** – helps maintain memory in the short term.

1.1 What is Age-Associated Memory Impairment (AAMI)? What is dementia?

Most people will experience a decline in certain cognitive abilities as they age such as to think quickly and to recall from short-term memory. This decline is usually not pathological and coincides with a number of common decreases in physiological function that occur in conjunction with normal developmental processes. The three main changes that occur in normal aging brains are:\n
- The accumulation of yellowish brown “wear and tear” pigment, loss of axon myelin that interferes with effective transmission of electrical impulses along the axon, and general shrinkage.
- A reduction in connections between neurons and the number of properly functioning connections between neurons.
- Diminished blood flow and blood volume.

This type of decline, called **Age Associated Memory Impairment (AAMI)** or **Age Related Cognitive Decline**, may affect as much as 45% of the population aged 50-59 and clearly increases in prevalence with age². It interferes with an individual’s ability to perform demanding everyday memory tasks and can severely interfere with work for those in intellectually demanding careers. The rate of this age-related decline can be slowed by physical and mental exercise and especially proper nutrition.

Unfortunately, people with age related cognitive decline have a greater risk of developing more severe forms of decline which go beyond what is considered "normal". They are progressively robbed of their memories, intellect, and eventually their abilities to recognize spouses or children, to maintain basic personal hygiene,
and to speak understandably. These more serious forms of cognitive deterioration are caused by a variety of neuropathological conditions and dementing diseases.

The word “dementia” is Latin for “irrationality”. Dementia is a brain disorder that seriously affects a person's ability to carry out daily activities. The World Health Organization describes dementia as a syndrome due to disease of the brain, usually of a chronic or unpredictably progressive nature, in which there is disturbance of multiple higher cortical functions, including memory, thinking, orientation, comprehension, calculation, learning capacity, language and judgement.

Consciousness is not clouded. Impairments of cognitive function are commonly accompanied, and occasionally preceded by deterioration in emotional control, social behaviour, or motivation.

1.2 What causes dementia?

Dementia is caused by many conditions, some of which are treatable and reversible while others are not. Reversible conditions can be caused by a high fever, dehydration, vitamin deficiency and poor nutrition, reactions to medication, thyroid gland problems, or head injury. Medical conditions like these can be serious and should be treated by a doctor as soon as possible.

Irreversible conditions, which means they cannot be cured, include dementia with Lewy body (Pick’s dementia), Parkinson’s disease with dementia, Alzheimer’s disease and multi infarct dementia (sometimes called vascular dementia). The later two are the most common forms in older people.

Alzheimer's disease is more common than multi infarct dementia. It involves the parts of the brain that control thought, memory, and language. Within these areas of the brain, nerve cells change resulting in death of a large number of cells. Symptoms of Alzheimer’s disease begin slowly and become steadily worse. As the disease progresses, symptoms range from mild forgetfulness to serious impairments in thinking, judgment, and the ability to perform daily activities. Eventually, patients may need total care. These symptoms coincide with the appearance of protein plaques consisting of amyloid β-peptide and nerve fibre tangles in the brain. Formation of these protein aggregates may release reactive oxygen species leading to oxidative or free radical damage to surrounding tissues. The most prominent membrane fatty acid in the brain, docosahexaenoic acid is particularly vulnerable to this attack. (Docosahexaenoic acid is an omega-3 fatty acid found in fish oils.) Oxidative stress also damages nuclear DNA and exposes membrane phosphadidylserine (see Section 3.0). Eventually, this deteriorating process leads to neuronal impairment and cell death.

In multi infarct dementia, a series of strokes or changes in the brain’s blood supply may result in the death of brain tissue. The location in the brain where the strokes occur and the severity of the strokes determine the seriousness of the problem and the symptoms that arise. Symptoms usually begin abruptly and progress in a step-wise fashion with repeated strokes. Currently, there is no way to reverse damage that has already been caused by a stroke. However, treatment to prevent further strokes including dietary modification is essential.
In rare cases, dementia is genetically inherited – runs in families. In these cases symptoms usually occur before the age of 60 and progress rapidly. All currently known changes in genetic material (mutations) result in an overproduction of amyloid $\beta$-peptide, which destroys nerve cells in the brain. Several genetic factors are known to increase the risk, without themselves being the cause. These include a (normal) variant of the gene apolipoprotein E which encourages the deposition of the harmful protein as plaques.

1.3 How common is dementia?

With people now living longer than they ever have before, the probability of suffering from dementia increases along with that advancing age. Dementia predominantly occurs in the second half of our life, usually after age 65. Its frequency increases with rising age from less than 2% for the 65-69 year-olds, to 5% for the 75-79 year-olds, to more than 20% for the 85-89 year-olds and over 30% for those over 90 years of age. More than half of those affected by dementia suffer from Alzheimer's disease. Recently, the Delphi Consensus Study highlighted some alarming statistics regarding the global prevalence of dementia. According to this report, there are currently 24.3 million people with dementia with 4.6 million new cases confirmed every year (that’s one new case every 7 seconds!). China and its developing western-Pacific neighbours have the highest number of people with dementia (6 million), followed by western Europe with 4.9 million, and North America with 3.4 million. The seven countries with the greatest number of people with dementia in 2001 were: China (5.0 million), the European Union (5.0 million), the United States of America (2.9 million), India (1.5 million), Japan (1.1 million), Russia 1.1 million, and Indonesia (1.0 million).

The number of affected people is estimated to double every 20 years to 81.1 million by 2040. Most of these people live in developing countries (60% in 2001, rising to 71% by 2040). However, the rates of increase are not uniform such that numbers in developed countries are forecast to increase by 100% between 2001 and 2040, but by more than 300% in India, China and their south Asian and Pacific neighbours. As the number of afflicted continues to increase, the need for safe and effective therapies to prevent, delay or avert dementia symptoms becomes critical.

1.4 What are the risk factors for dementia?

Cerebrovascular disease associated with atherosclerosis, type 2 diabetes and hypertension is a particular risk for multi infarct dementia. The probability of developing Alzheimer's disease increases with advancing age and tends to run in families. It occurs more often in women and in people with a lower standard of education. Other factors include the presence of apolipoprotein E4 allele which causes plaques and tangles in the brain and an impaired cholinergic system in the brain (i.e. the nerve cells that respond to the neurotransmitter called acetylcholine) which impact on learning, memory and attention. Normal apolipoprotein E is
involved in the transport of cholesterol in the blood and neuronal repair. Therefore, any detrimental alteration in the gene will impact these two areas.

Recent research is pointing to low DHA status as a risk factor for developing cognitive decline and Alzheimer’s disease in particular. Presently, the reason for these low body levels of DHA is believed to be multifactorial and includes low dietary intake of DHA, free radical or oxidative degradation of DHA within the brain, impaired liver DHA shuttling to the brain or conversion of DHA to neuroprotective agents (neuroprotectin) in response to disease progression\textsuperscript{25}.

1.5 What are the most important early indications of dementia?

1. Forgetfulness – that happens more frequently and results in inexplicable states of confusion.

2. Language problems - often cannot remember simple words and instead use inappropriate fillers which make it difficult to understand the sentences.

3. Problems with spatial and temporal orientation - dementia sufferers might be on their own street and no longer know where they are, how they got there and how to get home again.

4. Impaired capacity of judgement - sometimes wear totally inappropriate clothes. For example, a bathrobe while shopping or several blouses on top of each other on a hot summer day.

5. Problems with abstract thinking - cannot recognise numbers nor carry out simple calculations.

6. Misplacing items - for example putting the iron in the oven and then forgetting where the item was placed.

7. Mood swings and behavioural changes – often these occur without discernible cause.

8. Personality changes – someone who has historically been kind hearted suddenly or gradually becomes abusive.

9. Loss of initiative – may loose interest in work and hobbies without replacement with new activities.

1.6 What are the symptoms of Age Related Cognitive Decline, Dementia and Alzheimer's disease?

People with Age Related Cognitive Decline are not demented. They have adequate intellectual functions but complain of gradual memory loss since early adulthood which interferes with important tasks of daily living, and this loss is measurable in performance tests\textsuperscript{2}.

Dementia comprises a range of symptoms including asking the same questions repeatedly; becoming lost in familiar places; being unable to follow directions; getting disoriented about time, people, and places; and neglecting personal safety, hygiene, and nutrition. Dementia sufferers have impairment in one of four areas:

1. Aphasia – an inability to express oneself, repeating words or phrases or an inability to understand what is being said.
2. Apraxia – an inability to perform a routine motor activity such as combing
one’s hair despite no paralysis or musculoskeletal abnormality.

3. Agnosia – an inability to recognize familiar objects through touch. For
example, a person with dementia cannot identify a pencil held in their hand
without looking at it.

4. Impairment in Executive Functioning – have difficulty with abstract reasoning
and in organizing things, schedules and activities.

**Alzheimer's disease** was first described in 1907 by the physician Alois Alzheimer.
The most commonly known symptoms of Alzheimer's disease include sudden or
progressive confusion, impaired memory and orientation, limitations in concentration,
planning and judgement, personality changes and later also perceptual, speech and
walking disorders. In the final stage, various body functions such as swallowing and
the excretion process are also affected. During the course of Alzheimer's disease,
patients lose their perception and social relationships and eventually independence in
managing everyday life.

Alzheimer's disease can proceed at different rates and through a different course
depending on the individual, but it generally occurs in three stages. The mild stage
typically includes impairments of mental abilities as well as mood swings. In the
moderate stage, behavioural disturbances increasingly develop, while physical
problems dominant the advanced stage.

### 1.7 How is Age Associated Memory Impairment/Cognitive Decline Diagnosed?

In 1985, experts appointed by the United States National Institute of Mental Health in
the United States developed a series of criteria similar to those employed in the
diagnosis of Alzheimer’s disease to define age related cognitive decline (Age
associated Memory Impairment – AAMI)². These criteria are objective and rigorous
but also reflect everyday problems such as “difficulty in remembering names
following introduction, multiple items to be purchased or multiple tasks to be
performed, telephone numbers or mailing codes, misplacing objects and difficulty
recalling information quickly or following a distraction”. This definition also has
exclusion criteria which are intended to eliminate people with any condition that
might cause adult-onset memory impairment such as Parkinson’s disease. The
American Psychiatric Association has also suggested a somewhat different criteria for
Age Associated Cognitive Decline (Aacd)⁹⁷.

### 1.8 How is Dementia Diagnosed?

Physical, neurological, and psychiatric evaluation are normally recommended. A
complete medical examination for memory loss may include gathering information
about the person’s medical history, use of prescription, over the counter and
recreational drugs, diet, past medical problems, and general health. Often the doctor
may also ask a family member for information about the person because correct
diagnosis depends on recalling these details accurately and this may not be possible for the person being tested.

Blood, urine tests and a brain CT scan may be completed to rule out other ailments and reversible conditions. Follow up brain scans may be necessary to detect changes over time. Mental abilities may be tested including memory, problem solving, counting, and language.

Diagnosing Alzheimer’s disease and multi infarct dementia is complicated by their frequent co-existence. Scientists once thought that multi infarct dementia and other types of vascular dementia caused most cases of irreversible mental impairment. They now believe that most older people with irreversible dementia have Alzheimer’s disease.

1.9 What are the Treatments for Dementia?

Alzheimer’s disease and multi infarct dementia are currently thought to be irreversible so medications are aimed at slowing disease progression or reducing disease symptoms particularly agitation, anxiety, depression, or sleeping problems.

In the early and middle stages of Alzheimer’s disease, the drugs tacrine (Cognex), donepezil (Aricept), rivastigmine (Exelon), and galantamine (Razadyne, formerly known as Reminyl) may be prescribed to possibly delay the worsening of some of the disease’s symptoms. These drugs are aimed at improving cholinergic brain function by increasing the concentration of the neurotransmitter, acetylcholine and/or by inhibiting acetylcholinesterase. Some of these treatments have also shown promise in moderate to severe cases. Memantine (Namenda) is often recommended for treatment of moderate to severe AD. These medications may also help other types of dementias.

It is very important for people with multi infarct dementia to prevent further strokes by controlling high blood pressure, monitoring and treating high blood cholesterol and diabetes, and not smoking. Dietary changes including use of fish oil supplements would be of particular assistance owing to their beneficial effects on circulation including reduced blood clotting, blood vessel plaque formation, triglyceride levels and blood pressure. This same advice applies to any aging person as a means of preventing age related cognitive decline.

The most recently studied preventive therapies have included nonsteroidal anti-inflammatory drugs (NSAIDs) which inhibit cyclooxygenase thereby preventing formation of anti-inflammatory fatty acid metabolites, and antioxidants which suppress free radical damage. The best researched anti-oxidant to date is α-tocopherol which may provide particular protection for highly unsaturated fatty acids such as docosahexaenoic acid and arachidonic acid. These treatments are aimed at reducing inflammation and oxidative damage to brain tissue – conditions that are apparent in various forms of age related cognitive decline, regardless of its origin.
2.0  What is the connection between Docosahexaenoic Acid (DHA) and Cognitive Decline?

2.1  Epidemiological Evidence

Population studies to determine if there is a link between DHA status and cognitive decline have provided mixed results, but the bulk of evidence certainly points to a connection. DHA status tends to be lower than normal in patients experiencing decline and development of the condition tends to occur more frequently in those with low DHA status. On the other side of the story, increased DHA intake from fish oils reduces the likelihood of losing cognitive function. A summary of these studies follows:

2.1.1  Low levels of DHA have been measured in various tissues from patients with Alzheimer’s disease.

- **Brain DHA levels** relative to normal controls have been somewhat conflicting. An early study showed that the phosphatidylethanolamine (PE) fraction (a specific membrane phospholipid) of the brain frontal grey matter, frontal white matter, hippocampus and pons all had lower than normal levels of DHA and arachidonic acid (AA)\(^8\). These results were confirmed in hippocampus derived PE while another phospholipids, phosphatidylinositol (PI) had lower than normal levels of AA\(^9\). Researchers suggested that these low levels of highly unsaturated fatty acids may contribute to oxidative stress in the brain that may enable degradation of brain phospholipids in Alzheimer’s disease. However, more recent studies have measured in total lipid fractions, either no change in DHA levels relative to controls in temporal and parietal cortex, hippocampus and cerebellum\(^10\), or higher than normal levels of DHA in the frontal cortex\(^11\). Since these results were obtained from total lipid fractions while earlier reports included studies of specific phospholipids, seemingly conflicting results could merely be the result of cellular or subcellular DHA redistribution. The significance of this to disease development or progression is yet unknown.

- **Blood DHA levels** have been consistently low relative to normal controls. The earliest report was from the Efamol Research Institute and included fatty acid analysis of plasma and RBC phospholipids from 36 patients with Alzheimer’s disease\(^12\). DHA levels were within the normal range in the plasma, but all Omega-3 fatty acids including DHA were about 65 % of normal in the RBC phospholipids. This pattern of normal levels in plasma, but reduced levels in RBC membranes suggests a problem in incorporating DHA into cell membranes. A more recent study measured the fatty acid composition of plasma total lipids, phosphatidylcholine (PC), lysophosphatidylcholine (lysoPC), and PE in patients diagnosed with Alzheimer’s disease, patients with other kinds of dementias, patients with cognitive impairment but not dementia and normal controls. Levels of DHA and eicosapentaenoic acid (EPA) were lower in all conditions relative to controls in total phospholipids, PE and PC. Patients with cognitive decline but not dementia also had lower than normal levels of DHA in lysoPC\(^13\). A case control study measured the fatty acid composition of serum cholesterol esters
in Alzheimer’s disease patients who were categorized into groups according to their clinical dementia rating (i.e. mild, moderate and severe)\textsuperscript{14}. Their DHA levels were progressively reduced with severity of clinical dementia and all were significantly below normal levels.

2.1.2 Evidence supporting the link between low DHA status and the development of cognitive decline are somewhat inconsistent but become more consistent as more studies become available.

- Two studies have shown a direct correlation between low DHA status and the development of dementia. The first involved 1188 volunteers who were followed for 10 years to assess development of Alzheimer’s disease. At the start of the study, DHA levels were measured in their serum PC fraction. Those with low DHA levels had a 67\% greater risk of developing Alzheimer’s disease\textsuperscript{15}. No relationship was found between serum levels of EPA and dementia. The second study included 246 participants followed for 4 years after testing to determine fatty acid levels in their red blood cell phospholipids\textsuperscript{16}. Those with higher EPA and DHA had a 40\% lower risk of cognitive decline.

- One study related the plasma phospholipids EPA and DHA status of volunteers to development of cognitive impairment and dementia during a 5 year follow up period\textsuperscript{17}. Higher EPA concentrations were associated with development of cognitive impairment while higher DHA levels were found in those with dementia. The authors noted that the differences among groups were relatively small and may be of little clinical significance.

- One study looked at the impact on cognitive decline that occurs before development of more debilitating dementia and showed that high Omega-3 status is associated with slower mental decline with aging. The study\textsuperscript{121} from the University of North Carolina included 2251 white adults with an average age of 57 years at the start of the study. Blood fatty acid composition was measured initially and cognitive function was determined three and nine years late to evaluate verbal learning, recent memory, psychomotor performance, linguistic impairment and global cognition. The results, taking into consideration a number of interfering variables, showed that higher blood levels of omega-3 LC-PUFA prevented deterioration in verbal fluency. This was particularly apparent in people with high blood pressure and high blood triglyceride and/or cholesterol levels.

- One study showed that people with high DHA levels are half as likely to develop dementia. Data collected through the Framingham Heart Study followed 899 initially healthy volunteers with a median age of 76 years\textsuperscript{123}. Blood samples were taken at the start of the study to determine their levels of various fatty acids. Their fish and DHA intake were also measured using a food frequency questionnaire. Over the course of the study, 99 people developed dementia including 71 cases of Alzheimer’s disease. Blood DHA levels averaged 3.6 percent among all volunteers and the top 25 percent of the group had DHA values above 4.2 percent. People in this upper range had a 47 percent lower risk of developing dementia, even after controlling for other
interference including body mass index, diabetes, high blood pressure, smoking and other known promoting factors. People who ate two or more servings of fish per week were 39 percent less likely to develop dementia, but those who ate less than that did not derive any benefit. Although oily fish contains many different fatty acids, it was only the DHA that was responsible for preventing dementia.

- In the Invecchiare in Chianti (InCHIANTI) study\textsuperscript{125}, plasma fatty acids were measured in 935 community-dwelling older persons randomly extracted from the population of two towns near Florence, Italy. Cognitive impairment was measured using the Mini-Mental Status Examination. Participants who scored \( \leq 26 \) underwent a detailed clinical and neuropsychological evaluation. The diagnosis of dementia was based on Diagnostic and Statistical Manual of Mental Disorders, Third Revision (DSM-III-R) criteria. The population was divided in three groups: persons with normal cognitive function, persons with cognitive impairment not demented, and persons with dementia. After adjustment for age, gender, education, body mass index, weight loss, smoking status, cholesterol and triglycerides levels, daily intake of alcohol, fatty acid and total energy intake, cardiovascular disease, depression and other fatty acid levels, participants with dementia had significantly lower omega-3 fatty acid levels, particularly alpha-linolenic acid, than did participants with normal cognitive function.

2.1.3 A link between high fish and more specifically high docosahexaenoic acid (DHA) intake and a reduced incidence of developing dementia has recently been discovered and evidence continues to mount.

- One of the earliest reliable prospective population studies\textsuperscript{18} (The Rotterdam Study) included 5,386 nondemented volunteers, age 55 years or older, assessed for dementia at baseline and following approximately 2.1 years\textsuperscript{19}. A food-frequency questionnaire was used to estimate their fatty acid intake throughout that time. After adjusting for age, sex, education and energy intake, high intake of fish was highly significantly related to a decrease in the development of dementia and in particular to Alzheimer’s disease. Results of this study were confirmed in the PAQUID epidemiological study on cognitive and functional aging which included 1416 participants followed for 7 years to assess their meat or fish intake relative to development of dementia during that time\textsuperscript{20} and the Zutphen Elderly Study involving 476 volunteers to related fish consumption to cognitive impairment and decline\textsuperscript{22}. Additional conclusions based on the Chicago Health and Aging Projects (CHAP) which included 3718 residents aged 65 years and older, indicated that the rate of cognitive decline with aging over a 6 year period as compared to people who ate fish less than once per week, was 10% slower in people who ate 1 fish meal per week and 13% slower in people who ate 2 or more fish meals per week\textsuperscript{23}.

- Two additional studies have focused on specific fatty acid intake and cognitive decline. A cross-sectional study involving 1613 subjects aged 45-70 years assessed memory, psychomotor speed, cognitive flexibility and overall cognition during a 5 year period while habitual food consumption was
measured using a food-frequency questionnaire. Intake of fatty fish and specifically eicosapentaenoic acid (EPA) and DHA were inversely related to the risk of impaired overall cognitive function and speed\textsuperscript{24}. The most definitive study included 815 subjects followed over 7 years showing a 60\% less risk of Alzheimer’s disease in people who consumed fish once per week compared to those who rarely ate fish\textsuperscript{25}. There was no link between low EPA intake and the development of Alzheimer’s disease. However, there was a direct correlation between low DHA intake and development of the disease. This could be interpreted to mean that low DHA is more directly associated with the development of Alzheimer’s related dementia while combined low EPA and DHA are more likely to be associated with dementia resulting from cardiovascular disease (multi infarct dementia).

- One observational study in 64 year olds compared cognitive function in a group of fish oil supplement users to non-users\textsuperscript{26}. Higher total omega 3 fatty acids and ratio of DHA to AA in red blood cell phospholipids in the fish oil supplement group was associated with better cognitive function later in life.

- A study included 867 healthy adults aged 70-79 years living in England and Wales who did not have dementia or diabetes, who scored 24 or greater on a Mini-Mental State Examination (MMSE) and who did not take fish oil supplements, showed that increased intake of omega-3 LC-PUFAs from fish is associated with better memory and brain function\textsuperscript{117}. Their brain function was assessed for memory, executive function, psychomotor speed and attention using a variety of validated tests including the California Verbal Learning Test (CVLT). Their psychological health was measured using the GHQ-30 questionnaire and they were asked to provide details of their habitual fish consumption and educational achieve (i.e. age at leaving full-time education). Results showed there were significant and consistent increases in CVLT scores with repeated increases in fish consumption. There were also significant improvements in global cognitive Z-scores, memory scores, executive function and delay scores in higher fish consumers that became less significant when confounding variables were taken into consideration.

- Two studies in healthy, aging adults have confirmed that omega-3 fatty acids from fish prevent cognitive decline associated with aging. The first study\textsuperscript{118} included 2031 men and women aged 70-74 years from Western Norway who underwent a variety of cognitive tests including the Kendrick Object Learning Test designed to assess dementia status and memory among noninstitutionalized elderly, the Trail Making Test to assess visual motor speed and attention, the Digit Symbol Test to measure focused attention, visuomotor coordination and psychomotor speed, the Block Design to measure visuospatial and motor skills, the Mini-Mental State Examination (MMSE) to measure various aspects of cognitive function including orientation to time and place, naming, repeating, writing, copying, instantaneous recall, short-term memory, backward spelling and performing a 3 stage command, and the Controlled Oral Word Association Test to test verbal fluency and psychomotor speed. Those who ate 10 grams or more of fish or fish products per day had significantly better test scores and a lower prevalence of poor cognitive performance than did those whose intake was less than 10 g/day. Most
cognitive functions were influenced by fish intake with nonprocessed lean fish and fatty fish having the strongest positive effect. In addition, the level of cognition was dependent on the dose of seafood with the maximum effect observed at an intake of about 75 g/day. The second study119 from the Universities of Wageningen and Maastricht in the Netherlands included 807 men and women aged 50-70 years who had their blood fatty acid levels measured. Cognitive performance for memory, sensorimotor speed, complex speed, information-processing speed and word fluency was assessed at baseline and after 3 years in 404 of those participants. Those who had higher omega-3 LC-PUFAs had less decline in sensorimotor speed and complex speed over the 3 years. There was no decrease in 3 year decline of memory, information-processing speed or word fluency associated with higher omega-3 LC-PUFA.

- One study looked at the impact on cognitive decline that occurs before development of more debilitating dementia and showed that high Omega-3 fatty acid intake slows mental decline associated with aging. The study120 from the Dutch National Institute for Public Health and the Environment included 210 men from the Zutphen Elderly Study who were not suffering from any form of dementia. Historical dietary intake was assessed when the men were 70-89 years old and cognitive function was measured using the Mini-Mental State Examination (MMSE). Men who consumed about 400 mg of omega-3 LC-PUFAs [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] per day had less cognitive decline in a five year period than those who ate only about 20 mg per day of these nutrients. The second study121 from the University of North Carolina included 2251 white adults with an average age of 57 years at the start of the study. Blood fatty acid composition was measured initially and cognitive function was determined three and nine years late to evaluate verbal learning, recent memory, psychomotor performance, linguistic impairment and global cognition. The results, taking into consideration a number of interfering variables, showed that higher blood levels of omega-3 LC-PUFA prevented deterioration in verbal fluency. This was particularly apparent in people with high blood pressure and high blood triglyceride and/or cholesterol levels.

- One study related the risk of cognitive decline in a group of older adults with hypertension to their dietary intake of omega-3 LC-PUFAs and the corresponding changes in their blood fatty acid profiles. The study122 included a group of men and women aged 50-65 years from the Atherosclerosis Risk in Communities (ARIC) study. Dietary assessment using a food-frequency questionnaire and plasma fatty acid profiles were completed in 1987-1989 (visit 1), while cognitive assessment with three screening tools--the Delayed Word Recall Test, the Digit Symbol Substitution Test of the Wechsler Adult Intelligence Scale-Revised and the Word Fluency Test (WFT) were completed in 1990-1992 (visit 2) and 1996-1998 (visit 4). Findings indicated that an increase of one standard deviation in dietary omega-3 LC-PUFA and balancing the ratio of omega-3/omega-6 decreased the risk of 6-year cognitive decline in verbal fluency among hypertensives. This corresponded with an increase in plasma levels of omega-3 LC-PUFAs.
One study investigated the association between fish consumption and subclinical brain abnormalities\(^{124}\). It included 3,660 participants age \(\geq 65\) from the population-based Cardiovascular Health Study who underwent an MRI scan in 1992-1994. Five years later, 2,313 were scanned. Food frequency questionnaires were used to assess dietary intakes. After adjustment for multiple risk factors, the risk of having one or more prevalent subclinical infarcts (strokes) was lower among those consuming tuna/other fish 3 or more times/week, compared to less than once month. Higher tuna/other fish consumption was also associated with trends toward lower incidence of subclinical infarcts and white matter abnormalities. No significant associations were found between fried fish consumption and any subclinical brain abnormalities.

One study analyzed the relationship between dietary patterns and risk of dementia or AD, adjusting for sociodemographic and vascular risk factors, and taking into account the ApoE genotype\(^{126}\). It included 8,085 nondemented participants aged 65 and over from the Three-City cohort study in Bordeaux, Dijon, and Montpellier (France) in 1999-2000 that had at least one re-examination over 4 years. Results showed that weekly consumption of fish was associated with a reduced risk of AD and all cause dementia but only among ApoE - 4 noncarriers. Regular use of omega-3 rich oils was associated with a decreased risk of borderline significance for all cause dementia. Regular consumption of omega-6 rich oils not compensated by consumption of omega-3 rich oils or fish was associated with an increased risk of dementia among ApoE - 4 noncarriers. Therefore, frequent consumption of fish, and omega-3 rich oils may decrease the risk of dementia and Alzheimer disease, especially among ApoE- 4 noncarriers. (ApoE is a gene that codes for a special protein which helps to transfer lipoproteins, fat-soluble vitamins and cholesterol into the lymph system and then into the blood stream. People with a particular form of APOE, called APOE-4 are prone to develop atherosclerosis, reduced cognitive ability and AD.)

The largest population based study to date from either developing or developed countries reported that increased intake of fish reduces the risk of dementia by about 20 percent. The study included 14,960 people aged 65 year sor older in low and middle income countries including China, India, Cuba, the Dominican Republic, Venezuela, Mexico and Peru. After adjusting for various confounders and pooling the data from all sites, there was a dose dependent inverse association between dementia and fish consumption\(^{157}\).

\[\text{2.2 Preclinical Biochemical Studies}\]

Most biochemical studies have aimed at identifying the cause of Alzheimer’s disease and other irreversible types of dementia excluding multi infarct dementia*. These studies have followed diverse pathways of investigation and there is still relatively little understanding of how all the research results to date tie together to provide a
clear understanding of these conditions. However, many lines of research highlight the preventive role that DHA may play. A summary of these discoveries follows:

* multi infarct dementia is caused by cardiovascular disease

- AA is evenly distributed throughout the white and grey matter of the brain while DHA is particularly rich in grey matter where it is concentrated in the neurons. These unsaturated fatty acids are highly susceptible to free radical damage. This destructive process converts AA to isoprostanes and DHA to neuroprostanes. Levels of these free radical products have been measured in temporal and parietal cortex, hippocampus and cerebellum in Alzheimer’s disease patients and normal controls\(^\text{10}\). Neuroprostanes but not isoprostanes were 40-70% lower in all brain tissues from the Alzheimer’s disease patients as compared to controls. Therefore, DHA in particular is being destroyed as part of the disease process.

- Research highlighting the oxidative destruction of AA and DHA in Alzheimer’s disease has accumulated through studies using nonsteroidal anti-inflammatory drugs (NSAIDs) and antioxidants to treat the condition\(^\text{6}\). Although NSAIDs effectively inhibit enzyme catalyzed oxidation (through cyclooxygenase) and antioxidants suppress free radical mediated damage, both types of treatments have produced unexpected toxicity in the elderly\(^\text{6}\).

- One group of researchers has speculated that high DHA levels in the brain may contribute to oxidative damage to nearby proteins\(^\text{11}\). Looking at the problem from exactly the opposite direction, another research group has partially blamed the oxidative destruction of brain AA and DHA on overproduction of reactive oxygen species following aggregation of amyloid-β protein\(^\text{27}\). This research group has also measured the formation of an important DHA derivative called neuroprotectin created through the lipoxygenase pathway. They have speculated that neuroprotectin reduces inflammation and promotes survival of stressed brain cells in Alzheimer’s disease. Therefore, both oxidative damage and use of DHA as a substrate for neuroprotectins may contribute to low DHA levels observed in Alzheimer’s disease.

- Faulty blood supply and flawed nerve cell signalling converging to and diverging from oxidative metabolism has been proposed to contribute to initiation and maintenance of inflammatory responses and neuronal degeneration in Alzheimer’s disease. The cell signalling errors may be the result of modified membrane composition effecting receptor response as well as alterations in lipid derived messengers\(^\text{28}\).

- Existing drug medications for Alzheimer’s disease either increase the concentration of the neurotransmitter acetylcholine and/or inhibit the enzyme that breaks down acetylcholine (acetylcholinesterase)\(^\text{29,30}\). Alternate drug targets that also impact on memory have included the neurotransmitters glutamate and serotonin as well as the function of calcium and potassium ion channel\(^\text{31}\). The impact of abnormal fatty acid composition of brain cell membranes could profoundly affect all of these mechanisms through altering ion channel and receptor function and second messenger formation.
A review article published in 2008 proposed the following mechanisms of action for the effects of DHA in particular on AD:

(i) **Increased Cell Membrane Fluidity** – DHA is highly concentrated in the nerve cells in the brain and retina of the eye. Both of these tissue types require high fluidity within their membranes to ensure flexibility that allows proper enzyme, carrier molecule and receptor activity. Since DHA has six double bonds, it is a high flexible molecule and is responsible for providing the membrane fluidity required in these membranes.

(ii) **Altered Lipid Raft Composition and Function** – Lipid rafts are specialized organizational sites (sort of like little factories) within cell membranes that are responsible for separating different cell functions, acting as super highways to transport and process proteins within cells, and for enabling communication between cells (cell signaling). One type of lipid raft in particular is involved in the formation of amyloid precursor protein. This protein is produced in excess in the brains of people with AD and leads to the development of damaging protein plaques within their brain tissue. DHA may modify the lipid raft composition sufficiently to reduce formation of amyloid precursor protein.

(iii) **Altered Formation of Inflammatory Mediators and Regulators** – Both EPA and DHA give rise to compounds that have strong anti-inflammatory effects.

(iv) **As a Precursor of Bioactive Compounds** – It has recently been discovered that DHA can be made into compounds called docosanoids. One of these, Neuroprotectin D1, provides anti-inflammatory and neuroprotective activity within the brain.

(v) **As a Modulator of Cell Death (apoptosis) within the Brain** – DHA reduces the activity of an enzyme called caspase which is involved in progressing brain cell death in AD. In addition, DHA enhances the accumulation of phosphatidylserine (PS) within nerve cells which protects them from cell death.

(vi) **Inhibitor of Oxidative Stress within the Brain** – Oxidative stress occurs within the brains of people with AD before beta-amyloid protein deposition and formation of nerve fiber tangles. DHA protects against this oxidative damage.

(vii) **Altered Brain Gene Expression** – Increases in brain DHA alter the expression of a variety of genes including those controlling the cytoskeleton and membrane associations, raft formation, signal transduction, ion channel formation, energy metabolism and regulatory proteins. Many of these impact brain cell function.

(viii) **Reduced Beta-Amyloid Peptide Formation** – DHA reduces the accumulation of beta-amyloid peptide formation in the brain. The build up of this protein to eventually form plaques is the key physical brain change in patients with AD.
2.3 Preclinical Intervention Studies

Preclinical intervention studies have provided further understanding of the physiological mechanisms involved in dementia and have shown that DHA supplementation can slow disease progression and protect against cognitive deterioration.

- Amyloid-β peptide infusion into the brain can cause development of Alzheimer’s disease. Pretreatment with DHA can prevent associated learning deficiencies that normally occur as the disease progresses\(^{32}\). DHA supplementation can also reduce Amyloid-β peptide plaque formation by up to 50% in genetic conditions where this excessive protein accumulation occurs\(^{33}\).

- In genetic conditions where Amyloid-β peptide accumulates, DHA supplementation can increase DHA levels in the cortico-hippocampal regions of the brain, and reduce lipid peroxides and reactive oxygen species in the cerebral cortex and hippocampus; suggesting that DHA increases antioxidative defences in the brain\(^{34}\). Reduced lipid peroxides in the hippocampus have also been measured in normal aging populations supplemented with DHA as compared to those not treated with DHA\(^{35}\). These low lipid peroxide levels were directly correlated with decreased loss of reference and working memory, indicating that DHA protects against lipid peroxidation which results in sustained memory function.

- The brain is a massive network of electrically active cells (neurons) that communicate with each other through specialized cell junctions (synapses). Synapses consist of a presynaptic neuron that releases a chemical messenger (neurotransmitter) across a narrow gap to stimulate the postsynaptic neuron. Throughout development and in adult life, the brain responds to experience by adjusting the strength of communication at individual synapses and by changing the physical pattern of synaptic connections between neurons. In this way, information can be stored by the nervous system in the form of altered structure and chemistry of synapses and/or by the formation of new synapses and the elimination of old ones. This “plasticity” of brain synapses is believed to be the basis of learning and memory.

The architecture of synapses and proteins interacting with each other within the junction site on the postsynaptic neuron are fundamental to information processing and storage. Some of these proteins are receptors for specific neurotransmitters while others are scaffold proteins and enzymes that mediate postsynaptic signalling. Scaffold proteins maintain the three dimensional configuration of the site thereby ensuring that all relevant signal components are within proximity to one another in order to achieve successful message transmission. DHA supplementation protects against the loss of certain proteins within the junction site on the postsynaptic neuron including segments of enzymes\(^ {36}\) and scaffold proteins\(^ {37}\). This selective protection of postsynaptic proteins by DHA can have powerful preventive implications for Alzheimer’s disease where synaptic loss is critical.
- Long-term fish oil treatment enhances functional recovery of parts of the brain following ischemic damage\textsuperscript{111}.

- In mature animals, dietary supplemented DHA is incorporated into brain phospholipids and is associated with delay in cognitive decline\textsuperscript{112}.

- Dietary EPA preadministered protects against the impairment of learning ability in rats infused with beta peptide. Analysis revealed that altered genes included those that control synaptic signal transduction, cell communication, membrane-related vesicular transport functions, and enzymes and several other proteins were modified and that EPA, by acting as a precursor for DHA, ameliorates learning deficits associated with Alzheimer’s disease and that these effects are modulated by the expression of proteins involved in neuronal plasticity\textsuperscript{113}.

- A DHA-enriched diet significantly increases spatial learning ability, decreases hippocampal oxidized proteins and synaptic plasticity affecting learning and memory. These effects are enhanced by exercise\textsuperscript{114}.

- Fish oil supplementation from conception through to adulthood resulted in significant enhancement of both reference and working memory, whereas DHA depleted animals had significantly poorer reference and working memory. However, FO supplementation partially rescued both performances in depleted animals. The hippocampus and olfactory bulbs accumulated more DHA, were more resistant to dietary DHA deprivation, and showed better DHA recovery than the visual cortex, frontal cortex, and cerebellum. These results suggest that DHA is critical for the development and maintenance of learning memory performance\textsuperscript{115}.

- Living in an enriched environment during development enhances memory function in adulthood while living in an impoverished environment impairs memory function. However, DHA + uridine monophosphate supplementation ameliorates memory deficits associated with rearing under impoverished conditions, and that this effect may be mediated in part through enhanced synthesis of brain membrane phospholipids\textsuperscript{116}.

2.4 Clinical Trials using DHA Supplementation

- The first human intervention study investigated the effects of DHA supplementation on moderately severe multi infarct dementia\textsuperscript{38}. Twenty patients were treated with either 0.72 g of DHA per day or placebo for 12 months. All were subjected to psychometric tests (Mini-Mental State Examination – MMSE), Hasegawa’s Dementia Rating (HDS-R), clinical evaluation and blood fatty acid analyses at baseline and after 3, 6 and 12 months of treatment. There were no significant differences between the two groups at the start of the study. Blood DHA and eicosapentaenoic acid (EPA) was significantly higher in the active group compared to placebo by month 3.
This correlated with significant improvements in dementia scores (both HDS-R and MMSE) while the placebo group continued to deteriorate.

- The Japan Functional Food Research Association has reported improvements or slowing of disease progression in people with multi infarct dementia and Alzheimer’s disease when supplemented with a DHA concentrate. Patients showed improvements in communication skills, will power, motivation, delirium, the tendency to wander, emotional disorders and mental depression.

- A double-blind, placebo controlled study including 49 healthy men and women aged 22 to 51 years provides support for use of DHA supplementation in those with dementia. A fish oil providing 1.6 g of EPA, 0.8 g of DHA and 0.4 g of other omega-3 fatty acids per day improved their ability to control anger and repress unsuitable responses. In addition, they had less anxiety, fatigue and depression and had increased vigour. These improvements (that did not occur in the placebo group) were directly correlated with increased omega 3 content in their blood.

- The first double-blind, placebo controlled trial to describe the effects of Omega-3 supplementation in AD included 204 patients with mild to moderate AD as assessed by the Mini-Mental State Examination (MMSE). Patients were treated with either 1.7 g of DHA and 0.6 g of EPA or a placebo for 6 months, after which all received the fish oil treatment for another 6 months. Cognitive function was measured using the MMSE and cognitive portion of the Alzheimer Disease Assessment Scale, global function was assessed with the Clinical Dementia Rating Scale, and blood fatty acid levels were assessed at baseline following 6 and 12 months supplementation. At 6 months, in a subgroup of 32 mildly effected patients treated with Omega-3 fatty acids, there was a significant reduction in decline rate compared with the placebo group. A similar arrest in decline rate was observed between 6 and 12 months in the placebo subgroup when receiving the active treatment. Blood fatty acid levels of DHA and EPA were significantly below normal at baseline. After six months of active treatment there was a 2.4 and 3.6 fold increase in DHA and EPA respectively. After twelve months supplementation, both groups had similar blood levels of these two fatty acids. This study showed that Omega-3 supplementation delays the rate of cognitive decline in patients with very mild Alzheimer Disease and stresses the importance of early supplementation as it could halt progression of the disease.

- A randomized, double-blind, placebo-controlled clinical study, led from Taipei City Hospital, Taiwan included 23 people with mild or moderate AD and 23 with MCI that took either 1.8 g/day of Omega-3 (1080 mg EPA + 720 mg DHA) or an olive oil placebo for 24 weeks. Cognitive tests were performed at baseline and at weeks 6, 12, 18 and 24 and included the AD Assessment Scale (ADAS-cog) and the Clinician’s Interview-Based Impression of Change Scale (CIBIC-plus) which measures disease severity and progression of illness. Blood fatty acids were assessed at baseline and weeks 12, 18 and 24. Results showed that Omega-3 LC-PUFAs may improve general clinical function in patients with mild or moderate AD and MCI, but
not their cognitive function. The cognitive effects of omega-3 LC-PUFAs might be more apparent in patients with MCI rather than those with AD.

- A randomized, double-blind, placebo-controlled clinical study\textsuperscript{128} from The Netherlands included 302 cognitively healthy adults aged 65 years or older that took either 400 mg Omega-3 (226 mg EPA + 176 mg DHA), 1800 mg Omega-3 (1093 mg EPA + 847 mg DHA) or olive oil placebo for 26 weeks. Cognitive performance was determined at baseline and after 13 and 26 weeks to assess attention, sensorimotor speed, memory and executive function using a variety of tests and blood fatty acid status was assessed. Blood EPA/DHA increased by 238% and 51% in the high dose and low dose groups, respectively and compared with placebo. In general, cognitive test scores in all three groups improved, but changes were not significantly different among the groups and were probably mostly due to learning effects. However, men in the low dose group improved compared to placebo and people who carried the APOE-4 allele showed improvement following treatment with both doses of EPA+DHA. APOE is a gene that codes for a special protein which helps to transfer lipoproteins, fat-soluble vitamins and cholesterol into the lymph system and then into the blood stream. People with a particular form of APOE, called APOE-4 are prone to develop atherosclerosis, reduced cognitive ability and AD.

- A randomized, double-blind, placebo-controlled clinical study\textsuperscript{129} investigated the effect of EPA and DHA on mental well-being in 302 independently living individuals aged $\geq$ 65 y. They were randomly assigned to consume 1800 mg/d EPA+DHA, 400 mg/d EPA+DHA, or placebo capsules for 26 wk. Changes in mental well-being were assessed as the primary outcome with the Center for Epidemiologic Studies Depression Scale (CES-D), Montgomery-Asberg Rating Scale (MADRS), Geriatric Depression Scale (GDS-15), and Hospital Anxiety and Depression Scale (HADS-A). Results showed that plasma concentrations of EPA+DHA increased by 238% in the high-dose and 51% in the low-dose fish-oil group compared with the placebo group. Treatment with neither 1800 mg nor 400 mg EPA+DHA differentially affected any of the measures of mental well-being after 13 or 26 wk of intervention compared with placebo.

- The first study to confirm that a combination of DHA and Omega-6 arachidonic acid (AA) improve mental function in elderly people with mild memory loss and those with brain trauma injuries was a collaborative study among eight different research centres in Japan\textsuperscript{130}. This double-blind, placebo controlled trial included 39 patients (12 male, 9 female) averaging 68 years of age. Ten of these had organic brain lesions resulting from haemorrhage or brain trauma injuries more than 5 years previous, eight had Alzheimers disease (AD) and twenty-one had mild memory loss associated with aging. Patients were randomly assigned to take either a placebo or a fatty acid supplement containing 240 mg/day of DHA and AA for 90 days. Cognitive function was measured before and after treatment using the Japanese version of the standard neuropsychological screening battery, RBANS, which measures five domains of cognitive function including: immediate memory, visuospatial/constructional, language, attention, and delayed memory. The
DHA/AA supplementation produced the greatest significant improvement in patients with brain damage, where immediate memory scores improved by 10 points and delayed memory scores improved by 14 points. There was also significant improvement in immediate memory and attention in those with mild memory loss associated with aging as compared to the AD patients and placebo treated subjects.

The results of the Memory Improvement with DHA Study (MIDAS), presented on July 12th at the 2009 International Conference on Alzheimer’s Disease (ICAD 2009) in Vienna showed that DHA supplementation can improve both memory function and heart health in healthy older adults. The randomized, multi-centered, double-blind, placebo controlled trial included 485 healthy older people of approximately 70 years of age who reported they had mild memory loss (age related cognitive decline). They were randomly assigned to take either 900 mg of algal sourced DHA concentrate per day or an identical looking placebo for 6 months. Before and after treatment, they were tested for working memory, memory retention, attention and executive function as the primary end points for the study. As a secondary interest, they were also tested for visual acuity and blood fatty acid status. The results showed that compared to baseline, those taking DHA made significantly fewer errors on the Paired Associate Learning (PAL) test that assesses memory, attention and cognitive function. In addition, their plasma levels of DHA doubled and this increase correlated with improvements in the PAL test. Interestingly, this group also experienced a significant drop in heart rate while blood pressure and weight was unchanged. This large scale clinical trial confirmed that 6 months of DHA supplementation improved memory function and decreases heart rate in healthy older adults with age related cognitive decline. The authors stated the improvement was the equivalent of a 3 year age drop.158.
3.0 What is the connection between Phosphatidylserine (PS) and Cognitive Decline?

3.1 What is Phosphatidylserine (PS) and how is it involved in brain function?

Phosphatidylserine (PS) is a naturally occurring acidic phospholipid that is part of our normal diet and is also part of our cell membranes. It makes up about 10% of the total phospholipids, but its greatest concentration is found in myelin from brain tissue. It is the only phospholipid that carries an ionic charge which enables its unique and essential capacity to anchor proteins within the cell membrane. These specific PS-protein associations may be the ultimate instruments for its global effects on brain function.

Although percentage wise it is a relatively minor membrane component, it is essential to achieve electrical potential in membranes and to enable movement of electrically charged particles (ions) within the neuron. Therefore, it is a brain-specific nutrient because of its relative importance to neuronal function. Its specific functions are as follows:

- **Maintenance of the Cell’s Internal Environment:** Nerve cells rely on ATPases to regulate sodium-potassium and calcium-magnesium balance which is directly involved in electrical activity. ATPases require the presence of PS in the surrounding membrane to function optimally.
- **Secretory Vesicle Release:** PS helps regulate the release of many different types of neurotransmitters such as acetylcholine. PS is located on the inner side of the plasma membrane where neurotransmitter vesicles attach in preparation for their release from the pre-synaptic neuron. If PS levels in the membrane are too low, this interferes with release of the neurotransmitters and ultimately prevents message transmission.
- **Signal Transduction:** PS seems to help neuronal membranes resist age-related changes and possibly reverses some of them. In aging brains, the neuronal membranes loose receptors, those that remain become less effective at receiving messages, while the cell membrane itself becomes increasingly rigid. All these changes make it difficult to pass messages between neurons. PS helps to stabilize the position of existing receptors in the membrane, thereby allowing more efficient communication.
- **Gene Expression:** Neuronal membrane PS activates an enzyme called protein kinase C that plays a critical role in learning and memory. Specifically, protein kinase C turns on genes producing long-term changes in memory. It is worth noting that protein kinase C activity decreases with age, perhaps because of age-related deficits in PS.
- **Cell Growth and Renewal:**
  - **Due to physical location:** PS most often resides on the surface of the cell membrane that faces towards the inside of the cell. As a cell ages, enzymes called amino-PL translocases flip PS onto the outer side of the membrane. This signals that the cell is worn out and should be recycled. This displacement of PS with age may negatively impact receptor function.
  - **Due to impact on growth factors:** Growth factors are small proteins that pass between cells, turning on or off specific receptors that
regulate cell proliferation and renewal. PS stimulates nerve growth factor synthesis and release and partially prevents nerve growth factor receptor decline related to aging

Evidence suggests that the concentration of PS in neuronal membranes is partly affected by DHA levels. DHA within the membrane promotes translocation and activation of a kinase that increases the concentration of PS in the membrane. Therefore if DHA levels are too low, PS levels will also be too low. This mechanism strongly supports simultaneous supplementation with these two nutrients to achieve maximum benefit.

3.2 Preclinical Intervention Studies

Long term dietary supplementation with PS reduces and sometimes eliminates many neuronal effects of aging. Studies have confirmed it specifically:

- Enhances the availability or release of acetylcholine and dopamine which may provide a modest improvement in cognitive function.
- Restores receptor numbers to normal in the aged.
- Helps neuronal membranes maintain their electrical charge.
- Maintains general structure and health of the neuron.
- Prevents aging brains from losing dendritic spines (needle like projections that act as connections to a multitude of surrounding cells in the communication network).
- Prevents severe learning deficits and enhances memory.

Essentially these studies have shown that PS helps to allow neurons in the network to keep effectively communicating with one another so that existing memories can be retained and new memories can be formed.

3.3 Clinical Trials using PS Supplementation

At least 16 clinical trials using PS to treat either dementia or impaired cognitive function have been published. Five of these were open trials and one was a cross-over study which is somewhat inappropriate for assessment of a condition with expected gradual deterioration. Results reported in the remaining double-blind, placebo-controlled trials ranged from modest to substantial improvements in various memory functions, learning, concentration, word skills and mood that all enhance quality of life. These studies were measuring treatment outcomes in patients with active and often severe mental deterioration. Therefore, one could argue that much lower doses of PS in healthy aging adults could provide significant preventive effects. Highlights of the double-blind, placebo-controlled studies that reported benefit follows:

- Six weeks supplementation with 300 mg/day of PS or placebo in 35 hospitalized demented patients demonstrated a trend towards behavioural improvements that the author felt benefited daily living and was useful for both the subjects and their families.
- Two multi-centred studies co-ordinated through the Memory Assessment Clinic at Vanderbilt University School of Medicine in the United States, supplementing with 300 mg/day of PS or placebo for 12 weeks have measured
improvements which were more pronounced in people with greater age related impairment \(^48\) and less pronounced in people more severely affected with Alzheimer’s disease\(^49\). This supports the likelihood that preventive effects in healthy aging adults could be achieved and should be initiated before disease progression reaches a stage where correction becomes more difficult due to irreparable structural changes in the brain.

- One study included 149 **normally aging adults** with age-associated memory impairment (AAMI) that improved on computerized and standard performance tasks related to learning and memory\(^48\). People with dementia, Alzheimer’s disease or other forms of cognitive deterioration were excluded from this study. A subgroup of 57 relatively more severely impaired subjects had significantly improved name face acquisition, name face delayed recall, facial recognition, telephone number recall and misplaced objects recall. These benefits were lost 4 weeks after PS supplementation was discontinued. **Results of this study confirm that PS is an effective preventive means of reducing cognitive decline in normal, healthy aging adults.**

- The other study included 51 probable Alzheimer’s disease patients that showed improvements on four of five cognitive measures within 3 weeks of starting treatment\(^49\). Those with greatest cognitive impairment were least likely to gain benefit.

These solid clinical findings confirming protective effects suggest that supplementation in mid-life would slow age-related cognitive decline which is well underway in otherwise-healthy people by age 50. This preventive approach should be initiated before development of more severe forms of dementia which respond less favourably to treatment.

- The largest and longest running multi-centred study to date included 425 elderly patients recruited from 23 institutions in Italy with moderate to severe cognitive decline that were treated with 300 mg/day of PS or placebo for 6 months\(^50\). Patients were tested using the Plutchik Geriatric Rating Scale and the Buschke Selective Reminding Test prior to treatment and at 3 and 6 months. Statistically significant improvements in behavioural (withdrawal and apathy) and cognitive (memory and learning including word-list recall) parameters were measured in the PS treated group as compared to the placebo group. In addition, clinical evaluations and laboratory tests showed that PS was well tolerated.

- The Max-Planck-Institute in Germany has completed two studies to compare the effects of PS to a number of other treatments in patients with probable Alzheimer’s disease\(^51,52\).

  - The first study included 40 patients treated with either social support, cognitive training or cognitive training in combination with either pyritinol or 300 mg/day PS for six months\(^51\). The patients underwent neuropsychological testing and measures of regional cerebral metabolic rate achieved by a visual recognition task before and after treatment. At the end of the study, the cognitive training + PS group showed a significant enhancement in cerebral metabolic rate during the
visual recognition task and an improvement in cognitive function as compared to other groups.

- The second study included 70 patients randomly allocated to the same treatment groups as described for the first study and subjected to the same types of testing at multiple intervals throughout the study\(^5^2\). The dosage of PS in this study was 400 mg per day and it had effects on different measures of brain function than the other treatments. The neuropsychological improvements that were achieved were most notable after 8 and 16 weeks. However, they faded towards the end of the treatment period, possible because they were shadowed by the progressive pathological changes that were occurring in this group of severely diseased patients.

- A multi-centred study in Italy involved 87 subjects with moderate cognitive deterioration taking 300 mg/day of PS or placebo\(^5^3\). Measures of attention, concentration, short-term memory and daily living skills were accessed before treatment, after 60 days of treatment and 30 days after treatment was stopped. In the PS group, word acquisition and recall was highly significantly improved as were measures of self-sufficiency, sleep disturbances, disadaptive behaviour, initiative and overall behavioural deficit. Improvements in the later three would translate into lessened apathy and withdrawal. The author concluded that PS appears to exert an action in two distinct contexts: one relating to the cognitive effects such as vigilance, attention, and short-term memory, and the other relating to behavioural aspects such as apathy, withdrawal and daily living.

- Another multi-centred trial in Italy included 170 subjects with mild to moderate age related cognitive deterioration taking 300 mg/day of PS or placebo for 90 days\(^5^4\). A battery of neuropsychological tests were given at baseline, day 45 and day 90 following treatment. After ninety days, statistically significant improvements had occurred in the PS treated group for 12 of the 24 tests completed. These included measures of attention, vigilance, word manipulations, immediate recall, and delayed recall which combined improve memory, learning, concentration and verbal ability. Several studies using EEG (Electroencephalography) and PET (Position Emission Tomography) have shown that PS may enhance global brain performance. Both of these techniques have previously shown value in accessing drug availability and action in this organ.

- One study including 33 patients with mild primary degenerative dementia treated for 8 weeks with 300 mg/day of PS and 8 weeks with placebo in a cross over study. EEG mappings showed that patients initially had higher power values in all frequency bands (except alpha) when compared to younger, healthy controls. PS treatment reduced these values compared to placebo and shifted them towards the normal level\(^5^5\). These positive shifts in values in the PS group correlated with improvements in global clinical ratings.

- Another study used PET to correlate clinical improvements with brain metabolism\(^5^6\). It included 40 subjects with mild to moderate cognitive
impairment that were treated with 400 mg/day of PS or placebo for 6 months. PS treated subjects had greater metabolic activation which was associated with improved results for neuropsychological test.

- PET has also been used to detect improvements of up to 14.8% in glucose metabolism in patients with probable Alzheimer’s disease treated with 500 mg/day of PS for 8 weeks\(^56\). These improvements were most apparent in subjects most affected and were important since memory impairments can be related to impairments in glucose metabolism and improvements in glucose utilization may prevent further cognitive decline.

One placebo-controlled, multi-dose, double-blind, balanced-crossover study compared the effectiveness of Gingko biloba extract singly or combined with PS\(^132\). It included 28 healthy young participants received 120 mg Gingko biloba extract singly or complexed with PS or a matching placebo on separate days 7 days apart. Cognitive performance was assessed using the Cognitive Drug Research (CDR) computerised test battery and Serial Subtraction tasks immediately prior to dosing and at 1, 2.5, 4 and 6 h thereafter. Short term treatment with 120 mg of Gingko biloba extract was not associated with markedly improved performance on the primary outcomes. However, administration of Gingko biloba extract complexed with PS resulted both in improved secondary memory performance and significantly increased speed of memory task performance across all of the post-dose testing sessions. Both treatments were associated with improved calmness. PS appears to potentiate the cognitive effects associated with a low dose of Gingko biloba extract.

### 3.4 Approved Health Claims pertaining to PS and Cognitive Decline

In Italy in the mid-1970s, PS was registered as a drug for memory deficits in the elderly. The licence was eventually suspended due to financial problems experienced by the pharmaceutical company that owned the rights\(^57\).

In May of 2003, the United States Food and Drug Administration authorized two health claims for phosphatidylserine (PS) for inclusion on food labels\(^46\). This permission was granted following their review of support documentation in a petition filed by a food manufacturer wishing to make health claims pertaining to this ingredient on their product labels and in advertising. FDA approval of the following claims adds significant justification for use of this ingredient in products designed for prevention of cognitive decline (Note: Given the litigious nature of the American public, the FDA always includes a disclaimer on qualified health claims to insure that they are not held responsible for their approval).

**Dementia claim and disclaimer:**

“Consumption of phosphatidylserine may reduce the risk of dementia in the elderly.

Very limited and preliminary scientific research suggests that phosphatidylserine may reduce the risk of dementia in the elderly. FDA concludes that there is little scientific evidence supporting this claim.”

**Cognitive dysfunction claim and disclaimer:**
“Consumption of phosphatidylserine may reduce the risk of cognitive dysfunction in the elderly.

Very limited and preliminary scientific research suggests that phosphatidylserine may reduce the risk of cognitive dysfunction in the elderly. FDA concludes that there is little scientific evidence supporting this claim.”
4.0 What is the connection between the B Vitamins and Cognitive Decline?

Vitamin B12 and folic acid impact indirectly on cognitive decline through their effects on blood homocysteine levels. Homocysteine is an amino acid produced in the metabolism of methionine, a process dependent on sufficient levels of vitamin B12 and folic acid58.

There are two hypotheses to explain how Vitamin B12 and Folic acid may positively impact on cognitive decline through their effects on homocysteine metabolism58 (i.e. through keeping the levels of homocysteine low):

1. **Vascular Hypotheses.** Elevated homocysteine levels have been recognized as an independent cardiovascular risk factor. Homocysteine induces atherogenesis by directly increasing formation of reactive oxygen species and by promoting oxidation of low-density lipoproteins. Reactive oxygen species alter smooth muscle function and promote proliferation of vascular smooth muscle cells thereby contributing to plaque formation within blood vessels. Homocysteine also increases platelet aggregation which contributes to the occurrence of clinically apparent and silent brain infracts and is an independent risk factor for recurrent stroke in patients with pre-existing coronary artery disease and in other populations. Numerous epidemiological studies have demonstrated an association between high homocysteine levels and the incidence of silent brain infarcts and white matter lesions and increased cortical and hippocampal atrophy.

2. **Neurotoxic Hypotheses.** Homocysteine activates the glutamate-binding site and partially blocks the glycine-binding site of the N-methyl-D-aspartate (NMDA) receptor in brain cells. This receptor regulates calcium ion influx into nerve cells and its proper functioning is therefore critical for efficient message transmission between nerve cells. Under conditions of elevated glycine concentrations, such as occurs in stroke or head trauma, elevated homocysteine has a neurotoxic effect through hyperstimulation of the NMDA receptor. This results in an excess influx of calcium ions into the nerve cell and production of reactive oxygen species. The end result is brain cell damage or death.

4.1 Epidemiological Evidence

Serum homocysteine levels are significantly higher among patients with dementia than among normal control subjects59,60,61,62,63, the levels are inversely related to cognitive function in people with dementia64 and are associated with lower performance of attention, constructional ability, sensomotor speed and executive function in the elderly133, 135. These elevated homocysteine levels are more common among people with vascular dementia than in those with Alzheimer’s disease65. Similar correlations between high homocysteine levels and cognitive impairment are also present in normal aging populations with varying degrees of cognitive decline where dementia is not yet present66,67,68.
Low serum levels of vitamin B12 and folic acid correlate with high levels of homocysteine. In addition, low serum levels of vitamin B12 and folic acid are directly correlated with cognitive impairment in normal aging people without dementia and stroke as well as in those with progressive dementia. Post-stroke patients have been found to have combined low folate and high homocysteine levels.

In one study, low folic acid irrespective of Vitamin B12 levels has been associated with episodic memory and language in high functioning elderly Chinese while low Vitamin B12 irrespective of folic acid levels was associated with cognitive impairment in older people with absence of anaemia. However, another study in seniors with low vitamin B-12 status showed that high serum folate was associated with anemia and cognitive impairment, but when vitamin B-12 status was normal, high serum folate was associated with protection against cognitive impairment. Further investigation of this phenomenon has shown that in vitamin B12 deficiency, high folate status is associated with impaired activity of the two vitamin B12 dependent enzymes, methionine synthase and MMA-coenzyme A mutase.

It is worth noting that people with low red blood cell folate levels also have low DHA status and that there is a direct positive correlation between blood levels of these two nutrients. Therefore, preventive treatments containing both of these nutrients would provide more benefit than either alone.

### 4.2 Clinical Trials using B Vitamins Supplementation

A meta-analysis of randomized, placebo-controlled, clinical trials has shown that folic acid reduces blood homocysteine concentrations by 25% in normal aging populations. Vitamin B12 produces an additional 7% reduction. This significant trend was also measured in Alzheimer’s disease patients treated with vitamin B12 and folic acid.

A number of individual randomized, double-blind, placebo-controlled clinical trial studies have been reported as follows:

- Healthy aging women supplemented with vitamin B12, folic acid and vitamin B6 had positive effects on some measures of memory performance as assessed by standardized tests of cognitive processing, memory, executive function, verbal ability and self-reported mood measures.

- 818 participants taking 800 ug/day folic acid for three years reported significant improvements in areas of cognitive function that tend to decline with age including memory, sensorimotor speed and information processing speed. These improvements correlated with a 576% increase in serum folate concentrations.

- 195 healthy elderly people taking either 1000 ug Vitamin B12 with or without the addition of 400 ug of folic acid for 24 weeks had a borderline significant improvement in higher memory performance, but this was not associated with
decreased concentrations of homocysteine, although homocysteine levels did decrease\textsuperscript{133}.

- Males and females aged >50 years with mild and moderate AD and normal blood concentrations of vitamin B12 and folic acid supplemented with 1 mg folic acid, 0.5 mg Vitamin B12 and 5 mg Vitamin B6 + other vitamins and iron or placebo for 26 weeks had decreased homocysteine levels following supplementation but there was no significant beneficial effect on cognition of AD function\textsuperscript{141}.

- Vitamin B12) supplementation for 40 weeks in 30 consecutive mild to moderate dementia cases aged over 60 years with low serum B12 did not improve cognition but reduced delirium\textsuperscript{142}. 
5.0 What is the connection between Ginkgo biloba and Cognitive Decline?

Ginkgo biloba extracts have been widely promoted for a variety of functioning including memory and learning enhancement, and prevention and treatment of age related cognitive decline and dementia. Preclinical studies strongly support this assertion and many have focused on determining the mode of action\textsuperscript{143-149}, but studies in humans have lacked statistical requirements to achieve any definitive conclusion. However, the most compelling evidence indicates that it may be more useful as a long term preventative treatment for dementia with some improvements possible after the development of the condition, with less benefit apparent in normal, healthy adults. Multiple mechanisms of action have been proposed. However, the exact nature of the effects of Ginkgo biloba have yet to be determined.

5.1 Clinical Trials using Ginkgo biloba supplementation

Meta-Analyses of Randomized, double-blind, placebo-controlled clinical trials
- Alzheimer’s disease
  - A 1998 study reported a small but statistically significant effect of Ginkgo biloba extract (120-140 mg per day) on objective measures of cognitive function\textsuperscript{78}.
- Alzheimer’s disease, multi-infarct dementia or mixed types
  - A 1999 study concluded that Ginkgo biloba extract is safe and more effective for dementia than placebo, both in terms of delaying clinical deterioration and often in bringing about symptomatic improvements\textsuperscript{79}.
- Cognitive effects in healthy people
  - A 2002 study concluded there is no marked or consistent positive effect on any particular objective measure of cognitive function except for in the trial having the longest treatment duration\textsuperscript{80}. This last observation highlights a particular need for further long-term trials as long-term use as a preventative treatment may be justifiable.
- Intermittent claudication
  - A 2000 report states that Ginkgo biloba extract is superior to placebo in the symptomatic treatment of intermittent claudication\textsuperscript{81}. This may be of relevance to prevention of multi-infarct dementia as vascular problems are one of the risk factors for its development.

Summary of Clinical Trial Results for Studies Published Subsequent to the above listed Meta-Analyses
- Alzheimer’s disease, multi-infarct dementia or age related memory impairment.
  - In an open study lasting 6 months, there was a slight benefit and no clinically evident further loss of function in patients with Alzheimer’s disease, multi-infarct and mixed dementia\textsuperscript{82}.
  - In a randomized, double-blind, placebo-controlled study supplementing with either 160 or 240 mg/d of Ginkgo biloba extract for 24 weeks there was no dose-effect relationship and no benefits\textsuperscript{83}.
- Cognitive effects in healthy people
  - Of the six\textsuperscript{84,85,86,87,88,89} randomized, double-blind, placebo-controlled clinical trials completed up to 2006 following the 2002 meta-analysis
mentioned above, only one of those found enhancement in memory processing\textsuperscript{84} and one reported improvements in mental flexibility in postmenopausal women, but only in those with poorer performance\textsuperscript{85}.

- A study providing acute and chronic doses showed that a single dose improved performance in tests of attention and memory while there were no effects after six weeks continues treatment, suggesting that tolerance develops in young, healthy participants\textsuperscript{90}.

- A community based prospective cohort study found no quantifiable benefit on memory performance during long-term use in healthy adults\textsuperscript{91}.

- A randomized, double-blind, placebo-controlled study including 78 healthy young subjects supplemented with 120 mg Gingko biloba extract reported improved memory performance 1 and 4 hour post-dose, but a negative effect on performance on the speed of attention at 1 and 6 hours post-dose\textsuperscript{150}.

- A 4-month, randomized, double-blind, placebo-controlled parallel study including 90 men and women (age range 65 to 84 years) without dementia or depression were randomly assigned to placebo or a ginkgo biloba-based supplement containing 160 mg ginkgo biloba, 68 mg gotu kola, and 180 mg DHA per day for 4 months. At baseline, the participants' cognitive function was above average. Following treatment, there were no differences between placebo and active except for one of six cognitive tests where placebo was slightly better than active. There were no significant differences in quality of life, platelet function, or adverse events\textsuperscript{151}.

- A randomized, placebo-controlled, double-blind, 42-month pilot study with 118 cognitively intact subjects randomized to standardized Gingko biloba extract or placebo reported neither altered risk of progression from normal to Clinical Dementia Rating (CDR) = 0.5, nor protected against a decline in memory function. However, secondary analysis taking into account medication adherence showed a protective effect of Gingko biloba extract on the progression to CDR = 0.5 and memory decline\textsuperscript{152}.

- **Prevention of Alzheimer’s disease**

  - 1462 community-dwelling elderly women were followed for seven years. Those who were given Ginkgo biloba for at least 2 years had a statistically significantly lower risk of developing Alzheimer’s disease\textsuperscript{92}.

  - The National Institute of Health funded a $15 million, randomized, double-blind, placebo-controlled clinical trial supplementing approximately 3069 normal elderly people with 120 mg Ginkgo biloba extract twice daily for 6.1 years to access the prevention of dementia and/or age related cognitive decline\textsuperscript{93} (The GEM study). Baseline data was published\textsuperscript{94} previous to the final results\textsuperscript{153}. The overall dementia rate was 3.3 per 100 person-years in participants assigned to G. biloba and 2.9 per 100 person-years in the placebo group. Therefore, was not effective in reducing either the overall incidence rate of dementia or AD incidence in elderly individuals with normal cognition or those with MCI.
A second large scale randomized, double-blind, placebo-controlled clinical trial of Ginkgo biloba, administered in a dose of 120 mg twice per day to test if it is effective in the prevention of dementia (and especially Alzheimer's disease) in normal elderly or those with early cognitive impairment (The GuidAge Study) is underway. While there are many similarities between the GEM and Guidage studies, there are also significant differences that have been published. The rationale, design and baseline data for the GuidAge Study has been published. It is the largest study carried out in Europe on the prevention of AD. A total of 2854 subjects were enrolled between March 2002 and September 2004. The age of the study population was 76.8 +/- 4.4 with mean MMSE at entry 27.8 +/- 1.7. Final results should be available in 2010.

6.0 Studies on the Efalex Active 50+ Formulation

6.1 Preclinical

Researchers at the Institute of Cell and Molecular Science, Barts and The London School of Medicine and Dentistry, London, UK, in conjunction with Efamol Ltd. have confirmed that the active ingredients in Efalex Active 50+ maintain nerve cell health in the presence of a damaging agent. This first cell culture study showed that DHA, EPA, PS, Vitamin B12, Vitamin E, folic acid and Gingko biloba extract can protect and totally reverse nerve cell injury. The study involved growing nerve cells in culture. The cells were then exposed to lipopolysaccharide in the presence or absence of the individual ingredients within Efalex Active 50+ and combined in the concentration ratio of the Efalex Active 50+ formulation. Lipopolysaccharide normally causes nerve cell injury similar to what occurs in the aging brain. Survival of this injury is measured by determining how many cells are alive following exposure, how many cells are still able to grow neurite branches (special protrusions from the cell body through which the nerve cell picks up signals from surrounding cells) and the length of those neurite branches (the longer the branches the more healthy the cell). In this study, lipopolysaccharide exposure killed 34% of the nerve cells, reduced neurite branching by 36% and reduce neurite length by 39%. However, nerve cells survived and flourished after exposure to lipopolysaccharide when they were grown in the presence of DHA, EPA, PS, Vitamin B12 and E, folic acid and Gingko biloba extract. In fact, the protective effect of each individual component was so powerful that it was not possible to determine if combinations of ingredients would have synergistic effects because all the cells survived even when only each one of the ingredient was present. Results of this study are extremely important because they confirm the protective capacity of Efalex Active 50+ ingredients for nerve cells and add strength to the human data already available pertaining to these individual ingredients.
7.0 Suggested Dosages of DHA, PS, B Vitamins and Ginkgo biloba for Cognitive Decline

DHA
Population studies have provided insight into effective doses of DHA for prevention of cognitive decline. One or more fish meals per week have protected against age related cognitive decline\textsuperscript{22} and Alzheimer’s disease\textsuperscript{24}. In the later study, as little as 60 mg of DHA/day resulted in 60% lesser risk of developing Alzheimer’s disease\textsuperscript{24}. According to the Scientific Advisory Committee on Nutrition (SACN) of the UK Food Standards Agency (FSA), 2 portions of fish per week provides 450 mg/day of combined EPA+DHA\textsuperscript{95}. Therefore, one fish meal per week would provide an effective dose of 225 mg/day of combined EPA+DHA.

Intervention studies have shown that combined 1600 mg of EPA and 800 mg of DHA can enhance cognitive function and mood in healthy people\textsuperscript{40}, while as little as 720 mg of DHA can improve moderately severe multi infarct dementia\textsuperscript{38}.

PS
Phosphatidylserine is reported to have nutritive value at doses of 75 – 500 mg per day and to augment the risk of cognitive dysfunction and dementia at doses of 50 -500 mg per day\textsuperscript{96}. Clinical trials using doses of 300 mg per day for 6 months have confirmed significant treatment outcomes in patients with active and often severe mental deterioration (see Section 3.3). Therefore, one could argue that much lower doses of PS, such as those found in Efalex Active 50+, in healthy aging adults could provide significant preventive effects through long term use.

B Vitamins
A meta-analysis of randomized, placebo-controlled, clinical trials showing that folic acid reduces blood homocysteine concentrations by 25% and Vitamin B12 by an additional 7 % in normal aging populations has concluded that daily supplementation to achieve this should be in the range of 500-5000 ug folic acid and 0.5 mg of Vitamin B12\textsuperscript{74}.

Ginkgo biloba
Dosages of 40 mg three times per day up to 120 mg twice daily (120-240 mg total) have been recommended in clinical trials for prevention and treatment of dementia.
8.0 Rationale for the Efalex Active 50+ formulation

A daily dose of Efalex Active 50+ contains 500 mg DHA, 60 mg PS, 500 ug folic acid, 10 ug Vitamin B12, 120 mg Ginkgo biloba and 10 mg vitamin E (d-alpha – tocopherol).

Efalex Active 50+ provides 500 mg/day of DHA. That falls roughly in the middle of the effective preventive dose of 225 mg/day EPA+DHA for age related cognitive decline and the successful therapeutic dose of 720 mg of DHA for moderately severe multi infarct dementia (see Section 6.0 above).

The PAQUID epidemiological study on cognitive and functional aging mentioned in 2.1 above, found that participants who ate the most fish and had the lowest incidence of developing dementia were more likely to develop dementia if their vitamin E levels were low. Therefore, combined intake of vitamin E and fish oil can reduce the incidence of dementia more than fish oil alone20. Hence, Vitamin E in the Efalex Active 50+ formulation may provide benefits in addition to its antioxidant properties.

Over the last twenty years our daily intake of PS has decreased from 250 mg to 180 mg in a meat rich diet97. Those on a reduced fat diet consume about 100 mg daily and vegetarians eat only 50 mg per day. The effective therapeutic dose in clinical trials involving moderate to severely affected people was 300 mg daily (see Section 3.3 above). Providing 62 mg/day of PS in Efalex Active 50+ brings the average person’s intake up from 180 mg/day to about 242 mg/day which was near our daily intake from twenty years ago and only 60 mg less than the effective short term dose in severely affected patients. This dose should provide sufficient PS to aid prevention of age related cognitive decline in normal populations through long term use.

Commercial sources of PS are derived from either bovine cortex or soy. Although bovine sourced PS has an excellent safety record, its popularity as a supplement ingredient in recent years has been replaced by soy derived PS. Numerous pre-clinical and clinical studies have confirmed their bioequivalence such that trials confirming beneficial effects for one source can be used to support efficacy of the other2, 98,99. Efalex Active 50+ contains soy derived PS.

Supplementation with 500-5000 ug of folic acid per day is proven to reduce homocysteine levels by up to 25%73. However, the tolerable upper intake level for folate is only 1000 ug/day. Therefore, it was deemed appropriate to include the lowest safe effective dose of folic acid in Efalex Active 50+ (i.e. 500 ug folic acid).

Supplementation with 0.5 mg of Vitamin B12 in combination with folic acid will reduce homocysteine levels by 7%73. Although Vitamin B12 is extremely safe and because of this there is no defined tolerable upper intake level, the National Academies, Food and Nutition Board, Institute of Medicine still only recommends a Dietary Reference Intake (DRI) of 2.4 ug/day. Therefore, it was deemed appropriate to include a quantity of Vitamin B12 in Efalex Active 50+ that fell between the DRI and the effective dose of 0.5 mg (i.e.10 ug).

Effective daily doses of Ginkgo biloba extract range from 120-240 mg. Therefore, it was decided to include the lowest therapeutic dose in Efalex Active 50+.
9.0 Safety

**DHA**

All polyunsaturated fatty acids, including DHA benefit the cardiovascular system by helping to reduce blood clotting. However, caution is warranted under certain circumstances such as when clotting disorders are present, or when anti-coagulant therapy is in use. In later occasions, monitoring platelet function and medication adjustment may advantageous.

There is little clinical evidence to support concerns that fish oil concentrates may increase the risk of bleeding, may reduce glycemic control in diabetics or increase the risk if cancer or serious infection associated with modified immune responses\(^1\). Intake of 2 g/day of combined EPA and DHA - that is 4 times the daily dose in Efalex Active 50+\(^1\) is similar to that seen in large sectors of the Japanese population and well below that of Greenland Inuit, both of whom suffer no ill effects from this routine consumption. Moderate increases in bleeding times, that are lower than those seen with acetylsalicylic acid (ASA) therapy, have been observed in individuals taking 3-4 g/day – that is 6-8 times the daily dose of Efamox\(^1\).

**PS**

Clinical trials to confirm product efficacy have routinely included 300 mg/day of PS in divided doses of 100 mg each (See Section 3.3) – that is 5 times the daily dose in Efalex Active 50+. These studies reported no ill effects. The largest study, which also had the longest treatment duration (6 months) reported no interaction with any pharmaceutical drugs that were being used during the trial\(^5\). However, patients taking antipsychotics, antidepressants, barbiturates, methyl-dopa, reserpine and bromocriptine were excluded from the study. Crook and Adderly recommended against taking PS during pregnancy and lactation and cautioned individuals taking anticoagulants, although no reason is given as to why either should be a concern\(^1\).

A trial aimed specifically at accessing the safety of PS in humans, included up to 600 mg per day in divided doses of 200 mg each. It reported no ill effects related to standard biochemical and haematological safety parameters, blood pressure or heart rate and no adverse events were reported during this trial\(^1\).

Long term toxicity studies using up to 70 g per day for one year have reported no apparent damage\(^1\).

The United States Food and Drug Administration has concluded that the use of phosphatidylserine as a dietary supplement is safe and lawful under 21 CFR 101.14 provided that bovine-derived sources, if used, are not derived from bovine tissue from cattle born, raised or slaughtered in any country where BSE exist\(^4\). Efalex Active 50+ uses soy sourced PS.

All evidence indicates that PS is safe at much higher doses than those provided by Efalex Active 50+.

**B Vitamins**

The National Academies, Food and Nutrition Board, Institute of Medicine recommends a Tolerable Upper Intake Level (UL) of 1000 ug/day of folate for males
and females 19 years of age and older – that is twice the daily dose in Efalex Active 50+.

The National Academies currently do not recommend a UL for Vitamin B12 because Vitamin B$_{12}$ has a very low potential for toxicity. The Institute of Medicine states that "no adverse effects have been associated with excess vitamin B$_{12}$ intake from food and supplements in healthy individuals". In fact, the Institute recommends that adults over 50 years of age get most of their vitamin B$_{12}$ from vitamin supplements or fortified food because of the high incidence of impaired absorption of B$_{12}$ from animal foods in this age group$^{104}$.

The Office of Dietary Supplements, National Institute of Health recommends that older adults and vegetarians may benefit from a vitamin B$_{12}$ supplement or an increased intake of foods fortified with vitamin B$_{12}$$^{104}$. In addition, they state that up to 30 percent of adults 50 years and older may have atrophic gastritis, an overgrowth of intestinal flora that prevents normal absorption of vitamin B$_{12}$ from food. Older individuals are, however, able to absorb the synthetic B$_{12}$ added to fortified foods and dietary supplements. Vitamin supplements and fortified foods may be the best sources of vitamin B$_{12}$ for adults over the age of 50.

**Ginkgo biloba**

In several large trials where people took standardized ginkgo biloba extract at doses of 120 mg to 240 mg daily for up to one year there were no serious side effects$^{105, 106, 107}$. Various minor side effects included headache, nausea and intestinal complaints.

Ginkgo may interact with anticoagulants (blood thinners). Therefore, it is advised not to take ginkgo if bleeding disorder exits or together with garlic, high doses of vitamin E, acetylsalicylic acid, rofecoxib, warfarin, nonsteroidal anti-inflammatory agents, steroids and trazodone,$^{108, 109}$ without monitoring platelet function as medication adjustment may be necessary.

Ginkgo may theoretically affect insulin and blood sugar levels. Therefore caution is advised in patients with diabetes or hypoglycemia, and in those taking drugs, herbs or supplements that affect blood sugar. Serum glucose levels may need to be monitored and adjustment may be necessary$^{110}$.

Use of Ginkgo is not recommended during pregnancy or breast feeding due to lack of reliable scientific studies in this area$^{109}$. 
10.0 References

15. Kyle DJ et al. Low serum docosahexaenoic acid is a significant risk factor for Alzheimer’s dementia. Lipids 1999; 34:S245.


42. http://www.lipidlibrary.co.uk/Lipids/ps/index.htm


Efalex Active 50+ is a dietary supplement with the following ingredients per capsule:

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>DDR %*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish Oil: 500 mg</td>
<td>500 mg</td>
</tr>
<tr>
<td>Fatty Acid Omega-3 350 mg</td>
<td>350 mg</td>
</tr>
<tr>
<td>Docosahexaenoic Acid 250 mg</td>
<td>250 mg</td>
</tr>
<tr>
<td>Eicosapentaenoic Acid 40 mg</td>
<td>40 mg</td>
</tr>
<tr>
<td>Ginkgo Biloba extract 60 mg</td>
<td>60 mg</td>
</tr>
<tr>
<td>Phosphatidylserine 31 mg</td>
<td>31 mg</td>
</tr>
<tr>
<td>Vitamin E 5 mg</td>
<td>5 mg</td>
</tr>
<tr>
<td>Folic Acid 250 µg</td>
<td>250 µg</td>
</tr>
<tr>
<td>Vitamin B 12 5 µg</td>
<td>5 µg</td>
</tr>
</tbody>
</table>

* Recommended Daily Dose    ** Average values

Composition per Capsule:

**Ingredients:** Fish oil rich in Omega-3 fatty acid: docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA); Ginkgo biloba extract, glyceryl monostearate, phosphatidylserine, vitamin E, folic acid and vitamin B 12.

**Capsule composition:** Gelatin, glycerine, ferric oxide red, ferric oxide yellow.

What is the Importance of Its Composition to the Body?

Efalex Active 50+ is a dietary supplement containing Omega-3 fatty acid, Ginkgo biloba extract, phosphatidylserine, folic acid and vitamins E and Vitamin B 12, to ensure sufficient amounts of such ingredients while responding to the body’s variable needs.

**Omega-3 fatty acid**, especially docosahexaenoic acid, are important ingredients for neuronal membranes (nerve cells), which are fundamental to process information in the brain. Eicosapentaenoic acid is involved in immune and inflammatory processes.

**Phosphatidylserine** is also a very important component for neuronal cell membranes and is necessary to ensure efficient transmission of nervous impulses.

**Vitamin B and Folic acid** help maintain cell integrity and optimize nerve cell metabolism. Together, those two substances help protect the brain from potentially damaging effects of a substance called homocysteine.

**Vitamin E** plays an important role in protecting different tissues, specifically brain tissue, against chemical reactions of peroxidation, which may occur from different
normal metabolic processes and exogenous toxic agents. Non-neutralization of compounds resulting from peroxidation may contribute to early aging of cells. Besides those anti-oxidant properties, the need for vitamin E is enhanced in individuals with diets that are rich in polyunsaturated fatty acids. Components derived from Ginkgo biloba also have important antioxidant properties.

**When Should One Take Efalex Active 50+?**

*Efalex Active 50+* is to be taken by adults in any situation nutrient deficiency is likely, and especially in individuals with altered metabolism.

*Efalex Active 50+* may help maintain brain function and performance, especially in elderly individuals or in situations of intellectual stress/overload (for example, students studying for final exams), when nutritional deprivation may have a larger impact.

**How to Take Efalex Active 50+?**

Initially, a daily dose of one or two capsules of *Efalex Active 50+* is recommended, together with food or non-alcoholic beverages.

Do not take more than the recommended dosage.

Nutritional supplements, such as *Efalex Active 50+*, must not be used to replace a varied food diet.

**Warnings**

Do not prescribe this product for pregnant women or those who are nursing.

*Efalex Active 50+* must not be administered in cases of known hypersensitivity to any of the ingredients. Its use while taking anticoagulant or platelet anti-aggregating medication is not recommended.

*Efalex Active 50+* contains soybeans and fish allergens.

In case of epilepsy, coagulation problems or diabetes, take this product only with your doctor’s or pharmacist’s permission.

If you are going to have surgery, inform the doctor you are taking this product.

**Possible Side Effects**
Some of the components of **Efalex Active 50+** may cause gastrointestinal upset, allergic skin reactions, headaches, and reduced blood clotting.
Use preferably before the expiration date on the package.
Store at 25°C or less.
Keep out of reach of children.

**Presentation**
Container with 30 capsules.