

Department of Neurobiology, Care Sciences and Society

Division of Clinical Geriatrics

Karolinska Institutet, Stockholm, Sweden

# **Omega-3 fatty acid treatment in mild to moderate Alzheimer's disease: Results from the OmegAD study**

Yvonne Freund-Levi, MD



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The author may be contacted via e-mail: [Yvonne.Freund-Levi@ki.se](mailto:Yvonne.Freund-Levi@ki.se)

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*To the future of  
Rebecca, Jacqueline, Alexander and all my family with love.  
In memory of our family that perished in the Holocaust.*

*One who saves a single human being is considered by the Torah as if he had saved a whole world.*

Mishna Sanhedrin 4:5

## ABSTRACT

Alzheimer's disease (AD) is a major public health concern in all countries with an increasing prevalence. It is expected to quadruple by the year 2047. AD is a progressive neurodegenerative disease with a multifactorial origin, where a body of genetic and biochemical evidence, inflammatory aspects as well as the pivotal role of soluble amyloid  $\beta$  peptide ( $A\beta$ ) may be the proximate cause of synaptic injuries and neuronal death leading to cognitive and neuropsychiatric decline leading to suffering in both the patient and the caregiver. Today there is no cure for AD, albeit we have access to pharmacotherapy that might improve cognitive and neuropsychiatric symptoms initially in the course of the disease. However, AD continues to progress despite treatment. It is therefore of utter importance to find other pharmacological strategies that might postpone the development of AD and also slow down the cognitive and neuropsychiatric decline in manifest AD. AD patients display lower levels of the omega-3 fatty acid (n-3 FA) docosahexaenoic acid (DHA) in both plasma and brain tissues as compared to age matched controls. Furthermore, epidemiological and animal studies suggest that high intake of DHA might have preventive properties against AD. Little is known, however, of the effects of n-3 FA on AD. Therefore we have conducted the first one year long randomised placebo-controlled double-blind trial with six months administration of 2.3 g/day n-3 FA (1.7 g DHA + 0.6 g EPA) or placebo (linoleic acid, LA) followed by six months administration of n-3 FA to patients with mild to moderate AD. In total 204 patients with mild to moderate AD already on stable medication with acetylcholinesterase inhibitors (AChEI) were included. One hundred and seventy four patients fulfilled the trial and the **papers I, II and IV** are based on these patients. In a subgroup of these patients lumbar puncture was performed (**paper III**). All patients were followed for one year with cognitive, neuropsychiatric and functional evaluations as well as blood tests.

In **paper I** we conclude that n-3 FA treatment did not delay the rate of cognition as measured with the Mini Mental State Examination (MMSE) and the cognitive subscale of the Alzheimer's disease Assessment Scale (ADAS-cog) nor global status measured with the Clinical Dementia Rating scale (CDR). However, positive effects on cognition were observed in a small group of patients with very mild AD (MMSE >27). Safety and tolerability was good as was compliance measured with plasma levels of DHA, EPA and LA.

In **paper II** we addressed the effects of supplementation of n-3 FA on the neuropsychiatric, behavioural and functional symptoms using the Neuropsychiatric

Inventory (NPI) and the Montgomery Åsberg Depression Rating scale (MADRS). Disability Assessment for Dementia (DAD) and Care givers burden (CGB). The relationship with *APOE* genotype was assessed. There was no overall treatment effect on neuropsychiatric, behavioural and functional symptoms, but n-3 FA appeared to have positive treatment effects on depression in non-*APOEε4* carriers and in agitation in *APOE ε4* carriers.

In **paper III** inflammatory markers in plasma (hs-CRP, IL-6, TNF- $\alpha$  and sIL-RII) and cerebrospinal fluid (CSF, IL-6, TNF- $\alpha$  and sIL-RII) and biological AD markers in CSF (A $\beta$ 42, T-tau and P-tau) were analyzed in a subgroup of 35 patients. A correlation at baseline between A $\beta$ 42 and sIL-RII was detected albeit no n-3 FA treatment effects were found in neither inflammatory nor biological AD markers.

In **paper IV** we investigated the hypothesis that supplementation with n-3 FA would affect appetite and weight in relation to inflammatory biomarkers and to *APOEε4* carrier ship. Mean weight and Body Mass Index (BMI) increased at 6 and 12 months in the n-3 FA group. When the placebo group was administered n-3 FA for six months, the weight and BMI also increased significantly within the group. However, there was no significant treatment effect on weight and BMI between the groups. Not carrying the *APOEε4* allele and increased DHA were independently associated with weight gain. Caregiver's assessed appetite improved in the n-3 FA treatment group over the treatment period.

In conclusion, our study gives some evidence that supplementation with 2.3 g/day n-3 FA to patients with mild to moderate AD may have effects on cognition in the early phases of AD, may reduce depression and agitation depending on *APOEε4* status and increase body weight loss in patients with mild to moderate AD. No effect on possible neuroinflammation in subjects with AD was observed.

Supplementation with n-3 FA may be a strategy to add to lifestyle prevention for postponing early cognitive symptoms in AD but more research is needed.

**Key words** Alzheimer's disease; progressive neurodegenerative disease; omega-3 fatty acid (n-3 FA) docosahexaenoic acid, DHA; eicosapentatenoic acid, EPA; linoleic acid, LA; acetylcholinesterase inhibitors, AChEI; Minimental State Examination, MMSE; ADAS-cog; Clinical Dementia Rating scale, CDR; Neuropsychiatric Inventory, NPI; Montgomery Åsberg Depression Rating scale, MADRS; Disability Assessment for Dementia, DAD; Care givers burden CGB, *APOEε4*; hs-CRP, IL-6, TNF- $\alpha$  and sIL-RII; A $\beta$ 42, T-tau and P-tau; weight; Body Mass Index, BMI.

## SAMMANFATTNING PÅ SVENSKA

Alzheimers sjukdom (AD) är en neurodegenerativ sjukdom som blir allt vanligare i hela världen. År 2047 beräknas antalet insjuknade i AD ha fyrdubblats. Trots att sjukdomen beskrevs i början av 1900-talet är fortfarande orsaken oklar. Sjukdomen förklaras av ett samspel av komplicerade genetiska, biokemiska och inflammatoriska mekanismer, såväl som lösligt amyloid  $\beta$  protein ( $A\beta$ ). Dessa skador i hjärnan anses vara orsak till de synapsskador och neuronöd som leder till minnesproblem och neuropsykiatriska symtom. Sjukdomen medför ett svårt och långvarigt lidande för både patient och anhörig. Det finns idag ingen bot för AD utan endast symtomlindrande behandling. Tidigt insatt kan en sådan behandling ha en stabiliserande effekt som dock är av övergående natur. Det är av yttersta vikt att hitta behandlingar som hindrar sjukdomen från att uppkomma och som kan lindra redan etablerade minnesproblem och neuropsykiatriska symtom.

Patienter med AD uppvisar lägre nivåer av omega-3 fettsyror (n-3 FS) i både plasma och hjärnvävnad jämfört med åldersmatchade friska kontroller. Såväl befolkningsstudier som djurstudier har visat att ett högt intag av n-3 fettsyran dokosahexaensyra (DHA) kan skydda mot att utveckla AD. Lite är dock känt om hur intag av n-3 FS påverkar redan etablerad AD. Vi har därför genomfört den första längre, 1 år, randomiserade placebo-kontrollerade dubbel-blinda studien (RCT) med tillskott av n-3 FS till patienter med mild till måttlig AD. Under 6 månader tillfördes 2.3 g/dag av n-3 FS, dvs. 1.7 g DHA+0.6 g eikosapentaensyra (EPA) eller motsvarande mängd placebo (linolsyra, LA), följt av 6 månaders behandling av n-3 FS till alla patienter. Tvåhundra fyra (204) patienter med pågående acetylkolinesteras-hämmare (AChEI)-behandling inkluderades, varav 174 fullföljde studien. Resultat från dessa patienter beskrivs i manuskript **I**, **II** och **IV**. I en mindre patientgrupp på 35 personer analyserades ryggmärgsvätska (CSF) vid studiestart och efter 6 månader, manuskript **III**. Samtliga 174 patienter genomförde tester av minnesfunktioner, neuropsykiatriska symtom, funktionsnivå samt lämnade blodprover vid 0, 6 och 12 månader. Anhöriga deltog vid samtliga undersökningstillfällen samt deltog i skattningar. Studien var godkänd av etiska prövningsnämnden. Patienterna tolererade n-3 FS-supplementeringen väl och 85 % av patienterna fullföljde studien. Lindriga mag-tarm besvär var den vanligast förekommande biverkningen. Följsamhet till behandlingen, mätt med förändring av plasmanivåer av DHA, EPA och LA, var god.

Data i manuskript **I** beskriver att tillägg av 2.3 g n-3 FS inte påverkade minnesfunktioner mätta med Mini Mental Test (MMT) eller den kognitiva delen av Alzheimers Disease Assessment (ADAS-cog). I en mindre patientgrupp med mycket milda minnesbesvär (MMT>27) noterades emellertid en bromsande effekt på försämringen i minnesfunktion hos de patienter som behandlades med n-3 FS jämfört med de patienter som fick placebobehandling. Positiva effekter sågs särskilt på episodiskt närminne, försenat ordminne samt uppmärksamhet. Behandling med n-3 FS påverkade inte den allmänna funktionsnivån mätt med Clinical Dementia Rating Scale (CDR global and sum of boxes). **Manuskript II** beskriver effekter av tillägg av n-3 FS på neuropsykiatriska, beteendemässiga och funktionella symtom mätt med Neuropsykiatriskt Inventorium (NPI) och Montgomery-Åsbergs depressionsskala (MADRS). Allmän funktionsgrad (ADL)

mättes med Disability Assessment for Dementia (DAD) och anhörigas börda mättes med Caregivers Burden (CGB)-formulär. Fynden relaterades till förekomst av sårbarhetsgenen *APOEε4*. 72 % var bärare av *APOEε4*. Någon generell lindrande effekt av n-3FAs kunde inte påvisas på de nämnda symtomen. Däremot noterades positiva behandlingseffekter vid depressiva symtom hos icke-bärare av *APOEε4*-genen, samt på agitationssymtom hos patienter som var bärare av *APOEε4*-genen.

Manuskript **III** baseras på data från en subgrupp av de första 35 inkluderade patienterna. I början samt efter 6 månader undersöktes de inflammatoriska markörerna interleukin(IL)-6, tumörnekrotisk faktor(TNF)- $\alpha$  och soluble(s)IL-RII samt biomarkörer för demenssjukdom,  $A\beta_{1-42}$ , T-tau och P-tau, i CSF. Samtidigt mättes i plasma de inflammatoriska markörerna C-reaktivt protein (CRP) med högkänslig (hs) metod, liksom IL-6, TNF- $\alpha$  och sIL-RII. Fyndet relaterades till bärarskap av *APOEε4*-genen. Vi kunde påvisa en signifikant korrelation mellan sIL-RII nivåer och nivåer av  $A\beta_{1-42}$  i CSF. Detta kan avspeglade det ömsesidiga växelspelet mellan olika nivåer av IL-1 $\beta$  och  $A\beta$  peptider. Eftersom sIL-RII binder till IL-1 $\beta$  och kan ökningen av sIL-RII utgöra en skyddseffekt för att begränsa uttrycket/nivåerna av IL-1 $\beta$  i hjärnan vid AD. Våra resultat kunde dock inte påvisa någon behandlingseffekt på de inflammatoriska markörerna i vare sig CSF eller plasma oavsett bärarskap av *APOEε4*.

I manuskript **IV** undersökte vi hur tillskott av 2.3 g n-3FAs på hela patientgruppen av 174 patienter påverkade aptit och vikt, också med hänsyn taget till bärarskap av *APOEε4*. Vikt, body mass index (BMI, kg/m<sup>2</sup>) och armantropometri; triceps skinfold (TSF) och mått på överarmsomfång (AMC) bestämdes vid de tre undersökningstillfällena. Dessutom mättes inflammatoriska biomarkörer i serum (hs-CRP, IL-6, IGF-1, albumin) samt nivåer av DHA och EPA. Aptiten skattades av anhörig. Efter 6 och 12 månader noterades en signifikant viktuppgång i behandlingsgruppen. I placebogruppen var vikten stabil efter 6 månader. När placebogruppens patienter efter 6 månader övergick till aktiv behandling med n-3FAs gick även dessa upp signifikant i vikt. Dock noterades ingen signifikant skillnad mellan grupperna. Aptit skattad av anhörig ökade signifikant efter 12 månaders hos de patienter som fick n-3 FA. Logistisk regressionsanalys visade att viktuppgång var relaterad till icke-bärarskap av *APOEε4*-genen samt till ökning i DHA-nivåer mellan 0-6 månaders behandling (OR=3,3 95 %, KI 1.0-4.6). En invers korrelation påvisades mellan viktuppgång och en sänkning av hs-CRP.

Sammanfattningsvis indikerar OmegAD-studien att tillägg med 2.3 g/dag av ett DHA-rikt n-3-tillägg hos patienter med mild till måttlig AD kan ha positiva effekter på närminnet i en tidig fas av sjukdomsförloppet, samt att tillägget möjligen kan påverka depression samt agitationssymtom hos vissa patienter. Inga effekter noterades på inflammationsmarkörer eller på biomarkörer för demenssjukdom i plasma eller ryggmärgsvätska. Möjligen kan vikt förlust och nedsatt aptit vid AD påverkas med n-3 FS-behandling. Tillägg av n-3 FS kan utgöra en av flera strategier att addera till nödvändiga livsstilsförändringar för att påverka tidiga närminnesstörningar vid AD. Fler kliniskt välkontrollerade studier krävs för att säkerställa dessa hypoteser.



## LIST OF PUBLICATIONS

- I. **Y Freund-Levi**, M Eriksdotter-Jönhagen, T Cederholm, H Basun, G Faxen-Irving, A Garlind, I Vedin, B Vessby, L-O Wahlund, J Palmblad.  
ω-3 fatty acid treatment in 174 patients with mild to moderate Alzheimer Disease: the OmegAD study.  
*Arch Neurol* 63: 1402-1408, (2006).
- II. **Y Freund-Levi**, H Basun, T Cederholm, G Faxen-Irving, A Garlind, M Grut, I Vedin, J Palmblad, L-O Wahlund, M Eriksdotter-Jönhagen.  
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- III. **Y Freund-Levi**, E Hjorth, C Lindberg, T Cederholm, G Faxen-Irving, I Vedin, J Palmblad, L-O Wahlund, M Schultzberg, H Basun, M Eriksdotter Jonhagen.  
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- IV. **G Faxén-Irving\***, **Y Freund-Levi\***, M Eriksdotter Jönhagen, H Basun, K Brismar, E Hjorth, J Palmblad, B Vessby, I Vedin, L-O Wahlund, T Cederholm.  
N-3 fatty acid supplementation effects on weight and appetite in patients with Alzheimer's disease. The OmegAD Study.  
*Submitted.*

## LIST OF ABBREVIATIONS

AA	Arachidonic Acid
Ach	Acetylcholine
AChEI	Acetylcholinesterase Inhibitor
AD	Alzheimer's disease
ADAS-cog	Alzheimer Disease Assessment Scale
A $\beta$	Amyloid $\beta$ -peptide
ADCS	Alzheimer disease Cooperative Study
ADL	Activity of daily living
ALA	Alpha linolenic acid, 18:3 n-3
AMC	Arm muscle circumference
APP	Amyloid Precursor Protein
APOE	Apolipoprotein E
BACE	B-secretase
BBB	Blood Brain Barrier
BMI	Body Mass Index
B6	Vitamin B 6
B12	Vitamin B12
BPD	Bipolar Disorder
BPSD	Behavioral and Psychiatric Symptoms in Dementia
BHT	Butylated hydroxytolvene
BuChE	Buturyl cholinesterase
CHD	Coronary heart disease
CDR	Clinical Dementia Rating Scale
CSF	Cerebrospinal fluid
CGB	Care givers burden scale
CMAI	Cohen-Mansfield Agitation Inventory
CNS	Central nervous system
COX	Cyklooxygenase
CT	Computed tomography
CV	Coefficients of variations
DAD	Disability Assessment for Dementia scale
DGLA	Dihommo gamma linoleic acid
DHA	Docosahexaenoic acid
DNA	Deoxyribonucleic acid
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition
EEG	Electroencephalography
ELISA	Enzyme-linked immunosorbent assay
EOAD	Early Onset Alzheimer's disease
EPA	Eicosapentaenoic Acid
FA	Fatty acid
FO	Fish oil
GABA	Gamma aminobutyric acid
GCP	Good Clinical Practice
GLA	Gamma-linoleic acid
GLC	Gas-liquid chromatography
GMP	Good Manufacturing Practise
hs-CRP	High sensitive plasma C-reactive protein

ICD-10	International classification of disease 10 <sup>th</sup> revision
IGFBPs	IGF binding proteins
IκB	Inhibitory protein
IL-1	Interleukin 1
IL-1Ra	Interleukin-1ra
IL-6	Interleukin-6
LA	Linoleic acid, 18:2 n-6
LT	Leukotrienes
LOAD	Late onset Alzheimer's disease
LOX	Lipoxygenase
MADRS	Montgomery Åsberg Depression Rating Scale
MCI	Mild cognitive impairment
MDD	Major depressive disorder
MMSE	Mini-Mental State examination
MRI	Magnetic Resonance Imaging
NFκB	Nuclear factor-kappa B
NFTs	Neurofibrillary Tangles
NINCDS ADRDA	Neurological Disorders and Stroke-Alzheimer Disease and Related Disorders
NMDA	N-methyl-D-aspartate receptor antagonist
NIPA	Near infrared immunoassay
NP	Neuritic plaque
NPI	Neuropsychiatric Inventory
NSAID	Non-steroidal-antiinflammatory drug
n-6 PUFA	Omega 6 polyunsaturated fatty acid
n-3 PUFA	Omega 3 polyunsaturated fatty acid
n-3 FA	Omega 3 fatty acid
ω-3 FA	Omega 3 fatty acid
PET	Positron emission tomography
PG	Prostaglandins
PIB	Pittsburgh compound B
PLA2	Phospholipases A2
PPARs	Peroxisome proliferator-activated receptors
PKC	Protein kinase C
P-tau	Phosphorylated tau
PUFA	Poly unsaturated fatty acid
RCT	Randomised controlled trial
RIA	Radioimmunoassay
RO	Rapeseedoil
RXR	Retinoid X receptor
s-IGF-1	Serum Insulin-like growth factor 1
sIL-RII	Soluble receptor antagonist type-II
TG	Triglycerides
TNF-α	Tumour necrosis factor alpha
T-tau	Tau protein
TSF	Triceps skin fold
VCAM-1	Vascular cell adhesion molecule 1
VLCPUFA	Very long chain polyunsaturated fatty acid
VaD	Vascular dementia
WHO	World Health Organisation

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# **1 ALZHEIMER'S DISEASE DEFINITION**

Alzheimer's disease (AD) is a progressive neurodegenerative disorder with specific characteristic clinical and neuropathological features. AD is heterogeneous and can be divided in early onset AD (EOAD) and late onset AD (LOAD). The EOAD can be mediated by mutations of chromosomes 1, 21 and 14 and other yet unknown causative factors. Diagnostic accuracy has improved by development of specific criteria for dementia, AD and other causes of dementias, but the sensitivity and specificity of these criteria are imperfect and definitive diagnosis is still achieved post-mortally as no specific biological marker to detect AD has yet unraveled. Still 100 years after the disease was discovered by the neurologist Alois Alzheimer in his first paper published in 1906 based on the 56 year old patient Auguste D, the mystery of how and why AD evolves is still not solved (Goedert, *et al.* 2007).

## **1.1 CLINICAL DIAGNOSIS**

The clinical diagnosis rely on mainly three criteria-based approaches to the, International Classification of Diseases, 10<sup>th</sup> revision (ICD-10), (WHO 1992), the Diagnostic and Statistical Manual, 4<sup>th</sup> edit, (DSM-IV) (American Psychiatric Association 1994) and the National Institute of Neurological, Communicative disorders and Stroke-Alzheimer's Disease and related Disorders (NINCDS-ADRDA) (McKhann, *et al.* 1984). The three definitions share many common features.

### **1.1.1 ICD-10**

The ICD-10 WHO criteria (WHO 1992) define dementia as a disorder with memory impairment which might impair the activities of daily living (ADL). The memory impairment is notably in the domains that control registration, storage and retrieval of new information, and there is a deficit in thinking and reasoning. Inclusive of the diagnosis is also an insidious onset, slow deterioration of cognition and absence of systemic illness. Subtypes of AD are early and late onset as well as atypical and mixed types (mixed-AD-vascular dementia).

### **1.1.2 DSM-IV**

The DSM-IV (American Psychiatric Association 1994) defines dementia as a syndrome characterized by multiple cognitive deficits including memory impairment and one of the following cognitive disturbances: aphasia, apraxia, agnosia or dysfunctional executive functioning. The deficits must be sufficient to cause impairment of occupational or social functioning and represent a decline of a previous higher level of functioning. AD is defined as a disease with insidious and continuous cognitive decline. Other systemic conditions or substance abuse must be ruled out as well as major psychiatric disorder. Subtypes of AD are early and late onset AD as well as AD with neuropsychiatric manifestations such as delirium, delusions or depressed moods.

### **1.1.3 NINCDS-ADRDA criteria**

The NINCDS-ADRDA criteria (McKhann, *et al.* 1984) define AD as definite, probable or possible. Criteria for definite AD require that the patients have met clinical criteria for probable AD while alive and histopathological evidence for AD obtained by autopsy or biopsy. Probable AD is characterized by the presence of dementia established by a questionnaire, confirmed by neuropsychological testing, deficits in two or more areas of cognition, progressive worsening of memory and other cognitive functions, no disturbance of consciousness, onset between ages 40 to 90 and no other systemic disorder that can account for decreased cognition. Possible AD is diagnosed when a) the patient has a dementia syndrome with no apparent cause, but there are variations in the onset, presentation or clinical course compared with typical AD b) the patient has a second brain disorder or systemic illness that is sufficient to produce dementia but is not the *cause* of dementia c) the patient has a single gradually progressive deficit in the absence of any other identifiable cause. The latter could indicate Mild Cognitive Impairment (MCI).

In all three classification systems, dementia is defined as a decline in memory and other cognitive functions in comparison with the person's previous level of cognition. Features that support the AD diagnosis (but is not required for the diagnosis) include: progressive deterioration of specific functions such as language (aphasia), apraxia (motor skills), agnosia (perception), impaired ADL, altered behavioral pattern, family history of similar disorders, normal routine cerebrospinal fluid parameters, normal or non-specific changes on EEG and evidence of cerebral atrophy on serial computerized tomography (CT). Clinical features that are



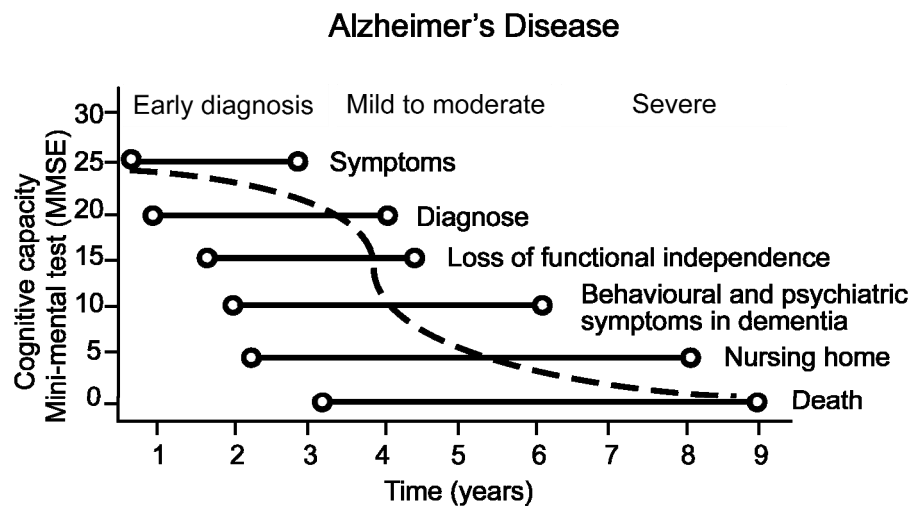
consistent with the diagnosis of AD (but not required for AD diagnosis) are: plateaus in the course of the disease progression, behavioral and psychiatric symptoms (BPSD) such as depression and delusions, motor signs such as cyclones or gait disturbances, seizures in the late course of the disease and a CT that is normal for age.

The three criteria systems share similar patterns and require that the patients shows symptoms of a dementia syndrome and require that memory loss is present and that the patients show impairment in at least one non-memory domain and that other causes of dementia are ruled out. The ICD-10 and DSM IV criteria share impairments of ADL or that occupational or social function should decline whereas the NINCDS-ADRDA criteria note that decline of ADL is supportive for the diagnosis but not necessary. The most inclusive criteria for dementia are considered to be the DSM-IV criteria and the least inclusive are the ICD-10 criteria. However, there are several limitations connected to these three criteria syndromes. The fact that the criteria are not operationalized open a wide window for the clinician to decide upon the degree of symptoms that leads to a diagnosis. Another problem is the lack of specified instructions on how to use the criteria and what exact "tools" to use. More specified and new investigational instruments such as newly developed ligands binding to  $\beta$ -amyloid, i.e. the Pittsburgh Compound B (PIB) in the brain, and new markers in CSF such as ubiquitin, neurofilament proteins and growth-associated protein 43 (neuromodulin) are less extensively studied.

#### **1.1.4 New research criteria in AD**

Due to the fact that new methods have been developed have put forward a proposal of revised research criteria for AD. New research criteria has recently been presented, the so called Dubois guidelines (Dubois, *et al.* 2007). The aim of these recommendations is to focus on distinctive and reliable biomarkers of AD that are available i.e. cerebrospinal fluid (CSF) analyses, magnetic resonance imaging (MRI), molecular neuroimaging with positron emission tomography (PET). The diagnostic criteria for probable AD are based on a clinical core of early and significant episodic memory impairment as reported by patient or caregiver. The episodic memory impairment must be gradual and progressive for a period of over 6 months and evidenced by testing. The memory impairment must be supported by either one or more supportive features i.e.

- 1) presence of medial temporal lobe atrophy
- 2) abnormal biomarkers in CSF
- 3) Specific patterns of neuroimaging with PET. Criteria for definite AD include either brain biopsy or a post-mortal autopsy and both clinical and genetic evidence (mutation on chromosome 1, 14 or 21) for AD. The criteria also wishes to eliminate the construct of mild cognitive impairment (MCI).



**Figure.** Clinical stages in Alzheimer's Disease. Cognitive capacity measured with MMSE. (Nordberg, Eriksson-Jonhagen, Garlind, Gut, Freund-Levi et al, 2006).

## **2 NEUROPATHOLOGY IN ALZHEIMER'S DISEASE**

### **2.1 ALOIS ALZHEIMER HISTORY**

The neuropathological hallmarks were first described in 1906 by Aloysius "Alois" Alzheimer (1864-1915), a German psychiatrist and neuropathologist in Frankfurt am Main at the "Städtische Anstalt für Irre und Epileptische (Asylum for lunatics and epileptics) and a colleague of Emil Kraepelin. In 1901 he observed a patient at the Fraralnkfurt Asylum named Mrs. Auguste Deter. The 51-year-old patient showed fear of people and had become jealous of her husband, and also showed a loss of short-term memory. This patient would become his obsession over the coming years. In April 1906, Mrs. D. died and Alzheimer had the patient records and the brain sent to Munich where he was working at Kraepelin's lab. In collaboration with others he used staining techniques to identify amyloid plaques and neurofibrillary tangles in her cerebral cortex.

At a speech 1906 the pathology and the clinical symptoms of presenile dementia was presented for the first time. Through fortunate circumstances the original microscopic preparations on which Alzheimer based his description of the disease were rediscovered and his findings could be reevaluated (Goedert, *et al.* 2007).

#### **2.1.1 Neuropathological hallmarks**

In AD there are two forms of protein aggregation: 1) the extracellular accumulation of amyloid- $\beta$ -protein polymers, leading to the  $\beta$ -amyloid plaque, whose main component consists of 40-42 amino acid peptides ( $A\beta$ ). It is a break down product from the APP. 2) the intracellular protein aggregates are called neurofibrillary tangles (NFTs), whose main constituent is a structural protein, tau, in a hyperphosphorylated state. Local inflammation elicits clustering and activation of microglia and astrocytes around the  $A\beta$  deposits, releasing inflammatory cells and cytokines. Moreover there is neuronal degeneration particularly in the temporal and parietal cortices, the entorhinal cortex, amygdala and in the hippocampus (Schaefer, *et al.* 2006). Preceding the neuronal degeneration there is a decrease in contact between the neurons. This synaptic loss explains the multiple neurotransmitter deficits noticed in AD (acetylcholine, glutamate, somatostatin, GABA, serotonin and dopamine, Braak, *et al.* 1991; LaFerla, *et al.* 2005).

### 2.1.2 A $\beta$ and Tau

There has to be an adequate number of  $\beta$ -amyloid plaques in order to establish the AD diagnosis. The precursor protein to amyloid  $\beta$ -protein (A $\beta$ ) is APP. APP is a Class 1 transmembrane glycoprotein that is expressed in most tissues in the body. The APP gene maps to chromosome 21q21.3-21. At present the biological function of the APP is unknown. It has been suggested that it regulates trophic functions, cell adhesion, neurite outgrowth and migration, and the induction of apoptosis (Russo, *et al.* 2005). Proteolytic processing in vivo is a normal physiological process. There are two major APP processing pathways: i.e. the amyloidogenic and the non-amyloidogenic. In the amyloidogenic pathway APP is cleaved by  $\beta$ -secretase (BACE) so that the extracellular part of APP is secreted. Thereafter APP is cleaved by  $\gamma$ -secretase and free  $\beta$ -amyloid is produced. In the non-amyloidogenic pathway APP is cleaved by  $\alpha$ -secretase. As this latter metabolic pathway includes a cleavage through the  $\beta$ -amyloid sequence no precipitation of  $\beta$ -amyloid will occur. In the AD brain there is an ongoing development of several stages of  $\beta$  amyloid (A $\beta$ ) that initially is formed by A $\beta$  oligomers that self aggregate and forms larger insoluble  $\beta$  pleated sheets forming diffuse plaques that then coalesce and mature into insoluble fibrillar A $\beta$  that forms senile plaques or neuritic plaques in later phases with formation of A $\beta$  induced neuritic dystrophy and reactive sprouting. The overproduction of A $\beta$  occurs in both sporadic and familial early onset AD (EOAD) cases and it is probably A $\beta$ 42 rather than A $\beta$ 40 that is predominantly deposited in the neuritic plaques (NPs). The other major neuropathological findings in AD are the intracellular deposited neurofibrillary tangles (NFTs) in the neuronal cytoplasm that are caused by an abnormal phosphorylation of Tau.

Normally Tau binds to microtubules to facilitate microtubular and cytoskeletal stability in the axons. When tau is hyperphosphorylated it aggregates abnormally and forms paired helical filaments and then NFTs that lead to decreased axonal transport to pre-and post synaptic terminals. Cognitive dysfunction in AD is primarily correlated to the NFTs and most important to the loss of the presynaptic terminals and not to the NPs (LaFerla, *et al.* 2005).



## 2.3 RISK FACTORS FOR DEVELOPMENT OF AD

EOAD can develop as early as in the third decade in life and is caused by the autosomal dominant mutation in the APP gene on chromosome 21, the presenilin-1 gene (PS1) on chromosome 14 or the presenilin-2 gene (PS2) on chromosome 1 (St George-Hyslop 2000). Onset of sporadic and familial disease with onset after 65 years of age, LOAD, is associated with the presence of apolipoprotein E (*APOE*) of the  $\epsilon$ -4 allele (Mayeux, *et al.* 1993).

The prevalence of AD is doubled every five years after 65 years of age up to 90 and then remains stable. The most important risk factor for the development of LOAD is increasing age. No specific environmental risk factor has been definitively identified as associated with AD. However, AD has been shown to be associated with a history of traumatic head injury, cardiovascular disease, hypertension in midlife, hypotension, smoking, stroke, diabetes and hyperinsulinaemia as well as hypercholesterolemia in midlife, obesity which is linked to vascular disorder which could be the link to AD, low education and low physical activity (Farlow 1998; Luchsinger, *et al.* 2004; Kivipelto, *et al.* 2008).

### 2.3.1 Apolipoprotein E

Apolipoprotein E (*APOE*) is a plasma cholesterol transport molecule. The gene has three major alleles (i.e.  $\epsilon$ 2,  $\epsilon$ 3, and  $\epsilon$ 4) which translates into 3 isoforms of the protein. E3 (60-70 % frequency in the general population), E4 (15-20 % frequency in the general population) and E2 (5-10 %). The gene for ApoE (*APOE*) is located on chromosome 19. *APOE* has been implicated as neurotrophic factor in the growth and repair of the CNS during development or after injury as an anti-oxidant and immune response mediator. Subjects who are homozygous for the  $\epsilon$ 4-allele are more likely to develop both sporadic, familial and LOAD. *APOE* $\epsilon$ 4 is thought to play a pathogenetic role in approximately 50 % of AD cases and to be second only to aging in pathogenetic importance (Laws, *et al.* 2003; Donahue, *et al.* 2008). *APOE* $\epsilon$ 4 has been shown to be a risk factor for vascular disease; both stroke and atherosclerosis and prolonged recovery of closed head injury (Farlow 1998). The presence of *APOE* $\epsilon$ 4 decreases the age of onset of AD in a dose dependent manner. When the number of *APOE* $\epsilon$ 4 increases from 0 to 2, the risk for developing LOAD increases from 20 to 90 % and the mean age at onset decreases substantially (Donahue, *et al.* 2008).

The role of *APOE* in AD is not fully resolved but it plays a major role in the transport and metabolism of cholesterol and triglycerides in humans. *APOE* $\epsilon$ 4 has been shown to bind and facilitate deposition of A $\beta$  plaques and NFTs in AD brains. It is found in blood vessels and is necessary for normal blood-brain-barrier (BBB) development. It has been suggested that the presence of the  $\epsilon$ 4 allele results in a decreased permeability and “leaky” BBB in turn leading to increased and higher brain A $\beta$  levels which could predispose to AD (Donahue, *et al.* 2008). *APOE* $\epsilon$ 4 carriers may as well potentially respond differently to drug treatment, and carriership may be one of the predictors to be taken into consideration when patients with mild cognitive impairment (MCI) will convert to AD (Lane, *et al.* 2005; Religa 2008).

### **3 PHARMACOTHERAPEUTIC STRATEGIES IN AD**

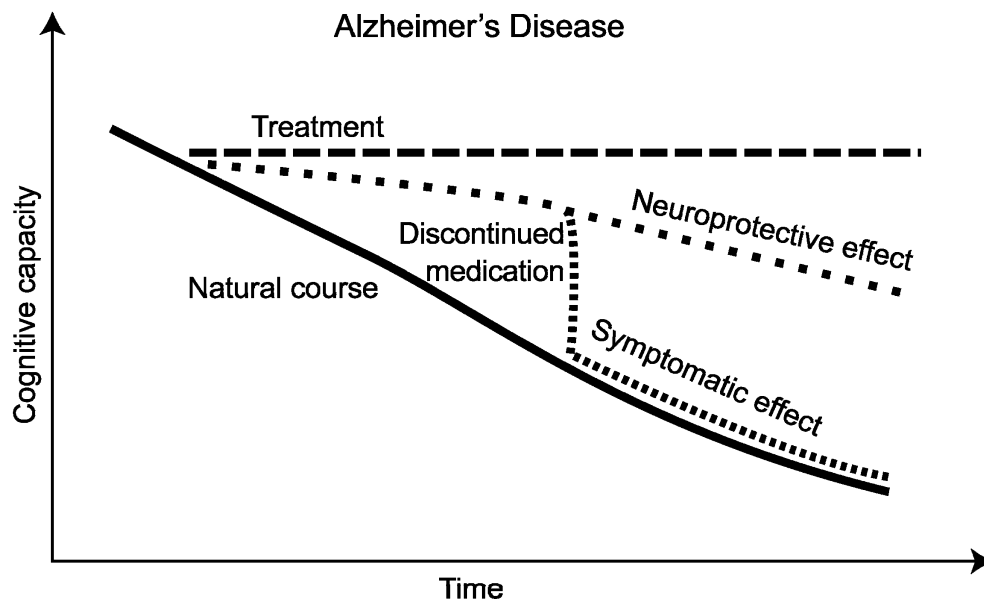
Current therapies for AD operate at the symptomatic level trying to increase levels of acetylcholine. The cholinergic hypothesis of AD concludes that the cognitive deterioration that occurs is associated with progressive loss of cholinergic neurons and decreasing acetylcholine (ACh) in the brain.

#### **3.1 ACETYLCHOLINESTERASE INHIBITORS**

To benefit from treatment with Acetylcholinesterase inhibitors (AChEI) it is important to establish an early diagnosis and to initiate treatment for AD. The goals for this include temporary improvement, stabilization and less-than expected decline of cognitive as well as neuropsychiatric symptoms. Accomplishing these goals may reduce institutionalization and care givers burden. First line treatment in mild to moderate AD have focused on selective inhibition of acetylcholine esterase (AChE) which occurs with donepezil, rivastigmine inhibits both AChE and butyrylcholinesterase (BuChE) and galantamine selectively inhibits AChE and modulates nicotinic receptors. On the basis of results from double-blind, randomized placebo-controlled trials all these three are approved for treatment of mild to moderate AD in Sweden. Six month long trials have shown beneficial effects on the cognitive and global functioning of AD patients in mild to moderate stages (Feldman, *et al.* 2001). Decline of cognition, improved or delayed cognitive decline from trials with a duration of 21-26 weeks showed statistically significant benefits in ADAS-cog and MMSE. Global function in 21-26 weeks trials showed stabilization/improvement in 64-70 %. Reduction of emergency neuropsychiatric symptoms and improved effects on already existing neuropsychiatric symptoms. AChEI have shown preserved functional ability up to one year. They have reduced need for psychotropic medications and reduced caregivers burden, and in some studies delayed nursing home placement (Nordberg, *et al.* 2006). Cognitive benefits sustained over a period of 3 years compared with projected rates of decline expected in untreated AD patients (Wallin, *et al.* 2007). In conclusion the trials provide evidence that AChEI therapy in AD can help the patients to maintain near pretreatment baseline levels for at least 6 months or more. The effects are less for those with severe dementia although there is very little evidence for other than mild to moderate dementia (Birks 2006; Farlow, *et al.* 2007). There are also studies with



AChEI showing effects on cytokines (Reale, *et al.* 2005; Tabet 2006). However, there is obviously a great need for more advanced neuroprotective treatment is obviously great in this progressive neurodegenerative disorder.



**Figure.** Medication with AChEI leads to stabilised cognitive capacity initially in mild to moderate AD compared to untreated or discontinued medication in patients with Alzheimer's Disease (Nordberg, Eriksson-Jonhagen, Garlind, Gut, Freund-Levi et al, 2006).

### 3.1.1 Memantine

The NMDA receptor antagonist memantine have in randomized clinical trials (RCT) showed positive treatment effects in more advanced stages of AD with beneficial effects on cognition, neuropsychiatric symptoms, ADL and caregivers burden. The addition of memantine to a stable dose of donepezil in patients with moderate to severe AD results in better outcomes compared to donepezil alone (Farlow, *et al.* 2007).

### **3.1.2 Concomitant therapy in AD**

Many patients are treated with alternative drugs and supplements for which a theoretical framework exists, but with low levels of support based on double-blind placebo-controlled trials (RCT).

Gingko biloba is safe but have shown inconsistent results (Fillenbaum, *et al.* 2005). Vitamin E is a dietary compound that functions as an anti-oxidant protecting from free radicals. Oxygen free radicals contain oxygen atoms with unpaired electrons and are highly reactive damaging proteins, DNA and cell membranes unless rapidly reduced by antioxidants. The brain contains a high proportion of fats. Since vitamin E is fat soluble it enters the brain and exerts antioxidant activities in cell membranes especially inhibiting the process of lipid peroxidation which damages the polyunsaturated fats (PUFAs) which are essential in the cell membranes. In humans with AD there have been reports of lower levels of Vitamin E in both CSF and plasma compared to normal and an association between low blood levels of Vitamin E and impaired cognitive function. According to the Cochrane Institute there are some beneficial effects of Vitamin E supplementation in AD treatment but further RCTs are needed (Tabet, *et al.* 2000; Fillenbaum, *et al.* 2005). Other agents for which supportive data are limited or I suggest no effect include vitamin C, hormone replacement therapy, melatonin, vitamin B12 and B6 and nonsteroidal anti-inflammatory effects (Feldman, *et al.* 2007).

### **3.1.3 Nonsteroidal anti-inflammatory drugs (NSAIDs) in AD**

The observation that patients with rheumatoid arthritis (RA) medicated with NSAIDs had a lower incidence of dementia is more than 20 years old. This finding is also supported by epidemiologic data from long term studies suggesting that the prevalence of AD was around 50 %less in individuals using NSAIDs. This observation is also supported by data from cell cultures, showing that ibuprofen and indomethacin lowered total A $\beta$  with >80 % (Weggen, *et al.* 2001).

NSAIDs can also decrease the inflammatory process through decrease of prostaglandins that are derived from arachidonic acid through the action of cyklooxygenase (COX) leading to induction of cytokines and other inflammatory cells. NSAIDs inhibit the enzymatic activity of COX. Indomethacin, which crosses the blood–brain barrier, is a potent non-selective COX inhibitor and has been shown to reduce inflammatory reactions by decreasing the levels of IL-6 and Il-1. Based on these data is seems as NSAIDs might have antiinflammatory effects decreasing the

risk for AD but not the progression of manifest AD. Very few RCT studies of NSAIDs in AD have been performed. There is one RCT using indomethacin for 6 months in 44 patients with mild to moderate AD using doses of 100-150 mg/day that could not show supportive evidence based on statistical differences supporting the use in mild to moderate AD (Tabet, *et al.* 2002). Another NSAID that has been investigated for treatment of AD but has not been recommended for treatment of AD is ibuprofen that seems to be safer, sold over the counter and is more widely used compared to Indomethacin. In transgenic mouse models ibuprofen reduces synthesis of prostaglandins by inhibition of COX and have shown a reduction of beta-amyloid deposits and IL-1B levels and levels of A $\beta$ 42. In human neuronal cells ibuprofen decreased secretion of total A $\beta$  and prevented accumulation of A $\beta$ 42. As no RCT have been published on ibuprofen, it can not be recommended for treatment of AD (Tabet, *et al.* 2003; Standridge 2004).

### **3.2 N-3 FA TREATMENT IN AD**

Several mechanisms have been suggested for the putative protective role of n-3 PUFAs in dementia some of which will be discussed here. To our knowledge only 4 RCT trials (including the OmegAD study) have been performed in patients with manifest AD

#### **3.2.1 Neuroprotective effects of n-3 PUFAs in dementia**

Cardiovascular disease has been shown to increase the risk of dementia and its major subtypes AD and VaD. The n-3 PUFAs beneficial effects of the reduced vascular risks include their antiarrhythmic effects, antithrombotic effects, antiinflammatory and antiatherogenic effects. They also decrease levels of triglycerides, lower blood pressure and improve endothelial function. Secondly they might reduce dementia through their anti-inflammatory effects by reduction of pro-inflammatory cytokines. Thirdly they (DHA) can modulate the physicochemical properties by modulation of membrane phospholipids properties, fluidity, elasticity thickness and fusion ability and structural features (Prasad, *et al.* 1998). This might even increase neurotransmitter levels as well as have anti-oxidative effects as have been discussed primarily in epidemiological studies (Florent-Bechard, *et al.* 2007). In addition the n-3 PUFAs can modulate gene expression in liver and adipose tissue through PPAR and RXR receptors that in turn can modulate target genes (de Urquiza, *et al.* 2000; Ray, *et al.* 2007). The perhaps most important role is data from

both cell cultures and animal data showing decrease of total A $\beta$  and A $\beta$ 42 (Lim, *et al.* 2006). The last years the published literature on the efficacy of n-3 FA on cognitive function in aging and dementia has been overwhelming (Issa, *et al.* 2006; Schaefer, *et al.* 2006; Barberger-Gateau, *et al.* 2007). The fatty acid composition of brain phospholipids in AD brains in postmortal studies shown a decrease of DHA (Söderberg, *et al.* 1991).

The population based Rotterdam-study (Kalmijn, *et al.* 1997b) was one of the first large prospective studies that showed that fish consumption is associated with a reduced risk of developing dementia and in particular AD. The favourable fish consumption was observed at an intake of >18.5 g/day. High total and saturated fat intakes were most strongly associated with an increased risk of dementia with a vascular component (vascular dementia and AD with cerebrovascular disease). Other population based prospective studies (Morris, *et al.* 2003) reported that participants who consumed fish once per week or more had 60 % less risk to develop AD compared to those who rarely or never ate fish. The total intake of n-3 PUFAs was associated with a reduced risk of developing AD as was the intake of DHA but this was not observed with EPA. Participants were followed on average of 2.3 years. However, new data from the Rotterdam study has been presented. This study by Engelhart (Engelhart, *et al.* 2002) could not reproduce data from previously performed studies which had shown a protective effect of fish consumption and dementia. Data from this trial that followed patients for 6 years could not show any association with increased risk of subtypes of dementia with high intake of total, saturated, and trans fat and cholesterol and low intake of n-3 PUFA, n-6 PUFA, MUFA and PUFA. Data from the Framingham Heart study have presented data showing significant links between low levels of plasma DHA and dementia. No such relationship between dementia and levels of EPA in plasma could be detected. Data are based on 899 subjects including both gender who were free of dementia at baseline and then followed for a period of 9.1 years with food frequency questionnaire to assess dietary DHA and fish intake and levels of plasma levels of FAs. No correlation was found to *APOE* $\epsilon$ 4 in this study (Schaefer, *et al.* 2006). Another prospective study measuring fish intake by food frequency questionnaires in >2000 subjects showed that consumption of fatty fish twice a week in non- carriers of *APOE* $\epsilon$ 4 was associated with a reduced risk of dementia and AD (Huang, *et al.* 2005).

In a large Norwegian population based study, recently published (n=2031 aged 70-74 years) an association between cognitive performance and intake of the main types of consumed seafood and fish oil was studied and showed a dose-response relationship between intake of seafood and cognitive function. The cognitive functions were influenced by fish intake with more pronounced effects for non-processed lean and fatty fish. Subjects whose mean daily intake of fish or fish products was  $\geq 10$  g/day had significantly better mean test scores and cognitive performance than those whose intake was  $\leq 10$  g/day. Maximum effects were observed at an intake of around 75 g/day. Most associations remained significant after adjustment for several factors (sex, *APOE* $\epsilon 4$  education) (Nurk, *et al.* 2007). The need for large randomised placebo-controlled clinical trials with various dosages of n-3 PUFAs especially DHA are needed.

### **3.2.2 Randomized placebo-controlled double-blind trials on n-3 PUFAs in AD**

Only few double-blind placebo-controlled RCT exists. The first short term double-blind study was an Israeli study performed by Yehuda ((Yehuda, *et al.* 1996) with 100 patients with AD. Sixty patients received 0.25 ml  $\alpha$ -linoleic acid and 40 patients received placebo for 4 weeks. All patients that received active treatment showed improvement in cognition and mood. One small Japanese study used 240 mg/day DHA and AA as active treatment. The placebo group received 240 mg /day olive oil for 3 months. The 21 patients that were included had mixed cognitive diagnosis, i.e. 8 patients had AD. Only patients with mild cognitive impairment and organic brain lesions showed improvement in immediate memory (Kotani, *et al.* 2006).

One of the early RCT studies was performed in 20 nursing home patients (average age of 83 years) with vascular dementia (VaD). Cognitive functions were measured using MMSE and Hasegawa's dementia Rating Score (HDS-R). Ten patients received 6 capsules of DHA, 4,32 g/day or placebo for 12 months. Inclusion scores was moderate to severe dementia with MMSE between 15-22. Scores of MMSE was evaluated at baseline after 3, 6 and 12 months. Comparisons between groups were significant and in favor of the DHA group at 3 and 6 months follow up. However, in this study no drop-outs or withdrawals were reported and

the procedure for how the randomization occurred was reported (Terano, *et al.* 1999). In conclusion very few studies have yet been performed that could confer or prove the utility of omega 3 PUFA in either preventing cognitive impairment or dementia (Lim, *et al.* 2006; Florent-Bechard, *et al.* 2007). These three RCTs all have a very small number of patients. The OmegAD study presented in this thesis is the largest RCT on n-3 PUFAs in the field of dementia.

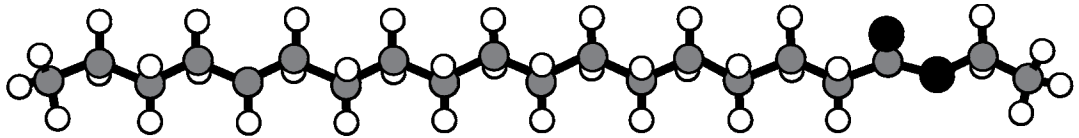
We are expecting results from the ongoing NIH sponsored randomized double-blind RCT. This study enrolls 400 patients with mild to moderate AD (MMSE 14-26 points) receiving 200 mg DHA/daily for 18 months and is evaluating cognitive decline in AD as measured by ADAS-Cog (NIH website, 2008).

## 4 FATTY ACIDS

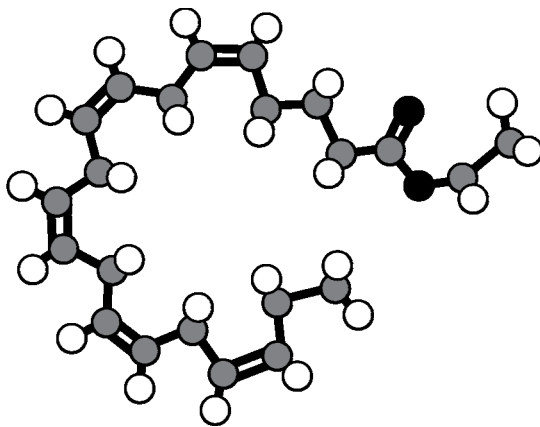
### 4.1 FATTY ACID NOMENCLATURE

The fat found in foods consists largely of a heterogeneous mixture of triacylglycerols (triglycerides)-glycerol molecules that each is combined with three FAs. The FAs can be divided into two categories: primarily based on chemical properties:

1. Saturated FA, usually solid at room temperature.



2. Unsaturated FA, liquid at room temperature.



The term “saturation” refers to a chemical structure in which each carbon atom in the fatty acyl chain is bound to (saturated with) four other atoms. These carbons are linked by single bonds, and no other atoms or molecules can attach.

Unsaturated FAs contain at least one pair of carbon atoms linked by a double bond, which allows the attachment of additional atoms to those carbons (resulting in saturation). Despite their differences in structure, all fats contain approximately the same amount of energy (37 kilojoules/g or 9 kilocalories/g). The class of unsaturated FAs can be further divided into;

- 1 Monounsaturated fatty acid, (MUFA). The primary constituents of olive and canola oils, contain only one double bond.

- 2 Polyunsaturated fatty acids, (PUFA). The primary constituents of corn, sunflower, flax seed, and many other vegetable oils contain more than one double bond.

FAs are often referred to using the number of carbon atoms in the acyl chain, followed by a colon, followed by the number of double bonds in the chain (e.g., 18:1 refers to the 18-carbon monounsaturated fatty acid, oleic acid; 18:3 refers to any 18-carbon PUFA with three double bonds).

PUFAs are further categorized on the basis of the location of their double bonds. An omega or n notation indicates the number of carbon atoms from the methyl end of the acyl chain to the first double bond. Thus, for example, in the omega-3 (n-3) family of PUFAs, the first double bond is 3 carbons from the methyl end of the molecule.

Finally, PUFAs can be categorized according to their chain length. The 18-carbon n-3 and n-6 shorter-chain PUFAs are precursors to the longer 20- and 22-carbon PUFAs, called very-long-chain PUFAs (VLCPUFAs).

#### **4.1.1 Fatty acid metabolism**

Saturated FA and monounsaturated FA can be made in all mammalian cells from non-fat precursors such as glucose or amino acids. This is seldom occurring in humans who consume a Western diet since the consumption of saturated and monounsaturated FA is very high. However, mammalian cells can introduce double bonds into all positions in the FA chain except in the n-3 and n-6 position. Thus we cannot convert oleic acid (18:1 n-9) to linoleic acid (18:2 n-6) as mammals lack the 12-desaturase which only is found in plants. Likewise mammals cannot convert linoleic acid (LA, 18:2 n-6) to  $\alpha$ -linolenic acid (ALA, 18:3 n-3) as only plants have access to 15-desaturase. Because these two FAs cannot be synthesised by mammals they are called essential FA. Thus, the shorter-chain ALA and LA are essential FAs. No other FAs found in food are considered 'essential' for humans, because they can all be synthesized from the shorter chain fatty acids. Plant tissues and plant oils tend to be rich in LA and ALA.

Following ingestion, ALA and LA can be converted in the liver to the long chain, more unsaturated n-3 and n-6 VLCPUFAs by a complex set of synthetic pathways. LA will be converted into di-hommo- $\gamma$ -linoleic acid (20:3 n-6) and from here to arachidonic acid (AA, 20:4 n-6). Using the same pathway dietary ALA (18:3 n-3) can be converted to 20:4 n-3 and then to eicosapentatenoic acid (EPA, 20:5 n-3) and further on to docosahexaenoic acid (DHA 22:6 n-3).



However, there is a competition between the n-6 and n-3 FAs for the enzymes which metabolize them. Animal studies show that an increasing availability of n-3 PUFAs in the diet (mainly from fat fish such as herring, mackerel, tuna and sardines) results in decreased proportions of AA and an increased proportion of n-3 FA in the phospholipids of the cell membranes in various blood cells. The incorporation of the n-3 FA act at the expense of AA and is considered to be AA antagonists. AA gives rise to inflammatory mediators (prostaglandins series 2, leukotrienes series 4) and through these regulates the activities of inflammatory cells and the production of cytokines, while the n-3 FA exerts less pro-inflammatory actions.

The key link between FAs and immune function is a group of hormone-like mediators called the eicosanoids. Because the membranes of most cells contain large amounts of AA (compared to DGLA and EPA) AA is usually the principal precursor for eicosanoid synthesis. However, both EPA (20:5n-3) and AA (20:4n-6) can act as precursors for the formation of eicosanoids. AA in cell membranes can be mobilized by various phospholipase enzymes, Phospholipase A2 and the free AA can act as a substrate for COX forming the series-2 prostaglandins and act as a substrate for lipoxygenase (LOX) enzymes forming the series-4 leukotrienes.

Eicosanoids are rudimentary hormones that are involved in modulating the intensity and duration of inflammatory and immune responses. However, unlike endocrine hormones, which travel in the blood stream to exert their effects at distant sites, the eicosanoids are autocrine or paracrine factors, which exert their effects locally in the cells that synthesize them or adjacent cells. They help with movement of calcium and other substances into and out of cells, relaxation and contraction of muscles, inhibition and promotion of clotting, regulation of secretions including digestive juices and hormones, and control of fertility, cell division, and growth.

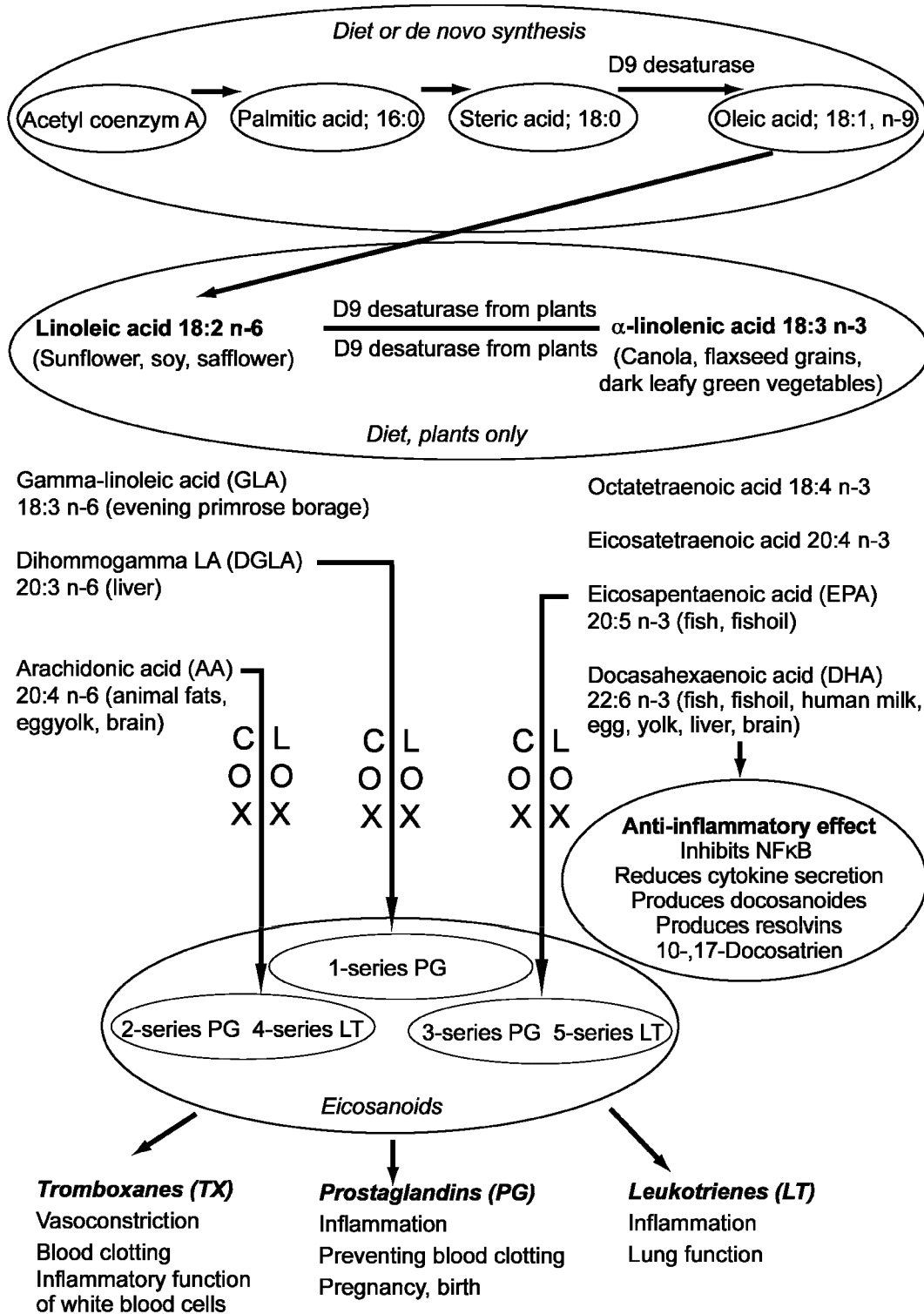
The eicosanoid family includes subgroups of substances such as the prostaglandins, the leukotrienes, and the thromboxanes. The long-chain omega-6 FA, AA (20:4n-6), is the precursor of a group of eicosanoids that include series-2 prostaglandins and series-4 leukotrienes. The n-3 FA, EPA (20:5n-3), is the precursor to a group of eicosanoids that includes series-3 prostaglandins and series-5 leukotrienes. The AA-derived series-2 prostaglandins and series-4 leukotrienes are often synthesized in response to injury or stress, whereas the

EPA-derived series-3 prostaglandins and series-5 leukotrienes appear to modulate the effects of the series-2 prostaglandins and series-4 leukotrienes usually on the same target cells. More specifically, the series-3 prostaglandins are formed at a slower rate and work to attenuate the effects of excessive levels of series-2 prostaglandins. Production of the series-3 prostaglandins seems to protect against heart attack, stroke and certain inflammatory diseases like arthritis and asthma (Farooqui, *et al.* 2007).

Since increased consumption of fish or fish oil results in a decrease of the amount AA in the cell membranes there will be less substrate available for the synthesis of AA produced eicosanoids. Furthermore, EPA competes with AA and inhibits the oxygenation of AA by COX. Thus fish oil consumption will result in decreased production of eicosanoids produced from AA and an increased production of eicosanoids from EPA as EPA in addition can act as a substrate for both COX and 5-LOX giving rise to series-3 prostaglandins and series-5 leukotrienes that are different from the AA-derived series-2 prostaglandins and series-4 leukotrienes. Therefore an increase of EPA derived eicosanoids will appear and there will be a suppression and decrease of eicosanoids that are derived from AA. The eicosanoids produced from EPA are less pro-inflammatory compared to the eicosanoids produced from AA. The reduction of AA derived mediators which accompany a consumption of fish oil has promoted the idea that fish oil is anti-inflammatory and might enhance immune functions.

EPA also affects lipoprotein metabolism and decreases the production of cytokines, interleukin 1 $\beta$  (IL-1 $\beta$ ), and tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ) which have pro-inflammatory effects. DPA (22:5n-3), the elongation product of EPA, is metabolized to DHA (22:6n-3). DHA (22:6n-3) is the precursor of a newly-described metabolite called 10,17S-docosatriene, which is part of a family of compounds called “resolvins”. Resolvins in the brain counteract the pro-inflammatory actions of infiltrating leukocytes by blocking interleukin 1-beta-induced NF-kappaB activation and COX-2 expression. DHA also plays a role in retinal rod outer segments by influencing membrane fluidity. The mechanism responsible for the suppression of cytokine production by n-3 FA remains unknown, although suppression of n-6 FA-derived eicosanoid production by n-3 FA may be involved, because the n-3 FA and n-6FA compete for common enzymes in the fatty acid metabolic pathway and the rate-limiting enzymes in the eicosanoid pathway phospholipases A2 (PLA2), COX and LOX. Along with AA,

DHA is the major PUFA found in the brain and is thought to be important for brain development and function (Mazza, *et al.* 2007).



#### **4.1.2 Other functions of n-3 PUFA**

Independent of the production of eicosanoids, the n-3 PUFAs also have other major effects. They can modulate leukocyte function through controlling proliferation, production of pro-inflammatory cytokines and adhesion molecules (Seierstad 2008). The n-3 PUFA can also change intracellular signalling pathways or lipid regulated transcription factors such as peroxisome proliferators –activated receptors (PPARs).

Some recent studies have shown that n-3 PUFAs like DHA can prevent neuroinflammation by decreased activation of the transcription factor NF- $\kappa$ B possibly by activation of PPAR and decreased phosphorylation of I $\kappa$ B which exert inhibitory effects on NF- $\kappa$ B. In the nucleus NF- $\kappa$ B mediates the transcription of many genes involved in inflammatory and immune responses such as, TNF- $\alpha$ , Il-1 $\beta$ , Il-6, vascular adhesionmolecule-1 (VCAM-1) and COX-2. Decreased activation of NF- $\kappa$ B will then lead to a decreased production of inflammatory cytokines TNF- $\alpha$ , Il-1 $\beta$ , Il-6 and VCAM-1. DHA is also important in modulation of neurotransmission.

#### **4.1.3 Dietary Sources**

Several lines of research have suggested that the high ratio of n-6 FA to low levels of n-3 FA currently consumed promotes a number of chronic diseases (Calder 2002). Whether or not the relatively high intake of n-6 FAs independently contributes to this problem is currently uncertain. Because of the slow rate of elongation and further desaturation of the essential FA, the importance of PUFAs to many physiological processes, and the overwhelming ratio of LA (n-6 FA) to ALA (n-3 FA) in the average diet, people interested in nutrition are recognizing the need for humans to augment the body's synthesis of n-3 PUFAs by consuming food that is rich in these compounds. The major dietary sources of n-3 FA are fish, fish oil, seaweed, vegetable oils (canola and soybean), walnuts, wheat germ, and some dietary supplements. The primary dietary sources of n-6 PUFAs are meats and dairy products.

The present western diet has a ratio between the n-6 FAs (AA ) to the n-3 FA (DHA) of about 15:1. This represents a historical change compared to the Paleolithic era from when humans have evolved and lived for most of our existence. At that time the ratio between AA and DHA was 1:1. The decreased consumption of DHA enriched foods and increased consumption of food enriched with n-6

PUFAS (mainly vegetable oils) is responsible for this shift in intake of PUFA (Farooqui, *et al.* 2007; Mazza, *et al.* 2007).

**Table.** Content of n-3 FA (g) and n-6 FA (g) per 100g fish (Palmlblad, Eriksdotter-Jonhagen, Freund-Levi, Cederholm, 2007 (Palmlblad, *et al.* 2007)).

<b>Fish</b>	<b>Amount fat per 100 g fish</b>	<b>n-3 family (g)</b>	<b>n-6 family (g)</b>
Herring	14 - 18	3 - 3.5	0.2 - 0.5
Tuna	5 - 10	1.3 - 3.4	0.1 - 0.5
Mackerel	15 -16	2.9	0.4 - 0.5
Salmon	5 -19.5	2.3 - 4.3	0.5 - 0.9
Halibut	5	1.2	0.1 - 0.2
Eel	33	4.7 - 4.8	1 - 4
Cod	0.7	0.2 - 0.3	0 - 0.02

#### **4.1.4 Safety considerations of n-3 PUFAs fish oil capsules**

Fish oil capsules may be associated with side effects including abdominal discomfort (belching of fish, loose stool and nausea), which are dose dependent. Fish oil can also prolong bleeding time slightly due to the incorporation of EPA instead of AA in platelet membranes which will lead to a decline in platelet counts and an increased vasodilatation. There has been reports of increased liver enzymes and deterioration of glycemic control in patients with type 2 diabetes during supplementation with n-3 PUFAs but mechanisms remain unclear and are presumed to be of no clinical significance (Eritsland 2000).

#### **4.1.5 Dietary source for fish**

A recently published thesis from Norway (Seierstad 2008), showed that after 16 months the salmons that were brought up with pure marine food contained almost three times as much EPA and DHA compared to salmon that were fed rapeseed oil and 50 % more EPA and DHA compared to salmons that were fed on a diet containing 50 % rapeseed oil and 50 % marine food. In a small double-blind intervention study, 60 patients with coronary heart disease (CHD) were randomly allocated to three groups consuming approximately 700 gr per week for 6 weeks of differently fed Atlantic salmon: 100 % fish oil (FO), 100 % rapeseed oil (RO) or 50 % of each (FO/RO) resulting in fillets with high, intermediate and low levels of n-3 PUFAs. Patient analyses before and after intervention included serum FA profile, serum lipoproteins and markers of vascular inflammation. The serum FA profiles of

the patients after the intervention mirrored those of the corresponding salmon filets and the respective salmon fed. Significant differences between the groups were obtained which was markedly increased in the FO group compared to the two other groups. The level of n-3 PUFAs and n-3/n-6 FA ratio was increased in the FO group and serum-triglycerides, vascular cell adhesion molecule-1 (VCAM-1) and IL-6 were significantly reduced. These findings imply that tailor-made atlantic salmon fillets fed with very high levels of n-3 PUFAs of marine origin seems to impose favourable effects in patients with CHD when compared to ingestion of fillets fed with rape seed oil. These data are important in putting focus on the fact that what we feed our selves with is as important as what we feed the fish we consume with. The combination of weather patterns such as El Nino which dramatically reduced the fish catch in concurrence with the global increase in aquaculture production with an increased amount of fish oil required for fish feed production which is increasing and today about 25 % of the world's total production of fish oil is used in fish feed. This also implies that salmon production (in particular) can only continue if suitable alternatives to fish oil can be found. This is important for us as fish consumers and consumers of fish oil. The medical and nutritive authorities today discuss that an excessive intake of n-6 FA linoleic acid, (LA) derived from vegetable seed oils can predispose for the incidence of various disorders (e.g. cardiovascular) and discusses the balance in intake of n-3 FA and n-6 FA. The volatile supply of fish oils recent years have promoted inclusion of various levels of vegetable oils in salmon diets and some studies have shown that vegetable oils can constitute a significant portion of the oil added to salmon diets without comprising the fish performance (Seierstad 2008).

#### **4.1.6 Lipids and human brain**

Lipids are the primary structural component of the adult brain making up to 50 % to 60 % of the brain dry weight. The most abundant FA in the phospholipids of the cerebral gray matter is DHA, which represents 45-65 % of the total phosphatidylserine in the mitochondria. In the areas of the brain which has the highest metabolic activity DHA is mostly concentrated in the neurons of the cerebral cortex, synaptosomes and mitochondria (Morris 2006). DHA is found in significant concentrations only in the brain, the retina and the testes (Connor, *et al.* 1988). All these areas are characterized by a high metabolic activity. Studies have revealed that DHA is essential to neurocognitive development. These studies were

driven from the fact that infant formulas did not contain n-3 FA, yet there was a selective uptake of DHA in utero. Furthermore, human milk contains high levels of DHA. These studies also found that DHA was important to maintain structure and function of neuronal membranes, neuronal membrane fluidity and flexibility, neurotransmission, and modulation of ion channels, receptors, and ATPase.

The levels of DHA in the brain have been shown to be very important for learning ability and memory in early life in studies of rodents, baboons and humans (Gamoh, *et al.* 1999; Ikemoto, *et al.* 2001). It is only recently that the role of n-3 FA has been investigated in the aging brain. The DHA composition of the brain decreases with age as a result of increased oxidative damage to the lipid membranes. The frontal lobe has the highest levels of DHA (Hibbeln, *et al.* 2006).

## **5 INFLAMMATION IN THE BRAIN**

### **5.1 NEUROINFLAMMATORY LINKS**

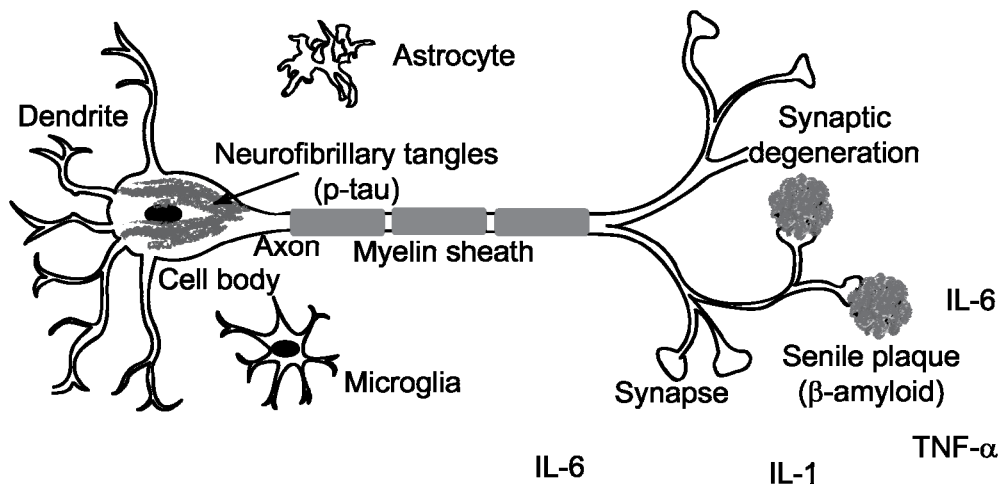
Neuroinflammation is an important part of diseases in the brain. Normally during immune surveillance there is a balance kept between proinflammatory and anti-inflammatory activators. The main mediators are microglia which initiate a rapid response that involves cell migration, proliferation, release of cytokines/chemokines and trophic factors and activation of astrocytes. Microglia is important for normal brain immunological function by its role in supporting neuronal antigen presentation, phagocytosis of debris and secretion of cytokines, and other various immune effectors. When microglia is immunologically active, it is transformed from a resting state to an activated macrophage and then migrate to sites of neuronal injury to remove injured cells and their debris by phagocytes.

Equally important are astrocytes that act as antigen presenting cells and provide structural and nutritive support to neurons and also are involved in clearance of debris. When an injury occurs, activated astrocytes, microglia and neurons engage in a complex interaction that leads to the release of proinflammatory substances such as cytokines which further activate astrocytes and induce neuronal and synaptic damage as well as further activation of inflammatory cells (Weisman, *et al.* 2006).

### **5.2 NEUROINFLAMMATION IN ALZHEIMER'S DISEASE**

AD brains show more diffusely activated microglia surrounding and clustering inside the plaques. The colocalization of both microglia and astrocytes with the NPs suggests that they are activated by direct contact with fibrillar A $\beta$ , however soluble A $\beta$  has also been shown to stimulate microglial production of proinflammatory cytokines. Microglia is activated in the early phase of formation of diffuse plaques in increasing the clearance of A $\beta$  burden. When insoluble NPs are aggregated, microglia activation and migration markedly increase and phagocyte insoluble A $\beta$  allowing for an additional accumulation of A $\beta$  in plaques. An increase in production of inflammatory cytokines, activation of complement factors and release of oxygen radicals (that provokes oxidative damage) accelerate the inflammatory reactions and neuronal damage.





Increased production of A $\beta$  oligomers and NPs induce activation of both microglia and astrocytes which both release the cytokines IL-1, IL-6 and TNF- $\alpha$ . They also induce the hyperphosphorylation of tau that in turn leads to decreased axonal transport and defects in synaptic transmission, synapse loss, and cholinergic deficits and neuronal loss (Engelhart, *et al.* 2004; Jia, *et al.* 2005).

### 5.2.1 Cytokines in AD pathology

The biological activity of cytokines is complicated and depends on target cell types, state of activation, the local cytokine concentration, receptor types and precise effect of cytokines. Cytokines are divided in different groups. Interleukins is one group which act as cell messengers that facilitate communication between cells both in the peripheral immune system (macrophages, blood leukocytes) as well as in the immune system in the brain coordinating signalling between neurons and astrocytes. Failure of coordinated activity of cytokines leads to inflammatory disorders and is detrimental to neurons. In AD brains several cytokines are up regulated such as IL-1, IL-6, IL-8 and some are down regulated such as IL-10 in areas that correspond to AD lesions (Patel, *et al.* 2005; Oprica, *et al.* 2007). The actions of cytokines require a complex network that involves feedback loops. The overall cytokine response is dependent on the different synergistic or antagonistic effects of various cytokines (Engelhart, *et al.* 2004).

#### Interleukin-1 (IL-1), IL-1 Ra and decoy receptor sIL-RII in AD

In microglia, that are associated with NPs the pro-inflammatory cytokine IL-1 is increased (Kuhn, *et al.* 2007). IL-1 levels analysed both in CSF as well as in plasma

show levels that are everything from unchanged to increased or decreased. This is partly due to small sample sizes or methodological difficulties in detecting low levels of these cytokines. IL-1 receptor antagonist IL-1Ra, has been shown to be increased in neurons around plaques in AD. Other molecules that bind to IL-1 are the decoy receptor soluble receptor antagonist type II (sIL-1RII). sIL-1RII acts as a signalling decoy receptor that prevents stimulation of IL-1 receptor that mediates intracellular signalling while IL-1Ra directly inhibits IL-1. These "double" antagonisms of IL-1 serve to balance the proinflammatory effects of IL-1. Some studies have showed elevated levels of sIL-RII in mild to moderate AD (Garlind, *et al.* 1999) as well as unchanged levels in MCI as in late stage AD (Lindberg, *et al.* 2005).

### **IL-6 in AD**

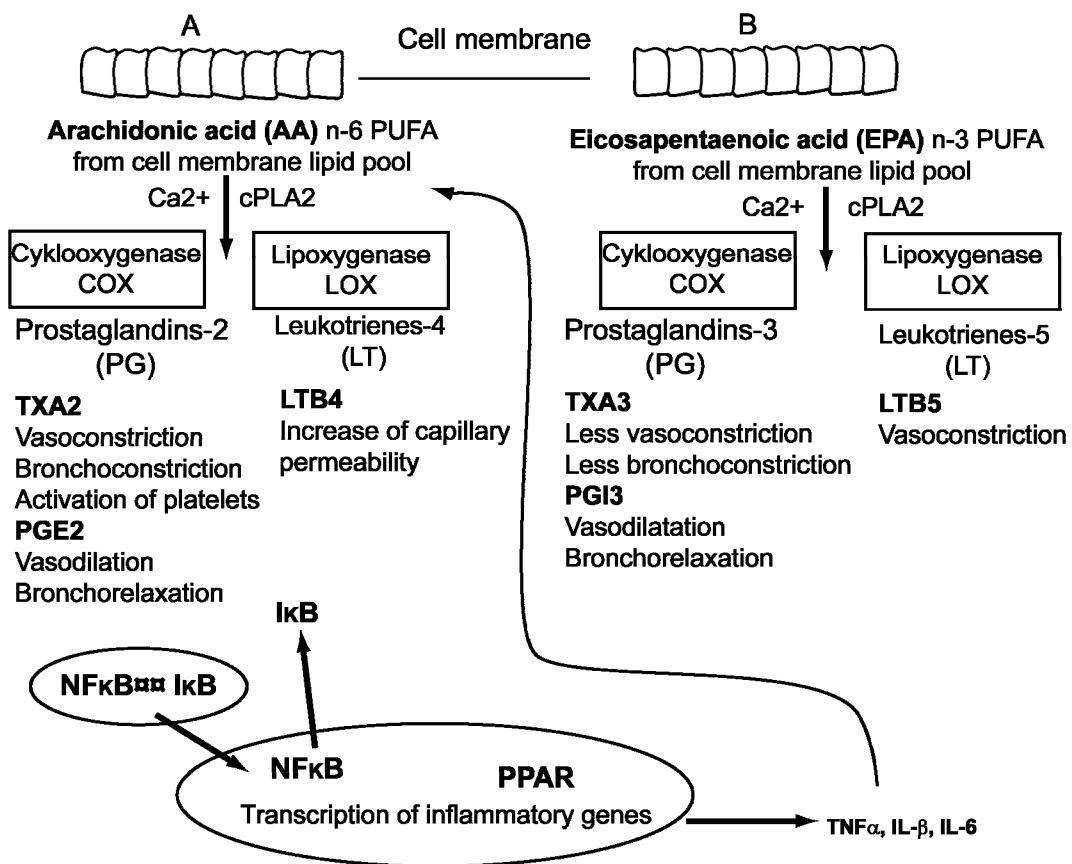
IL-6 is a proinflammatory cytokine which can be analysed in CSF and plasma. The effects of IL-6 are more dependent on the cellular environment where IL-6 either exerts a damaging pro inflammatory effect in CNS or show an anti-inflammatory neuroprotective and immunosuppressive effect necessary for normal neuronal growth. In AD, IL-6 seems predominantly to have pro-inflammatory properties and is produced by microglia in response to injury of A $\beta$  and promotes early development of NPs which might explain why IL-6 is found in a high proportion in diffuse plaques in AD brains. Similar to the IL-1 levels IL-6 levels in CSF and plasma show inconsistent results. In AD, CSF levels have been shown to be increased (Martinez, *et al.* 2000). In the periphery increased IL-6 levels show a correlation with the severity of the disease (Licastro, *et al.* 2000).

### **TNF- $\alpha$ in AD**

TNF- $\alpha$  is a non-interleukin cytokine which also is produced by activated microglia in response to A $\beta$  and binds to multiple receptors expressed on astrocytes, neurons and microglia and induces neuronal injury (Tarkowski, *et al.* 1999). Treatment for modulation of increased levels of TNF- $\alpha$  have been reported (Tobinick, *et al.* 2006).

### 5.3 N-3 PUFAS, CYTOKINES AND INFLAMMATION IN ALZHEIMER'S DISEASE

The main mediators in neuroinflammation are the microglia and astrocytes. They initiate a rapid response involving cell migration, proliferation and release of cytokines IL-1, IL-6 and TNF- $\alpha$  and chemokines. The cytokines stimulate multiple forms of phospholipase A2 (PLA2) and COX and LOX that break down the neuronal membrane glycerophospholipids with release of AA which produces pro-inflammatory PG, TX and LT.



**Figure.** A and B are alternative pathways for lipid mediator syntheses involved in an inflammatory reaction. In states of inflammation EPA is released (B) and competes with AA (A) for metabolism of COX and LOX. The metabolites from EPA are less biologically potent and less pro-inflammatory compared to AA metabolism.

Enrichment in diet of DHA and EPA competitively inhibits the oxygenation of AA by COX thus suppressing the production of pro-inflammatory cytokines and eicosanoids. EPA is a substrate for both COX and 5- LOX and gives rise to 3-series PG and tromboxanes and 5-series leukotrienes. DHA is not a substrate for COX.

Actions of 15-LOX however, on DHA produces the neuroinflammatory compounds 17S-resolvins and 10,17S-docosatrienes/neuroprotectin. They have anti-inflammatory effects and antagonize the effects of AA produced eicosanoids, modulate leukocyte trafficking and down regulate the cytokines from microglia cells and slow down the production of inflammatory cytokines from astrocytes. This suggests that the n-3 PUFAs have internal anti-inflammatory protective effects for preventing brain damage in neurodegenerative diseases. This process can modulate the expression of the pro-inflammatory genes for COX-2.

EPA and DHA resemble each other in some biochemical effects including the decrease of production of cytokines (TNF- $\alpha$ ) but they differ from each other in other ways e.g. expression of specific genes and biochemical effects. EPA has hypotriglyceride effects which DHA does not. Both EPA and DHA produce neuroprotectins and resolvins, reduce chronic inflammation by inhibition of NF $\kappa$ B activation (by decreasing the phosphorylation of I $\kappa$ B), thereby modulating the availability of NF $\kappa$ B and thereby the availability of expression of the proinflammatory cytokines Il-1 and TNF- $\alpha$  as well as COX-2 activation down-regulating pro-inflammatory gene induction.

Altogether increasing EPA and DHA produces numerous effects including

1. decrease in lymphocyte proliferation
2. suppressed production of pro-inflammatory cytokines
3. reduced gene-expression of COX-2
4. reduced natural killer cell activity
5. increased membrane fluidity
6. affect signal transduction
7. modulate gene expression
8. modulate antigen presenting capacity

The interactions and relations between EPA, DHA, cytokines, eicosanoids, resolvins and neuroprotectins are of a very complex nature and seem to be associated with neuroprotective effects in inflammatory processes. It has been suggested that a moderate intake of AA and a balanced ratio intake of AA and DHA can be important in modulation of neuroinflammatory processes. The optimal ratio between intakes of AA and DHA is still not clear.

### **5.3.1 N-3 PUFAs and Transcription factor peroxisome proliferator-activated receptors; PPARs**

The expression of genes which are involved in the inflammatory response is controlled transcriptionally and post-transcriptionally. One group of transcription factors is the transcription factor NF $\kappa$ B (see chapter 5.3). The second group of transcription factors which is involved in neuroinflammation is the PPARs which are members of the nuclear hormone receptor family. PPARs have three major isoforms and are found in neural tissue: PPAR- $\alpha$ , PPAR- $\gamma$  and PPAR- $\delta$ . N-3 PUFAs act as endogenous ligands and in the presence of PPAR heteromerize with retinoid X-receptors (RXR) (Calderon, *et al.* 2007) which then directly modulates the expression of target genes thus decreasing the inflammatory response.

### **5.3.2 Neuroinflammation and NSAIDs in AD**

In A series of events in AD include the activation of microglia and astrocytes by different factors including  $\beta$ -amyloid and pro-inflammatory cytokines. Other early signs are also hippocampal synaptic dysfunction (Selkoe 2002) associated with neuronal apoptosis and aggregation of fibrillar  $\beta$ -amyloid forming NPs. The primary actions of NSAIDs is inhibition of the COX enzymes which exist in inducible form (COX-2) that has been found to be increased in AD brains and a constitutive form (COX-1). The theoretical background for the antiinflammatory effects of NSAIDs is that the inflammatory processes in the brain can be decreased through inhibition of COX-2 enzyme which is critical for the production of proinflammatory prostaglandins. NSAIDs can also reduce A $\beta$  42 production by direct modulation of  $\gamma$ -secretase (Weggen, *et al.* 2001).

Epidemiological studies, have found a reduced risk of developing AD that is associated with long term treatment with non-steroidal-anti-inflammatory drugs (NSAIDs) (Tabet, *et al.* 2002). But clinical trials have only yielded limited results. In vitro studies have shown that NSAIDs (ibuprofen) (Lim, *et al.* 2000) directly can influence the processing of APP. Transgenic mouse models of AD have shown a reduction of  $\beta$  amyloid plaques and a decrease of production of Il-1 $\beta$  and glial fibrillary acid proteins. Altogether ibuprofen suppresses a limited subset of inflammatory markers in transgenic mice. In human neuronal cells, ibuprofen treatment has shown a decrease of total A $\beta$  from cytokine stimulated cells by 50 % and a decrease of A $\beta$ 42 and 40. Naproxen does not lower A $\beta$ 42 in vitro

(Blasko, *et al.* 2001; Tabet, *et al.* 2003). Using clinically relevant dosing, brain levels of NSAIDs were too low to implicate a number of pharmacological dose targets that were demonstrated *in vitro* (Cole, *et al.* 2004; Rojo, *et al.* 2008). So far clinical studies indicate that NSAIDs cannot clinically improve patients with manifest AD regardless what has been shown in animal studies on the effects on the A $\beta$  load and on microglia. Although COX-2 inhibitors also show promising effects in animal studies and enhance cognitive deficits in animal models, clinical trials with COX-2 inhibitors have been negative for treatment of AD. A principal limitation of long-term treatment with NSAIDs is also gastro-intestinal side effects that will occur (in t' Veld, *et al.* 2001).

#### **5.4 NEUROINFLAMMATION AND DHA**

Epidemiological studies suggest that intake of a diet enriched with n-3 PUFAs like EPA and DHA is associated with a reduced the risk of AD (Kalmijn, *et al.* 1997a; Kalmijn, *et al.* 1997b).

In AD the neuroinflammatory changes are primarily characterised by reactive microglia and astrocytes, IL-1, IL6 and TNF- $\alpha$  and complement factors that are triggered by the production of A $\beta$  and hyperphosphorylation of tau. Some reports have shown that DHA levels are lower in serum and brains of AD patients (Söderberg, *et al.* 1991) which could be the results of a low dietary intake of n-3 PUFAs or oxidation. Several mechanisms have been postulated for the protective role n-3 PUFAs may have in dementia such as the reduction in cardiovascular disease (an increase in cardiovascular diseases leads to an increase in subtypes of AD and vascular dementia), the anti-arythmic, anti-thrombotic, anti-inflammatory and anti-atherogenic effects. N-3 PUFAs also lower the levels of triglycerides, blood pressure and improve endothelial function (Farooqui, *et al.* 2007). N-3 PUFAs may also reduce anti-inflammatory effects by the synthesis of pro-inflammatory cytokines, by incorporation into neuronal membranes in the brain thus maintaining membrane stability, fluidity and flexibility. In animal studies, dietary DHA show increased levels of neurotransmitters and modulation of ion channels and reduced neuronal damage (Mazza, *et al.* 2007). Animal studies from Lim *et al.* (Lim, *et al.* 2005) have in repeated trials in both aged transgenic mice and young mice fed on DHA enriched diets shown interesting data pointing to a reduction of over 70% of total A $\beta$  a decrease of A $\beta$ 42 and an overall reduced

plaque burden with 40 % with the largest reduction in the hippocampus and parietal cortex (Lim, *et al.* 2006). To date there are no published data on humans on intake of fish or n-3 fish oil products on inflammatory markers. To our knowledge there are two short-term clinical trials of n-3 PUFAs focused on cognition and mood in patients with manifest AD (Yehuda, *et al.* 1996; Kotani, *et al.* 2006) and our own study. The choice of DHA for patients with AD is of clearly clinical significance since the brain contains a high proportion of DHA which exerts neuroprotective effects on PPAR and RXR receptors and enhances membrane fluidity and neuroprotection (Mukherjee, *et al.* 2007). Also EPA can be used since EPA to a certain extent can be metabolized to DHA.

## 6 WEIGHT AND NUTRITION IN AD

### 6.1 WEIGHT LOSS

Weight loss is a major problem in the geriatric population that increases mortality (Dey, *et al.* 2001). Weight loss might precede mild to moderate AD and is a common feature in patients with AD (Barrett-Connor, *et al.* 1996; Cronin-Stubbs, *et al.* 1997). Weight loss is only included in the diagnostic set up in the NINCDS-ARDRA criteria for probable AD. Reasons for weight loss in AD are of multifactorial aetiologies and include (White, *et al.* 1996; White, *et al.* 1998; Guerin, *et al.* 2005):

1. Inadequate energy intake.
2. Abnormally high energy expenditure (Rheaume, *et al.* 1987)
3. Difficulties in shopping or preparing food.
4. Decreased appetite due to decline of the olfactory system (Schiffman, *et al.* 2002). Neurofibrillary pathology appears first in the olfactory system where pathological changes may occur years before the onset of cognitive impairment and AD pathology is also known to impair olfactory functions particularly in *APOEε4* carriers (Graves, *et al.* 1999; Vanhanen, *et al.* 2001). There are losses in the ability to identify, recognise and detect odours (Bacon, *et al.* 1998). Medial temporal lobe atrophy where the earliest anatomical changes occurs in AD is associated with low body weight in AD patients.
5. Concomitant acute and chronic somatic serious diseases e.g. heart disease, depression, hypertension, stroke, alcoholism.
6. In more advanced stages of AD other reasons might include behavioural disturbances such as pacing, wandering and agitation and physical activity. Additionally the availability of food and food preferences (Morris, *et al.* 1989).
7. Cytokines may inhibit feeding by acting on the gastrointestinal system or indirectly on the central nervous system. Elevated levels of cytokines such as TNF- $\alpha$  have been related to weight loss and cachexia in patients with AD. Higher levels of circulating TNF- $\alpha$  produced by microglia surrounding and invading plaques in the CNS might pass through the blood-brain-barrier in AD into systemic circulation (Zuliani, *et al.* 2007), or elevated levels of TNF- $\alpha$  could result from a systemic reaction in patients with AD (Fillit, *et al.* 1991; Carrero, *et al.* 2007).



The association between weight loss and AD is well known. However, it seems as the body weight regulation system in AD is dysfunctional (Faxen-Irving 2004; Faxén-Irving, *et al.* 2005). Aging alone can impair mechanisms of energy regulation leading either to weight loss or weight increase. The clinical importance of weight gain is unknown but weight gain in old age might be protective (Björkelund, *et al.* 1997). IL-1, IL-6, TNF- $\alpha$  (also called cachectin) and PG2 have all been implicated in cancer-induced cachexia. Cytokines may inhibit feeding by causing nausea and vomiting and by decreasing gastric motility and emptying, intestinal motility or by modifying gastric acid secretion. The cytokines (e.g., IL-1 and TNF- $\alpha$ ) can exert direct action on the gastro-intestinal system or indirectly mediate effects on the central nervous system. IL-1 $\beta$  can also stimulate serotonin and their metabolites which in turn suppresses food intake and IL-6 and TNF- $\alpha$  can inhibit gastric emptying. TNF- $\alpha$  can also induce the production of both IL-1 and IL-6 (Yeh, *et al.* 1999). N-3 FAs have been suggested to reduce advanced anorexia and cachexia, increase of weight, and improve quality of life in patients with malignant diseases, although this hypothesis is still not proven (Fearon, *et al.* 2003). N-3 FAs can interfere with COX and LOX metabolic pathways in PGE2 production and reduce the production of PGE2 which in turn will lead to a decreased production of cytokine synthesis and activity e.g., IL-1 and thereby perhaps reverse anorexia and cachexia (Wouters-Wesseling, *et al.* 2002).

### **6.1.1 High-sensitivity C-reactive protein**

Other established sensitive markers of systemic low grade inflammation are levels of serum concentrations of high-sensitivity C-reactive protein (hs-CRP, Pearson, *et al.* 2003). C-reactive protein has been found in and around  $\beta$ -amyloid plaques in the brain of patients with dementia (Kuo, *et al.* 2005). Increased levels have been associated with poor memory, AD and vascular dementia (VaD) although other studies have shown no such correlation. Low serum levels are hs-CRP <1.0 mg/l, average 1.0-3.0 mg/l and high >3.0 mg/l. Hs-CRP is a marker that has been shown to identify individuals at an increased risk for decline in cognition (Komulainen, *et al.* 2007).

### **6.1.2 Insulin-like growth factor IGF-1 and serum-albumin**

Insulin-like growth factor IGF-1 and albumin in serum are commonly used in the assessment of nutritional status in addition to body mass index (BMI). Both IGF-1

and albumin are negative acute-phase reactants which means that serum concentrations of both can drop precipitously in response to acute severe stress such as sepsis and major surgery as a result of increased vascular permeability. Therefore these markers are more linked to co-morbidity and inflammation than to nutritional status. Levels of serum albumin have been found to be a predictive indicator of mortality (Hilding, *et al.* 1995).

## **6.2 ANTHROPOMETRY**

The commonly used anthropometric measures to estimate body fat and muscle are body weight measurements and recording of upper arm circumference. The body mass index (BMI), kg/m<sup>2</sup> is currently the most used weight and height index. The cut-off values for adults lies between 20-25 and for elderly a BMI <23.5 defines underweight in community and hospital settings (Faxen-Irving 2004; Faxén-Irving, *et al.* 2005). Low values of BMI predicts mortality in the elderly in both sexes. On the other hand weight gain has been found not to be a risk for mortality after age 70 in both sexes. A BMI <23 was related to reduced 7-year survival in patients with various dementia diagnoses. Maintenance of stable body weight seems to be good for survival in the elderly (Dey, *et al.* 2001).

The reliability of anthropometric measures has been discussed as variations occur depending on e.g. variations in fluid balance. Using discriminant analyses measures of skinfold thickness and weight lost during the past year is a good predictor of 1-year mortality in geriatric patients (White, *et al.* 1998).

Identification of weight loss of 5 % or more often concerns physicians because of the risk for cancer and other diseases especially if the weight loss occurs in the preceding 3 months or a 10 % weight loss or more if occurring during the past 6 months (Faxen-Irving 2004; Faxén-Irving, *et al.* 2005).

Many AD patients have a lower weight and lower BMI that occurs more frequently compared to cognitively normal control subjects. Increased severity and progression of AD correlates with greater weight loss. Weight loss in patients with AD is predictive of increased mortality whereas weight gain appears to have a protective effect. Within 8 years of onset of AD 50 % of patients need help with feeding or artificial nutrition (Cederholm 1994; Faxen-Irving 2004).

### 6.3 DIETARY PATTERNS IN MIDLIFE AND RISK FOR DEMENTIA

Recent research has revealed that lifestyle-related factors and vascular risk factors dementia such as elevated blood pressure and high serum cholesterol levels in mid-life, type-2 diabetes and obesity are of importance in the development of AD and dementia (Kivipelto, *et al.* 2008). Epidemiological studies show that aging, inflammation and cerebrovascular disease might lead to AD in combination with low dietary intake of B6, B12, folate and low intake of unsaturated fats and high intake of saturated fats, but reports are inconsistent (Laitinen, *et al.* 2006). A modest intake of alcohol intake can be protective as well as a mediterranean diet (Scarmeas, *et al.* 2006). The effects have in some studies been more pronounced in non-carriers of *APOE* $\epsilon$ 4 allele. Randomised clinical trials of supplementation of vitamin B6 B12 and folate have shown no cognitive benefit (Luchsinger, *et al.* 2007). Other factors that might coincide with the dietary factors and incidence of AD are such factors as socioeconomic status, e.g. education smoking and alcohol drinking. The only known genetic risk factor in LOAD is the occurrence of the  $\epsilon$ 4 allele of Apolipoprotein E (*APOE*) which is involved in cholesterol metabolism (Laitinen, *et al.* 2006; Barberger-Gateau, *et al.* 2007).

## **7 NEUROPSYCHIATRIC AND FUNCTIONAL SYMPTOMS IN ALZHEIMER'S DISEASE**

In AD cognitive failure is not a sufficient explanation for functional disability or impaired quality of life. Neuropsychiatric symptoms such as depression, apathy, delusions and aggressive are common. Apathy, depression (Vercelletto, *et al.* 2002) and anxiety appear early in the course and delusions, hallucinations and agitation appear in the middle to late stages. Quality of life for both patients and care givers are negatively affected by the presence of neuropsychiatric symptoms (McKeith, *et al.* 2005). Occurrences of neuropsychiatric symptoms are also important determinants in the prescribing of psychotropic drugs and allocation of nursing-home placement. Impairment of ADL and the presence of neuropsychiatric symptoms are also strongly linked. ADL functions are closely linked to a person's ability to cope with the environment in terms of basic adaptive tasks (e.g. clothing, bathing). These are maintained late in the course of the disease. Performances that require higher cortical functioning are dependent on frontal-subcortical structures mediating executive functioning (preparing a meal, shopping, driving and handling your economy) and are affected earlier in the course. Neuropsychiatric symptoms are also mediated by frontal brain regions and the high correlation between neuropsychiatric symptoms and impairment of ADL may reflect this shared regional neuropathological base. There is also a link with cholinergic deficiency. The cognitive deficits play an important role in ADL function of AD patients where loss of memory, language difficulties visuspatial abilities, apraxia, or agnosia contributes to decreased ADL function. Also problems with physical health might influence the level of ADL activity. In summary it is obvious that ADL-activity has a complex relationship with both cognition and neuropsychiatric symptoms where more prominent neuropsychiatric symptoms such as agitation, psychosis, agitation, apathy and aberrant motor behaviour seem to have a greater impact on the level of ADL compared to less prominent neuropsychiatric symptoms (Tekin, *et al.* 2001).

AD features a complex array of neuropsychiatric symptoms including apathy, agitation, depression, anxiety, elusions and irritability; less common symptoms are elation and hallucinations. There are three basic behavioural syndromes in AD: one patient group with few neuropsychiatric symptoms, a second group with prominent psychotic symptoms and a third group with more affective symptoms.

Agitation and apathy are seen in combination with other neuropsychiatric symptoms (McKeith, *et al.* 2005).

Imaging studies suggest that patients with neuropsychiatric symptoms tend to have a lower perfusion and metabolism in the frontal and temporal lobes compared to patients with less neuropsychiatric symptoms (McKeith *et al.* 2005). A high burden of NFTs at autopsy is associated with both agitation and psychosis compared to patients without these symptoms.

Scales that measure neuropsychiatric symptoms include The Neuropsychiatric Inventory (Cummings, *et al.* 1994), Cornell depression rating scale and Cohen-Mansfield agitation inventory (CMAI) (Alexopoulos, *et al.* 1988; Cohen-Mansfield, *et al.* 1989). Scales can be multi dimensional in assessing several symptoms in dementia such as NPI or uni-dimensional focusing on only agitation (Cohen-Mansfield, *et al.* 1989). In the NPI, questions are answered by proxy. Questions to both caregiver and patient are included in the Cornell depression rating scale and in Montgomery Åsberg-Depression Rating scale (MADRS) (Montgomery, *et al.* 1979) only the patient answers the questions.

## **7.1 PHARMACOLOGICAL TREATMENT OF NEUROPSYCHIATRIC SYMPTOMS IN AD**

There have been very few randomised double-blind placebo-controlled trials of psychotropic drugs in patients with dementia. Despite the lack of systematic evidence and regulatory approval the prescription of psychotropic drugs to patients with dementia is currently widespread.

Antipsychotic drugs, mood stabilisers, antidepressants, anxiolytics and sedative hypnotics are today commonly used psychotropic drugs for patients with dementia to treat symptoms such as agitation, psychosis, depression, anxiety and sleeping disorders. The only approved medication for treating behavioural and psychiatric symptoms in dementia (BPSD) in Sweden is the atypical antipsychotic risperidone for treatment of severe agitation in patients with dementia. In UK and USA no such treatment is at the moment prescribed for patients with BPSD. Recently it has been reported a two to three times increased risk for cerebrovascular events with the use of both atypical antipsychotics and typical antipsychotics limiting the use (Madhusoodanan, *et al.* 2007).

Antidepressants are widely used although few trials with short follow-up time have been performed (Herrmann, *et al.* 2007). Several trials have not shown any

significant differences between active drug and placebo treatment. The most commonly used anti-depressive psychotropic drugs are the selective serotonin-reuptake inhibitors (SSRI) that have shown positive treatment effects and also a favourable profile of side effects in this age group. Another often used psychotropic group of medication in patients with more pronounced anxiety and sleeping problems are the combined serotonin and adrenalin reuptake inhibitors which are not well studied in patients with dementia. The group of tricyklics is today avoided mainly due to their well known anti-cholinergic side-effects that impose problems in patients with cognitive deficits already with low levels of acetylcholine. Anticonvulsant drugs with mood stabilising properties have an old history of treatment in this patient group for treatment of agitation and are most often used as second line treatment although few are studied in clinical trials. In order to avoid well-known side effects in sedation, ataxia and falls few patients are on a constant regime of benzodiazepine anxiolytics and hypnotics (McKeith, *et al.* 2005).

Very few studies have been performed with neuropsychiatric symptoms as the primary outcome. There is an immediate need to design studies with neuropsychiatric symptoms as primary outcome.

### **7.1.1 Acetylcholinesterase inhibitors (AChEI), and treatment of neuropsychiatric symptoms in AD**

The drugs that are used for treatment of cognitive symptoms in mild to moderate AD in Sweden today are the AChEIs, donepezil, galantamine and rivastigmine (Cummings, *et al.* 2004). All these three compounds have been tested in clinical trials where the primary outcome has been cognition and secondary outcome has been the neuropsychiatric symptoms. These trials have shown positive effects on the emergence of newly developed neuropsychiatric symptoms during the trials as well as positive treatment effects on already established symptoms which might be stabilised (Rösler, *et al.* 1998; Feldman, *et al.* 2001). A relatively new drug on the market is the N-methyl-D-aspartate receptor antagonist Memantine which also been showed to have effects on agitation in patients with AD (McKeith, *et al.* 2005).

## **7.2 APOE AND NEUROPSYCHIATRIC SYMPTOMS IN AD**

Since *APOEε4* is a risk factor for the development of AD the relationship between neuropsychiatric symptoms and *APOEε4* in AD has been studied (Pritchard, *et al.* 2007).

However, contradictory results have been reported. The occurrence of neuropsychiatric symptoms in dementia seems to be influenced by numerous factors such as gender, age, severity of the dementia, which dementia diagnosis the patient has, time of observation and also general medical health where the status of *APOEε4* might be one of several risk factors. In one study no close relationship between *APOEε4* and depressive/dysphoric symptoms in a homogenous north Irish population was found (Craig, *et al.* 2005). In the Cache County study (Scarmeas, *et al.* 2002) where a small sample of 87 AD patients were followed up to 9.3 years (mean 5.5 y) data showed that the presence of one or more ε4-allele was a significant predictor for the incidence of delusions in the course of AD but not on depression or illusions. The relationship of various neuropsychiatric symptoms show a tendency of clustering that in turn may be altered depending on the stage and the severity of the disease.

### **7.3 N-3 FATTY ACIDS MODE OF ACTION IN PSYCHIATRIC SYMPTOMS**

The supplementation of n-3 PUFAs has been proven to be beneficial in treatment in a number of psychiatric disorders such as bipolar disorders, major depression, schizophrenia and postpartum depression (Su, *et al.* 2003; Boston, *et al.* 2004; Bourre 2005; Peet, *et al.* 2005). The modern research on therapeutic benefits of n-3 PUFAs was initially carried out as an effect of observations that levels of n-3 PUFAs was found to be reduced in cell membranes of patients with schizophrenia and depression. These findings were further supported by epidemiologic studies (Conquer, *et al.* 2000; Young, *et al.* 2005) showing that fish consumption was inversely correlated with rates of major depression (Hakkarainen, *et al.* 2004) and bipolar disorders (Montgomery, *et al.* 2008). A decreased ratio of n-3 PUFAs to n-6 PUFAs in plasma and erythrocytes of patients with major depression (Peet, *et al.* 1998) and a decreased level of n-3 PUFAs in erythrocytes have been associated with an increased severity of depression (Freeman, *et al.* 2006).

Presently the true mode of action of the n-3 PUFAs is not known. Data from trials for both depression and schizophrenia currently suggest that EPA rather than DHA is more effective. This is somewhat surprising since DHA is the most abundant FA in the brain. The major part of DHA is present in the phospholipids of the cerebral gray matter. If EPA is the most important active agent out of these two n-3 PUFAs that would suggest that the biological effects are different from those

which are expected from DHA. However, both EPA and DHA can to a small extent be converted to each other. They can modulate leukocyte function through controlling proliferation, production of pro-inflammatory cytokines and adhesion molecules (Tanskanen, *et al.* 2001). The n-3 PUFA can also change intracellular signalling pathways or gene expression by lipid regulated transcription factors such as peroxisome proliferators –activated receptors (PPARs). Some recent studies have shown that n-3 PUFAs like DHA can prevent neuroinflammation by decreased activation of the transcription factor NF- $\kappa$ B possibly by activation of PPAR and decreased phosphorylation of I $\kappa$ B which exert inhibitory effects on NF- $\kappa$ B. EPA has been suggested to inhibit phospholipase A2 which is elevated in patients with schizophrenia (Freeman 2000; Peet, *et al.* 2005).

The mood stabilising effects in bipolar and major depressive disorders of the n-PUFAs have been proposed to be an inhibiting effect on protein-kinase C (PKC) in a similar way that lithium and valproic acid act in vitro studies (Mirnikjoo, *et al.* 2001; Seung Kim, *et al.* 2001; Logan 2003).

PKC represents a family of enzymes highly enriched in the brain where it plays an active role in regulation of neuronal excitability, neurotransmitter release, regulation of synaptic plasticity and memory and learning. Both lithium and valproate can decrease levels of PKC in humans with bipolar disorders (BPDs, Zarate, *et al.* 2007). In rats it has been shown that an increased intake of EPA and DHA as well as the combination of the two inhibit levels of PKC (Seung Kim, *et al.* 2001). This might represent a potential site of action of the n-3 PUFAs effects in BPD.

### **7.3.1 Epidemiological trials and n-3 PUFAs in mood disorders**

In cross national analyses Hibbeln (1998) has found 30-60 fold higher prevalence rate of major depression, post-partum depression, and BPDs in countries with a low per capita consumption of fish (Noaghiul, *et al.* 2003). In a majority of studies high fish consumption has been associated with a lower prevalence of depressive symptoms. Cross-national and country-specific epidemiologic studies suggest that consumption of at least 2 to 3 seafood meals per week is associated with a decreased risk of mood disorders (Freeman, *et al.* 2006).

Data from the cross-sectional Rotterdam study including 3884 adults both gender older than 60 years who were screened for depressive symptoms that underwent analyses of % of fatty acids in plasma phospholipids showed that patients with



depressive symptoms showed a higher ratio of n-6 PUFAs to n-3 PUFAs (Tiemeier, *et al.* 2003).

### **7.3.2 Clinical trials and n-3 PUFAs in mood disorders**

To date there has been 3 double-blind randomised placebo-controlled studies using EPA without DHA or a combination of EPA and DHA as an adjunctive treatment for anti-depressant refractory major depressive disorder. The treatment with DHA immunotherapy 2.0 g/day for 6 weeks showed no significant depressive effect in 36 patients with major depressive disorders (MDD) (Marangell, *et al.* 2003). Another study performed in patients with MDD in a 12 week long trial with 70 patients on doses of only EPA ranging from 1.0, 2.0 or 4.0 g/day showed significantly positive effects with the 1g/day dose (Peet, *et al.* 2002). Also add on treatment with EPA 2 g/day on patients with MDD already treated with SSRIs showed significantly better effects compared to placebo (Nemets, *et al.* 2002).

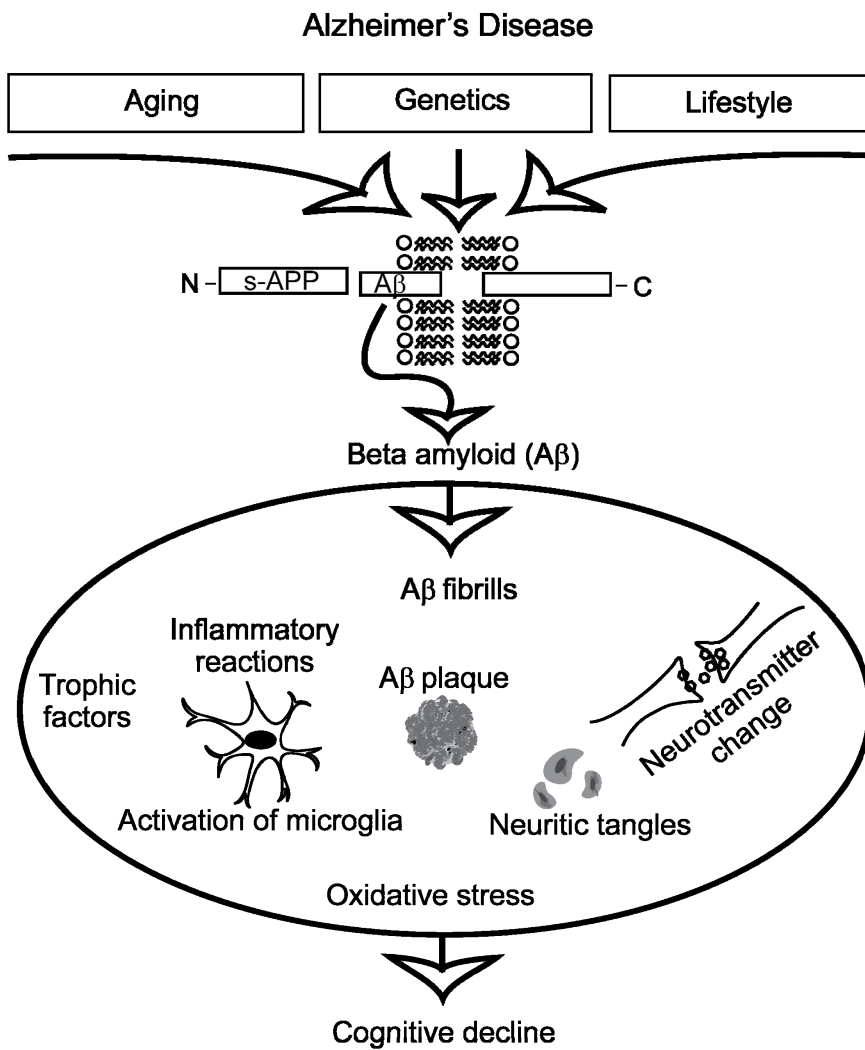
Treatment with a mixture of EPA+DHA 3.0 g/day, (2.4 g DHA and 0.6 g EPA) or placebo as add on treatment in MDD showed improvement in both groups, and no significant better outcome in either group (Silvers, *et al.* 2005).

In conclusion DHA showed no antidepressive effect when used alone and EPA at doses varying between 1.0 and 2.0 g/day or fish oil in higher doses seems to improve depressive symptoms (Freeman, *et al.* 2006).

In bipolar disorders, Stoll *et al.* conducted a double-blind placebo-controlled study of 9.52 g/day of EPA and DHA (6.16 g EPA and 3.36 g DHA) in 30 patients for 4 weeks where he found that the duration on of remission was significantly greater with active treatment compared to placebo (Stoll, *et al.* 1999).

Frangou reported in a double-blind placebo-controlled study of adjunctive EPA in participants with BPD who either received 1.0 g EPA or 2.0 g EPA but no DHA for 3 months. The results showed no significant difference benefit between the two different doses of EPA (1.0 g vs. 2.0 g) but both groups receiving EPA showed improved effects compared to the placebo group (Frangou, *et al.* 2006).

A double-blind placebo-controlled study of adjunctive ethyl ester EPA 6.0 g/day for 4 months in patients with BPD showed no significant treatment effects of PUFAs on psychiatric symptoms (Keck, *et al.* 2006). In conclusion results of clinical trials in BPSD show inconsistent results.



**Figure.** Risk-factors, pathophysiological, neurochemical mechanisms leading to AD (Nordberg, Eriksson-Jonhagen, Garlind, Grut, Freund-Levi et al, 2006).

## 8 AIMS OF THE THESIS

This thesis examines how the supplementation of n-3 FA in patients with mild to moderate AD treated with a stable dose of AChEI influences cognition, neuropsychiatric symptomatology, inflammatory markers CSF and plasma and biomarkers in CSF as well as weight and nutritional parameters. The study was performed as a randomized double-blind placebo-controlled study (RCT).

The specific aims of the different papers were:

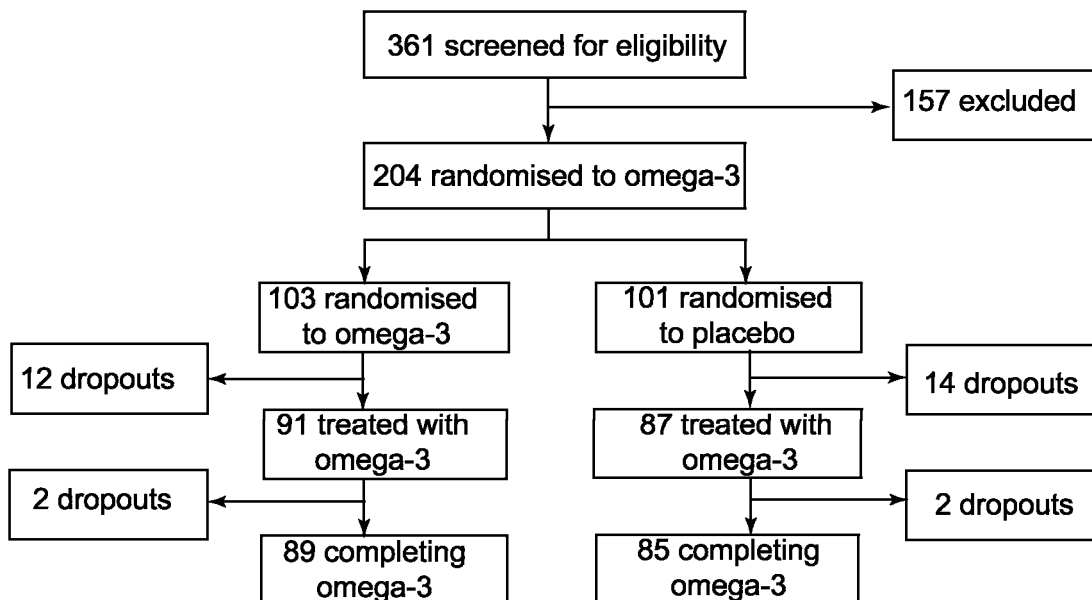
- I. To determine dietary effects of supplementation with n-3 FA on cognitive functions, global functions, safety, tolerability and compliance in 174 patients with mild to moderate AD.
- II. To study effects of supplementations of n-3 FA on psychological and behavioral symptoms, activities of daily living with (ADL) and care giver's burden in 174 patients with mild to moderate AD. The possible relation between carriers of *APOEε4* alleles and treatment effects on neuropsychiatric symptoms was also investigated.
- III. To study effects of n-3 FA supplementation on the inflammatory biomarkers IL-6, TNF- $\alpha$ , sIL-RII in CSF and IL-6, TNF- $\alpha$ , sIL-RII in plasma and on the dementia biomarkers A $\beta$ <sub>1-42</sub>, T-tau and P-tau in 35 patients.
- IV. To study effects of supplementation with n-3 FA in 174 patients with mild to moderate AD on weight, BMI, measures of arm anthropometry and appetite, and the inflammatory and nutritional parameters clinically used in patients, s-albumin, s-IGF-1 hs-CRP. A possible relationship between carrier ship of the *APOEε4* alleles and nutritional parameters was also investigated.

## 9 SUBJECTS AND METHODS

An overview of the demographic characteristics and global cognitive status of the patients included in this study is presented below. Comprehensive description of the study population as well as methods and statistics employed can also be found in the specific papers.

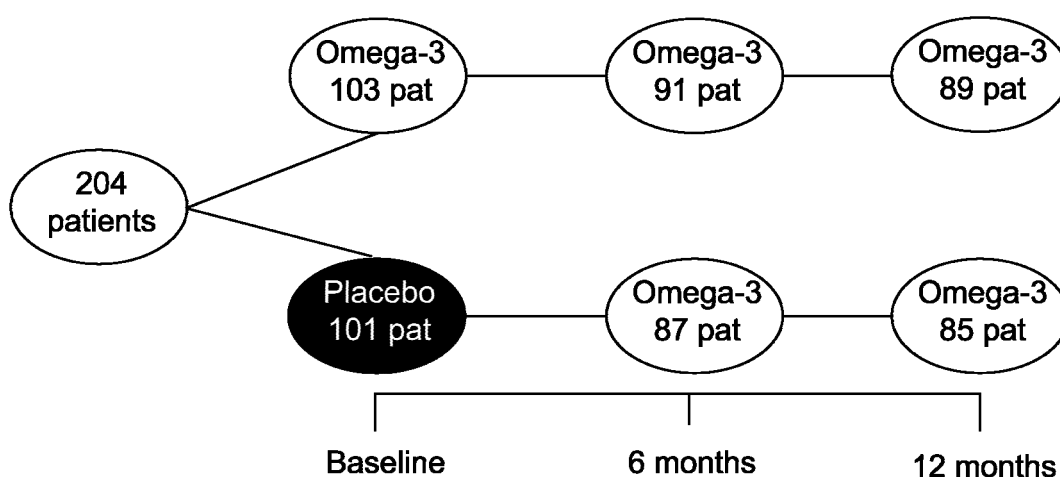
### 9.1 STUDY POPULATION PAPER I-IV

361 patients were screened for eligibility and the study population consisted of 204 patients (110 women and 94 men, mean age  $74 \pm 4$  years) diagnosed with mild to moderate AD according to the DSM-IV classification. The clinical diagnosis of AD was consistent with the ICD-10 classification. Patients were recruited from specialist memory clinics at Karolinska University Hospital Huddinge, Danderyds Hospital and St Göran Hospital in Stockholm, Sweden between the 13<sup>th</sup> of December 2000 and 25<sup>th</sup> of March 2004. Thirty patients dropped out during the study and 174 patients underwent assessments at baseline and after 6 and 12 months and data in paper I, II and IV are based on these patients. A subgroup of the first consecutively 40 patients are described in paper III and were assessed at baseline and after 6 months.



## 9.2 STUDY DESIGN AND PROCEDURE

This study was designed as a double-blind randomized placebo-controlled study. Patients were randomized using sealed envelopes in blocks of four to four 1 g capsules daily, each containing either 430 mg DHA and 150 mg EPA (EPAX 1050TG™ from Pronova Biocare A/S Lysaker, Norway) or an isocaloric placebo oil (containing 1 g of corn oil, including 0.6 g of linoleic acid) for 6 months, followed by 6 months open treatment with n-3 FA for all patients. EPAX 1050TG™ is a 60 % n-3 FA concentrate in triglyceride form produced according to Good Manufacturing Practise (GMP). 4 mg vitamin E (tocopherol) was added to each EPAX 1050TG™ capsule and placebo capsule.



The included 174 patients underwent the following evaluations at baseline and after 6 and 12 months: testing of cognition with MMSE and ADAS-Cog, neuropsychiatric testing with MADRS and NPI and testing of ADL using the DAD and care givers burden from 3 items from the CGB, length, weight, BMI, triceps skin fold and arm circumference, systolic and diastolic blood pressure and blood and urine sampling. Lubar puncture (LP) was also performed on 35 patients at baseline and at 6 months follow-up.

## 9.3 CLINICAL EVALUATION OF PATIENTS

All patients had undergone extensive medical examination at their specialist memory clinic before inclusion, including a detailed history from the patient and a close informant as well as general physical, neurological and psychiatric status examination. Computerised tomography (CT) or magnetic resonance imaging (MRI), psychometric testing of cognition, routine blood sampling including

hemoglobin, glucose, sodium, potassium, calcium, creatinine, albumin, thyroid hormones, vitamin B12, folic acid and Borrelia were also performed. Based on this information the patients received the AD diagnosis according to the ICD-10 classification by specialists in geriatrics at each specialist memory clinic.

### **9.3.1 Inclusion of patients**

The inclusion criteria required that the patients were diagnosed with AD according to the DSM-IV criteria and therefore were rediagnosed by an experienced rater (Yvonne Freund-Levi) before entering the study. All recruited patients had undergone a previous clinical work up as described in 9.3. The included patients had a MMSE score inclusive of 15-30 points, were living in their own homes and were treated with a stable dose of AChEI for a period of at least 3 months and planning to continue treatment during the study period.

### **9.3.2 Exclusion of patients**

Patients were not included in the study if they did not fulfill the inclusion criteria and also if they were treated with non-steroidal-anti-inflammatory drugs (NSAIDs), although low-dose acetylsalicylic acid was accepted. Also patients were excluded if they already were treated with n-3 FA anti-coagulantia agents; (low-dose acetylsalicylic acid was accepted) abused alcohol or suffered from a concomitant serious disease or did not have a caregiver.

### **9.3.3 Scales**

In clinical trials concerning AD, certain scales are used more often than others. The most common “Golden standard scales” in AD trials were included in our study in order to compare the results from our trial with previously performed trials with medications such as the AChEI and Memantine regarding effects on cognitive and neuropsychiatric symptoms. In testing of cognitive function the MMSE which ranges from 0-30 points, higher levels indicate a high cognitive performance (Folstein, *et al.* 1975). The cognitive portion of ADAS-Cog was also used which has a range between 0-85 points, in which higher levels indicate a decline in cognitive functions (Mohs, *et al.* 1997). Global functions were assessed using the Clinical dementia Rating Scale (CDR, sum of boxes and total sum, (Morris JC, *et al.* 1993). Neuropsychiatric symptoms were rated using the NPI which includes 12 different neuropsychiatric domains rated by the caregiver including, depression,

agitation, hallucination and agitation as well as anxiety and apathy (Cummings, *et al.* 1994). The total sum score of the NPI is 144 points and each domain has a maximum score of 12 points (0-12 p) where high points indicate a high neuropsychiatric load. Depressive symptoms were rated directly from the patient by using the MADRS which ranges from 0-30 points, higher points indicate a higher load of depressive symptoms (Montgomery, *et al.* 1979).

The Activities of Daily living was rated using the DAD scale (Gelinias, *et al.* 1999) and the care givers burden was assessed by direct questions to the caregiver with 3 questions from the Care givers burden scale CBS; 1) emotional overload 2) economical overload and 3) if the caregiver finds herself/himself captured in a role (Pearlin, *et al.* 1990).

### **9.3.4 Involvement of caregivers**

Patients with a progressive neurodegenerative disorder such as AD, have, due to Ethical reasons, a caregiver, who has a moral and caretaking responsibility not to expose the included patient in a clinical trial for unnecessary interventions. Also in order to establish a good relation to both patient and caregiver according to Good Clinical Practice (GCP) we wanted the caregiver to be involved in the trial to enhance the quality for the patient and to give information used in evaluations of treatment efficacy. For paper II, the caregiver validated the neuropsychiatric symptoms. In the trial performed in a subgroup we evaluated the intake of nutritive compounds by making interviews in focus groups consisting of both caregiver and patient (data not presented here). We also asked each caretaker about how the patient's appetite changed during the trial and at baseline and after 6 and 12 months. The NPI covers 12 different domains e.g. apathy, depression, sleep and appetite. The questions are put to the caregiver and in Paper IV appetite was specifically evaluated. The caregiver rated the appetite of the study participant as increased, stable or decreased at baseline and after 6 and 12 months. Changes were then categorised into 4 frequencies ranging from 1 (=less than once a week) or 4 (=daily or constantly). Thus a 1-9 score was constructed where 1 e.g. constantly decreased appetite 5 e.g. stable appetite and 9 showed constantly increased appetite.

## 9.4 ETHICAL CONSIDERATIONS

The study was conducted according to GCP and ethical principles of the Declaration of Helsinki. Both patient and care giver gave written informed consent prior to entering the study. The local ethics committee of Karolinska University hospital Huddinge/ Karolinska Institutet approved the study # 291/00 for Paper I-IV.

## 9.5 BLOOD TESTS

In all patients the following blood analyses were performed at baseline and after 6 and 12 months: hemoglobin, glucose, creatinine, sodium, potassium, calcium, albumin, vitamin B12, folic acid, hs-CRP and white blood cells.

### 9.5.1 Analyses of serum FA, Paper I-IV

Blood samples for analyses of serum FA (Boberg, *et al.* 1985) were obtained in all 204 patients in order to assess compliance to the therapy with n-3 FA and placebo. The concentrations of the individual FAs were expressed as relative percentages of the sum of investigated FA. The following FAs were evaluated at baseline and after 6 and 12 months: EPA, DHA and the placebo oil (LA).

For lipid extraction, 2.5 ml of methanol was added to 0.2-0.4 ml of serum. After thorough mixing, the extract of 5.0 ml chloroform (containing 0.005 % butylated hydroxytoluene as an antioxidant) was added followed by 7.5 ml of 0.2 mM sodium dihydrogen phosphate ( $\text{Na}_2\text{H}_2\text{PO}_4$ ) and the extract was left at + 4°C over night. The chloroform phase was evaporated to dryness and the lipid esters were transmethylated at 60°C overnight after addition of 2.0 ml 5 %  $\text{H}_2\text{SO}_4$  in methanol. The methyl esters were extracted into 3.0 ml of petroleum ether containing 0.005 % butylated hydroxytoluene after addition of 1.5 ml distilled water. The phases were separated after thorough mixing and centrifugation at 1500 g for 10 min. The petroleum ether phase was pipetted off and the solvent was evaporated under nitrogen. The methyl esters were then redissolved in Uvasol, grade hexane. The FA methyl esters were separated using gas-liquid chromatography (GLC) on a 30-m glass capillary column coated with Thermo TR-FAME (Thermo Electron Corporation, USA), with helium gas as a carrier gas. A Hewlett-Packard system (Avondale, PA, USA) consisting of model GLC 5890, integrator 3396 and auto sampler 7671 A was used. The temperature was programmed to 150-230°C. The



FAs were identified by comparing each peak's retention time with FA methyl ester standard Nu Check Prep (Elysian, MN, USA). The FA composition was expressed as relative percentages (Figure 2, paper I) of individual FA.

### **9.5.2 Analyses of APOE, Paper I-IV**

Patient DNA samples for *APOE* genotype was collected from all patients from peripheral white blood cells using standard methods (Hixson, *et al.* 1990).

*APOE* genotypes were determined by a microsequencing method on micro titer plates (AffiGene ApoE, Sangtec Medical, Bromma, Sweden).

## **9.6 ANALYSES OF PLASMA DATA**

### **9.6.1 Inflammatory samples IL-6, TNF- $\alpha$ and sIL-1RII, Paper III**

The levels of IL-6, TNF- $\alpha$ , and sIL-1RII were analysed in plasma samples by ELISA using commercially available kits (R&D systems, Abingdon, Oxon, UK). The detection range was 0.16 – 10 pg/ml for IL-6 and 31.3 – 2000 pg/ml for sIL-1RII in plasma. For TNF- $\alpha$ , the detection range was 15.6 – 1000 pg/ml in plasma samples.

The levels of hs-CRP were analysed in plasma samples using a commercially available kit from LX 20 (Beckman AB, Bromma) and performed at the Department of Clinical Chemistry, Karolinska University Hospital. A near infrared immunoassay (NIPA) rate method based on the binding of an anti-CRP antibody-coated particle that binds to CRP in the presence of a patient sample was used. The detection range was 0.2-380 mg/l.

### **9.6.2 IGF-I, Paper IV**

IGF-I was determined in plasma by RIA after separation of IGFs from IGFbps by acid ethanol extraction and cryoprecipitation (Bang, *et al.* 1991). The intra- and interassay CV were 4% and 11% respectively. Plasma levels of IGF-I decrease with age. Thus IGF-I values were also expressed as SD scores calculated from the regression of the values of 247 healthy adult subjects (Hilding, *et al.* 1995).

## 9.7 CSF DATA

### 9.7.1 Inflammatory markers in CSF; TNF- $\alpha$ , IL-6 and sIL-1RII, Paper III

CSF samples from 35 patients were collected at baseline and at 6 months. The LP was performed in a standardized manner with all patients in a sitting position, and the tap performed between 11 am and 1 pm with a non-traumatic cannula placed in the intervertebral space L 3/L 4 or L 4/L 5.

Five ml of CSF were collected in sterile polypropylene tubes and put on ice and centrifuged at 3000 rpm for 10 minutes at 4°C. The supernatants were aliquoted and stored at -70°C until analysis. The levels of IL-6, TNF- $\alpha$ , and -1RII were analysed in CSF by ELISA using commercially available kits (R&D systems, Abingdon, Oxon, UK). The detection range was 0.16-10 pg/ml for IL-6 and 31.3-2000 pg/ml for sIL-1RII in CSF. For TNF- $\alpha$ , the detection range was 0.038-0.191 pg/ml in the CSF samples. All inflammatory biomarkers were analysed at the Accredited Laboratory Karolinska University Hospital Laboratory.

### 9.7.2 Biomarkers in CSF A $\beta$ <sub>1-42</sub>, T-tau P-tau, Paper III

Frozen CSF samples were sent on dry ice to the Clinical Neurochemistry Laboratory, Mölndals Lasarett, Gothenburg, Sweden for analysis of A $\beta$ <sub>1-42</sub>, and tau protein. The total levels of CSF tau protein (T-tau) (Andreasen, *et al.* 1999b) was determined using a sandwich ELISA constructed to detect both normal and hyperphosphorylated tau (P-tau) (Blennow, *et al.* 1995). CSF P-tau levels were determined using a sandwich ELISA, constructed to specifically detect tau phosphorylated at Thr181 (Vanmechelen, *et al.* 2000). The levels of A $\beta$ <sub>1-42</sub> were analyzed in CSF samples using a sandwich ELISA, constructed to specifically measure A $\beta$ <sub>1-42</sub> (Andreasen, *et al.* 1999a).

## 9.8 NUTRITIONAL ASSESSMENTS PAPER I-IV

Nutritional assessments were performed by antropometric and biochemical assessments at baseline and after 6 and 12 months in all patients that included measurements of weight, length and Body Mass Index (BMI (kg/m<sup>2</sup>), triceps skinfold (TSF mm) measured by Harpenden skinfold calliper and arm muscle circumference (AMC cm). To estimate body fat and subcutaneous fat mass measurements of skin fold thickness the triceps muscle is used, triceps skinfold

(TSF). This is measured on the non-dominant arm between the acromion and the olecranon. From the recordings of TSF and mid-upper arm muscle circumference, the AMC can be calculated according to the formula  $AMC (cm) = \text{arm circumference (cm)} - \pi \times TSF (cm)$ . AMC delineates muscle mass (Jelliffe 1966).

## **9.9 BLOOD PRESSURE PAPER I-IV**

Systolic and diastolic blood pressure after 5 minutes rest was assessed at baseline after 6 and 12 months.

## **9.10 DATA ANALYSIS**

All statistical analysis was performed in the Statistica<sup>®</sup> 7.0 software package (Stat soft Inc 2004 , Tulsa, OK, USA) or SAS, version 9.1.3 (SAS Institute, Cary, NC, USA for Paper III).

### **9.10.1 Statistical analyses I-IV**

With a statistical significance level of 0.05 and 80 % power, 200 patients were needed to detect a difference of more than 2.5 points between the patients treated with  $\omega$ 3 FA and placebo using the ADAS-Cog scale. The primary outcome in the OmegAD study was cognition. The study was thus not powered specifically for outcomes of neuropsychiatric symptoms, inflammatory markers or weight. In all papers both intention to treat (ITT) and per protocol analyses were performed. In the ITT analyses the last observation was carried forward to subsequent registration. Since there was no difference in outcomes between the two methods we chose to present the data using the per protocol mode.

From Paper I longitudinal changes in MMSE and ADAS-Cog scales were assessed using repeated measures ANOVA. Fishers LSD-test was used for post-hoc analyses. For variations in cognitive decline rates between the two groups at 6 and 12 months Student t-tests were performed. Data were given as means and 95 % confidence intervals (CI). Longitudinal changes in blood pressure and measurements of the FA were analyzed using repeated measures ANOVA.

Paper II: Longitudinal changes in NPI, MADRS, Caregiver's burden and DAD scales were assessed using repeated measures ANOVA. Inter-group analyses of skewed variables were performed using the Mann-Whitney U-test. Data were given as means and 95% CI. When analysing changes over time (0-6 months),

treatment effect was controlled for gender and *APOE* frequency using general regression models (GRM).

Data from Paper III are presented as means and 95% CI. Between and within-group analyses of skewed variables were performed by the Mann-Whitney U-test. Due to missing data at either baseline or after 6 months, the effects of  $\omega$ -3 FA and time on IL-6, sIL-1RII, TNF- $\alpha$ , P-tau, T-tau and A $\beta$ <sub>1-42</sub> were analysed using mixed linear effect models. In all models, gender, age, *APOE*, MADRS, NPI, ADAS-cog, treatment group, time and the interaction treatment by time were controlled for. A backward selection was performed to evaluate these effects with an exclusion criterion of  $p > 0.05$ . Cook's distance was used to reveal divergent observations in the data material. One such observation was found in the analysis of IL-6, P-tau, T-tau and A $\beta$ <sub>1-42</sub> in one patient and was excluded from the analyses.

Paper IV: As cognition was the primary overall outcome of the study no specific power analysis with weight as outcome measure was performed. Data are presented as mean  $\pm$  SD, 95% CI or median (25<sup>th</sup>–75<sup>th</sup> percentile). To analyse the variations between two groups the Student's t-test, the Mann Whitney U-test and Chi-2 test were used in accordance with the type and distribution of the variables. Possible changes at 6- and 12-months follow-up were evaluated by Student's paired t-test or Wilcoxon's Matched Pair Test. To analyse longitudinal changes within and between the two groups ANOVA repeated measures was used. Fisher least significant difference (LSD) test was used for post-hoc analyses. For correlation analyses, Pearson's and Spearman's correlation coefficients were calculated depending on the type and the distribution of the variables. Logistic regression was performed to evaluate the independent relation between different relevant variables and weight gain. For the statistical analyses *APOE4* was dichotomized into carriers and non-carriers of the *APOE* $\epsilon$ 4 allele and appetite was trichotomized into increased, stable and decreased appetite.

## 10 RESULTS AND DISCUSSION PAPER I-IV

### 10.1 PAPER I

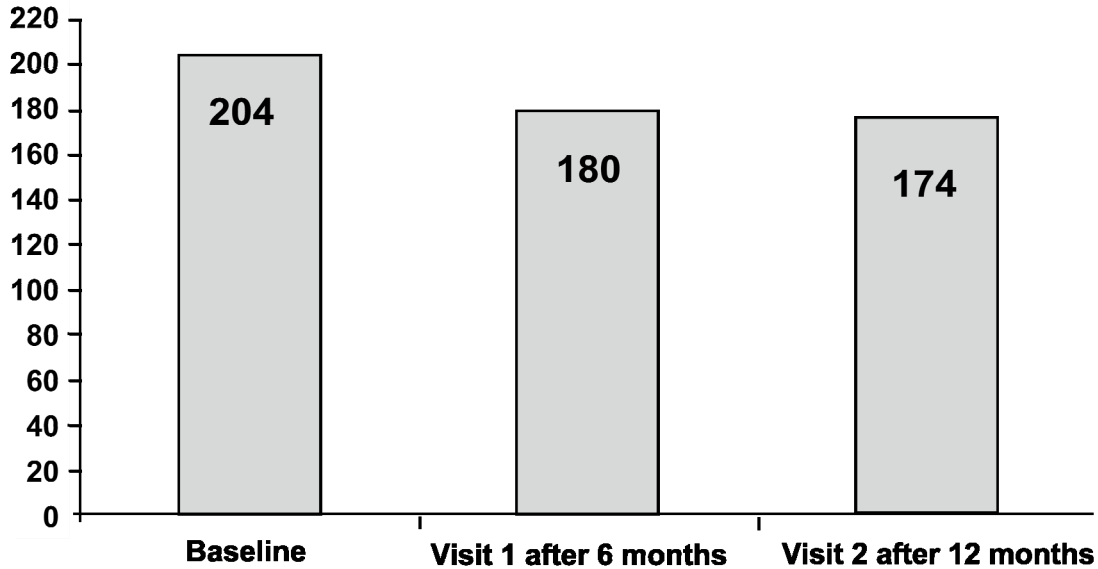
#### *Effects of n-3 FA supplementation on cognitive symptoms, safety and tolerability and compliance in mild to moderate AD.*

The aim of the first study was to determine the effects of supplementation of 2.3 g n-3 FA on cognitive functions to patients with mild to moderate AD. We also studied compliance by measuring levels of the FAs EPA, DHA and placebo oil. 204 patients were included at baseline (110 women and 94 men, mean age  $74 \pm 4$  years). 103 patients were randomized to treatment with n-3 FA and 101 patients to placebo oil for 6 months. Then all received n-3 FA for 6 more months.

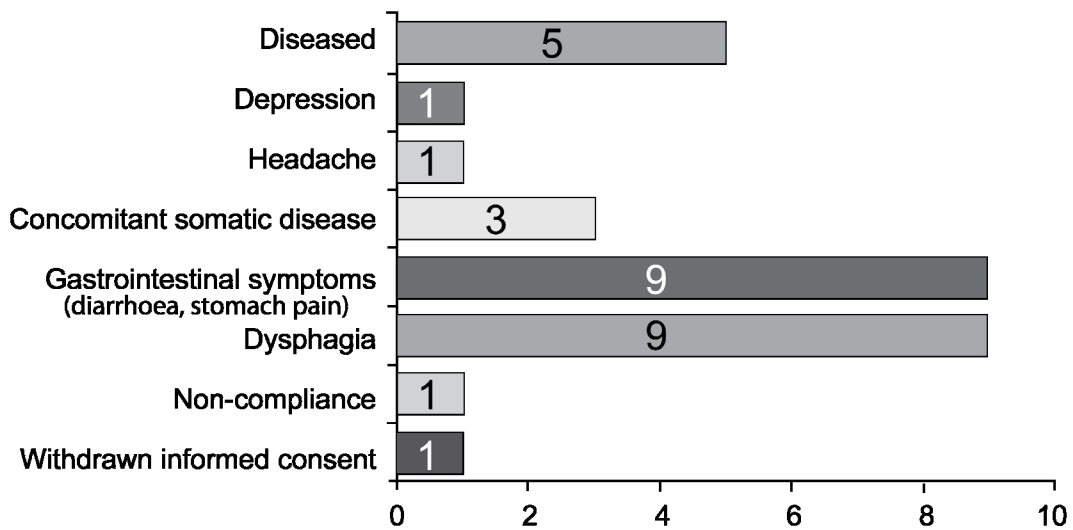
The primary efficacy variables were cognitive functions assessed by using MMSE and the cognitive portion of ADAS-Cog. Secondary outcomes were safety and tolerability, blood pressure, and global function as assessed by the CDR, global (0-3) and summary of boxes. Compliance was checked with assessment of EPA, DHA and placebo oil (LA). All measurements were performed at baseline and at 6 and 12 months. At all the visits patients and care givers were asked about possible adverse reactions (AE) related to the received treatment.

There were no differences in demographic variables at baseline; mean values of MMSE; ADAS-Cog and CDR values in the two randomised groups were similar. This was also shown relating to the *APOE* $\epsilon$ 4 group frequency in non-carriers in the n-3 FA group (21 %) and in the placebo group (28 %) and also in the *APOE* $\epsilon$ 4 carrier group (68 % versus 57 %). All patients were treated on stable doses of the three different AChEI available on the market (donepezil, galantamine and rivastigmine). There was a slight increase of females included in the n-3 FA group (51 %) compared to the placebo group (39 %). Out of the whole patient group a few patients were smokers (7 % in n-3 FA versus 6 % in the placebo group). Around 40 % of the patients in the n 3 FA group were medicated with anti-depressants compared to the placebo group, 32% but few were medicated with neuroleptics or herbal medication and statins. Blood pressures were stable during trial in both groups. No differences in global or total CDR-scores between the two groups were seen (Paper I Table 2). There were no significant changes in routine blood and urine tests. Safety and tolerability was good.

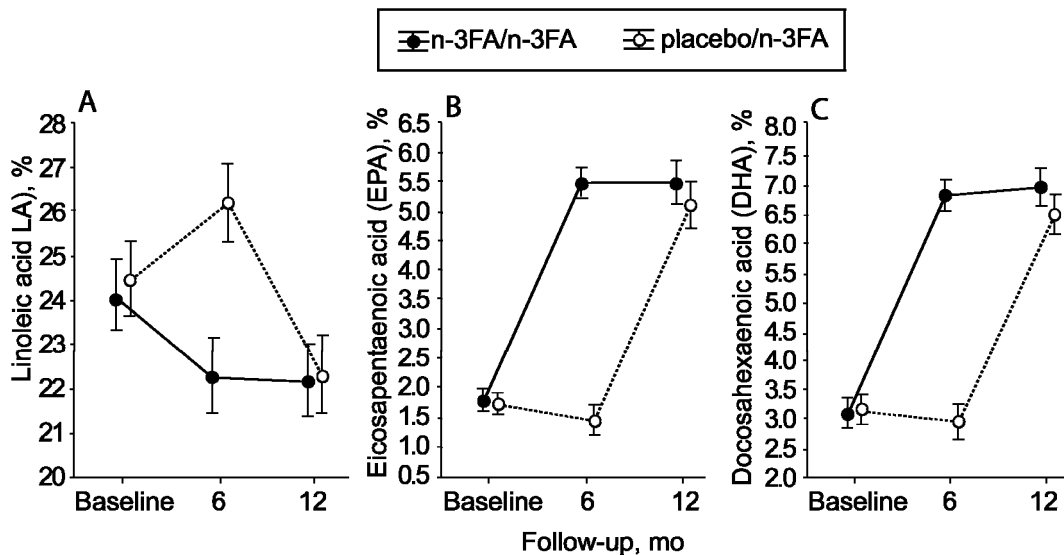
**Table.** 85 % of the 204 included patients completed the Omeg AD study (174 patients out of 204 patients).



Reasons for drop-out are given in the table below. Out of the 30 patients that did not conclude the trial 14 patients were treated with n-3 FA and 16 patients treated with the placebo oil.



**Figure:** Reasons for drop put in the OmegAD study.

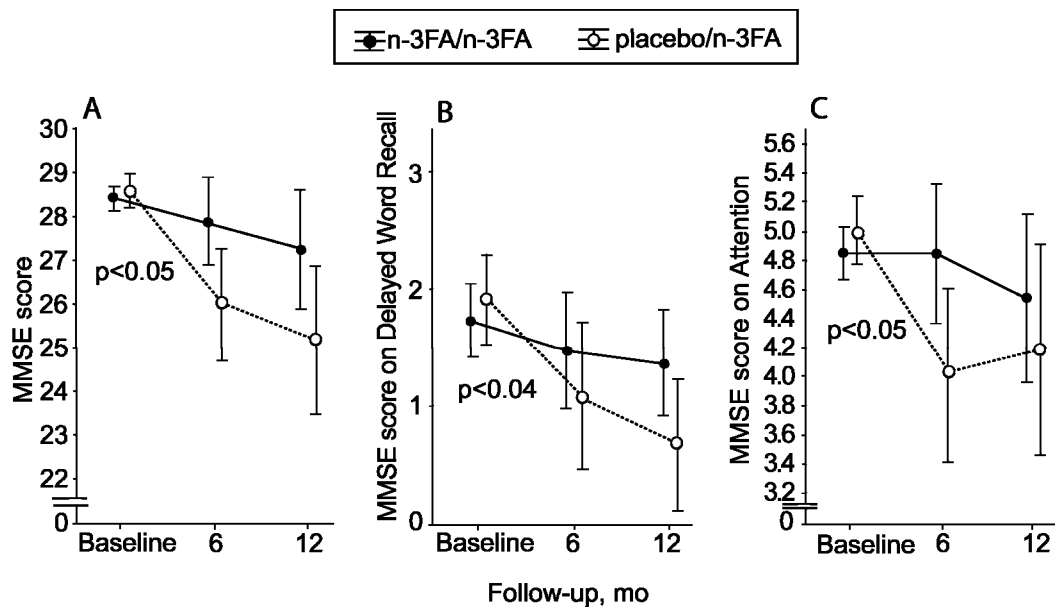


**Figure:** Compliance was measured with levels of A) LA (dotted line) in the placebo group, patients were switched to active treatment of n-3 FA after 6 months whereas levels of LA rapidly decreased. B) Increased levels of EPA (black line) in the active treatment group receiving n-3 FA from baseline compared to the placebo group receiving LA (dotted line) at baseline. After 6 months the placebo group received active treatment with n-3 FA and then increased levels of EPA. C) Increased levels of DHA (black line) in the active treatment group receiving n-3 FA at baseline compared to the placebo group receiving LA (dotted line). After 6 months the placebo group received active treatment with n-3 FA and then increased in levels of DHA.

There was no significant difference over 6 and 12 months between the n-3 FA (figure) and the placebo groups in MMSE or ADAS-cog (see Paper I, Table 2). 174 patients fulfilled the trial. At baseline, MMSE, and ADAS-cog scores in the two randomized groups were similar. At 6 months the decline in cognitive functions did not differ between the groups. However, in a subgroup (n=32) with very mild cognitive dysfunction, i.e. MMSE >27 points, a significant (p<0.05) reduction in MMSE decline rate was observed in the n-3 FA group compared to the placebo group. A similar arrest in decline rate was observed in this placebo subgroup when receiving n-3 FA between 6 and 12 months (fig A, next page).

Moreover, when analyzing each subitem of the MMSE, significant treatment effects were found over time in the subitems “delayed word recall” of the MMSE

( $p=0.036$ ; (fig B) and “attention” ( $p=0.047$ ; (fig C), where the placebo group showed a significant reduction between 0 and 6 months for both items ( $p=0.003$  and  $0.002$ , respectively) and stabilized between 6 and 12 months, whereas the n-3 FA group showed no significant decline at all. There was a significant difference in decline rate for “attention” in the ADAS-Cog scale too in favour of the n-3 FA treated group.



**Figure:** A) A reduction in MMSE decline rate was observed in a subgroup of patients (MMSE >27) with very mild cognitive dysfunction receiving n-3 FA compared to the placebo group. B) Significant treatment effects were found over time in the subitems “delayed word recall” of the MMSE and for C) “attention”.

Notwithstanding the negative results for the whole group of patients, our study indicated that the n-3 FA preparation conferred a slower decline of cognition in those with the mildest impairment (MMSE >27) compared with placebo treated controls with similar degree of cognitive dysfunction at start. This was also observed in the second part of the trial, when all patients were given the n-3FA preparation, since the decline rate of the previously placebo treated patients was reduced to become similar to those given the n-3 FA preparation during the whole trial. Although, these findings, found in post-hoc analyses, were based on a small number of patients with very mild AD (n=32) and need to be confirmed in large



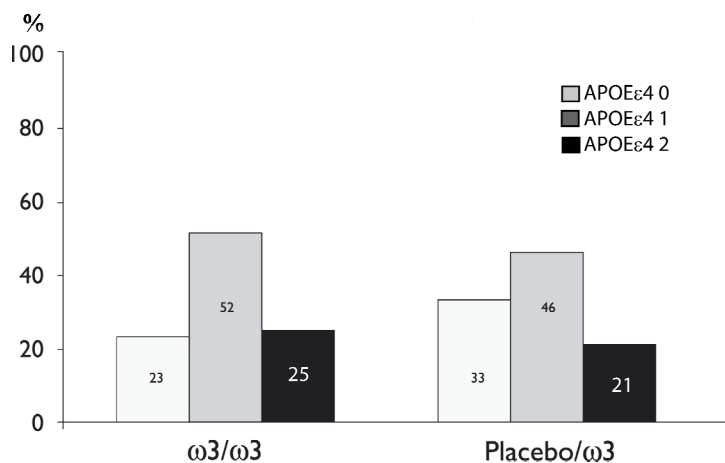
patient cohorts. It is however important to emphasize the similarities found in our post-hoc analyses of the very mild AD patients. Both in the MMSE and ADAS-cog, the improvement was found in the memory component reflecting a key symptom in AD, the episodic memory.

Our findings cannot serve as a basis for general recommendations for treatment of AD with dietary DHA rich fish oil preparation. However, studies on larger cohorts with mild cognitive impairment, including those at risk for developing AD, are needed to explore the possibility that n-3 FA treatment might be beneficial to halt cognitive decline in manifest AD and perhaps postpone cognitive decline.

## 10.2 PAPER II

### *Effects of n-3 FA supplementation on neuropsychiatric symptoms, caregiver's burden and ADL function in relation to APOE $\epsilon$ 4.*

To determine effects of dietary n-3 FA supplementation to AD patients with mild to moderate disease on psychiatric and behavioral symptoms, daily functions and a possible relation to carriership of *APOE $\epsilon$ 4*. Neuropsychiatric symptoms were assessed using NPI and MADRS. Caregiver's burden was evaluated using three items from Caregivers burden scale (CGB): 1) emotional overload; 2) economic overload and 3) if the caregiver felt himself captured in a role. ADL was assessed using DAD. When calculating total scores on the NPI at baseline in both the n-3 FA group and placebo group low total scores were found (15.6 in the n-3 FA group vs. 14.9 in the placebo group). These patients did not show a high load of neuropsychiatric symptoms, reflecting the mild to moderate stage of the disease. Less than 10 % of the patients showed psychotic features such as delusions and hallucinations. Symptoms of apathy and depression were more common. (see Table 2, paper II). 45 % of the patients in the n-3 FA group were medicated with antidepressants compared to the placebo group 38 % ( $p=0.36$ ) and 10 % in the n-3 FA group and 6 % in the placebo group ( $p=0.31$ ) medicated with neuroleptics. 72 % of the included 174 patients were *APOE $\epsilon$ 4* carriers (figure). No significant overall treatment effects on neuropsychiatric symptoms, on ADL or CGB were found. However, significant positive treatment effects on the scores on agitation in the NPI domain in *APOE $\epsilon$ 4* carriers ( $p<0.006$ ) and in MADRS scores in non-*APOE $\epsilon$ 4* carriers ( $p<0.005$ ) were found.



**Figure.** Percentages (%) of patients with *APOE $\epsilon$ 4* alleles in the OmegaAD study in the n-3 FA group (=  $\omega 3/\omega 3$ ) and in the placebo group (= placebo/ $\omega 3$ ). 0= No *APOE $\epsilon$ 4* alleles, 1= one *APOE $\epsilon$ 4* alleles, 2= two *APOE $\epsilon$ 4* alleles.

**Table:** Scores from the rating scales NPI, MADRS and CGB (three items) and ADL functions, DAD at baseline and after 6 and 12 months.

	<b>Baseline</b>	<b>6 months</b>	<b>12 months</b>	<b>0-6</b>	<b>6-12</b>
	<b>Mean</b>	<b>Mean</b>	<b>Mean</b>	<b>months</b>	<b>months</b>
	<b>(95 % CI)</b>	<b>(95 % CI)</b>	<b>(95 % CI)</b>	<b>p-value, n-3/n-3 vs. placebo/n-3</b>	
<b>NPI (0-144 p)</b>					
n-3/n-3	15.6(12.9-18.2)	16.6(13.9-19.2)	16.1(12.9-19.2)	0.45	0.35
placebo/n-3	14.9(12.1-17.7)	16.0 (12.9-19.2)	13.5(10.4-15.6)		
<b>MADRS (0-30p)</b>					
n-3/n-3	1.8(1.3-2.3)	1.5(1.0-1.9)	1.2(0.8-1.6)	0.49	0.33
placebo/n-3	1.9(1.5-2.4)	1.6(1.2-2.1)	1.1(0.7-1.5)		
<b>CGB;</b>					
<b>Emotional overload</b>					
n-3/n-3	8.6(6.3-10.9)	8.2(7.6-8.8)	8.8(8.1-9.5)	0.11	0.10
placebo/n-3	8.2(7.5-8.8)	8.2(7.5-8.9)	8.3(7.7-8.9)		
<b>Economic overload</b>					
n-3/n-3	7.5(7.3-7.8)	7.3(7.0-7.6)	7.4(7.1-7.7)	0.72	0.025
placebo/n-3	7.9(7.5-8.2)	7.5(7.3-7.8)	7.0(7.0-7.5)		
<b>Captured in a role</b>					
n-3/n-3	5.3(4.8-5.7)	5.9(5.3-6.4)	6.0(5.4-6.6)	0.74	0.74
placebo/n-3	6.0(5.4-6.5)	6.4(5.8-6.9)	6.4(5.9-7.0)		
<b>DAD (0-46 p)</b>					
n-3/n-3	33.5(31.6-35.3)	31.8(29.7-33.9)	28.3(25.7-30.8)	0.34	0.85
placebo/n-3	33.1(31.0-35.2)	30.5(28.3-32.8)	26.9(24.3-29.5)		

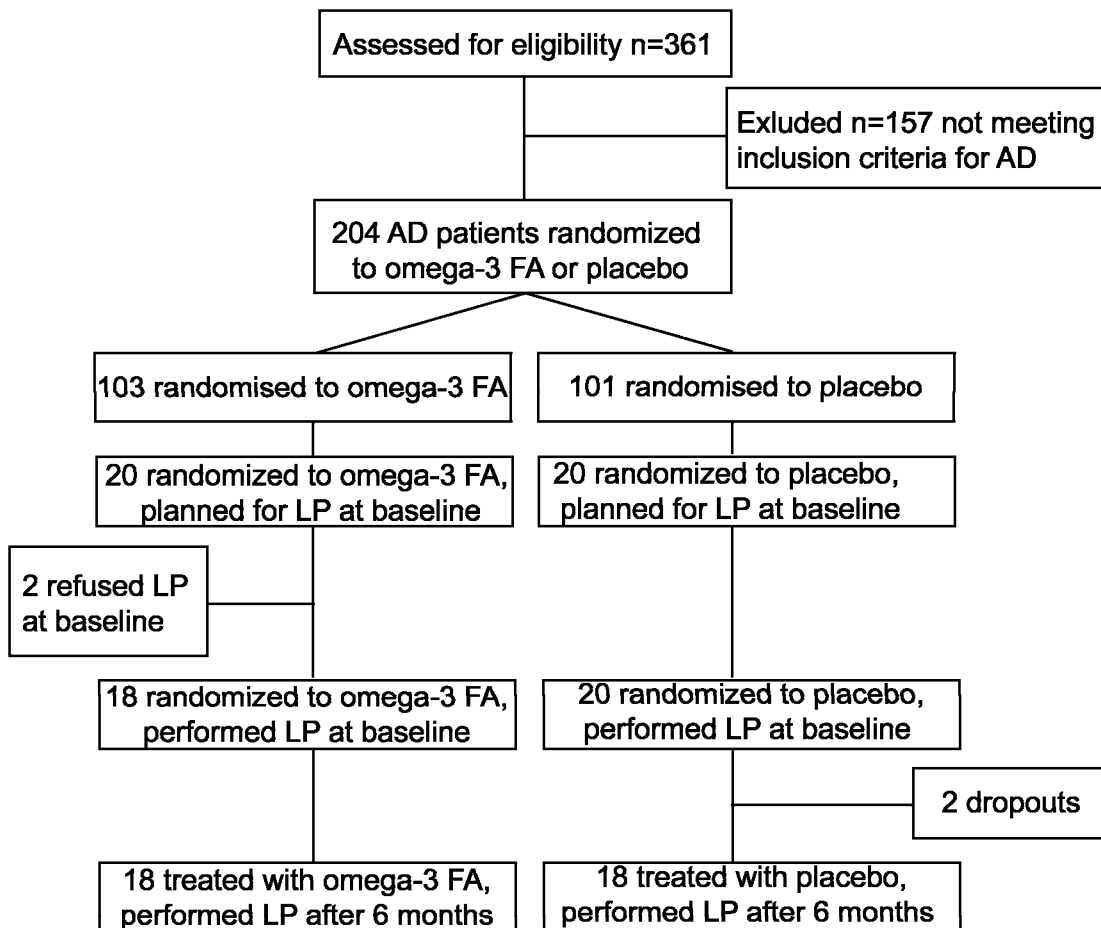
In conclusion supplementation with n-3 FA in patients with mild to moderate AD did not result in marked effects on overall neuropsychiatric symptoms except for possible positive effects on depressive symptoms (assessed by MADRS) on non-carrier of *APOEε4* and agitation symptoms (assessed by NPI) in *APOEε4* carriers. Nor did it seem to influence care givers burden or functional ability. In order to explore n-3 FA treatment effects in neuropsychiatric domains in AD future studies should include patients with more prominent symptoms. However, this will be a challenge as these patients then might be more cognitively impaired and thus more difficult to include and evaluate in clinical trials. The n-3 FA doses for treatment of neuropsychiatric symptoms might have been too low. Also all patients were treated with a stable dose of AChEI that may have reduced the

symptoms and as the patients all were in a mild to moderate stage the neuropsychiatric symptoms were not that prominent. There is mounting evidence that dietary n-3 FA supplementation may be beneficial in various psychiatric conditions as have been described previously. The majority of the small RCTs that have been performed in depression and BPD in non-demented patients seem to suggest more positive effects when combining both DHA and EPA. Based on this study recommendation on supplementation of n-3 FA for treatment of neuropsychiatric symptoms in mild to moderate AD cannot be advised. However, addition of n-3 FA in adjunctive treatment of depressive symptoms can be tried.

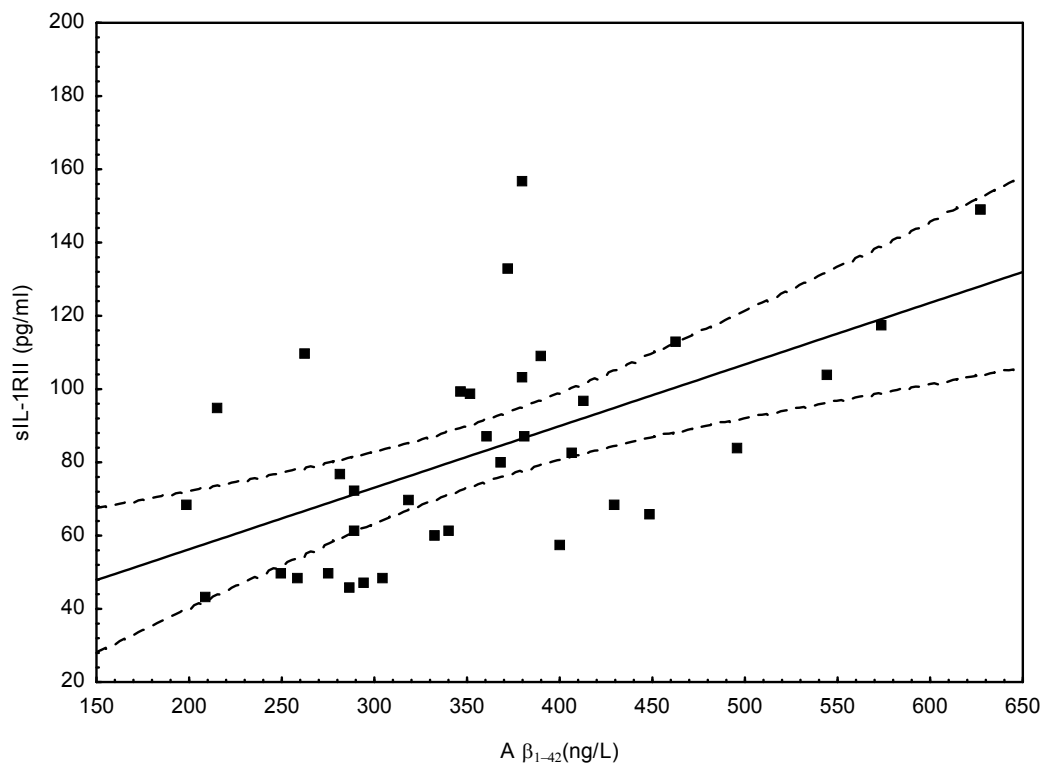
### 10.3 PAPER III

#### *Effects of n-3 FA supplementation on inflammatory markers and biomarkers in CSF and on inflammatory markers in plasma.*

Data from Paper III originate from a subgroup of the patients participating in the OmegAD study. The first consecutively 40 recruited patients with a diagnosis of mild to moderate AD were randomized to a daily intake of 2.3 g n-3 FA or placebo for 6 months. From this group 4 patients dropped out, one patient was excluded from the analyses and then 35 patients ( $70 \text{ years} \pm 8.2$ ) underwent LP at baseline and after 6 months.



CSF and plasma at baseline and at 6 months were obtained and the inflammatory markers IL-6, TNF- $\alpha$  and sIL-1RII analyzed. The AD markers T-tau, P-tau, and A $\beta_{1-42}$ ) were assessed in CSF. Hc-CRP was assessed in plasma. A possible relation to the *APOE $\epsilon$ 4* genotype was investigated. Treatment effects with n-3 FA were not found in inflammatory or in AD biomarkers in CSF. There were no treatment effects on inflammatory markers in plasma detected, nor was there any relation to *APOE*. However, a significant correlation was observed at baseline between sIL-1RII and A $\beta_{1-42}$  levels in CSF which might reflect the reciprocal interactions with IL-1 and A $\beta$  peptides.



In summary this is the first RCT that has studied these n-3 FA effects in patients with mild to moderate AD. Analyses of the inflammatory markers and dementia biomarkers in relation to the *APOE $\epsilon$ 4* could not detect any association but the treatment and placebo groups as well as the number of non-*APOE $\epsilon$ 4* carriers may have been too small to detect correlations. The OmegAD study was powered to detect changes in cognition and not on of treatment effects on inflammatory or biomarkers in CSF or plasma. However, animal studies as well as studies on cell cultures with both NSAID and n-3 FA have shown a reduction of both total A $\beta$  and A $\beta_{1-42}$ .

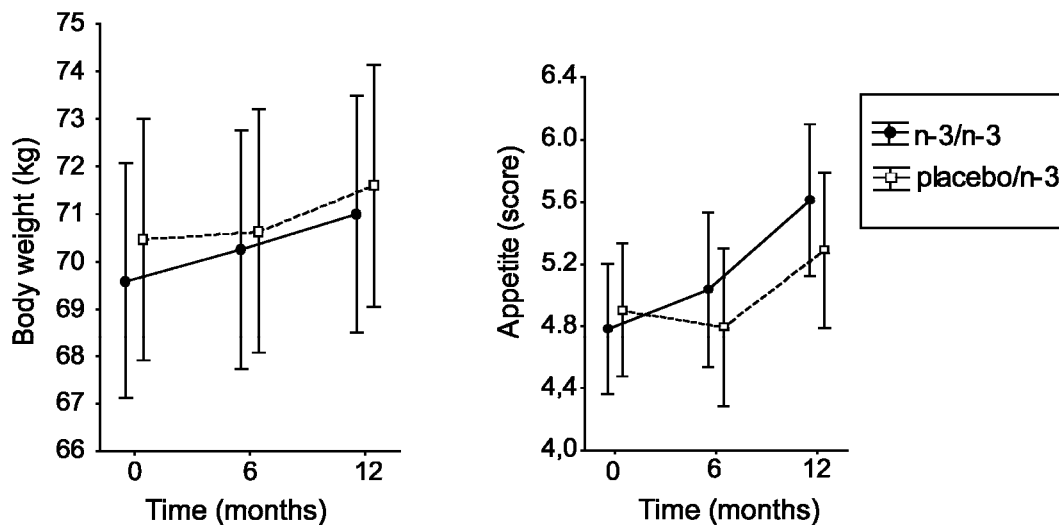
Correlation analysis between inflammatory markers and the AD biomarkers showed a positive correlation at baseline between sIL-RII and  $A\beta_{1-42}$  in the CSF. Since sIL-RII binds to IL-1 $\beta$  and acts as a "sink" for IL-1 $\beta$ , the increase in sIL-RII shedding might be an attempt to limit negative effects of the IL-1 $\beta$  expression of in the brain.

Future research with RCT is needed with different doses of DHA in patients with MCI and AD including analyses of inflammatory markers in CSF and plasma.

## 10.4 PAPER IV

### *N-3 FA supplementation, effects on weight and appetite in relation to APOEε4.*

At 6 and 12 months follow-up the weight had increased by  $0.7 \pm 2.5$  kg ( $P=0.015$ ) and  $1.4 \pm 2.9$  kg ( $P<0.0001$ ) in the n-3 FA group. In the placebo-group the weight was unchanged at 6 months, but increased ( $P=0.011$ ) at 12 months follow-up after n-3 supplementation was initiated. Appetite improved in the n-3 FA over the treatment period ( $P=0.012$ ).



In logistic regression analyses, not carrying *APOEε4* and elevation of plasma DHA concentrations were independently related to weight gain in the combined group of patients at 6 months follow up.

In conclusion in patients with mild to moderate AD receiving supplementation weight was increased. However, no firm conclusion can be drawn from this data since there was no significant differences between the patients treated with active n-3 FA treatment compared to the placebo group. However, supportive of the assumption that supplementation of n-3 FA may improve appetite and weight in non-*APOEε4* carriers was the positive association between elevations in plasma DHA and weight gain. Limitations of this study is that power calculations was not performed with weight as a primary outcome variable and also that the study lacks information of food intake data. Weight loss is already a fact before AD is evident. In the future research must focus on long term RCT with different doses of n-3 FA in patients with MCI and risk factors for AD and careful assessment of food frequency questionnaires and nutritional parameters.



## 11 CONCLUSIONS

Understanding how the brain functions in healthy and aging brains in order to understand how the potentially sick brain develops in neurodegenerative disorders is one of man's greatest challenges. This has implications for improving health in patients with devastating disease such as AD. AD affects the intellectual and emotional "core" of the human brain and affects a whole society including care givers in a way that is very often kept in silence. The individual path of each and every patient is clouded by a progressive memory decline and followed by a network of cognitive and neuropsychiatric symptoms that ultimately leads to a premature death where the diagnostic set up still is within the frame of the diagnose put by Alois Alzheimer himself in 1906. There is still no cure for AD albeit we have access to only two classes of drugs that produce modest symptomatic improvement initially in the course of the disease. It is therefore of utter importance to find other pharmacological strategies that might postpone the prevalence of AD and also slow down the cognitive and neuropsychiatric decline in established AD.

The findings of the work presented in this thesis have provided unique new insights into how the brain and body in patients with mild to moderate AD from a clinical setting react to supplementation with n-3 FA. Thus the findings presented in this placebo-controlled randomised double-blind trial with supplementation of 2.3 g/day n-3 FA (1.7 g/day DHA+0.6 g/day EPA) for 6 months is to our knowledge the first to be published on the effects of n-3 FA supplementation mainly with DHA for AD. 204 patients were included and 174 patients fulfilled the one year long trial with measurements analyzed at baseline and after 6 and 12 months in both groups. All patients were diagnosed according to the DSM-IV criteria and were treated with stable doses of AChEI. Patients included in the placebo group (receiving LA) at baseline were switched to the active treatment with n-3 FA at 6 months for an additional 6 months compared to the group receiving active n-3 FA with the same dose of 2.3 g/day n-3 FA for 12 months.

The results and the general conclusion of his study can be summarized as follows:

- There seems to be an effect on delayed rate of cognitive decline including effects on episodic memory, delayed word recall and attention in a subgroup of very mild AD (MMSE >27) in patients treated with 6 months of n-3 FA.
- The dosage of n-3 FA treatment seems to be well tolerated and safe which also accounts for a low drop-out rate, 15 % in the whole group.
- No significant changes in routine blood and urine tests were seen and systolic and diastolic blood pressure remained unaltered.
- When analyzing global functions with CDR no difference was seen in ratings of global or total CDR.
- A high level of compliance with plasma levels of n-3 FA as well as placebo has been confirmed during the whole trial by measuring levels of LA (placebo group) and DHA and EPA at baseline. At 6 months in the LA there was a shift to active treatment showing sharp elevations of both DHA and EPA which then remained stable during the additional 6 months.
- Furthermore, when analysing neuropsychiatric symptoms (controlling for age, *APOEε4* genotype and gender) a positive n-3 FA treatment effect was demonstrated on agitation symptoms (NPI) in patients being *APOEε4*-carriers and on depressive symptoms (MADRS) in non-*APOEε4*-carriers.
- No significant differences between the groups on ADL nor on the caregivers burden were found.
- We further have demonstrated that the effects of n-3 FA predominantly with the DHA-enriched n-3 FA supplement may positively affect weight and appetite in patients with mild to moderate AD. Mean weight and BMI showed an increase after 6 and 12 months in the n-3 FA group (non-significant between groups) and in the placebo group weight and BMI was increased after 12 months.
- Not carrying the *APOEε4* allele and increased DHA were independently associated with weight gain.

- Caregiver's assessed appetite improved in the group receiving active n-3 FA treatment at the end of the study implying that increased appetite might have a positive outcome on weight gain after 1 years of treatment.
- We have also data from 35 patients out of the 174 included patients from CSF showing that there is a correlation at baseline between A $\beta$ 42 and sIL-RII
- We can also establish that no detectable n 3-FA treatment effects were perceived in inflammatory markers in plasma (hs-CRP, IL-6, TNF- $\alpha$  and sIL-RII) or CSF (IL-6, TNF- $\alpha$  and sIL-RII) nor on any biological AD markers, (A $\beta$ 42, T-tau and P-tau) after 6 months treatment in 35 patients.

## 12 FUTURE PERSPECTIVES

The results from this thesis have raised additional new questions and research perspectives as well as opened possibilities for new projects. Future research perspectives will be discussed in this section of my thesis with suggestions for new clinical research within the field of neuroscience and medicine. Considering the fact that there are still several unknown mechanisms underlying the basic understanding of how the progressive neurodegenerative disease AD is developed, there are many research questions unanswered.

A body of genetic and biochemical evidence, inflammatory aspects as well as the pivotal role of soluble amyloid  $\beta$  peptide ( $A\beta$ ) may be the proximate cause of synaptic injuries and neuronal death leading to cognitive and neuropsychiatric decline in turn leading to suffering in both the patient and the caregiver as well as society in the end. We need the complex cooperation within the worldwide network of research society and pharmaceutical industry and their curiosity together with the individual patient and caretaker to try to solve this multifaceted mystery that is AD and leads to premature death. This can only be accomplished by cross faculty expertise in various scientific disciplines and areas of expertise with an advanced level of technical and clinical skills.

In addition, in the field of nutritional approach of prevention and delay in cognitive and neuropsychiatric decline in AD the importance of understanding how nutrients affect the normal and aging brain cannot be stressed enough. Furthermore, more research is needed to elaborate on how the metabolism of DHA in the brain works, what the molecular bases of the neuroprotective effects of DHA including gene expression are, the effects on inflammatory processes and oxidative stress, enrichment and physicochemical properties of neuronal membranes and signalling pathways. Further studies are necessary to identify the preferential mechanisms with the view to optimize and provide a scientific rationale and insight.

## 13 RECOMMENDATIONS FOR FUTURE CLINICAL RESEARCH

The following recommendations for future clinical research can be drawn from the results of the OmegAD study:

1. One extremely important issue is if the n-3 FAs enter the brain and if so to what extent is this possible and what are the underlying mechanisms for this passage? Analyses of FAs in CSF from the OmegAD study are ongoing and thus the question if n-3 FAs enter the brain will soon be answered.

2. How do different levels of the various n-3 FA treatments affect cognition and neuropsychiatric symptoms depending on gender and *APOEε4* carrier ship. Based on the results described in this thesis we conclude that in a subgroup of AD patients there was an effect on delayed word recall and attention. Our data has shown that future clinical studies using n-3 FA should focus on more selected population of AD patients, namely those with prodromal AD who has a high risk of developing AD within a few years. Treatment duration if possible should be longer than in our study (longer than six months) in order to draw more scientific information about important parameters on cognition and neuropsychiatric symptoms. This can only be done by performing multicenter trials with higher number of patients with risk factors for AD and also *APOEε4* carrier ship. Our data also suggest that *APOEε4* carrier ship influence the prevalence of certain neuropsychiatric symptoms and weight. Since *APOE4* genotype is a major genetic risk factor for AD it would also be of great interest to perform clinical trials in this patient group

There are also many indications that cardiovascular diseases play an important role in the etiology of AD. Epidemiological studies on midlife hypercholesterolemia and high blood pressure have been shown to increase the risk for developing AD later in life. Patients with prodromal vascular dementia (VaD) were excluded from our OmegAD study. Patients with cardiovascular risk factors and patients with prodromal VaD would also be interesting to study.

Therefore, long-term studies in these patients groups would lay a base for prevention of a reduced risk in the area of reducing both vascular disorders as well

as AD. Techniques that would add new knowledge in this field would be using Magnetic Resonance Imaging (MRI) in order to measure atrophy of the hippocampus and temporal lobes as well as incidence and prevalence of silent brain infarcts and stroke.

3. As a consequence of our results in paper II focusing on neuropsychiatric symptoms it would be of even more interest to measure levels of neurotransmitters in CSF such as acetylcholine, serotonin, dopamine and noradrenalin in order to see how they would correlate with the positive and negative treatment effects of DHA supplementation on various neuropsychiatric symptoms in AD. To study n-3 FA supplementation in the future it would mean to include a study population with more severe neuropsychiatric symptoms compared to the study population included in the OmegAD study. However, our findings showed possible effects in mild AD so this would create a problem in inclusion as patients with more severe neuropsychiatric symptoms would have more severe cognitive decline and ADL dysfunction thus representing another group of patients than those with very mild cognitive dysfunction.

4. In future studies of n-3 FA the food intake must be monitored closely by including food frequency questionnaires. This would enhance the knowledge of how intake of saturated and unsaturated fats and flavonoids from consumption of vegetables and fruit affect the AD risk. In the OmegAD study we have analyzed plasma levels of DHA, EPA and LA and shown that compliance was good. Food frequency questionnaires have been given to a subset of patients in the OmegAD and care givers and are now being analyzed, but information of food intake for all patients in the study would have been even better.

5. In our conclusion from Paper III on treatment with n-3 FA on biological AD markers ( $A\beta_{42}$ , T-tau and P-tau) in CSF and inflammatory cytokines from both plasma and CSF we could not confirm data from animal studies and cell cultures showing a decrease of  $A\beta_{42}$  after treatment with antiinflammatory treatment albeit we did not see any difference between the treatment groups. This could be due to a limited number of patient samples and/or that six months of treatment was not long enough to detect any difference. Also, data on animals are often not to be directly translated to similar effects in man.

6. Another interesting point is the question of dose. Maybe the dose of DHA or the proportion of DHA/EPA is not sufficient to affect the inflammatory parameters and thus did not lead to an overall effect on cognition (paper I) or neuropsychiatric symptoms (paper II) and in inflammation (paper III). Thus, a larger dose of DHA/EPA in future studies must be taken into consideration.

7. Data from our Paper IV on weight and appetite implies interesting data on levels of DHA and appetite. In the treatment group receiving one year of active treatment a substantial increase of weight occurred, implying that weight loss (that even might precede AD) is of a complex nature and will take long time to regulate. This adds substantial new information on the presumed anti-inflammatory and other still unknown effects of supplementation with n-3 FA.

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