# THE EFFECTS OF DIETARY DOCOSAHEXAENOIC ACID SUPPLEMENTATION ON PATHOLOGY AND COGNITION IN A MOUSE MODEL OF ALZHEIMER'S DISEASE


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# Abstract

Alzheimer's disease (AD) is the most common form of dementia in the elderly. Despite over 100 years of research, there is no cure for the disease. Thus, ways of preventing its onset and/or slowing its progression are of particular interest. Evidence from epidemiological and animal studies has suggested that dietary docosahexaenoic acid (DHA) may reduce the incidence of AD and, more specifically, attenuate β-amyloid (Aβ) pathology and improve cognitive symptoms associated with the disease. However, the efficacy of such an intervention remains controversial. Some clinical trials and animal studies have shown limited or no effect of DHA supplementation on behaviour or pathology. Therefore, further research is required to test the hypothesis that dietary DHA supplementation improves cognition and alleviates Aβ pathology.

The strategy adopted in this thesis was to evaluate dietary DHA supplementation on cognition and pathology in a mouse model of β-amyloid pathology. Tg2576 transgenic mice (Tg), which overexpress the human APPswe mutation, and wild type littermates were fed a diet containing approximately 1.8% DHA or a control diet from the age of 4 months. The mice were tested at different times (8, 12 and 16 months of age) using two different spatial memory tasks. Lipid analyses were carried out on plasma and specific brain regions and the distribution of Aβ was analysed using immunohistochemisty and enzyme-linked immunosorbent assay.

The results showed that the levels of DHA were increased in plasma and in cortex, hippocampus and cerebellum of DHA-fed mice. In addition, the brain lipid analysis showed that phosphatidylethanolamine (PE), a major phospholipid in brain, was one of the main DHA-containing phospholipids and was the phospholipid that was most clearly affected by dietary DHA and Aβ pathology. However, long-term DHA supplementation had only a mild positive effect on learning and memory in the Tg mice. There was no statistically significant effect of DHA supplementation on the accumulation of soluble and insoluble Aβ1-40 and Aβ1-42 in the cortex and the hippocampus of Tg mice. These findings suggest that DHA may improve cognitive functions in Tg2576 mice, perhaps by reducing the inflammatory and oxidative effects caused by Aβ, rather than reducing the accumulation of the Aβ peptide *per se* and that PE may have a key role in this process.

# Résumé

La maladie d'Alzheimer est la première cause de démence chez les personnes âgées. Malgré plus de cent ans de recherche, il n'existe actuellement aucun traitement efficace contre cette maladie. Les moyens permettant de prévenir la maladie et/ou de ralentir sa progression présentent donc un intérêt particulier.

Des études épidémiologiques et animales ont suggéré que la presence d'acide docosahexaénoïque (DHA) dans l'alimentation peut réduire l'incidence de la maladie d'Alzheimer et, en particulier, atténuer la pathologie liée au peptide β-amyloïde (Aβ) et améliorer les troubles cognitifs associés à la maladie. L'efficacité d'une telle intervention reste cependant controversée. Quelques essais cliniques et des études animales ont montré un effet limité voire nul des suppléments alimentaires à base de DHA sur la mémoire ou la pathologie. Par conséquant, de nouvelles recherches sont necessaires pour tester l'hypothèse selon laquelle la supplementation en DHA dans l'alimentation améliore la mémoire et atténue la pathologie liée au peptide Aβ.

Afin d'évaluer l'effect de la supplementation en DHA sur les fonctions cognitives et la pathologie, la stratégie adoptée dans cette thèse a été la suivante : des souris transgéniques (Tg2576) surexprimant le gene humain codant pour la protéine précurseur du peptide Aβ avec la mutation dite « Swedish mutation » (APPswe) et des souris normales ont reçu une alimentation contenant environ 1,8 % de DHA ou un régime témoin ne contenant pas de DHA, à partir de l'âge de 4 mois. Deux méthodes ont été utilisées pour élavaluer la mémoire spatiale des souris à huit, douze et seize mois. Des analyses de lipides ont ensuite été effectuées sur le plasma et des régions spécifiques du cerveau, et la distribution du peptide Aβ a aussi été analysée par deux methodes d'immuno-detection.

Les résultats ont montré que le taux de DHA avait augmenté dans le plasma, le cortex, l'hippocampe et le cervelet des souris qui avaient reçu l'alimentation riche en DHA. De plus, l'analyse des lipides du cerveau de ces souris a montré que la phosphatidyléthanolamine, un des phospholipides les plus abondants dans le cerveau, était un des phospholipides contenat le plus de DHA et était aussi le phospholipide le plus affecté par la présence de DHA dans l'alimentation et par la pathologie liée au peptide Aβ. La consommation de DHA à long terme n'a cependant qu'un effet limité sur la mémoire des souris transgéniques et n'a pas eu d'effet statistiquement significatif sur l'accumulation des peptides Aβ1-40 ou Aβ1-42, solubles ou

insolubles, dans le cortex ou l'hippocampe de ces souris. Ces résultats suggèrent que la supplémentation en DHA peut améliorer les fonctions cognitives des souris Tg2576, possiblement en reduisant l'inflammation et l'oxidation causées par la presence du peptide Aβ, plutôt qu'en réduisant l'accumulation de ce peptide. De plus, la phosphatidyléthanolamine joue peut-être un rôle clé dans ce processus.

# Abbreviations

AA – Arachidonic acid (20:4n-6)

Aβ – β-amyloid

Aβ1-40 – β-amyloid 1-40

Aβ1-42 – β-amyloid 1-42

AD – Alzheimer's disease

AFSSA – Agence Française de Sécurite Sanitaire des Aliments

ALA – α-linolenic acid (18:3n-3)

ANSA – 8-anilino-1-naphthalene sulphonic acid

ANOVA – Analysis of variance

APOE – Apolipoprotein E

APP – Amyloid precursor protein

APPs – Secreted amyloid precursor protein

BACE1 – Beta-site APP-cleaving enzyme 1

BCA – Bicinchonic acid

BHT – butylated hydroxytoluene

bp – base pair

BSA – Bovine serum albumin

BSAT-DPBS – BSA-Tween-20-Dulbecco's phosphate buffered saline

°C – Degree Celsius

CA1 – Cornu ammonis 1

CAA – Cerebral amyloid angiopathy

COT – Committee on Toxicology

COX – Cyclooxygenase(s)

COX-1 – Cyclooxygenase 1

COX-2 – Cyclooxygenase 2

cPLA2 – Cytosolic phospholipase A2

CSF – Cerebrospinal fluid

DAB – 3,3-diaminobenzidine

DHA – Docosahexaenoic acid (22:6n-3)

DNA – Deoxyribonucleic acid

dNTP – Deoxyribonucleotide triphosphate(s)

DPA – docosapentaenoic acid (22:5n-3)

DSM-IV – Diagnostic and statistical manual of mental disorders – revision 4

EDTA – Ethylene-diaminetetra-acetic acid

EGCG – Epigallocatechin-gallate

ELISA – Enzyme-linked immunosorbent assay

EOAD – Early-onset Alzheimer's disease

EPA – Eicosapentaenoic acid (20:5n-3)

ESI-MS-MS – Electrospray ionisation tandem mass spectrometry

FA – Fatty acid(s)

FAME – Fatty acid methyl ester(s)

FCA – Forced choice alternation

GLC – Gas liquid chromatography

HPLC – High performance liquid chromatography

HSV1 – Herpes simplex virus 1

IL-1β – Interleukin-1β

IL-6 – Interleukin-6

ISSFAL – International Society for the Study of Fatty Acids and Lipids

LR11 – Low-density lipoprotein receptor 11

kb – kilo base

kDa – kilo Dalton

LA – Linoleic acid (18:2n-6)

LOAD – Late-onset Alzheimer's disease

LOX – Lipoxygenase(s)

LOX-5 – Lipoxygenase-5

LysoPC – lysophosphatidylcholine

MAPT – Microtubule-associated protein tau

MMSE – Mini-Mental State Examination

MRI – Magnetic resonance imaging

MUFA – Monounsaturated fatty acid(s)

NINCDS-ADRA – National Instituteof Neurological and Communicative Diseases and Sroke, and Alzheimer's Disease and Related Disorders Association

NMDA – N-methyl-D-aspartate

NPD1 – Neuroprotectin D1

NSAID – Non-steroidal anti-inflammatory drug(s)

ORF – Open reading frame

pH – Potential hydrogen

PBS – Phosphate buffered saline

PE – Phosphatidylethanolamine

PET – Positron emission tomography

PC – Phosphatidylcholine

PCR – Polymerase chain reaction

PG – Phosphatidylglycerol

$PGE_2$ – Prostaglandin $E_2$

PGH – Prostaglandin H

PI – Phosphatidylinositol

PiB PET – Pittsburgh compound-B positron emission tomography

$PLA_2$ – Phospholipase $A_2$

PrP – Prion protein

PS – Phosphatidylserine

PS1 – Presenilin 1

PS2 – Presenilin 2

PUFA – Polyunsaturated fatty acid(s)

RNA – Ribonucleic acid

r.p.m. – Rotation per minute

SACN – Scientific Advisory Committee on Nutrition

SDS – Sodium dodecyl sulphate

SEM – Standard error of the mean

SFA – Saturated fatty acid(s)

Sph – Sphingomyelin

TAE – Tris-acetate-EDTA

TAG – Triacylglycerols

TBS – Trizma-base sodium chloride

TE – Tris-EDTA

TES – Tris-EDTA-SDS

Tg – Transgenic

TLC – Thin layer chromatography

TNF-α – Tumor necrosis factor-α

TNS – Tris non-saline

TTR – Transthyretin

TXTBS – Triton X-100 TBS

UK – United Kingdom

USA – United States of America

UV – Ultraviolet

VLDL – Very low density lipoprotein

WT – Wild type

# CHAPTER 1

## General introduction

Alzheimer's disease (AD), which is the most frequent cause of dementia, is a major medical, social and economic concern in our society. Although symptomatic treatments are available to the patients, there is currently no cure for this fatal neurodegenerative disease. In addition, there is growing concern that once AD is clinically diagnosed, it is already too late to initiate effective treatment. Hence, strategies to reduce the risk or to prevent AD are of particular relevance and various nutritional approaches are gaining a lot of interest. One of the most important nutrients for the brain that has been associated with AD is the n-3 polyunsaturated fatty acid (PUFA) known as docosahexaenoic acid (DHA, 22:6n-3) which is a significant component of oily fish. Although many studies have already been carried out in humans, in animal models and *in vitro*, the mechanisms by which DHA may interact with (and potentially alleviate) AD pathogenesis are not fully understood. However, there is some evidence that DHA has a beneficial effect on the β-amyloid pathology, one of the main neuropathological features of AD. Therefore, the main aim of this thesis was to test the hypothesis that DHA may alleviate the Alzheimer's β-amyloid pathology in a mouse model of the pathology.

The first part of this introduction chapter describes Alzheimer's disease, its neuropathology, the risk factors, the current methods of diagnosis, treatments and possible future treatments. The structure of DHA and some of its well known properties are presented in the second part. Finally, the potential connection between AD and DHA is shown through a literature review of *in vivo* studies carried out in humans and animal models.

## 1.1 Alzheimer's disease

### 1.1.1 Alzheimer's discovery

Alzheimer's disease was first described just over a century ago. The disease is named after the German psychiatrist, Aloïs Alzheimer who presented the clinical and neuropathological characteristics of the disease for the first time in 1906. In the following year, Aloïs Alzheimer published his findings, describing the case of a 51 year old patient Mrs. Auguste Deter who developed symptoms such as short-term memory loss, speaking and comprehension difficulties, hallucination and disorientation (Alzheimer, 1907; Stelzmann et al., 1995). A *post-mortem* histological examination of the brain was carried out and revealed an evenly atrophic brain. Alzheimer also described the presence of "thick bundles of fibrils" and "miliary foci" known as fibrillary tangles and β-amyloid plaques, respectively.

### 1.1.2 Prevalence of the disease

Alzheimer's disease is the most common neurodegenerative disease accounting for 50-60% of all cases, currently affecting about 25 million people worldwide (Brookmeyer et al., 2007; Wimo et al., 2007). In 2001, the worldwide prevalence of AD was evaluated at 24 million cases with 4.6 million new cases every year, leading to 42 million cases by 2020 and 81 million by 2040. Although representative data for developing countries are sparse, about 60% of people with AD live in this part of the world where the increase rate of cases was predicted to be three times higher than in the developed countries (Ferri et al., 2005). In 2000, almost half of the AD population lived in Asia (about 4.6 million in China and 1.5 million in Japan), which is more than in Europe and North America taken together. About 4.6 million were afflicted in the European Union and 2.8 million in the United States (Wimo et al., 2003).

The disease appears to be more prevalent in women, with a proportion evaluated at 62% of the worldwide cases occurring in females (Brookmeyer et al., 2007). The incidence of AD also increases with age, affecting about 1% of the population at 60-64 years, 1.5% at 65-69 years, 3% at 70-74 years, 6% at 75-79 years,

13% at 80-84 years, 24% at 85-89 years, 34% at 90-94 years and 45% at 95 years and over (Wimo et al., 2003). With the phenomenon of aging of the population, the disease has dramatic consequences for public health and healthcare with an annual economic cost estimated at £7 billion in the UK and over $100 billion in the USA (Love, 2005b).

### 1.1.3 Clinical symptoms

In its early stage, short-term memory loss is the most characteristic symptom, usually manifesting as minor forgetfulness that becomes more pronounced with illness progression. At this stage, patients also experience minor confusion in completing everyday tasks, have difficulties making new memories, making decisions and also show less motivation and have trouble finding words. As the disorder progresses, cognitive impairment extends to the domains of skilled movements, recognition, social behaviour and other functions such as judgment and logic. Eventually, even simple tasks, such as maintaining personal hygiene, cannot be performed by the patients who become completely socially dependent. The symptoms may also include mood and behavioural changes, such as outbursts of violence or excessive passivity, depression, paranoia and hallucinations (Understanding stages and symptoms of Alzheimer's disease, ADEAR, 2008; (Weiner et al., 2005).

### 1.1.4 Diagnostic

Diagnosing AD is often difficult, particularly in the early stages. However, the disease can be diagnosed using information about the patient and family history, psychological tests as well as medical imaging and analysis of cerebrospinal fluid (CSF) biomarkers.

The diagnostic criteria commonly used for the clinical diagnosis of AD were published in 1984 by the National Institute of Neurological and Communicative Diseases and Stroke, and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) (McKhann et al., 1984). Similar to the NINCDS-ADRDA Alzheimer's criteria DSM-IV (Diagnostic and statistical manual of mental disorders - revision 4) are criteria published by the American Psychiatric Association in 1994.

To evaluate the cognitive impairments, neuropsychological screening tests such as the Mini Mental State Examination (MMSE) are widely used (Folstein et al., 1975). Neuroimaging is used to provide more information on some features of the disease and also allows the exclusion of alternative causes of dementia. MRI can show cortical and hippocampal (Teipel et al., 2008) atrophy while PET is used to detect glucose metabolism changes and more specifically, hypometabolism in cortical regions of Alzheimer's patients (Prince et al., 2008). A new technique known as "PiB PET" has been developed for direct imaging of Aβ deposits *in vivo* using a contrasting tracer that binds selectively to the deposits (Jack Jr et al., 2008; Nordberg, 2008; Rowe et al., 2008). Another recent diagnosis tool is the analysis of biomarkers present in the cerebrospinal fluid. Total tau and phosphorylated tau concentrations are increased while Aβ1-42 concentration is decreased in Alzheimer's patients CSF (Bouwman et al., 2008). However, a definite diagnosis may only be confirmed at *post-mortem* when brain material is available and can be examined histologically and histochemically (McKhann et al., 1984).

## 1.1.5 Neuropathological abnormalities

### 1.1.5.1 Neuronal and synaptic loss

At a macroscopic level, AD is characterised by degenerative changes in specific brain regions including the temporal and parietal lobes and restricted regions within the frontal cortex and cingulate gyrus. The disease is also characterized by degenerative changes in a variety of neurotransmitter systems. These include alterations in the function of the glutamatergic system and the monoaminergic neural systems that release norepinephrine and serotonin as well as a reduction of cortical choline acetyltransferase and cholinergic neuronal loss (Davies and Maloney, 1976; Wenk, 2003).

### 1.1.5.2 The two protein hallmarks of Alzheimer's disease and related pathology

At the molecular level, AD is characterised by two hallmark proteins in the brain: amyloid-β (Aβ) and tau. These proteins accumulate in the brain and form insoluble bodies called β-amyloid plaques and neurofibrillary tangles, respectively.

The accumulation of these proteins interferes with normal neuronal function, leading to cell death.

#### *1.1.5.2.1 Generation of β-amyloid plaques*

The Aβ peptide is a small protein of about 4 kDa generated from the proteolysis of the transmembrane amyloid precursor protein (APP) (LaFerla and Oddo, 2005). The APP gene, localised on chromosome 21, is expressed in a variety of tissues. After synthesis of the precursor protein, a small fraction of APP reaches the plasma membrane where proteolysis occurs. APP can be metabolized by two competing pathways, a non-amyloidogenic and an amyloidogenic pathway (Figure 1.1). In the first case, the precursor protein is successively cleaved by the α-secretase within the Aβ domain, thereby preventing the formation of Aβ, and subsequently by γ-secretase, releasing APPs-α and P3 peptides. In the second case, APP is first cleaved at the amino terminus of Aβ by the β-secretase. This cleavage results in the release of a large secreted derivative, APPs-β and a membrane-bound β-cleaved carboxy-terminal fragment of APP (C99). Then, cleavage of C99 by γ-secretase results in the production of the Aβ peptides with different C termini. Aβ1-40 and Aβ1-42 are the most common forms generated with Aβ1-42 being the most abundant in the *post-mortem* brains of AD patients (Naslund et al., 2000; Wang et al., 1999). Aβ proteins then aggregate and form pro-inflammatory and neurotoxic Aβ oligomers (Akama and Van Eldik, 2000; Akiyamaa et al., 2000). After oligomerisation, Aβ proteins accumulate in the brain, mainly in the form of deposits known as Aβ plaques. Aβ plaques are compact spherical extracellular deposits mainly composed of the Aβ peptide. These extra-cellular lesions are usually found in different areas of the brain such as the frontal cortex, parietal cortex, occipital cortex, temporal cortex, and hippocampus (Masuda et al., 1988). Aβ proteins also accumulate in the blood vessels of the cerebral cortex. This deposition of Aβ proteins called cerebral amyloid angiopathy (CAA) affects over 90% of patients with AD and creates an important risk of stroke (Love, 2005b).

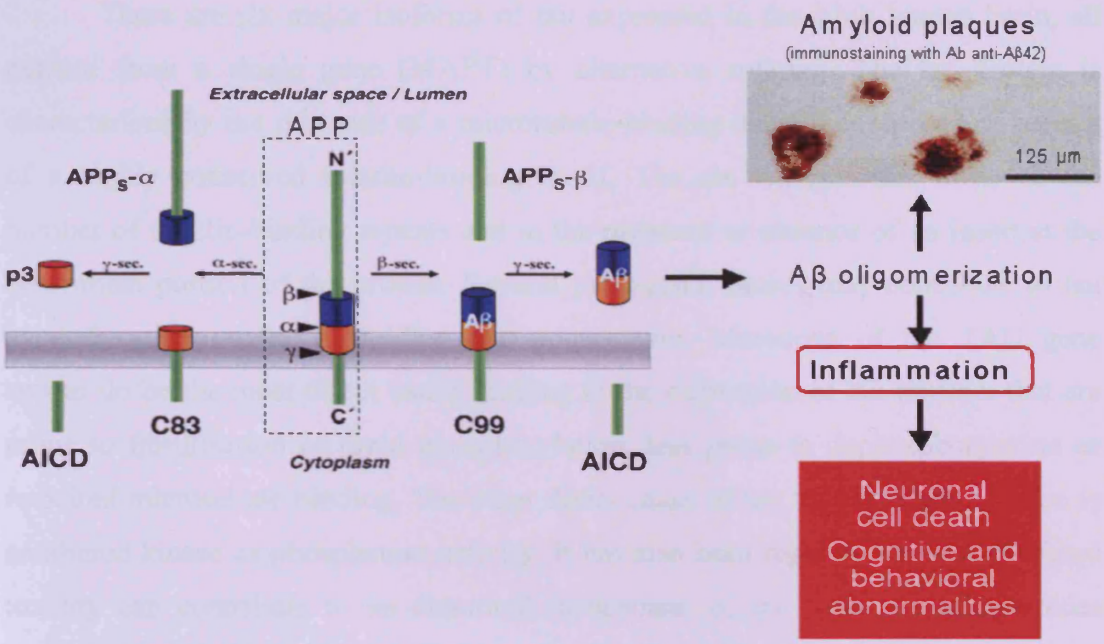


**Figure 1.1.** Generation of β-amyloid plaques in Alzheimer's disease and effects of Aβ oligomers (adapted from (Gandy, 2005; Kaether and Haass, 2004; LaFerla and Oddo, 2005)). APP, amyloid precursor protein; APPs-α, secreted APP cleaved by the α-secretase; APPs-β, secreted APP cleaved by the β-secretase; AICD, APP intracellular domain; α-sec., α-secretase; β-sec., β-secretase; γ-sec., γ-secretase.

#### *1.1.5.2.2 Generation of neurofibrillary tangles*

Tau is a widely-expressed protein from the microtubule-associated family. The main function of tau is to maintain microtubule stability. It is mainly located in neurons and, more specifically, in the axons where it modulates microtubule dynamics contributing to structural functions and axonal transport. The ability of tau to bind microtubules is mainly regulated by its state of phosphorylation which modulates the affinity of tau to microtubules. Under normal physiological conditions, the binding of tau to microtubules is in constant dynamic equilibrium, controlled by kinases and phosphatases responsible for the phosphorylation and dephosphorylation of the protein. In the case of AD, the tau protein, in a hyperphosphorylated form, is the main constituent of intraneuronal bundles known as neurofibrillary tangles. Hyperphosphorylation of the tau protein reduces its ability to bind microtubules and leads to cytoskeletal degeneration and neuronal death (Ballatore et al., 2007; Lovestone and Reynolds, 1997).

There are six major isoforms of tau expressed in the adult human brain, all derived from a single gene (MAPT) by alternative splicing. The tau protein is characterised by the presence of a microtubule-binding domain composed of repeats of a highly conserved tubulin-binding motif. The six tau isoforms differ in the number of tubulin-binding repeats and in the presence or absence of an insert at the N-terminal portion of the protein. Several pathogenic causes may contribute to tau hyperphosphorylation, misfolding and aggregation. Mutations of the TAU gene appear do be the most direct cause, leading to the expression of tau mutants that are prone to fibrillisation or rapid phosphorylation, less prone to dephosphorylation or impaired microtubule binding. The other direct cause of tau hyperphosphorylation is an altered kinase or phosphatase activity. It has also been reported that Aβ-mediated toxicity can contribute to an abnormal detachment of tau from the microtubules (Lovestone and Reynolds, 1997). Neurofibrillary tangles are mainly located in the hippocampus and the temporal cortex (Giannakopoulos et al., 1994). They are not specific to Alzheimer's disease, and are also found in a variety of other neurodegenerative conditions such as Parkinson's disease (Goedert and Spillantini, 2006).

### 1.1.6 Risk factors

Ageing is the main risk factor for AD since the prevalence of AD increases considerably with age, from 1% at 60-65 years of age to 24% or more at 85 years of age and over (Wimo et al., 2003). Although the causes of Alzheimer's disease are not fully understood, there is some evidence that the development of the disease may be influenced by a combination of factors including predisposing genetic polymorphisms and mutations as well as lifestyle, environment and pathological conditions. Familial Alzheimer's disease is a very rare autosomal dominant disease with early onset, called early-onset Alzheimer's disease (EOAD) and caused by mutations in the amyloid precursor protein or presenilin genes, both linked to Aβ metabolism. By contrast, sporadic Alzheimer's disease, also called late-onset Alzheimer's disease (LOAD), is the most common form of AD and may be caused by ageing in concert with a complex interaction of both genetic polymorphisms and environmental risk factors.

### 1.1.6.1 Genetic risk factors

Molecular analysis of families with EOAD allowed the identification of rare autosomal dominant mutations occurring in three separate genes encoding for the amyloid precursor protein (APP), presenilin 1 (PS1) and presenilin 2 (PS2) (Tanzi and Bertram, 2005). The APP gene is located on chromosome 21 which is also involved with Down syndrome constituting an additional risk for the pathology (Folin et al., 2003; Masters et al., 1985; Robakis et al., 1987). The autosomal dominant mutations in APP, associated with EOAD, cluster around the β- and γ-cleavage sites. For example, the two point mutation, called the Swedish mutation, at amino acids 670 Lys-Met and 671 Asp-Leu is located upstream of the β-cleavage site and increases the formation of both Aβ1-40 and Aβ1-42 by five to eight fold. Two different single-point mutations, called the London mutation and the Indiana mutation, at amino 717, adjacent to the γ-cleavage site, specifically increase the production of Aβ1-42 (Chapman et al., 2001). The genes coding for PS1 and PS2 are respectively located on chromosome 14 and chromosome 1 (Tanzi and Bertram, 2005). More then 160 mutations in the presenilin genes have been identified. Presenilin gene mutations increase the ratio of Aβ1-42 to Aβ1-40 and this appears to be due to a reduced γ-secretase activity (Goedert and Spillantini, 2006) or an increased concentration of γ-secretase substrate (Ye et al., 2007).

Other genes have been identified as risk factors. The APOE gene, located on chromosome 19, encodes for the apolipoprotein E (Hardy, 2006). APOE is a major serum protein involved in cholesterol metabolism, transport and storage. The APOE gene has three major isoforms, APOE2, APOE3 and APOE4; APOE3 being the most common. Although the two alleles, APOE2 and APOE3, are not associated with AD, the APOE4 variant has been identified as a risk factor for late onset AD (LOAD) (Chapman et al., 2001; Hardy, 2006). With a reduction of APOE concentration in brain tissue, APOE4 is associated with amyloid plaque accumulation and tau phosphorylation, the main features of AD (Corder et al., 1998; Poirier, 2005).

Mutations of PS1 are the most common cause of EOAD, APP mutations account for a smaller percentage and PS2 mutations are rare. However, EOAD accounts for only a minority of AD cases, with prevalence of less than 5% of all AD cases. The majority are sporadic cases of AD as the disease generally occurs after 65

years of age. Mainly associated with APOE4 allele, LOAD also has risk factors related to lifestyle and environment (Hoenicka, 2006).

#### 1.1.6.2 Lifestyle and environmental factors

While it is clear that genetic factors play an important role in the development of AD, there is some evidence that life style and environmental factors also have a major impact (Grant et al., 2002; Hooijmans and Kiliaan, 2008; Jansson, 2005; McDowell, 2001).

As reviewed by Blennow et al. (2006), conditions affecting blood circulation may increase the risk of AD. These conditions include illnesses directly related to the vascular system such as high blood pressure, hypertension and cerebral hypoperfusion, cardiovascular disease, atherosclerosis, heart failure, strokes and cerebrovascular lesions. Other conditions, also affecting the vascular system, such as obesity, diabetes, hyperlipidemia and high intake of saturated fat, inflammation, excessive alcohol consumption and smoking, are also risk factors for AD (Blennow et al., 2006). The social environment and life style also seem to have an impact on the development of dementia. Low mental and physical activity as well as low education and poor social network may be risk factors (Qiu et al., 2007). However, all these lifestyle and environmental factors are so closely connected that it is difficult to determine the exact relationship with the disease and the impact of each factor in isolation on the disease pathogenesis.

Several exposures appear hazardous such as intake of aluminium from food or drinking water which confers excess risk (Andrási et al., 2005; Gupta et al., 2005; Jansson, 2001, 2005; Kawahara, 2005) and viral infections such as the presence of HSV1 also seem to increase the risk of AD (Grant et al., 2002).

### 1.1.7 The β-amyloid pathology

#### 1.1.7.1 The amyloid cascade hypothesis

Although amyloid deposition was described by Alzheimer over a century ago, it was more recently that the toxicity of Aβ was described and recognised as a key component in the pathogenesis of AD (Selkoe, 2001). In the "amyloid cascade

hypothesis" of AD, it is considered as the primary event in the development of the disease (Hardy and Selkoe, 2002), leading to the formation of neurofibrillary tangles, neuronal damage, cell death and ultimately dementia (Kowalska, 2004). Strong support for this hypothesis has been derived from genetic studies of the APP, PS1 and PS2 genes that are linked with EOAD. In addition, APOE4 is a major risk factor for LOAD and affects the rate of Aβ deposition (Bales et al., 2009). However, the hypothesis has been challenged on the grounds that there is a weak correlation between elevated levels of Aβ plaques in the brain and cognitive decline. Due to the abundance of Aβ plaques in the brain of AD patients, it was first hypothesised that Aβ deposits were the cause of neuronal degeneration in AD brains (Anderson et al., 1996; Estus et al., 1997). However, neither the rate of dementia nor the extent of neurological damage correlates with the plaque-associated Aβ. In contrast, the soluble forms appear to correlate better with the severity of AD (Lue et al., 1999; McLean et al., 1999; Naslund et al., 2000). In addition, brain and cerebrospinal fluid levels of Aβ oligomers have been reported to correlate with cognitive status (Georganopoulou et al., 2005; Gong et al., 2003). Moreover, in Tg2576 mice that over-express a human APP mutation associated with EOAD, memory deficits correlate with Aβ elevation rather than plaque formation and, therefore, suggest that soluble forms of Aβ are neurotoxic (Hsiao et al., 1996). Numerous *in vitro* studies have shown that soluble Aβ species are significantly more toxic than insoluble Aβ (Kim et al., 2003; Lambert et al., 1998; Walsh et al., 2002). Such studies therefore provide evidence that Aβ-mediated neurodegeneration in AD may be the result of toxic soluble Aβ species and that extracellular aggregates of insoluble Aβ species, such as Aβ plaques, may represent end-products of the pathology (and may be neuroprotective). It has also been shown that Aβ1-42 tends to aggregate more than Aβ1-40 and Aβ1-42 is believed to initiate the formation of oligomers, fibrils, leading to the formation of plaques (Kirkitadze et al., 2001; Walsh et al., 1997).

Although, it is still unclear whether the generation of Aβ is the cause of the development of AD or a final by-product, it is clear that Aβ peptides are neurotoxic and lead to synaptic dysfunction and ultimately neuronal death (Crouch et al., 2008). Therapeutic strategies that aim to inhibit the Aβ pathway are, therefore, an area of intense research focus. Although mechanisms of Aβ-induced neuronal death remain unclear, hypotheses relating to oxidative stress and neuroinflammation have been proposed.

### 1.1.7.2 Oxidative stress

Considerable evidence indicates that initiation of oxidative stress may be one of the earliest events and may contribute to the neurodegenerative process in AD (Markesbery and Carney, 1999). *Post-mortem* analysis of AD brains has revealed elevated markers of oxidative stress including: protein oxidation (indicated by elevated levels of protein carbonyls and nitration of tyrosine), lipid peroxidation (indicated by elevated levels of thiobarbituric-acid reactive substances, malondialdehyde, 4-hydroxy-2-trans-nonenal, isoprostanes and altered phospholipid composition), DNA and RNA oxidation (indicated by elevated hydroxylated guanosine) and reactive oxygen species formation (Aksenov and Markesbery, 2001; Butterfield and Lauderback, 2002; Moreira et al., 2005). In addition, the role of oxidative stress in the pathogenesis of AD has been connected to the neurotoxicity of Aβ (Mucke et al., 2000). The apoptotic cell death induced by soluble Aβ oligomers may proceed through an early reactive oxygen species-dependent perturbation of the cytoskeleton and of the plasma membrane of cortical neurons (Pillot et al., 1999; Sponne et al., 2003). Furthermore, Aβ has been proposed to have a metalloenzyme-like activity generating hydrogen peroxide through its superoxide dismutase activity and it has been further suggested that the oxidative stress may promote the amyloidogenic pathway (Opazo et al., 2002). The resulting increase in Aβ can in turn generate more hydrogen peroxide leading to further oxidative damage, and subsequently enhance the development of AD.

### 1.1.7.3 Neuroinflammation

Inflammation is a process that has been related with the onset of several neurodegenerative disorders such as AD, Parkinson's disease and multiple sclerosis. Moreover, there is some evidence that Aβ proteins are involved in the inflammation process of AD (Heneka, 2006; Sastre et al., 2006). Astrocytes and microglia have a major role. It has been described that cytokines, including interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), secreted by microglial cells, astrocytes or neuronal cells may induce the production of Aβ (Blasko et al., 2000; Bo et al., 1995), and Aβ can also induce the expression of IL-1β, TNF-α and IL-6 in astrocytes and microglial cells *in vitro* (Chong, 1997; Gitter et al., 1995).

Moreover, the expression of IL-1β, TNF-α and IL-6 is upregulated in the brain of patients with AD (Cacabelos et al., 1994; Dickson et al., 1993; Wood et al., 1993) and was also increased in the hippocampus and the cortex of Tg2576 mice over expressing a mutated human APP gene (Tehranian et al., 2001). In addition, it has been shown that the expression of IL-1β, TNF-α and IL-6 by microglial cells in the cortex of the same mouse model is associated with Aβ deposits (Benzing et al., 1999), also suggesting that Aβ induces the expression of pro-inflammatory cytokines.

There is also considerable evidence that phospholipid metabolism is altered in AD and that membrane defects contribute significantly to disease pathology (Farooqui et al., 1997). Increases in free fatty acids, eicosanoids and products of lipid peroxidation are well known to occur early during progression of AD, leading to the hypothesis that phospholipases play a role in the production of second messengers involved in neurodegenerative disorders (Sun et al., 2004). In a gene array study, profiling 12,633 genes in the hippocampal CA1 area of AD patients, an increased expression of cytosolic phospholipase $A_2$ ($cPLA_2$) and cyclooxygenase-2 (COX-2), was observed. Both are involved in the synthesis of pro-inflammatory lipid mediators known as eicosanoids (Colangelo et al., 2002). In addition, another study showed that prostaglandin $E_2$ ($PGE_2$) levels in the cerebrospinal fluid of patients with AD correlated with the level of dementia (Combrinck et al., 2006). COX-2 was stimulated by IL-1 and TNF-α produced by microglia and astrocytes in AD, while cyclooxygenase-1 (COX-1) is only mildly up-regulated in AD brain (Yasojima et al., 1999). Exposure of cortical neurons to soluble Aβ oligomers *in vitro* induced the activation of the $cPLA_2$ in the perinuclear region and was associated with an increased release of arachidonic acid (Kriem et al., 2004) which may then be converted to potent bioactive mediators including pro-inflammatory prostaglandins, thromboxanes and leukotrienes. It was also shown by Hull et al. (2006) that Aβ induces the expression of COX-2, which is involved in the conversion of fatty acids to prostaglandins and thromboxanes, and increased the release of $PGE_2$ by astrocytes (Hull et al., 2006). Moreover, in the Tg2576 mouse model, inhibition of COX-2 improved Aβ-mediated neurodegeneration (Kotilinek et al., 2008). In addition, the expression of lipoxygenase-5 (LOX-5), involved in the conversion of fatty acids to leukotrienes, is upregulated in Tg2576 mice (Firuzi et al., 2008). This also suggests that Aβ may induce an eicosanoid-mediated neuroinflammatory response.

Although it is clear that Aβ can induce a neuroinflammatory response by increasing the production of cytokines or eicosanoids, all the implications of neuroinflammation in AD have not been fully elucidated. Although inflammation may be induced by Aβ, it has, itself, also been proposed as a cause of AD i.e., inflammation may also trigger the development of Aβ pathology. Alternatively, neuroinflammation could be a simple by-product of the disease process or even a beneficial reaction that could slow down the disease progression, as discussed by Tony Wyss-Coray in his review (Wyss-Coray, 2006).

## 1.1.8 Treatment

Although there is currently no cure for AD, five drugs that treat the symptoms of the disease are currently on the market. These drugs are classified into two categories. Donepezil (Aricept; Eisai/Pfizer), galantamine (Razadyne; Johnson & Johnson), rivastigmine (Exelon; Novartis) and tacrine (Cognex; First Horizon Pharmaceuticals) are cholinesterase inhibitors while memantine (Namenda; Forest/Lundbeck) is a N-methyl-D-aspartate (NMDA) receptor antagonist (Lleo et al., 2006).

### 1.1.8.1 Current treatments

#### *1.1.8.1.1 Cholinesterase inhibitors*

As AD leads to neuronal damage and cell death, it is not surprising that a reduction of the production of acetylcholine is observed in patients. From this observation, cholinesterase inhibitors were the first approved treatment for dementia symptoms. Although cholinesterase inhibitors appear to have a beneficial impact on the symptoms, they cannot stop the course of brain cell damage. Consequently, as the disease progresses and cells die, the efficiency of cholinesterase inhibitors declines (Lleo et al., 2006).

#### *1.1.8.1.2 N-methyl-D-aspartate receptor antagonist treatment with Memantine*

Another approach to the treatment of AD is to block glutamatergic neurotransmission. Glutamate is the main excitatory neurotransmitter in the brain. One of its receptors, NMDA, has been implicated in the neuronal mechanism responsible for learning and memory. Too much activation of this receptor complex, however, leads to neuronal dysfunction and death due to high intracellular concentrations of calcium. Memantine is a specific, low- to moderate-affinity, uncompetitive NMDA receptor antagonist and prevents calcium influx. Like the cholinesterase inhibitors, memantine appears to offer AD patients modest symptomatic benefits with minimal adverse effects (Lleo et al., 2006).

### 1.1.8.2 Drug candidates targeting the Aβ pathology

Among the efforts directed towards new treatments for Alzheimer's disease, drugs that target amyloid pathology have been a major focus.

#### *1.1.8.2.1 Secretase modulators*

The resolution of the structure of the APP-cleaving enzyme 1 (BACE1), the first of the two sequential enzymes leading to the production of Aβ, allowed the generation of various inhibitor compounds that could be developed into drugs. Inhibitors are currently being tested on cell culture and animals (Fu et al., 2008; Ghosh et al., 2008).

γ-secretase is the second enzyme involved in the generation of Aβ. It has been demonstrated that γ-secretase is not a unique molecule but a protein complex composed of four proteins: presenilin, nicastrin, aph-1 and pen-2, all required for proteolytic activity (Sato et al., 2007). Some inhibitors of γ-secretase have toxic effects due to inhibition of Notch cleavage. However, new γ-secretase inhibitors having no effect on Notch signalling have been developed (Petit et al., 2001) and have shown good tolerability in phase I trials (Siemers et al., 2006). Interestingly, some non-steroidal anti-inflammatory drugs (NSAID) have been found to modulate γ-secretase and to selectively reduce Aβ1-42 levels without affecting Notch receptor cleavage (Imbimbo, 2008). Activation of the non-amyloidogenic processing of APP

also appears to be a potential alternative strategy to reduce cerebral amyloidosis (Bandyopadhyay et al., 2007). The success of these drugs in mitigating AD pathogenesis remains to be evaluated.

#### *1.1.8.2.2 Anti-amyloid immunotherapy*

The vaccination of an APP transgenic mouse with Aβ1-42 attenuated Aβ deposition (Schenk et al., 1999). Similar results were also obtained with passive immunisation of transgenic mice using antibodies against Aβ (Dodel et al., 2003). With active immunisation, Aβ proteins seem to be cleared by microglial cells via anti-Aβ antibodies binding to Aβ plaques. Alternatively, with passive immunisation, antibodies against Aβ may bind to soluble Aβ in the periphery, changing the equilibrium of Aβ between brain, CSF and plasma causing an efflux of Aβ from the brain (Dodel et al., 2003). The success of Aβ immunotherapy in animal models led to the initiation of clinical trials of an active anti-Aβ vaccine composed of pre-aggregated Aβ1-42 (AN1792 from Elan/Wyeth). After showing good tolerability and immunological response in a phase I clinical trial, the drug candidate was taken to phase II clinical trials. This study of AN1792 in human patients with mild to moderate AD had to be prematurely interrupted when 6% of inoculated patients developed encephalitis (Dodel et al., 2003; Schenk et al., 2004). AN1792 was then subsequently withdrawn from human trials. One explanation of the encephalitis seen in the trial could be a contamination by external pathogens due to an alteration of the blood-brain barrier caused by the vaccine. It has also been suggested that the side effect was due to an autoimmune response mediated by T-lymphocytes (Dodel et al., 2003; Schenk et al., 2004). To address the issues raised by AN1792, a novel peptide carrier protein conjugate using an amino-terminal fragment of Aβ (ACC-001) has been developed to avoid potentially harmful T-cell responses, while maintaining a similar antibody response to that of AN1792. Immunotherapeutic trials using this treatment approach started in 2005 with a phase 1 trial and are currently in a phase II trial (Alzheimer's Research Forum, Drugs in clinical trials, 2009). A second generation of vaccines using the passive immunisation approach is currently in development. Three antibodies against different domains of Aβ are currently being tested clinically (Melnikova, 2007).

#### *1.1.8.2.3 β-amyloid fibrillisation inhibitors*

Small molecules binding Aβ peptides can prevent fibrillisation, thereby preventing the formation of amyloid plaques. Tramiprosate (Alzhemed™) from Neurochem is a glycosaminoglycan mimetic designed to interfere with the Aβ aggregation. Tramiprosate treatment in a transgenic mouse model resulted in a dose-dependent reduction of Aβ levels in plasma as well as soluble and insoluble Aβ1-40 and Aβ1-42 in brain (Gervais et al., 2007). In a recent phase III clinical trial, the drug was safe, reduced CSF Aβ1-42 levels after 3 months of treatment and improved cognition after a longer term treatment (Aisen et al., 2008).

### 1.1.8.3 Other potential treatments

#### *1.1.8.3.1 Non-steroidal anti-inflammatory drugs*

Epidemiologic studies showed that non-steroidal anti-inflammatory drugs (NSAID) such as ibuprofen or indomethacin may protect against the development of AD (Etminan et al., 2003). Moreover, experiments on cell cultures and transgenic models have shown that NSAID reduce the accumulation of Aβ (Townsend and Praticó, 2005).

#### *1.1.8.3.2 Cholesterol-lowering drugs*

Current human studies dealing with the use of cholesterol-lowering drugs known as statins are conflicting with regard to their neuroprotective effects on cognitive impairment; some have shown that these compounds can reduce the risk of dementia while others have shown no benefit (see Kandiah and Feldman, 2009).

#### *1.1.8.3.3 Nutritional approaches*

There is some concern that once AD is clinically diagnosed it is already too late to initiate effective treatment to ameliorate the cognitive and neuronal pathology. Hence, there is a growing interest in preventive nutritional approaches to reduce the cognitive decline before it becomes clinically diagnosed. Amongst the various

nutritional approaches, one that has received a lot of attention relates to the intake of fish, or more specifically, omega-3 fatty acids or DHA. As this is the subject of the present work, the relation between the risk of AD and the intake of fish, omega-3 fatty acids or DHA will be developed in the following sections. Prior to this, the effect of other dietary factors that may influence AD development will be briefly outlined.

Several other protective dietary factors have been identified in epidemiological studies or supplementation studies in humans. Vitamin $B_6$, vitamin $B_{12}$ and folate, vitamin E, curcumin, *ginkgo biloba* extracts and red wine in moderate quantities or resveratrol and alpha-lipoic acid are associated with the risk of AD or were protective in supplementation studies. A low plasma level of vitamin E was associated with AD (Helmer et al., 2003; Zaman et al., 1992), while increased consumption of vitamin E from foods was correlated with a lower risk of AD, although this effect was confined to APOE4 negative persons (Morris et al., 2002). However, vitamin C, beta-carotene, and vitamin E from supplements were not associated with a reduced risk of AD, unless vitamin C and vitamin E were used in combination (Morris et al., 2002; Zandi et al., 2004). Vitamin E supplementation has also been demonstrated to slow down the progression of AD (Sano et al., 1997) but was not systematically protective (Masaki et al., 2000; Petersen et al., 2005). In addition, vitamin E supplementation reduced the brain level of Aβ and improved the cognitive status in mice over-expressing a human APP gene (Nishida et al., 2006; Sung et al., 2004). Low blood levels of vitamin $B_{12}$ or folate have also been associated with AD but supplementation did not have a significant effect (Sun et al., 2007).

Curcumin consumption was also associated with a reduced risk of AD (Ng et al., 2006) and clinical trials are currently being carried out (Goel et al., 2008). Numerous studies on rodents and *in vitro* models have shown that curcumin is a potent anti-inflammatory, anti-oxidant and neuroprotective dietary supplement that reduces the accumulation of Aβ and alleviates cognitive impairments (Bala et al., 2006; Begum et al., 2008; Frautschy et al., 2001; Garcia-Alloza et al., 2007; Giri et al., 2004; Lim et al., 2001; Pan et al., 2008; Shukla et al., 2003; Sreejayan and Rao, 1994, 1996, 1997; Yang et al., 2005). *Gingko biloba* extract supplementation has also been reported to have a moderate effect on cognitive dysfunction in AD (Oken et al., 1998). Moderate alcohol consumption is also associated with a lower risk of

developing dementia (Deng et al., 2006; Huang et al., 2002; Lindsay et al., 2002; Luchsinger et al., 2004; Mukamal et al., 2003; Orgogozo et al., 1997; Ruitenberg et al., 2002; Truelsen et al., 2002). This may be related to the presence of the polyphenol known as resveratrol, which is present in the skin of red grapes and blueberries. Flavonoids present in wine, as well as berries and peanuts, have been shown to lower the levels of the amyloid-β peptides (Marambaud et al., 2005; Wang et al., 2006).

In addition other dietary components and supplements including caffeine, pomegranate juice, apple juice, aged garlic extract, alpha-lipoic acid, green tea extracts, coenzyme Q10 and luteolin have also been shown to have some beneficial effects in animal models of AD pathology. Moreover, caloric restriction also appears to have a neuroprotective effect (Love, 2005a; Wang et al., 2005b) by decreasing the accumulation of Aβ deposits (Patel et al., 2005).

## 1.2 Docosahexaenoic acid

### 1.2.1 Docosahexaenoic acid as a fatty acid

Docosahexaenoic acid (DHA) is a polyunsaturated fatty acid (PUFA) that is highly abundant in brain and particularly in its membrane phospholipids.

Fatty acids consist of a hydrocarbon chain with a terminal carboxylic acid ($CH_3$-$(CH_2)_n$-COOH), most frequently containing an even number of carbon atoms, between 14 and 22. Fatty acids can be distinguished by their content in double bonds. Fatty acids with all the carbon atoms bonded to the maximum number of hydrogen atoms do not contain double bonds and are known as saturated fatty acids (SFA). In contrast, unsaturated fatty acids contain one or more double bonds (usually *cis*). Monounsaturated fatty acids (MUFA) contain only one double bond while polyunsaturated fatty acids (PUFA) such as n-6 and n-3 PUFA contain more than one double bond in their carbon chain. It is the position of the first double bond within the hydrocarbon chain (numbering from the methyl end) that gives the n-6 and n-3 PUFA their name and properties. The n-6 PUFA have their first double bond on the sixth carbon from the methyl (non-carboxyl) end of the fatty acid molecule whereas in an n-3 PUFA the first double bond is located at the third carbon. DHA is an n-3 PUFA composed of twenty two carbons and containing six double bonds which are all methylene-interrupted (i.e. 3 carbons apart).

### 1.2.2 Source of docosahexaenoic acid

Although mammals can synthesize fatty acids *de novo*, they cannot generate linoleic acid (LA, 18:2n-6) or α-linolenic acid (ALA, 18:3n-3). Therefore these two fatty acids have to be provided in the diet and are both classified as essential fatty acids (Yehuda et al., 2002). In contrast, arachidonic acid (AA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3) and DHA can be synthesized from their respective precursors. As shown in Figure 1.2, LA can be converted to AA, and ALA to EPA and DHA by the action of several elongases and desaturases that add additional carbons and double bonds. However, the rate of conversion of ALA to DHA is very low. It has been estimated that less than 8% of ALA is metabolised to

EPA and only between 0.02% and 4% of ALA is metabolised to DHA (Burdge and Calder, 2005; Pawlosky et al., 2001; Vermunt et al., 2000). Therefore most of the DHA needs to be provided by the diet. Studies in animal and humans have established that dietary deficiency of n-3 fatty acids, or LA in combination with ALA, results in decreases in brain phospholipid AA and DHA, with concomitant increases in brain n-9 and n-7 MUFA and other PUFA (Uauy and Dangour, 2006).

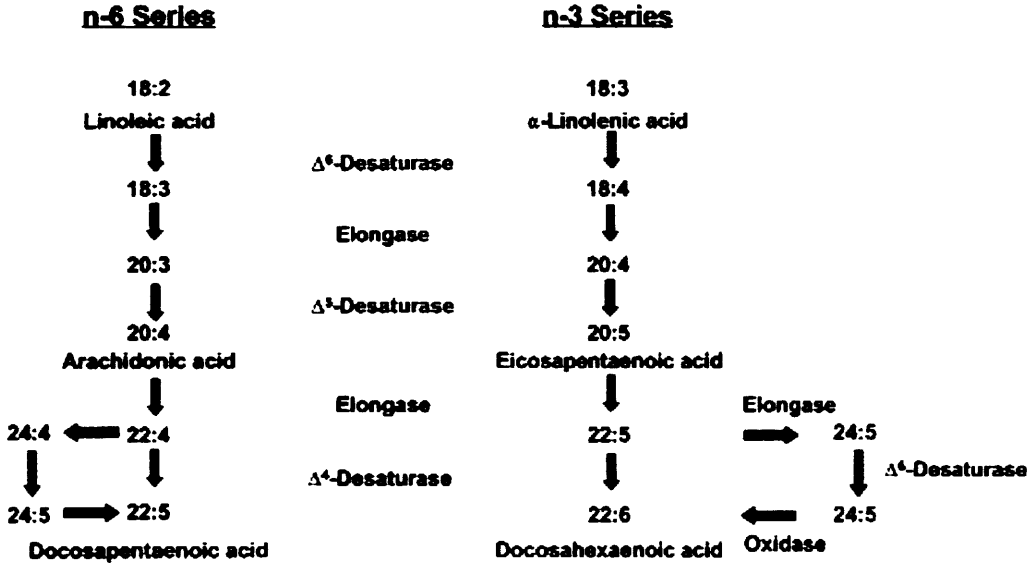


**Figure 1.2.** Biosynthesis of very long-chain n-3 and n-6 series polyunsaturated fatty acids from their 18-carbon precursors α-linolenic acid (18:3n-3) and linoleic acid (18:2n-6) (Lauritzen et al., 2001).

The three main n-3 PUFA found in human diet are ALA, EPA and DHA. In contrast to mammals, plants possess the necessary desaturases to convert oleic acid (18:1) to both 18:2n-6 and 18:3n-3 so ALA is mostly found in nuts, seeds and vegetable oils such as soybean and flaxseed oils. The main source of dietary DHA is fatty fish, such as salmon, mackerel and sardines, as they eat algae and plankton that are the primary source of DHA (Lunn and Theobald, 2006). DHA can also be provided from dietary supplements including cod liver oil, fish-based products and also DHA-rich microalgal oil that was authorized for release into the market by the European Commission in 2003 (Commission Decision 2003/427/EC authorising the placing on the market of oil rich in DHA (docosahexaenoic acid) from the microalgae Scizochytrium sp. as a novel food ingredient, Regulation (EC) No 258/97). Recently, new kinds of foods, enriched with very long chain PUFA such as EPA,

docosapentaenoic acid (DPA, 22:5n-3) and DHA, named "functional foods", have also been developed to prevent nutritional deficiencies (Lunn and Theobald, 2006).

## 1.2.3 Properties of n-3 PUFA and DHA

Fatty acids have three major roles. (1) In the form of triacylglycerols (triglycerides), they provide an important form of energy storage. (2) As components of phospholipids, they have structural functions in cell membranes and (3) also play an important role in signalling through the synthesis of derivative products and the induction of gene expression (Gurr et al., 2002).

### 1.2.3.1 Plasma membrane structure and functions

The neuronal membrane fatty acid composition and more specifically, its DHA content, may affect many membrane properties such as membrane fluidity and elasticity, receptor affinities, ion fluxes, and activities of membrane-bound enzymes (Bruno et al., 2007; Stillwell and Wassall, 2003). Moreover, dietary PUFA may reduce membrane-bound cholesterol that can cause neural membrane rigidity when present in excess (Horrocks and Farooqui, 2004).

### 1.2.3.2 Expression of cytokines

There is some evidence that DHA has an anti-inflammatory effect by reducing the expression of pro-inflammatory cytokines. Several studies have shown that n-3 fatty acids, including DHA, decrease the production and activity of pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α (Blok et al., 1997; Chavali and Forse, 1999; Hughes and Pinder, 1997; Yano et al., 2000), while n-6 fatty acids have the opposite effect (Caughey et al., 1996; Grimble, 1998; James et al., 2000). Fish oil has also been shown to decrease IL-6, IL-10, Il-12 and TNF-α in cell cultures (Denisova et al., 2001).

### 1.2.3.3 Synthesis of eicosanoids

In the nervous system, PUFA can be released from membrane phospholipids when neurones are stimulated with neurotransmitters and can be metabolized in the brain, giving rise to a series of twenty carbon oxygenated active products known as eicosanoids (Gurr et al., 2002). Eicosanoids include prostaglandins, leukotrienes, thromboxanes, and a variety of hydroxyl and hydroperoxy fatty acids. These products may act in the intracellular environment as neuronal secondary messengers and may be released in the extra-cellular space, regulate membrane ion channels, protein kinases, ion pumps and interact with G-protein-coupled receptors on neurones and glial cells. As such, they may influence neuromodulation and synaptic plasticity (Piomelli, 1994).

The enzymes, phospholipase $A_2$ ($PLA_2$), cyclooxygenases (COX) and lipoxygenases (LOX) are involved in the generation of eicosanoids from PUFA released from the membrane phospholipids. $PLA_2$ liberates fatty acids from the phospholipids to be converted by COX or LOX. COX convert PUFA into prostaglandin H (PGH) and other enzymes further convert PGH into pro-inflammatory prostacyclins, thromboxanes and prostaglandins. When converted by LOX, PUFA are derived into other pro-inflammatory leukotrienes. Although eicosanoids are often pro-inflammatory, eicosanoids derived from n-3 PUFA are much less potent then those derived from n-6 PUFA. Indeed, DHA has been reported to be a potent inhibitor of highly pro-inflammatory prostaglandin biosynthesis. EPA and DHA competitively inhibit the oxygenation of arachidonic acid (AA) by COX and LOX (Figure 1.3). Increases in daily consumption of n-3 PUFA are quickly reflected in elevated concentrations of these fatty acids, mainly EPA and DHA, in plasma and membrane of red and white blood cells. A major effect of such changes in membrane alters the composition of eicosanoids formed through the action of the enzymes COX and LOX. As well as increased production of weakly inflammatory prostanoids and leukotrienes from EPA, there is also inhibition of the production of strongly inflammatory eicosanoids from AA (Gurr et al., 2002; Horrocks and Farooqui, 2004). In the context of this work, dietary DHA might reduce the production of pro-inflammatory eicosanoids induced by Aβ.

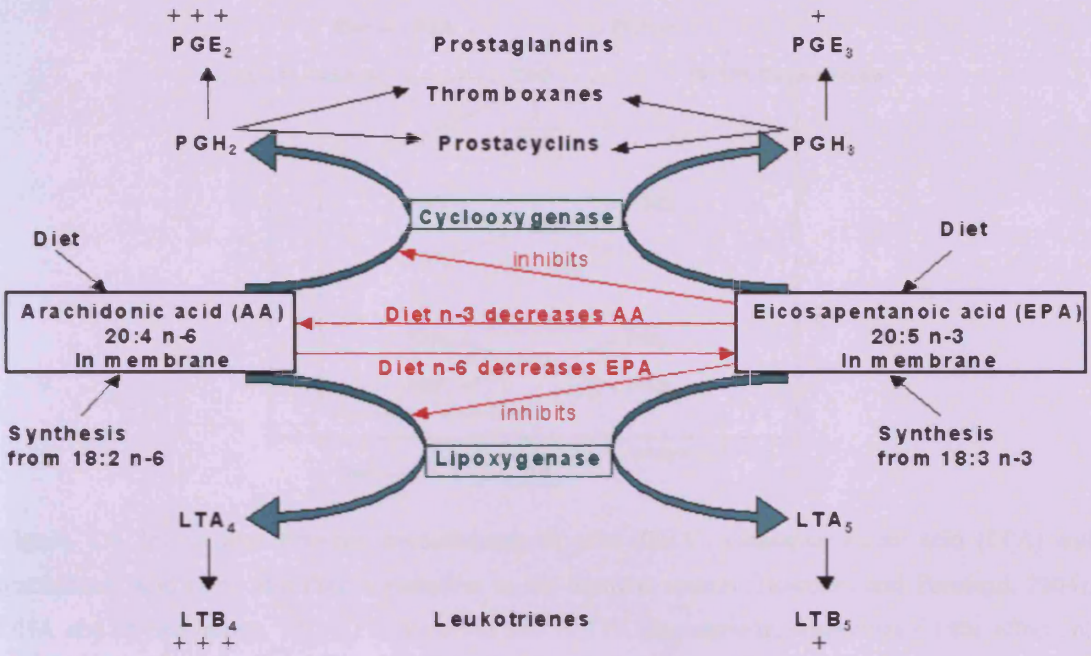


**Figure 1.3.** Influence of dietary n-3 and n-6 polyunsaturated fatty acids on inflammatory eicosanoid production (Gurr et al., 2002). +, weakly inflammatory; +++, strongly inflammatory; PGE and PGH, prostaglandins; LTA and LTA, leukotrienes.

### 1.2.3.4 Synthesis of novel anti-inflammatory mediators

A new series of bioactive fatty acid derivatives, E-series resolvins derived from EPA, and D-series resolvins and protectins derived from DHA, have been recently elucidated (Bannenberg et al., 2007; Serhan et al., 2004). Several studies have shown the anti-inflammatory and immunoregulatory properties of the 10,17S docosatriene derived from DHA by LOX, and also called neuroprotectin D1 (NPD1) (Butovich, 2005; Hong et al., 2003; Schwab et al., 2007) (Figure 1.4). Moreover, in primary co-cultures of human neurons and glial cells, DHA treatment was associated with a 20-25% decrease in Aβ production, NPD1 synthesis and 50% decrease in apoptosis caused by Aβ1-42 (Lukiw et al., 2005). In addition, there was some evidence that NPD1 induced anti-apoptotic and anti-inflammatory gene expression which suppressed Aβ1-42 induced neurotoxicity, suggesting that DHA may alleviate the β-amyloid pathology through the synthesis of NPD1.

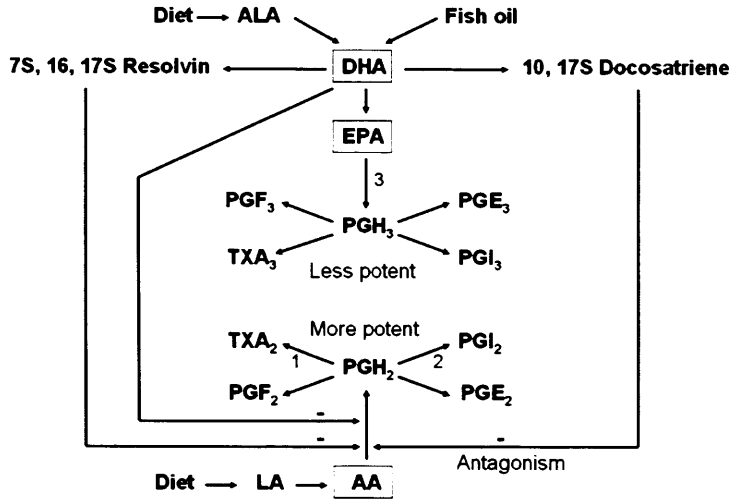


**Figure 1.4.** Interactions between docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (AA) and their metabolites in the immune system (Horrocks and Farooqui, 2004). DHA and its derivatives, 7S,16,17S Resolvin and 10,17S Docosatriene, antagonize (-) the affects of prostaglandins (PGE) and thromboxanes (TXA). LA, linoleic acid; ALA, $\alpha$-linolenic acid; (1) thromboxane synthase; (2) prostaglandin I synthase; and (3) cyclooxygenase-1 and -2.

## 1.2.4 Recommended intake and consumption of DHA

In 1992, the Scientific Committee for Food of the European Union recommended a daily intake of 1 g of very long chain n-3 PUFA, representing 0.5% of the total nutritional energy consumption (Reports of the Scientific Committee for Food, 1992). In 2001, the recommendation for DHA intake proposed by official committees, such as the Agence Française de Sécurite Sanitaire des Aliments (AFSSA) and scientific societies such as the International Society for the Study of Fatty Acids and Lipids (ISSFAL), was 0.05-0.1% of the energy intake, corresponding to 100-200 mg and 120-240 mg per day for females and males, respectively (Alessandri et al., 2004). In 2004, based on the assessment of the health impact by the Scientific Advisory Committee on Nutrition (SACN) and the Committee on Toxicology (COT), the UK government recommended that people should eat at least two portions of fish per week, of which one should be oil-rich. This equates to approximately 450 mg very long chain n-3 PUFA per day. However, the average consumption of fish in Western countries such as the UK is well below the recommendation. The mean consumption of oily fish by adults was estimated at 53 g per week, about a third of a portion (Henderson et al., 2002). The average intake of the UK population has been estimated to be approximately 244 mg of long chain n-3

PUFA per day with 131 mg per day of the total provided by oil-rich fish (Givens and Gibbs, 2006). These figures show that the very long chain n-3 PUFA intake is considerably below the recommended two portions of fish per week or 450 mg very long chain n-3 PUFA per day. Therefore, dietary habits may have to be changed and the consumption of fatty fish may have to be encouraged in persons who never or rarely eat fish.

In addition to the level of dietary very long chain n-3 PUFA, the n-6 to n-3 PUFA ratio appears to be another important factor of a balanced diet. As the metabolic pathways of these two families of fatty acids share some of the same enzymes, dietary n-6 PUFA may affect the metabolism of the n-3 PUFA. Moreover, although n-3 and n-6 PUFA may be processed by the same enzymes, their metabolites tend to have opposite biologic effects: n-6 derived metabolites tend to be pro-inflammatory, whereas n-3 derived metabolites tend to be less or even anti-inflammatory, so increased consumption of n-6 PUFA-rich food may have a detrimental effect on health. The ratio was estimated at 1:1 during the prehistoric times; however the current Western diet provides a n-6 to n-3 ratio around 10:1 and probably even higher in some individuals (up to 15:1 or 20:1), according to estimates in the USA (Simopoulos, 2002; Simopoulos et al., 2000). The fall in fish consumption associated with an increase of n-6 fatty acid-rich vegetable oil and meat consumption are the main causes of the imbalance between n-6 and n-3 PUFA, and may account for the incidence of diseases for which n-3 PUFA appear to be beneficial.

### 1.2.5 Importance of docosahexaenoic acid in brain functions

Dietary n-3 PUFA and DHA play an important role in cerebral development and the maintenance of brain lipids. In humans, deficiency of n-3 FA results in several neuronal-related defects that include learning and visual impairments (Connor et al., 1992). In contrast, supplementation improves cognitive and visual function of young children (Dunstan et al., 2006; Judge et al., 2007a; Judge et al., 2007b). The decline in memory and learning with age may also be partially related to decreased brain levels of DHA (Horrocks and Farooqui, 2004). Indeed, a diet high in n-3 PUFA can reverse cognitive decline (Beydoun et al., 2007; Dullemeijer et al., 2007; Heude et al., 2003; Nurk et al., 2007). In addition, alterations of the PUFA status have also

been associated with other neurological diseases such as multiple sclerosis, epilepsy and Huntington disease, and psychiatric disorders including depression, bipolar disorder, schizophrenia, hyper-activity and autism (Alessandri et al., 2004).

In rats, dietary restriction of DHA and the α-linolenic acid precursor also results in decreased DHA levels in brain phospholipids (Calon et al., 2004), learning and memory deficits (Catalan et al., 2002; Gamoh et al., 1999) and increased depressive and aggressive behaviour (DeMar et al., 2006). Yoshida et al. (1997) have suggested that dietary α-linolenic acid deficiency affects synaptic vesicle turnover in the hippocampal CA1 region and induces loss of learning ability (Yoshida et al., 1997). Deficits of DHA in the two main phospholipids in foetal guinea pig brain and severe neurological dysfunction were partially remedied by maternal fish oil supplementation during pregnancy (Burdge, 1997). In rats, DHA supplementation after long-term n-3 PUFA deficiency reversed the altered learning behaviour (Ikemoto et al., 2001). DHA supplementation in old rats also reversed the decrease of DHA in the hippocampus and age-related impairments in long-term potentiation and depolarization-induced glutamate transmitter (McGahon et al., 1999). In mice, dietary DHA also improved learning in a maze task (Lim and Suzuki, 2000). However, there is an agreement in the literature that mammalian brain accumulates its DHA during specific periods of intra-uterine and postnatal life and, after these periods, there is less ability to alter its fatty acid composition (Farkas et al., 2000). For instance, a study on rats showed that an n-6/n-3 fatty acid imbalance early in life leads to persistent reductions in DHA in glycerophospholipids in the hypothalamus, even after long-term n-3 fatty acid repletion (Li et al., 2006).

## 1.3 Docosahexaenoic acid and Alzheimer's disease

There is clearly good evidence for the benefits of dietary DHA on mental and physical health, including cardiovascular diseases, asthma, cancer and mental disorders. This section will examine the evidence for a beneficial effect of dietary DHA on Alzheimer's disease with reference to both human and animal studies.

### 1.3.1 Human studies

Two main scientific methods have been used to examine the role of DHA in mental health in AD. The first are epidemiologic methods that focus on fish or DHA intake, DHA levels in blood or in brain, and the incidence of AD. The second are clinical trials that have investigated the effects of DHA dietary supplementation on the incidence and symptoms of AD.

#### 1.3.1.1 Dietary DHA

The association between the risk of AD and dietary DHA emerged from prospective epidemiological studies that have shown an inverse association between the risk of AD and the consumption of fish or omega-3 fatty acids. The Rotterdam Study was the first to report that fish intake protected against the risk of AD (Kalmijn et al., 1997). In 5,386 non-demented participants aged 55 years and over at baseline, fish consumption was inversely related to incidence of dementia and AD in the average 2.1 years follow-up. High total fat, saturated fat and cholesterol intake appeared to increase the risk of dementia. However, in a 6-year follow-up of the same participants, there was no association between n-3 PUFA intake and the risk of dementia or AD (Engelhart et al., 2002). In an epidemiological study carried out in France on 1,416 subjects living at home, without dementia and aged 68 and over at the start of the study, 170 subjects developed dementia during the seven years of follow-up, including 135 cases of AD (Barberger-Gateau et al., 2002). This study revealed that participants who ate fish or seafood at least once a week had a lower risk of developing dementia, including AD for which the hazard ratio was 0.69 with borderline significance. The association of fish and omega-3 fatty acid intake and the

risk of AD was also analysed in 815 participants aged 65 and over of the Chicago Health and Aging Project (Morris et al., 2003). After a mean follow-up of 3.9 years, 131 subjects developed AD. Participants who ate fish once a week or more had 60% less risk of AD compared to those who rarely or never ate fish. Total intake of n-3 PUFA and intake of DHA were associated with a reduced risk of AD, but not intake of EPA.

In another study, carried out on 3,718 participants of the Chicago Health Aging Project, fish intake was associated with a slower rate of cognitive decline among subjects who consumed two or more fish-based meals per week, with a borderline significance over 6 years (Morris et al., 2005). However, there was no association with n-3 PUFA, EPA or DHA intake. In a population study carried out on 2,233 participants over four US communities, consumption of lean fish did not have a significant effect while consumption of fatty fish twice to four times a week was associated with a reduced risk of dementia by 28% and AD by 41% in comparison to those who ate fish less than once a month (Huang et al., 2005). This effect appeared to be selective to subjects without the APOE4 allele and was attenuated with adjustment by education and income. In the Three City Study carried out in France on a total of 8,085 non-demented participants aged 65 and over, weekly consumption of fish was associated with a reduced risk of AD and all causes of dementia, but only among non-APOE4 carriers (Barberger-Gateau et al., 2007). Regular use of omega-3-rich oils was also associated with a decreased risk of borderline significance for all causes of dementia. Regular consumption of omega-6-rich oils not compensated by consumption of omega-3-rich oils or fish was also associated with an increased risk of dementia among non-APOE4 carriers.

In the Zutphen Elderly Study, analysis of data on the fish consumption of 210 male participants who were aged 70-89 years at the start of the study revealed that fish consumers had significantly less cognitive decline in the subsequent 5 years than did non-consumers and there was a significant inverse relationship with EPA + DHA intake and cognitive decline (Van Gelder et al., 2007). The association between fish and meat consumption with dementia was also investigated recently in low and middle income countries of Latin America, China and India, and revealed a significant dose-dependent inverse association between fish consumption and dementia that was consistent across all sites except India and a less consistent dose-dependent direct association between meat consumption and prevalence of dementia

(Albanese et al., 2009). In a case-control study including 27 AD patients, 15 patients with vascular dementia and 49 age-matched controls, the analysis of nutritional status revealed a higher n-6/n-3 ratio in the diet of AD patients and a lower intake of fish and n-3 PUFA in female AD patients, compared to controls (Otsuka et al., 2002).

Although consumption of fish or n-3 PUFA was associated with better cognitive performance or a lower risk of AD in most studies, this type of study cannot ascertain causality. Indeed, dementia is accompanied by a loss of autonomy in daily routine activities that can lead to malnutrition as the patients cannot feed themselves properly or even forget to eat. Moreover, other lifestyle factors including education, exercise, culture, social and economic environment and other nutrition factors may also be linked to the fish or n-3 PUFA intake. Hence, the value of epidemiological studies in understanding the link between intake of fish or DHA and the risk of cognitive decline due to AD depends a lot on the design of the studies.

#### 1.3.1.2 Blood DHA

Since the risk of AD may be associated with low DHA or fish intake, and because of the positive association between blood levels of DHA and intake of DHA or fish (Arterburn et al., 2006; Cao et al., 2006; Harris et al., 2007; Meyer et al., 2007; Philibert et al., 2006; Schaefer et al., 2006), the levels of DHA in the blood is expected to correlate with the risk of AD. Indeed, several studies have found that low blood levels of DHA are associated with an increased risk of developing the disease and AD patients have lower levels of DHA in their blood compared to controls (Conquer et al., 2000; Kyle et al., 1999; Schaefer et al., 2006; Tully et al., 2003). However, the reliability of these observations remains a matter of concern. Other studies have not shown this relationship. One study showed a direct relationship between plasma levels of n-3 PUFA and dementia with increased levels of DHA in plasma phospholipids of demented participants (Laurin et al., 2003) and recent studies found no significant associations between total n-3 PUFA or DHA in plasma or erythrocyte membranes and AD (Kröger et al., 2009; Samieri et al., 2008). The studies relating AD with levels of DHA in blood are presented in greater detail in the introduction of Chapter 4.

### 1.3.1.3 Brain DHA

If the risk of AD is increased with low DHA intake and low DHA levels in blood, it may also be associated with low levels of DHA in the brain of AD patients. As described in the following examples, a number of studies have investigated the association between AD and omega-3 fatty acids or DHA levels in *post-mortem* autopsy samples of human brain tissue. The evidence indicates there are changes in a major brain phospholipid, phosphatidylethanolamine (PE), which is one of the main DHA-containing phospholipids. PE total fatty acids are significantly decreased in the parahippocampus gyrus and the inferior parietal lobule of patients with AD compared to controls (Prasad et al., 1998). The levels of DHA are significantly decreased in PE from the frontal gray matter, the frontal white matter, the hippocampus and the pons (Prasad et al., 1998; Soderberg et al., 1991) and from the parahippocampus gyrus (Prasad et al., 1998) of AD patients. In another *post-mortem* analysis, PE molecular species were analysed by mass spectrometry in different brain regions (Han et al., 2001). The level of total PE was significantly lower in the cortex from AD subjects than controls, which was mainly caused by lower levels of plasmalogen PE, including DHA-containing species such as 18:0/22:6 and 18:1/22:6 and other molecular species such as 18:1/18:1, 16:0/22:4 or 18:0/20:4 and 18:0/22:4. In the other major brain phospholipid, phosphatidylcholine (PC), the relative amount of DHA was much lower than in PE and was only significantly decreased in the frontal gray matter of AD patients (Prasad et al., 1998; Soderberg et al., 1991) and in the cerebellum (Prasad et al., 1998). The fatty acid analysis of cardiolipin, which represents 1-3% of total phospholipids in brain, also revealed a significant decrease of DHA in temporal cortex from AD cases, and this was the only fatty acid for which a significant change was observed (Guan et al., 1994). Moreover, levels of unesterified DHA and neuroprotectin D1 (NPD1), a metabolite of DHA were analysed by ESI-MS-MS in the CA1 hippocampal region, the superior temporal lobe, the thalamus and the occipital cortex of 6 AD subjects and 6 controls. The levels of DHA and NPD1 were significantly reduced by about half and one twentieth, respectively, in the CA1 and the superior temporal lobe of AD subjects compared to controls, but not in thalamus and occipital cortex (Lukiw et al., 2005). However, two additional investigations have not reported significant changes in DHA concentrations in the grey and white matter of the frontal, parietal and parahippocampal regions of 15 AD patients; except for a

higher proportion of DHA in AD parietal white matter (Skinner et al., 1993) or in the parahippocampal cortex of 8 AD patients (Corrigan et al., 1998). The studies relating AD and levels of DHA in brain are presented in greater detail in the introduction of Chapter 5.

In the context of the study, these findings suggest that alteration in DHA levels in blood and brain may occur with AD, and that dietary DHA has a beneficial impact on the development of the disease. Therefore, such changes may also be observed in the mice used in this study.

#### 1.3.1.4 DHA supplementation

If the risk of AD correlates to low DHA intake and low levels of DHA in the brain, it is plausible that DHA supplementation may have a beneficial effect by compensating for the lack of DHA. Although there are no published reports of brain DHA levels in humans after DHA supplementation, there is evidence that DHA supplementation increases brain levels of DHA in monkeys (Connor et al., 1990), in rats (Gamoh et al., 1999; Marteinsdottir et al., 1998) and in mice (Carrie et al., 2000; Lim and Suzuki, 2000; Suzuki et al., 1998). A number of clinical trials have been carried out and despite promising findings from epidemiological studies (reviewed in sections 1.3.1.1, 1.3.1.2 and 1.3.1.3), very little or no beneficial effects of omega-3 supplementation were observed.

To date, only a single clinical trial has focussed on the prevention of cognitive decline (Van De Rest et al., 2008). In this study, 302 cognitively healthy participants aged 65 years or older were randomly assigned to 1,800 mg per day EPA + DHA, 400 mg per day EPA + DHA, or placebo capsules for 26 weeks. Although plasma concentrations of EPA + DHA increased by 238% in the high-dose and 51% in the low-dose fish oil group compared with placebo, there were no significant changes in cognitive function for either low-dose or high-dose fish oil supplementation.

Other studies have focussed on patients suffering from AD or other types of dementia. A clinical trial carried out on 174 AD patients receiving acetylcholine esterase inhibitor treatment, a daily intake of 1.7 g DHA and 0.6 g EPA compared to a corn oil control reduced cognitive decline significantly after 6 months of treatment. However, this effect was limited to only those with very mild cognitive dysfunction

(Freund-Levi et al., 2006). In another trial carried out on 39 participants, including 8 with AD, cognitive function was evaluated before and 90 days after the start of supplementation with 240 mg per day arachidonic acid (AA) + DHA or 240 mg per day olive oil as a control. Once again this study showed no significant beneficial effects of AA + DHA supplementation (Kotani et al., 2006). In another study that included 35 participants with either mild to moderate AD or mild cognitive impairment, 1080 mg EPA + 720 mg DHA per day (versus 1800 mg per day olive oil as placebo), led to improvements over the 24 week follow-up, on the Clinician's Interview-Based Impression of Change Scale. However, improvements in ADA-cog test were only observed in participants with mild cognitive impairment ($p = 0.03$) (Chiu et al., 2008).

These clinical studies highlight several challenges associated with their design. In all four of the clinical trials, DHA supplementation was associated with supplementation of another potentially active fatty acid, EPA or AA. So the question as to whether the effect is due to DHA alone, EPA, AA or the combination of DHA and EPA or AA remains unanswered. Questions about the most effective dose and the length of the treatment also arise, with doses ranging between 1.8 g to 2.4 g of DHA + EPA or AA per day and treatment length ranging between 3 and 6.5 months. Furthermore, cognitive status of the participants at the start of the study differs from one study to another and the treatment may interact with severity of cognitive dysfunction, as it seemed more successful in mildly-affected patients. Moreover, cognitive performance was assessed using different tests in different studies and DHA may only have a greater influence on some cognitive functions than others.

### 1.3.2 Studies based on animal models

More recently animal models, including rats and mice, have been used to study the effect of dietary omega-3 PUFA supplementation on the Aβ pathology. The advantages of the animal studies include the use of a relatively homogeneous genetic pool and age range, known dietary and environmental history, etc.

Hashimoto et al. (2002) gave rats 300 mg dietary DHA per kg of body weight per day for 12 weeks prior to Aβ1-40 injection into the cerebral ventricle. DHA had a beneficial effect on avoidance learning performance and it was suggested that the mechanism of action of DHA was via its anti-oxidative properties (Hashimoto et al.,

2002). In other studies by this group, decreased levels of Aβ1-40 and cholesterol in the cortex lipid rafts fraction were observed in DHA-fed rats and were also associated with improved performance in an eight-arm radial-maze task. This group also reported increased DHA/AA ratio and levels of DHA in cortex and hippocampus as well as an increased fluidity of synaptosomal membranes (Hashimoto et al., 2005a; Hashimoto et al., 2006). In a recent study, where DHA was replaced by EPA, similar results were observed with significantly improved performance in an eight-arm radial maze task, increased levels of EPA and DHA in cortex, hippocampus and plasma, decreased oxidative stress and altered expression of diverse range of genes (Hashimoto et al., 2008).

Several APP over-expressing mouse models have been used to study the effect of dietary DHA on Aβ pathology. These mouse models include the Tg2576 mouse expressing a mutant of APP, the APPswe/PS1dE6 mouse expressing mutants of APP and PS1, and the 3xTg-AD mouse expressing mutants of APP, PS1 and microtubule-associated protein tau (MAPT). A description of the mouse models will be further developed in Chapter 2.

In a study based on the Tg2576 mouse model, 17 month-old WT and Tg mice were fed with a control diet, a safflower oil-based n-3 PUFA depleted diet or a safflower oil-based n-3 PUFA depleted + 0.6% DHA diet (Calon et al., 2004). After 4-5 months of diets, Tg mice on the n-3 PUFA depleted diet were significantly impaired relative to Tg mice on the DHA supplement, in a spatial water maze hidden platform task (latency, $p < 0.005$) but not in a visible platform version of the task. After about 103 days of diets, DHA level was significantly decreased in the frontal cortex of n-3 PUFA depleted Tg mice compared to WT mice on the same diet ($p < 0.05$) and Tg mice on the control diet ($p < 0.01$). By adding DHA to the n-3 PUFA depleted diet, cortical levels of AA were significantly decreased ($p < 0.01$) while cortical levels of DHA were significantly increased ($p < 0.01$), compared with the mice on n-3 PUFA depleted diet. These effects were accompanied by exacerbated dendritic and synaptic pathology in DHA-depleted Tg mice, while DHA supplementation showed anti-apoptotic properties. Furthermore, Calon et al. (2005) showed that dietary DHA supplementation protected Tg mice from the loss of NMDA receptor subunits (Calon et al., 2005).

In 22.5 month-old female and male Tg2576 mice fed with special diets from the age of 17-19 months, immunochemical analysis using an Aβ1-13 antibody

revealed that plaque burden and number were reduced by around 40% with high DHA diet (0.6% DHA) compared to low DHA diet (0% DHA) (Lim et al., 2005). Aβ ELISA analysis on the cortex also showed a reduction of total insoluble Aβ levels with the high DHA diet compared to the low DHA diet and the control diet (0.09% DHA). Aβ1-42 was significantly reduced with the DHA-rich diet compared to the remaining diets. Aβ1-40 was significantly reduced with the high DHA diet compared to the low DHA diet but not compared to the control diet. The high DHA diet also did not reduce the level of total soluble Aβ in cortex, compared to the two other diets (Lim et al., 2005). It was also shown that, in the cortex of low DHA-fed Tg mice, cytosolic APP was significantly increased, while membrane-bound APP, βCTF and αCTP levels were significantly decreased compared to control and high DHA Tg mice. These findings suggest that DHA decreased APP processing in Tg2576 mice. As the expression of BACE1 was not significantly affected by DHA treatment, DHA may have had an effect on APP trafficking and secretase activity rather than an effect on BACE expression *per se* (Lim et al., 2005).

The mechanisms by which DHA supplementation reduces the accumulation of Aβ were further investigated in a more recent study from the same group that focussed on the expression of LR11. LR11 is a neuronal sorting protein that reduces APP trafficking to secretases that generate Aβ. Seventeen month-old Tg2576 mice were fed with a control diet, a safflower oil-based n-3 PUFA-depleted diet or a safflower oil-based n-3 PUFA-depleted + 0.6% DHA. After about 103 days of diets, it was shown that n-3 PUFA-depleted diet significantly decreased LR11 levels in brain compared to the control diet and the decrease was prevented by DHA. These results indicate that DHA can reduce the accumulation of Aβ by up-regulating LR11 (Ma et al., 2007).

In 9 month-old APPswe/PS1dE6 female mice fed with test diets from 6 months of age, the hippocampal level of total Aβ1-42 was significantly reduced with a fish oil supplemented diet (with a n-6/n-3 ratio of 1.4) compared to soy oil or corn oil-supplemented diets (with respective n-6/n-3 ratios of 8 and 70) (Oksman et al., 2006). However, no dietary effect was found on the average number of amyloid plaques in the hippocampus. In the same study, 10 month-old APPswe/PS1dE6 male mice were fed with a soy oil-supplemented diet (with a n-6/n-3 ratio of 8), a "lipid neutral diet" (with a n-6/n-3 ratio of 23), a "typical Western diet" (with a n-6/n-3 ratio of 23) or a DHA-enriched diet (with a n-6/n-3 ratio of 3 and 0.5% DHA) from 6

months of age. The DHA group had lower levels of hippocampal total Aβ1-40 and total Aβ1-42 than the "typical Western diet" group. In addition, these mice had a decreased membrane n-6/n-3 ratio in the cerebellum compared to the other diet groups and significantly decreased plasma cholesterol levels relative to the "lipid neutral diet" and the "typical Western diet" groups. However, the average plaque load did not differ between the diet groups and there was no significant effect of the diets on performance in the Morris water maze (all $p > 0.3$) (Oksman et al., 2006).

In another study using the same animal model, the mice received a "typical Western diet", a DHA diet (0.4% DHA) or a standard diet from the age of 6 months. At 18 months of age, immunohistochemical analysis of the mice brain using an anti-human Aβ4-10 antibody showed a significant regional variation of Aβ plaque load with a high Aβ plaque load in dentate gyrus where it was significantly reduced with the standard diet but not with the DHA diet compared to the "typical Western diet" (Hooijmans et al., 2007). It was also shown that the relative cerebral blood volume was increased with high DHA diet, with no effect on blood flow. DHA and AA levels in brain phospholipids were not significantly affected in Tg mice compared to WT mice on the standard diet. However, the DHA diet increased significantly the level of DHA and decreased significantly the level of AA in brain phospholipids of Tg mice compared to Tg mice on the standard diet.

In a follow-up study from the same lab, the mice received the experimental diets from the age of 2 months. DHA levels were significantly increased and AA levels were significantly decreased in the brain of mice that received the DHA diet compared to mice on the standard diet or the "typical Western diet". At 15 months of age, the DHA-containing diet decreased Aβ deposition in the cingulate gyrus and the amount of vascular Aβ, and improved spatial memory in the Morris water maze task, compared to the standard diet group. However, at 8 months of age, no dietary effects were found on Aβ deposition or performance in the Morris water maze task. In addition, no dietary effects were found at either age in the reversal learning in water maze or the 12 circular hole board (Hooijmans et al., 2009).

3xTg-AD mice were fed with different DHA containing diets (n-6/n-3 = 1:1, (1) DHA, (2) DHA-DPA, (3) DHA-ARA) or a control diet (n-6/n-3 = 10:1) from the age of 3 months. Fatty acid analysis of whole brain and red blood cells as well as brain PC, PE and PS showed increased levels of DHA and simultaneous decreased levels of AA with increased dietary DHA. Aβ levels in brain were then analysed by

ELISA using anti-Aβ35-40 and anti-Aβ35-42 antibodies, and by immunohystochemistry using an anti-Aβ antibody. At 6 and 9 months of age, DHA-containing diets significantly reduced the levels of soluble Aβ1-40 and Aβ1-42 in whole brain and more specifically in hippocampus and amygdala but the levels of insoluble Aβ1-40 or Aβ1-42 were not changed. At the age of 12 months, only the DHA diet (1) reduced soluble Aβ1-40 levels compared to the control. Dietary DHA appeared to reduce levels of soluble Aβ by reducing presenilin 1 (PS1) expression but did not affect APP expression, processing of APP by α- or β-secretase or Aβ degradation (Green et al., 2007). DHA also reduced the accumulation of tau and phosphorylated tau in the brain.

Despite the beneficial effects of dietary DHA seen in these studies, a recent study showed no effect of dietary DHA on the APPswe/PS1dE6 mouse model of AD (Arendash et al., 2007). Two month-old APPswe/PS1dE6 and WT littermates were fed a high omega-3 or a standard diet. At 6-9 months of age, a battery of behavioural tests, including Y-maze alternation and water maze tasks, showed no effect of high omega-3 diet on performance in the transgenic mice. Along with these results, the diets did not have a significant effect on the levels of soluble and insoluble Aβ1-40 and Aβ1-42 in the hippocampus of the Tg mice nor the percentage of saturated and monounsaturated fatty acids in the frontal cortex of Tg and WT mice. The high omega-3 diet only increased the levels of n-3 PUFA in the frontal cortex of WT mice ($p < 0.01$) but not in Tg mice and decreased levels of n-6 PUFA were found in both Tg and WT frontal cortex. The high omega-3 diet also increased the level of DHA by 92% and decreased the level of AA by 10% in the frontal cortex compared to the standard diet in WT mice but did not have a significant effect on the level of these two fatty acids in the Tg mice.

Although most studies on rodent models of the Alzheimer's Aβ pathology suggest that dietary DHA can alleviate cognitive impairments caused by the pathology and reduce the accumulation of Aβ, some studies showed no effect and mechanisms by which DHA may affect the pathology still need to be clarified.

# CHAPTER 2

# Rationale for the study of dietary docosahexaenoic acid in the Tg2576 mouse model of Alzheimer's β-amyloid pathology

The objective of this chapter is to explain the context and the aims of the study, as well as the design of the experimental work.

The first section sets the aims of the study in the context of current knowledge. The Tg2576 transgenic mouse model used for the experiments and the genotyping method are presented in the second and third sections. The set up and organisation of the mouse cohorts used in the study are presented in the fourth section. Finally, the nature of the experimental diets, the oil blend diet and the DHA diet, and their fatty acid composition as well as information on mouse body weight and diet consumption are presented in the last two sections of this chapter, before the discussion.

## 2.1 Aims of the study

### 2.1.1 Focus on β-amyloid pathology and dietary DHA

The β-amyloid pathology is unique to AD and Down's syndrome and, therefore, a prime target for therapeutic intervention. In Down's syndrome, the accumulation of Aβ is attributed to an excess expression of APP due to the extra copy of chromosome 21 (Folin et al., 2003; Robakis et al., 1987). Although, the reason for the accumulation Aβ in LOAD is unknown, neurofibrillary tangles appear in both syndromes and other neurodegenerative diseases. Another key argument of the amyloid cascade hypothesis is that mutations of APP, PS1 and PS2, associated with EOAD, enhance the production of Aβ. Therefore, according to the amyloid cascade

hypothesis, agents capable of reducing the production of Aβ should be useful for the treatment for AD. It has been shown that dietary DHA may reduce the production of Aβ by affecting the processing of APP via the non-amyloidogenic pathway (Green et al., 2007; Lim et al., 2005; Ma et al., 2007; Sahlin et al., 2007). Furthermore, the accumulation of Aβ in the brain of patients with AD activates a neuroinflammatory reaction along with the synthesis of pro-inflammatory prostaglandins and cytokines that may also be reduced by DHA supplementation. Finally, DHA may also improve the clearance of Aβ by affecting degradative enzymes or protein scavengers, such as transthyretin (TTR) (Puskas et al., 2003). This evidence leads to the hypothesis that dietary DHA should attenuate Aβ pathogenesis and associated inflammatory and cognitive abnormalities.

## 2.1.2 Need for further investigations

### 2.1.2.1 The choice for experimenting on an animal model

Published work on the role of omega-3 fatty acids or DHA in Alzheimer's disease and the β-amyloid pathology was presented in the General introduction. Although most of these studies, carried out on human, animal models or cell cultures, suggest that dietary DHA may have an impact on the development of AD, the mixed outcomes of the studies demonstrate that further investigation is required to elucidate the effects of dietary DHA supplementation on the β-amyloid pathology and its mechanism of action. The aim of the experiments reported in this thesis is to test the hypothesis that dietary supplementation influences the development of the Aβ pathology in a transgenic mouse model expressing an EOAD human genetic mutation.

The benefits of studying the effects of DHA supplementation in a rodent model of AD stem from several difficulties in assessing human epidemiological studies. These studies are frequently based on questionnaires in which the intake of omega-3 fatty acids or DHA is indirectly estimated. The species of fish consumed (Philibert et al., 2006; Weaver et al., 2008) as well as the cooking methods (Gladyshev et al., 2006), for example, can affect the omega-3 fatty acid content and these factors are not necessarily taken into account. The analysis of blood fatty acids

may be a better quantitative method but it does not directly quantify levels of omega-3 fatty acid intake (even if an increased intake of omega-3 fatty acids may increase omega-3 fatty acid levels in blood). Epidemiological studies therefore cannot represent a definitive assessment of the effect of dietary omega-3 fatty acids on the development of AD.

The other major difficulty with human studies is the heterogeneity of the sample population, including variations in genetic background, life style factors including substance abuse and particularly nutrition. These difficulties with human studies point out the necessity of animal studies where the parameters such as diet, intake and source of omega-3 fatty acids, genotype and environment can be controlled and therefore, allow a more controlled assessment of the effect of dietary DHA on Alzheimer's Aβ pathology. An alternative approach to whole animal studies is cell cultures procedures. However, this approach has the disadvantage that the compound tested, i.e. DHA, does not go through the digestive system. In addition, animal models of AD offer the possibility of studying early pathological changes that are not accessible in human AD patients. Moreover, animal models, such as rodents, can be used in behavioural experiments that can provide information on the cognitive benefits derived from this intervention approach. Therefore, a study using a rodent model of AD pathology is the most appropriate method to test the hypothesis under consideration.

### 2.1.2.2 The choice of experimental diets

Although animal studies may offer the benefit of a relatively uniform genetic and environmental background, the choice of the experimental conditions and control conditions remain complex and critical for the interpretation of the outcome of the study. This is illustrated by the range of approaches used in the animal literature, including variations in DHA supplementation or depletion and the choice of control diet.

In some studies, the effect of an n-3 PUFA-rich diet was compared to a control diet such as high n-6 PUFA diets or n-3 PUFA-depleted diets (Calon et al., 2005; Hooijmans et al., 2007; Hooijmans et al., 2009; Oksman et al., 2006). The risk with this kind of experimental design is the comparison with a control that does not represent the standard population diet. Moreover, at the opposite of n-3 PUFA, n-6

PUFA are known to have a pro-inflammatory effect. Therefore, this kind of study may show harmful effects of n-6 PUFA rich or n-3 PUFA depleted diets, rather than the benefit of n-3 PUFA supplementation. In the study by Arendash et al. (2007), the high DHA diet was also a high fat and high n-6 PUFA diet compared to the control diet. The risk in this study is that high content in fat and n-6 PUFA of the high DHA diet may overshadow the benefit of DHA supplementation. Indeed, it was found that the high DHA diet did not have a significant effect on cognitive performance, on cortical fatty acids levels or on levels of soluble and insoluble Aβ in the hippocampus of APPswe/PS1dE6 mice. In regard to the disparity between experimental diets used in published work, the choice of experimental diets for the present study appeared a central issue, in addition to the choice of animal model.

Due to all the discrepancies between study outcomes and experimental design issues, it was clear that further studies to investigate the effect of dietary DHA on the Aβ pathology are needed, and experimental diets and animal model have to be chosen carefully to address the question in rigorous scientific manner.

## 2.2 The Tg2576 mouse model of β-amyloid pathology

Different animal models have been developed to study the aetiology and evolution of the disease, and potential therapies for AD. Transgenic mouse models reproducing the Aβ pathology have mainly been generated with a mutated human APP gene. Several mouse models of β-amyloid pathology have been produced and it is beyond the scope of this thesis to review these models in detail. In brief, however, the PDAPP mouse expressing a human APP with the Indiana mutation $APP_{V717F}$ was the first transgenic mouse presenting with Aβ pathology (Games et al., 1995). The Tg2576 mouse was generated shortly after (Hsiao et al., 1996) and then, other APP transgenic mice including the APP23 mouse and the TgCRND8 mouse were also developed (McGowan et al., 2006). Transgenic mice carrying a mutant APP in combination with one or two other transgenes such as a mutant presenilin 1 (PS1) or a mutant microtubule-associated protein tau (MAPT) (McGowan et al., 2006) have also been recently produced.

The Tg2576 mouse, which is perhaps one of the most commonly used APP models in AD research, was chosen for the present series of experiments. The Tg2576 mouse model of AD was developed in 1996 by Karen Hsiao and colleagues at the University of Minnesota (Hsiao et al., 1996). Tg2576 mice carry a transgene coding for a mutated 695 amino acid isoform of the human APP ($HuAPP_{695}$. K670N-M671L). As the mutation was found in a Swedish family, it is also known as the Swedish mutation (APPswe). It is a double mutation occurring on the amino acids 670 (Lys-Asn) and 671 (Met-Leu). The gene of the human $APP_{695}$ containing the double mutation was inserted into a hybrid background of C57BL/6 × SJL mice using a hamster prion protein cosmid vector. The resultant transgenic mice express the APP mutant under the control of the hamster prion protein (PrP) promoter. To produce the animal used in the experiments, heterozygous male mice expressing the Swedish double mutation in the hybrid background of C57BL/6 × SJL were mated with female C57BL/6 × SJL F1.

The Swedish mutation is known to cause an increased production of both Aβ1-40 and Aβ1-42 (Cai et al., 1993; Citron et al., 1994; Scheuner et al., 1996). Previous work with Tg2576 mice has shown that these mice display both types of Aβ

with an age-dependent Aβ plaque deposition (Harigaya et al., 2006; Irizarry et al., 1997; Kawarabayashi et al., 2001). Changes begin at 6-7 months of age. Between 6 and 10 months of age, when SDS insoluble Aβ1-40 and Aβ1-42 are easily detected in every animal, histopathology is minimal; only isolated Aβ plaques can be identified. By 12 months of age, diffuse plaques are evident, and from 12 to 23 months, Aβ plaques increase to levels such as those observed in human AD brains. In addition to the development of Aβ histopathology, Tg2576 mice also develop age-dependent behavioural deficits in different tasks such as Y-maze, T-maze and Morris water maze procedures (Chapman et al., 1999; Hsiao et al., 1996; Lalonde et al., 2003; Westerman et al., 2002). Similar to AD patients, they also exhibit signs of neuroinflammation evidenced by high numbers of plaque-associated microglia and astrocytes as well as expression of inflammatory cytokines, including interleukin-1β, tumor necrosis factor-α and interleukin-6 (Benzing et al., 1999; Frautschy et al., 1998; Irizarry et al., 1997; Mehlhorn et al., 2000), oxidative stress (Pappolla et al., 1998; Smith et al., 1998), neuronal abnormalities (Chapman et al., 1999; Irizarry et al., 1997) and tau phosphorylation (Kawarabayashi et al., 2004). As the Tg2576 mouse model shows many features of the human AD pathology related to amyloid-β production, it therefore provides an opportunity to study the effect of drug treatments on the Aβ pathology in isolation. However, Tg2576 mice do not develop neurofibrillary tangles or neuronal loss like human AD patients (Irizarry et al., 1997). Although, this may be a drawback of the model, the absence of these pathological features may also help to clarify the effects of manipulations specifically on of Aβ pathology, without the added complication provided by, for example, the incorporation of tau mutations.

More detailed information on Aβ pathology and cognitive performance in the Tg2576 mouse model will be given, as needed, in the different experimental chapters.

The following section describes the procedures for breeding and genotyping of the transgenic mice, as well as detail of the experimental cohorts. In addition, an analysis of the lipid content of the diets used in the experimental procedures and an analysis of the effects of the diets on the body weight and food consumption of Tg and WT mice are also provided.

## 2.3 Genotyping

### 2.3.1 Introduction

Tg2576 mice and WT littermates are bred by crossing wild-type C57B6/SJL F1 females (WT) to Tg2576 heterozygous males (Tg) that carry the transgene, so the offspring is a mixture of Tg and WT mice. In order to determine whether a mouse is WT or Tg, genotyping has to be carried out. Genotyping was done for each mouse used in the experiments.

The genotyping is based on the deoxyribonucleic acid (DNA) construction of the transgene (Hsiao et al., 1995). The PrP-HuAPP$_{695}$.SWE transgene was generated by inserting human APP open reading frame (ORF) into a hamster prion protein (PrP) cosmid vector and by mutating the insert. To detect PrP-HuAPP$_{695}$.SWE fusion DNA, it was amplified using PCR with a pair of oligomer primers, 1503 and 1502, located in the 3' region of APP and the 3' untranslated region of PrP, respectively. The oligomer primer 1501 was used to amplify a fragment of murine PrP with the oligomer primer 1502. This second reaction was performed as a positive control.

### 2.3.2 Materials and methods

#### 2.3.2.1 Tissue preparation

Tail biopsies were taken from the mice and stored at -20°C until use. Tail biopsies were defrosted, 600 µl TES cell lysis buffer (10 mM Tris pH 8.0, 5 mM EDTA pH 8.0, 0.1% (w/v) SDS) was added and samples were placed at 55°C for 20 min. 1.8 µl of 25 mg/ml Proteinase K (catalogue number P2308, Sigma) was added and mixed by inverting the tubes. Then, samples were incubated overnight at 55°C.

### 2.3.2.2 Deoxyribonucleic acid extraction

Samples were mixed vigorously, in order to solubilise as much tissue as possible. 200 µl 5M ammonium acetate was added to each sample and mixed vigorously before centrifugation for 10 min. at 14,000 r.p.m., in order to pellet proteins. 650 µl of supernatant was transferred into 600 µl molecular biology grade isopropanol and mixed by inversion. Samples were centrifuged for 2 min. at 14,000 r.p.m. in order to pellet DNA. Supernatant was discarded, 150 µl ice cold 70% (v/v) molecular biology grade ethanol was added and samples were centrifuged for 2 min. at 14,000 r.p.m.. Supernatant was discarded and DNA was washed a second time using 150 µl ice cold 70% molecular biology grade ethanol. The supernatant was discarded, ethanol was evaporated and DNA resuspended in 60 µl TE buffer (10 mM Tris chloride pH 8.0, 1 mM EDTA pH 8.0). Samples were incubated overnight at 55°C and stored at -20°C until use.

### 2.3.2.3 Amplification of deoxyribonucleic acid by polymerase chain reaction

The polymerase chain reaction was prepared using reagents from Applied Biosystems, Foster City, CA, USA: 1.2 µl PCR buffer 10×, 1.2 µl $MgCl_2$ 25 mM, 1.25 µl dNTP 2.5 mM, 7.35 µl MilliQ$^{TM}$ water (Millipore, Bedford, MA, USA), 0.25 µl Primer 1501 (50 pM/µl), 0.25 µl Primer 1502 (10 pM/µl), 0.25 µl Primer 1503 (10 pM/µl), 0.25 µl Taq polymerase and 1 µl DNA sample.

The PCR reactions were set up in two segments as follows, with segment 1 repeated in 35 cycles:

- Segment 1, 35 cycles: 1 min. at 94°C, 1 min. at 62°C, 2min. at 72°C,
- Segment 2: 4°C until sample collection.

The primers are presented in Table 2.1.

**Table 2.1.** Oligonucleotide primers used for genotyping (Hsiao et al., 1995)

| PCR primer | DNA sequence | Product size (bp) |
|---|---|---|
| 1501 | 5' AAGCGGCCAAAGCCTGGAGGGTGGAACA | 600 bp |
| 1502 | 5' GTGGATAACCCCTCCCCCAGCCTAGACCA | |
| 1503 | 5' CTGACCACTCGACCAGGTTCTGGGTTGAC | 450 bp |

### 2.3.2.4 Deoxyribonucleic acid electrophoresis

A 1.5% (w/v) agarose gel was prepared using 3 g of NuSieve® molecular grade agarose (Flowgen, Ashby de la Zouch, UK) in 200 ml Tris-Acetate-EDTA (TAE) buffer (10 mM Tris base, 0.114% (v/v) glacial acetic acid, 1 mM EDTA) and 10 µl ethidium bromide solution (Sigma, Poole, UK) (10 mg/ml) . The DNA ladder was prepared using 2 µl loading buffer (0.25% Orange G, 15% Ficoll in MilliQ$^{TM}$ water) and 10 µl PCR marker (LMV 50-1,000 bp, Promega G3161, Promega Corporation, WI, USA). The samples were prepared by adding 2 µl Orange G to each 13 µl PCR product. 10 µl of DNA ladder and 8 µl of each sample were loaded on the gel. The gel was run at 100 V in TAE buffer for 20 min. and observed under a UV light.

## 2.3.3 Results

Figure 2.1 presents an example of result obtained for the genotyping of seven mice. As WT mice do not carry the PrP-HuAPP$_{695}$.SWE trangene, the electrophoresis of DNA amplified by the oligomer primers 1501,1502, 1503 will show a single band corresponding to the amplification of the mouse prion protein gene by the 1501 and 1502 oligomer primers. In addition to the band observed for the WT mice, the electrophoresis of the amplified DNA from Tg mice by the same oligomer primers will show a second band corresponding to the PrP-HuAPP$_{695}$.SWE transgene, product of primers 1502 and 1503.

According to the bands obtained after PCR and electrophoresis presented in Figure 2.1, the mice corresponding to the PCR reactions in lanes 2, 4, 6, 7 and 8 were Tg mice, whereas mice corresponding to the PCR reactions in lanes 3 and 5 were WT mice.

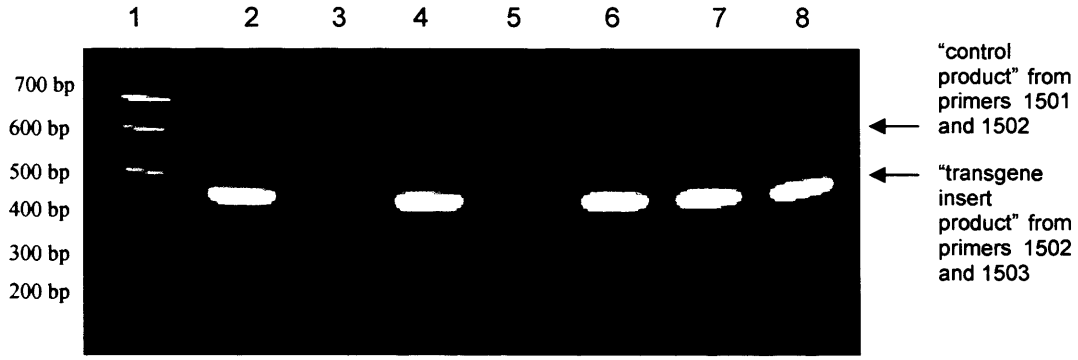


**Figure 2.1.** Electrophoresis of the mouse DNA amplified by PCR using 1501, 1502 and 1503 oligonucleotide primers. Lane 1: 10 µl of 1kb DNA ladder, lanes 2 to 8: 7 µl of PCR reaction, WT in lanes 3, 5, Tg in lanes 2, 4, 6-8.

## 2.4 Organisation of the mouse experimental cohorts

All the experiments, including behavioural experiments and biochemical analysis, were performed in full compliance with the United Kingdom Home Office guidelines, on Tg(HuAPP$_{695}$.K670N-M671L)2576 mice (Tg) and wild type littermates (WT). Mice were generated and established in accordance with standard procedures by mating Tg2576 males with hybrid strain C57B6/SJL F1 females, in order to compare Tg mice with the WT littermates controls of the same age and background strains.

Mice were housed in a temperature-controlled environment on a 12 hour light/12 hour dark cycle. Pups were weaned at 5-6 weeks of age and housed in same sex mixed genotype littermates groups of 2 to 4 mice per cage for the females and individually or in pairs for the males. Animals received *ad libitum* access to standard laboratory rodent chow and water during the first four months of their lives. Then, four month-old males and female Tg and WT mice were randomly divided in two treatment groups. Mice received either the DHA-enriched diet or the oil blend control diet, both manufactured by SDS (Special Diet Services, Essex, UK), from the age of four months. The experimental diets were given to young mice, as early as four months of age, in order to test whether dietary DHA supplementation before the appearance of Aβ plaques could prevent cognitive impairments and the development of the Aβ pathology.

Three cohorts of mice were used for the entire study:

- Cohort 1: The first cohort was composed of males and females. Some of these mice performed the foraging task presented in Chapter 3. Then, the whole cohort was sacrificed at 12 months of age, corresponding to the first time point of biochemical analysis. Blood samples were collected for plasma fatty acid analysis (Chapter 4) and brain tissue was collected for lipid analysis (Chapter 5) and Aβ analysis using ELISA (Chapter 6).
- Cohort 2: The second cohort was composed of males only. These mice performed the T-maze task at 8 months, 12 months and 16 months of age (Chapter 3). Then, the whole cohort was sacrificed at 16 months of

age, corresponding to the second time point of biochemical analysis. Blood samples were collected for plasma fatty acid analysis (Chapter 4) and brain tissue was collected for lipid analysis (Chapter 5) and Aβ analysis using ELISA (Chapter 6).

- Cohort 3: The third cohort composed of females only and was only used for biochemical analysis. The mice were sacrificed at 21 months of age. Blood samples were collected for plasma fatty acid analysis (Chapter 4) and brain tissue was collected for biochemical analysis of Aβ using ELISA and immunohistochemistry (Chapter 6).

## 2.5 The experimental mouse diets

### 2.5.1 Introduction

As previously discussed in section 2.1.2.2, the choice of the experimental diets, the DHA-enriched diet and the control diet, can be a crucial element of the study. In order to study the effects of dietary DHA supplementation, the only difference between the DHA-enriched diet and the control diet should be the content in DHA. Moreover, the fat content also had to be maintained equal in the two diets in order to be consistent. Therefore, it was decided to supplement a normal rodent chow with 5% of a DHA-rich oil for the "DHA diet", or 5% of an oil blend for the "oil blend diet" so that the DHA-rich oil was replaced by a blend of fat typical of an average U.K. diet. That way the fat content of the two diets was equal and DHA was mainly replaced by saturated or monounsaturated fatty acids which would not be expected to be either pro- or anti-inflammatory.

The food pellets, manufactured by SDS (Special Diet Services, Essex, UK), were made from a normal rodent chow containing sufficient amounts of n-3 and n-6 PUFA to satisfy normal mouse requirements for essential fatty acids. This chow was supplemented with either 5% of a DHA-rich oil (DHASCO, Martek Biosciences Corporation, Columbia, MD, USA) or 5% of an oil blend control (lard, palm oil, olive oil and coconut oil, 3:3:3:1 by weight). According to the manufacturer's details these pellets contained approximately 12.8% crude protein, 7.2% crude oil, 3.9% crude fibre and 5.6% ash and had roughly the same caloric value, 3409.10 kcal/kg for the DHA diet and 3407.08 kcal/kg for the oil blend diet. The fatty acid content of the diets is presented in Table 2.2. The main saturated fatty acid was 16:0 and small amounts of 12:0, 14:0 and 18:0 were also present. The major monounsaturated fatty acid was oleic acid (18:1n-9). The n-6 PUFA was mainly 18:2n-6 with small amounts of 20:4-6. EPA was present at less than 0.05% of the fresh diet weight whilst DHA composed 2.25% of the DHA fresh diet weight and less than 0.01% of the oil blend fresh diet weight.

Lipid extractions of the chows and fatty acid analysis by GLC were carried out in order to confirm the fatty acid composition of the diets.

**Table 2.2.** Fatty acid composition of the oil blend and DHA food pellets, information provided by the manufacturer (SDS, Essex, UK) as percentage of fresh diet weight.

| Fatty acids | Rodent chow + 5 % oil blend | Rodent chow + 5 % DHA rich oil |
|---|---|---|
| 12:0 | 0.26 % | 0.10 % |
| 14:0 | 0.24 % | 0.64 % |
| 14:1 | 0.00 % | 0.01 % |
| 16:0 | 1.34 % | 0.87 % |
| 16:1 | 0.08 % | 0.08 % |
| 18:0 | 0.31 % | 0.09 % |
| 18:1n-9 | 2.79 % | 2.04 % |
| 18:2n-6 | 1.13 % | 0.95 % |
| 18:3n-3 | 0.08 % | 0.08 % |
| 20:4n-6 | 0.11 % | 0.12 % |
| 22:5n-3 | 0.00 % | 0.01 % |
| 22:6n-3 | 0.00 % | 2.25 % |
| Total SAT * | 2.15 % | 1.70 % |
| Total MUFA * | 2.87 % | 2.13 % |
| Total PUFA * | 1.32 % | 3.41 % |
| Total n-3 * | 0.08 % | 2.34 % |
| Total n-6 * | 1.24 % | 1.07 % |
| n-3/n-6 ratio * | 0.06 | 2.19 |

* Calculated from the manufacturer's specification data

## 2.5.2 Materials and methods

Diets were stored in a refrigerated room at 4°C from the day of arrival and were analysed three weeks later. Pellets were crushed using a pestle and mortar and 0.5 g was used for lipid extraction. 0.5 ml MilliQ$^{TM}$ water (Millipore, Bedford, MA, USA) and 3.75 ml chloroform-methanol (1:2 by volume) were added and the mixture was sonicated at room temperature for 30 min. Lipids were extracted using the method of Garbus et al. (1963) as described in section 5.2.4 and resuspended in 500 µl of chloroform-methanol (2:1 by volume). 10 µl were used for fatty acid analysis by GLC. For this analysis, 50 µg of pentadecanoic fatty acids (15:0) (Nu-Chek Prep Inc., Elysian, MN, USA) were added as a standard. Then, samples were treated and analysed as described in sections 5.2.6, 5.2.7 and 5.2.8.

## 2.5.3 Results

Lipid extractions were carried out three weeks after arrival of the diets and for each diet, extractions were performed on three diet samples taken from three different bags. Then, all the lipid extracts were analysed by GLC. The results are presented in Table 2.3 and Table 2.4.

**Table 2.3.** Fatty acid composition of the oil blend diet (n = 3) and the DHA diet (n = 3). Results are represented as mean percentage of fresh diet (by weight) ± SEM. N.D., not detected; tr., trace (less than 0.05).

| Fatty acids | Rodent feed + 5% oil blend | Rodent feed + 5% DHA rich oil |
|---|---|---|
| 12:0 | 0.05 ± 0.01 | 0.04 ± 0.01 |
| 14:0 | 0.08 ± tr. | 0.38 ± tr. |
| 16:0 | 1.65 ± 0.07 | 0.86 ± tr. |
| 16:1 | 0.03 ± tr. | 0.08 ± tr. |
| 18:0 | 0.37 ± 0.01 | 0.07 ± tr. |
| 18:1n-9 | 2.87 ± 0.13 | 1.66 ± 0.01 |
| 18:2n-6 | 2.06 ± 0.09 | 1.60 ± 0.02 |
| 18:3n-3 | 0.20 ± 0.01 | 0.17 ± 0.01 |
| 20:0 | 0.01 ± tr. | 0.01 ± tr. |
| 20:1 | 0.02 ± tr. | 0.01 ± tr. |
| 20:2 | 0.01 ± tr. | N.D. |
| 20:4n-6 | N.D. | N.D. |
| 20:3n-3 | N.D. | N.D. |
| 20:5n-3 | N.D. | N.D. |
| 22:0 | 0.01 ± tr. | 0.01 ± tr. |
| 22:5n-3 | N.D. | 0.01 ± tr. |
| 22:6n-3 | N.D. | 1.86 ± 0.04 |
| 24:0 | N.D. | N.D. |
| Total SAT | 2.17 ± 0.08 | 1.37 ± 0.01 |
| Total MUFA | 2.93 ± 0.13 | 1.75 ± 0.01 |
| Total PUFA | 2.27 ± 0.10 | 3.64 ±0.05 |
| Total n-3 | 0.20 ± 0.01 | 2.04 ± 0.03 |
| Total n-6 | 2.06 ± 0.09 | 1.60 ± 0.02 |
| n-3/n-6 ratio | 0.1 ± tr. | 1.3 ± tr. |

**Table 2.4.** Fatty acid composition of the oil blend diet (n = 3) and the DHA diet (n = 3). Results are represented as mean percentage of total fatty acid (by weight) ± SEM. N.D., not detected; tr., trace (less than 0.05).

| Fatty acids | Rodent feed + 5% oil blend | Rodent feed + 5% DHA rich oil |
|---|---|---|
| 12:0 | 0.7 ± 0.1 | 0.7 ± 0.1 |
| 14:0 | 1.1 ± tr. | 5.6 ± 0.1 |
| 16:0 | 22.4 ± tr. | 12.7 ± 0.1 |
| 16:1 | 0.5 ± tr. | 1.1 ± tr. |
| 18:0 | 5.1 ± tr. | 1.0 ± tr. |
| 18:1n-9 | 39.0 ± 0.1 | 24.6 ± tr. |
| 18:2n-6 | 28.0 ± tr. | 23.6 ± 0.1 |
| 18:3n-3 | 2.7 ± tr. | 2.5 ± 0.1 |
| 20:0 | 0.2 ± tr. | 0.1 ± tr. |
| 20:1 | 0.3 ± tr. | 0.2 ± tr. |
| 20:2 | 0.1 ± tr. | N.D. |
| 20:4n-6 | N.D. | N.D. |
| 20:3n-3 | N.D. | N.D. |
| 20:5n-3 | N.D. | N.D. |
| 22:0 | 0.1 ± tr. | 0.2 ± tr. |
| 22:5n-3 | N.D. | 0.1 ± tr. |
| 22:6n-3 | N.D. | 27.5 ± 0.3 |
| 24:0 | N.D. | 0.1 ± tr. |
| Total SAT | 29.4 ± 0.1 | 20.3 ± 0.3 |
| Total MUFA | 39.7 ± 0.1 | 25.9 ± 0.1 |
| Total PUFA | 30.8 ± tr. | 53.9 ± 0.3 |
| Total n-3 | 2.7 ± tr. | 30.2 ± 0.3 |
| Total n-6 | 28.0 ± tr. | 23.6 ± 0.1 |
| n-3/n-6 ratio | 0.1 ± tr. | 1.3 ± tr. |

The fatty acid analysis of the rodent diets showed a similar pattern to the fatty acid composition provided by SDS with 16:0, 14:0 and 18:0 as the main saturated fatty acids, and 18:1 n-9 and 16:1 as the main monounsaturated fatty acids. Regarding the n-6 PUFA, 20:4n-6 was not detected in either of the diets while 18:2n-6 was the main n-6 PUFA, representing about 2.1% of the oil blend diet and about 1.6% of the DHA diet. Although the content in 18:2n-6 was slightly higher in the oil blend diet than in the DHA diet, the difference in n-6 PUFA content between the two diets was more limited than in previous studies (Calon et al., 2005; Green et al., 2007). However, the content of some fatty acids was a little lower than indicated by SDS. DHA represented approximately 1.8% of the DHA diet while no DHA was detected in the oil blend diet. DHA was also the main fatty acid in the DHA diet, with about 27.5% of the total fatty acid weight. In addition, the presence of DHA in the DHA diet was mostly compensated by "neutral" fatty acids, mono-unsaturated and saturated fatty acid.

## 2.6 Records of body weight and diet consumption

### 2.6.1 Introduction

Whether it is a phenotypic marker of AD or a consequence of malnutrition of AD patients, weight loss has been reported to be associated with AD in humans (Nourhashémi and Vellas, 2008). In Tg2576 mice, Lalonde et al. (2003) reported that 15-20 month-old Tg mice had normal body weight compared to control littermates. However, the effect of genotype combined with the two different diets used in our study may have an effect on mouse body weight. Monitoring food consumption may help to detect eating abnormal behaviour caused by the transgene or a particular preference or aversion to the experimental diets. Therefore, data on food consumption and body weight may help to interpret results presented in the following chapters.

### 2.6.2 Materials and methods

#### 2.6.2.1 Body weight

Body weight was measured before sacrificing mice from the three cohorts. The number of mice per group is indicated in Table 2.5.

Analysis of variance (ANOVA) was carried out with genotype, diet, age and gender as factors.

**Table 2.5.** Composition of the three cohorts of mice used to record body weight: Tg2576 and WT mice at 12, 16 and 21 months of age.

| Gender | Male | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|
| Diet | oil | | DHA | | oil | | DHA | |
| Genotype | Tg | WT | Tg | WT | Tg | WT | Tg | WT |
| Cohort 1<br>12 months | 6 | 10 | 6 | 9 | 13 | 14 | 14 | 14 |
| Cohort 2<br>16 months | 9 | 9 | 7 | 10 | - | - | - | - |
| Cohort 3<br>21 months | - | - | - | - | 6 | 5 | 6 | 6 |

### 2.6.2.2 Food consumption

In order to determine the average rate of food consumption per day, food consumption was first examined as a factor of gender and then, the effect of transgene and diet on food consumption was analysed only in male mice. The reason for this is that the male mice were housed in single cages (because of marked aggression in this background strain). Female mice were housed in group cages with mixed genotypes and thus could provide data related only to gender. Food consumption was recorded for the 12 month-old cohort, for 13 weeks between the age of 9 months and 12 months. The 13 measurements of food weight given to each mouse (or group of mice) were added and the weight of remaining food was subtracted. The daily consumption per mouse was calculated for each mouse or as an average when mice were housed together. For some mice, a large amount of food was found shredded at the bottom of the cage, therefore the food consumption was calculated on a reduced number of mice (compared to the number of mice used to record body weight), for the data to be as accurate as possible. The number of mice is indicated in Table 2.6.

**Table 2.6.** Composition of the cohort of mice used to record food consumption: Tg2576 and WT mice at 12 months of age.

| Gender | Male | | | | Female | |
|---|---|---|---|---|---|---|
| Diet | oil | | DHA | | oil | DHA |
| Genotype | Tg | WT | Tg | WT | Tg/WT | Tg/WT |
| Cohort 1<br>12 months | 6 | 7 | 6 | 6 | 8 | 12 |

## 2.6.3 Results

### 2.6.3.1 Body weight

Mouse body weights recorded at 12 months on both males and females, at 16 months on males only and at 21 months on females only are presented in Figure 2.2 as mean value of body weight and corresponding standard error of the mean for each group.

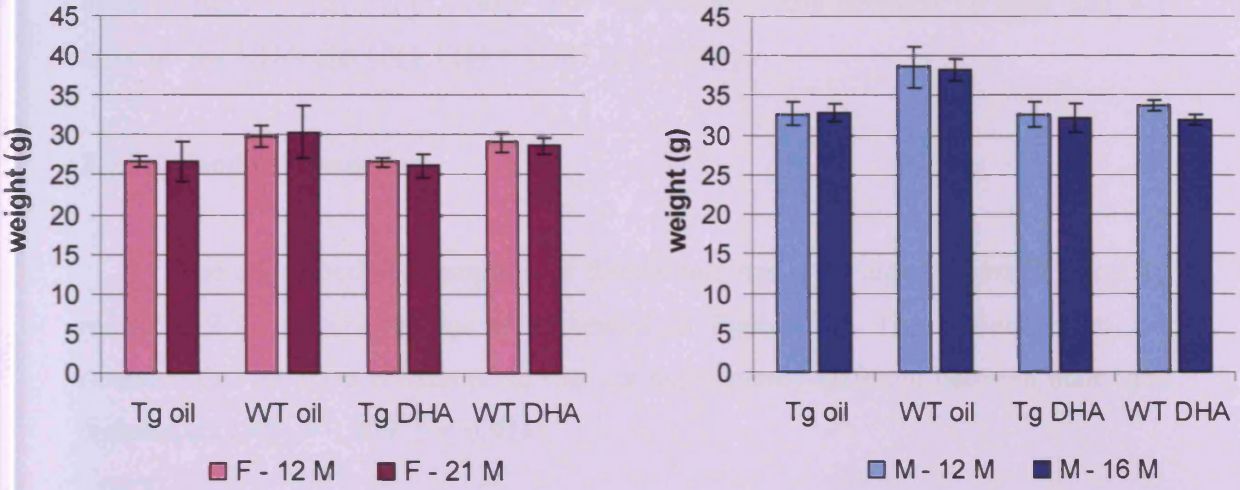


**Figure 2.2.** Mouse body weight. Mean values of mouse body weight and corresponding standard error to the mean are represented for male (M) and female (F) mice, Tg and WT mice on oil blend and DHA diets, at the age of 12 months (12 M), 16 months (16 M) and 21 months (21 M) (see Table 2.5 for mouse numbers).

The statistical analysis using ANOVA revealed that the weight of Tg mice was significantly lower than the weight of WT mice ($F(1,128) = 12.598$, $p = 0.001$) with an average of 29.13 (± 0.56) g and 33.49 (± 0.69) g respectively. The diet also had a significant effect on mouse weight ($F(1,128) = 5.251$, $p = 0.024$) with an average of 29.94 (± 0.49) g for the mice on the DHA diet and 32.92 (± 0.79) g for the mice on the oil blend diet. The females' weight was significantly lower than the males' ($F(1,128) = 41.927$, $p < 0.001$), with an average of 28.01 (± 0.50) g for females and 34.38 (± 0.62) g for males. However, age did not have a significant effect on mouse weight ($F(2,128) = 0.284$, $p = 0.753$). The average male weight was 35.03 (± 1.03) g at 12 months of age and 33.80 (± 0.73) g at 16 months and the female average weight was 28.05 (± 0.63) g at 12 months and was 27.87 (± 1.07) g at 21 months of age.

The statistical analysis also revealed a significant interaction of genotype by diet ($F(1,128) = 4.041$, $p = 0.047$). The test of simple main effect for the interaction showed that the weight of WT mice was higher when on the oil blend diet than on the DHA diet ($F(1,128) = 10.238$, $p = 0.002$), and the weight of mice on the oil blend diet was significantly higher for WT mice compared to Tg ($F(1,128) = 17.001$, $p < 0.001$) but there was no significant difference between Tg mice on the oil blend diet and Tg

mice on the DHA diet ($F(1,128) = 0.017$, $p = 0.898$) and between Tg mice and WT mice on the DHA diet ($F(1,128) = 1.387$, $p = 0.241$).

### 2.6.3.2 Food consumption

The daily food consumption of female and male, averaged across WT and Tg mice, at 9-12 months of age is presented in Figure 2.3. The statistical analysis revealed that the food consumption was not significantly different between males and females ($F(1,43) = 0.009$, $p = 0.924$).

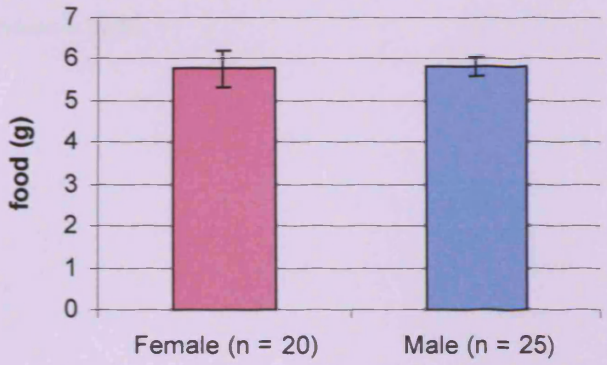


**Figure 2.3.** Daily food consumption of female and male Tg2576 and WT mice at 9-12 months of age. Mean ± SEM.

Figure 2.4 shows the daily food consumption of Tg and WT males on oil blend and DHA diet at 9-12 months of age. The statistical analysis revealed no significant effect of genotype ($F(1,21) = 2.057$, $p = 0.166$) or diet ($F(1,21) = 0.125$, $p = 0.728$) on the food consumption.

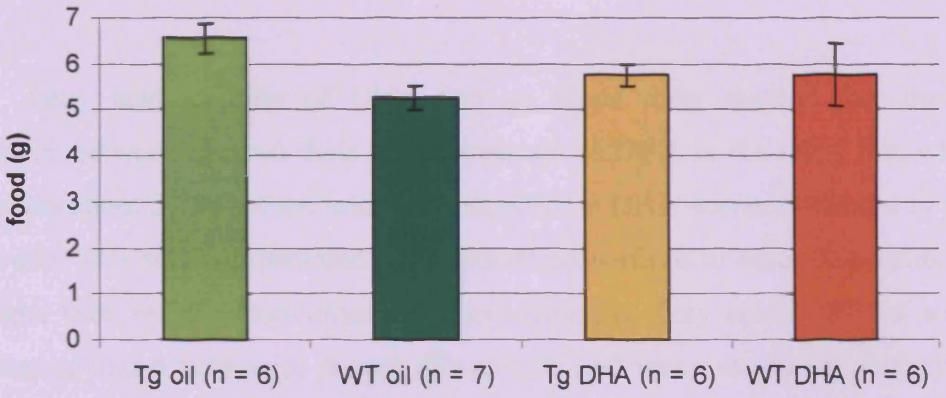


**Figure 2.4.** Daily food consumption of male Tg2576 and WT mice on the oil blend and the DHA diet at 9-12 months of age. Mean ± SEM.

## 2.7 Discussion

Fatty acid analysis of DHA and oil blend diets showed that the main difference between the two diets is the presence of DHA in the DHA diet where it represents about 27.5% of the total fatty acids while DHA was not detected in the oil blend diet. This was compensated by increased proportions of other fatty acids in the oil blend diet, mainly monounsaturated and saturated fatty acids. 18:2n-6 was the only source of n-6 fatty acids in both diet and 18:3n-3 was a source of n-3 fatty acids in both diets. Arachidonic acid (20:4n-6) was not detected in either diets and the n-3 to n-6 ratios of oil blend and DHA diet were respectively 0.1 and 1.3.

Together, the food consumption and body weight data gave some information about the effect of the diets and genotype on the mice general health. As the average food consumption of males and females was virtually the same (5.8 ± 0.2 and 5.8 ± 0.4 respectively), the difference of body weight observed between males and females, may be due to a difference of metabolism between the two genders, as seen in humans. Significantly lower body weight of Tg mice compared to WT mice may be due to the development of the disease. As observed in AD patients, Tg mice may loose weight as part of the development of the symptoms. However, age did not have a significant effect, suggesting that at 12 months of age, the APP transgene was influencing weight. Diet also had a significant effect on mouse body weight but with no significant difference of the food consumption between Tg and WT males on oil blend or DHA diet. This suggests that mice on the oil blend diet were heavier than mice on the DHA diet because of differences of diet composition, suggesting that DHA supplementation helps to maintain healthy weight. The absence of a significant effect of diet or genotype on food consumption in Tg and WT males on either diet also suggests that the mice accepted both diets equally and that Tg mice did not show any significant deterioration of their feeding behaviour.

According to the fatty acid analysis of the rodent diets (section 2.5.3), the DHA-enriched diet contained approximately 18 mg of DHA per gram of food so DHA-fed mice consuming about 5g of food would have an intake of about 90 mg of DHA per day. Considering that the average mouse body weight is about 30 grams, the DHA intake would be 3 g DHA/ kg/ day, which is well over the recommended DHA intake for humans, of 300 mg/ day (i.e. about 4.3 mg/ kg/ day for an average

human body weight of 70 kg). By comparing the DHA intake between mouse and human relative to the energy intake, the fact that mice should consume more DHA per kg of body weight than human makes more sense. For men the recommended energy intake is about 2,500 kcal per day (i.e. 36 kcal/ kg / day for a man of 70 kg). In this experiment the mouse average energy intake was about 568 kcal/ kg/ day for a 30 g mouse consuming 5 g of food per day, which is about 16 times more energy per kg than humans. Although this comparison is interesting and the fact that mice should consume more DHA per kg of body weight than human appears logical, this shows that it is not possible to make a direct comparison between human and animal experimentation. In addition, the food consumption may be over evaluated. As some of the food may have been shredded and mixed with the sawdust at the bottom of the cages, the data on food consumption were only an approximate measure of the amount of food consumed by the mice.

It is also interesting to note that the DHA content of our DHA diet, of approximately 1.8%, was higher than that of the DHA-rich diets used in most other published studies in which the effect of dietary DHA was studied in mice models over-expressing APP mutants. The DHA content was generally comprised between 0.4% and 0.6% of the diet weight (Hooijmans et al., 2007; Lim et al., 2005; Oksman et al., 2006) although in the study of Green et al. (2007), the DHA diet contained 1.27% of DHA.

# CHAPTER 3

# Effect of dietary docosahexaenoic acid on learning and memory in Tg2576 mice

## 3.1 Introduction

The aim of the work presented in this chapter was to investigate the effect of dietary DHA supplementation on cognition in both Tg and WT mice. The mice were fed with the experimental diets from the age of 4 months and then tested on spatial memory paradigms. This experiment was designed to determine whether exposure to the diets before the appearance of β-amyloid plaques in the brain could protect against the development of cognitive impairments in Tg2576 mice. In addition, WT mice also received the DHA-enriched diet, and thus the effect of DHA supplementation on cognition of normal animals was also assessed. The next section is a brief description of the cognitive deficits reported in Tg2576 mice and the effects of dietary DHA. The two main tasks used in the behavioural experiments, the open field foraging task and the T-maze forced choice alternation task are then described in the materials and methods section. Finally, the results of these experiments will be presented and discussed.

Age-related deficits in learning and memory have been well-documented in Tg2576 mice. One of the earliest studies carried out by Hsaio et al. (1996) reported that Tg2576 mice showed no impairment in Y-maze spontaneous alternation at 3 months of age but a significant deficit at 9-10 months of age. They also reported a progressive reference memory impairment in the Morris water maze task that was significant by 9-10 months of age (Hsiao et al., 1996). Since 1996, the Tg2576 mouse model has been used in a number of studies. In some cases, Tg2576 mice were impaired by the age of 3 months (King and Arendash, 2002) but most frequently by

the age of 6 to 8 months and later (Barnes et al., 2004; Chapman et al., 1999; Corcoran et al., 2002; Hale and Good, 2005; King et al., 1999; Lalonde et al., 2003; Middei et al., 2006; Ognibene et al., 2005). Several studies have shown that the onset of behavioural deficits coincides with the production of insoluble forms of Aβ that accumulate in cortical and hippocampal areas (Hsiao et al., 1996; Westerman et al., 2002). Thus, Tg2576 mice show age-related changes in spatial memory that make them suitable for the study of how dietary manipulations influence the development of cognitive abnormalities.

Indeed, Tg2576 mice have been used in several laboratories to examine the effects of dietary supplements on β-amyloid pathology and cognition. For example, supplementation of epigallocatechin-gallate (EGCG), one of the main flavonoids present in green tea, reduced Aβ deposition in cortex and hippocampus of Tg2576 mice, along with a promotion of the non-amyloidogenic α-secretase proteolytic pathway and cognitive benefits in a radial arm water maze task (Rezai-Zadeh et al., 2008). In another water maze study, Tg2576 mice showed a significant spatial learning impairment while Tg2576 mice treated with *gingko biloba* extract showed a performance equivalent to that of WT mice (Stackman et al., 2003). However, biochemical analysis revealed no significant treatment effect on cortical levels of soluble and insoluble Aβ1-40 and Aβ1-42 and plaque burden in hippocampus. Alpha-lipoic acid supplementation also improved learning and memory retention in the Morris water maze task and context fear conditioning in 10 month-old Tg2576 mice, without affecting brain Aβ levels significantly (Quinn et al., 2007). Eleven month-old Tg2576 mice that received Cabernet Sauvigon (final concentration of 6% ethanol in water) from the age of 4 months had significantly attenuated deterioration of spatial memory, compared to water control and ethanol control Tg mice. These mice also showed significantly reduced levels of Aβ1-40 and Aβ1-42 in neocortex and hippocampus, possibly due to a promotion of the non-amyloidogenic processing of APP (Wang et al., 2006). Finally, in another example of beneficial effects of dietary supplements, Tg2576 mice fed pomegranate juice from 6 to 12.5 months of age showed significantly improved spatial and non-spatial learning in a water maze task and reduced levels of soluble Aβ1-42 and plaques in hippocampus (Hartman et al., 2006).

To date only four studies, all from Greg Cole's lab, have examined the effects of dietary n-3 PUFA supplementation in the Tg2576 mouse line (Calon et al., 2005;

Calon et al., 2004; Lim et al., 2005; Ma et al., 2007). Among these studies, only Calon et al. (2004) investigated the effects of n-3 PUFA supplementation on behaviour. It was reported that 21-22 month-old Tg2576 mice that were fed a safflower oil-based n-3 PUFA depleted diet from 17 months of age were significantly impaired compared to Tg mice on the same diet + 0.6% DHA, in the hidden, but not the visible platform version of the water maze task. In another study, it was also shown that atherogenic diet (containing 15.75% fat, 1.25% cholesterol and 0.5% sodium cholate) augmented the spatial learning impairment in Tg2576 mice compared to Tg2576 on normal diet (Li et al., 2003). Although these studies suggest the importance of DHA in cognitive functions and the detrimental effect of certain fatty diets on Aβ pathology, they do not demonstrate unequivocally whether DHA supplementation *per se* is beneficial or not, against a normal baseline diet. In the study of Calon et al. (2004), the effect of DHA supplementation was compared to the effect of DHA depletion, so the observation of any improvement of cognitive performance with DHA supplementation is confounded with changes in the control condition. This shows the importance of the design of the experiment and choice of diets to study the effect of DHA supplementation, and also points out the need for further investigation, as discussed in Chapter 2.

Although the effect of dietary DHA supplementation on cognition in Tg2576 mice is not very well documented, behavioural studies on other rodents suggest that dietary n-3 PUFA supplementation can improve performance on spatial memory tasks. For example, pre-administration of 300 mg dietary DHA per kg of body weight per day for 12 weeks to 20 week-old Wistar rats prior to Aβ1-40 injection into the cerebral ventricle had a beneficial effect on avoidance learning performance (Hashimoto et al., 2002). Other studies by this group have shown that DHA decreased levels of Aβ1-40 and cholesterol in the cortex lipid raft fraction and that this was associated with significantly better performance in the eight-arm radial-maze task. (Hashimoto et al., 2005a; Hashimoto et al., 2006; Hashimoto et al., 2005b). In a recent study where DHA was replaced by EPA, similar beneficial behavioural findings were observed with significantly improved performance in the eight-arm radial maze task (Hashimoto et al., 2008). Although the findings of Hashimoto and colleagues are consistent with the view that DHA may be protective and beneficial to cognition, other studies have reported mixed results. For example, in a partially baited eight-arm radial maze experiment, administration of DHA for 10 weeks (300

mg/kg/day) significantly reduced the number of reference memory errors, without affecting the number of working memory errors of young male rats (Gamoh et al., 1999). Ten month-old APPswe/PS1dE9 mice that were fed with a DHA-enriched diet for 4 months showed significantly decreased concentration of hippocampal Aβ1-40 and Aβ1-42 levels compared to the mice on a "typical Western diet". However, the dietary intervention had no significant effect on the escape latency, wall swimming, swimming speed or spatial search bias in the Morris water maze task (Oksman et al., 2006). Another study showed no significant effect of high omega-3 diet on the cognitive performance of 6-9 month-old APPswe/PS1dE6 mice (Arendash et al., 2007), including no significant effect of genotype or diet in the Y-maze alternation task, in the Morris water maze acquisition and retention, circular platform and radial arm water maze tasks. However, a significant effect of genotype but not diet was found in the platform recognition task. In a recent study on the same mouse model, the mice received a "typical Western diet", a DHA diet (0.4% DHA) or a standard diet from the age of 2 months. DHA levels were significantly increased in the brain of mice that received the DHA diet, and this diet also decreased Aβ deposition and improved spatial memory in the Morris water maze task, in 15 month-old mice, compared to the standard diet group. However, at 8 months of age, no dietary effects were found on Aβ deposition or the performance in the Morris water maze task. In addition, no dietary effects were found at either age in a reverse Morris water maze task carried out after the Morris water maze task, using a different target quadrant, or in a 12-circular hole board task (Hooijmans et al., 2009).

These data suggest that Tg2576 mice present progressive spatial learning deficits in a range of spatial tasks and that DHA supplementation can reduce these impairments. However, negative findings and mixed behavioural changes suggests that this conclusion must not be accepted unequivocally. Thus, in order to establish whether dietary DHA supplementation influences Aβ pathology at both the neuronal and functional levels, the mice were tested on two different spatial memory tasks that are sensitive to the APPswe mutation. In order to test the hypothesis that dietary DHA supplementation alleviates the cognitive impairment of Tg2576 mice, the mice were tested on an open field foraging task (that is analogous to the radial arm maze task) and the T-maze forced choice alternation (FCA) task. Two different tasks were used to establish the generality of any behavioural changes across different tasks or sensorimotor requirements.

Experiment 1 was carried out using an open field foraging task. The mice were first habituated to the experimental environment and pre-trained to forage in pots baited with a food reward. Following habituation and pre-training, the mice were tested in an arena containing eight pots baited with a food reward. Animals needed to find the eight food rewards. The most effective strategy is to visit each pot only once as the food rewards are not replaced during a trial. Effective foraging may therefore make use of memory for the spatial locations visited within each trial. Performance was evaluated using the time taken to complete the task (retrieve all eight rewards), the total number of errors (total number of returns to previously visited and depleted pots), and the total number of repeated errors (total number of consecutive returns to previously visited and depleted pots). Experiment 2 was carried out using a T-maze FCA task in which animals are rewarded for selecting an arm not visited on a previous sample trial. Their performance was evaluated using the percentage of choices to the correct arm. Details of the design of Experiment 1 and Experiment 2 are provided in the following materials and methods section. The prediction was that DHA dietary supplements would reduce the performance deficit of aged Tg2576 mice on these tasks.

## 3.2 Materials and methods

### 3.2.1 Experiment 1: Open field foraging task

#### 3.2.1.1 Subjects

Mice were tested at 12 month of age, when Aβ plaques can be detected in Tg mice. The experimentally naive cohort of mice tested in the foraging task was composed of 12 Tg mice on oil blend diet (4 females and 8 males), 10 WT mice on oil blend diet (4 females and 6 males), 11 Tg mice on DHA diet (4 females and 7 males) and 11 WT mice on DHA diet (4 females and 7 males). These mice were part of Cohort 1, as described in section 2.4. During the experiments, the mice were housed at a maximum of two per cage. A full description of the breeding, genotyping and maintenance of the mice is presented in Chapter 2.

#### 3.2.1.2 Apparatus

A wooden grey-painted foraging arena (100 × 100 × 40 cm) was placed on the floor, in the centre of a light attenuated and quiet testing room (5.80 × 5.30 m), with a variety of extra maze cues, such as video recording equipment and doors. The position of the experimenter and the arrangement of the equipment within the experimental room remained constant throughout testing. The floor of the arena was covered with sawdust (approximately 3 cm depth). During the testing sessions, eight white ceramic pots (6.5 cm diam. × 3.5 cm ht.) full of sawdust and baited with one half coco-pop (Bellona ChocoRice, LIDL; approximately 18.4 mg per half coco-pop) at approximately 1 cm depth were placed in the arena according to the pattern shown in Figure 3.1. During the training sessions in the arena, only the two white ceramic pots placed in the middle of the arena were used. All trials were monitored by a camera (Digital CCD Camera, Sanyo) mounted at 120 cm above the centre point of the arena and attached to a video recorder (SVHS, Panasonic) and monitor (Vista, Norbain SD LTD).

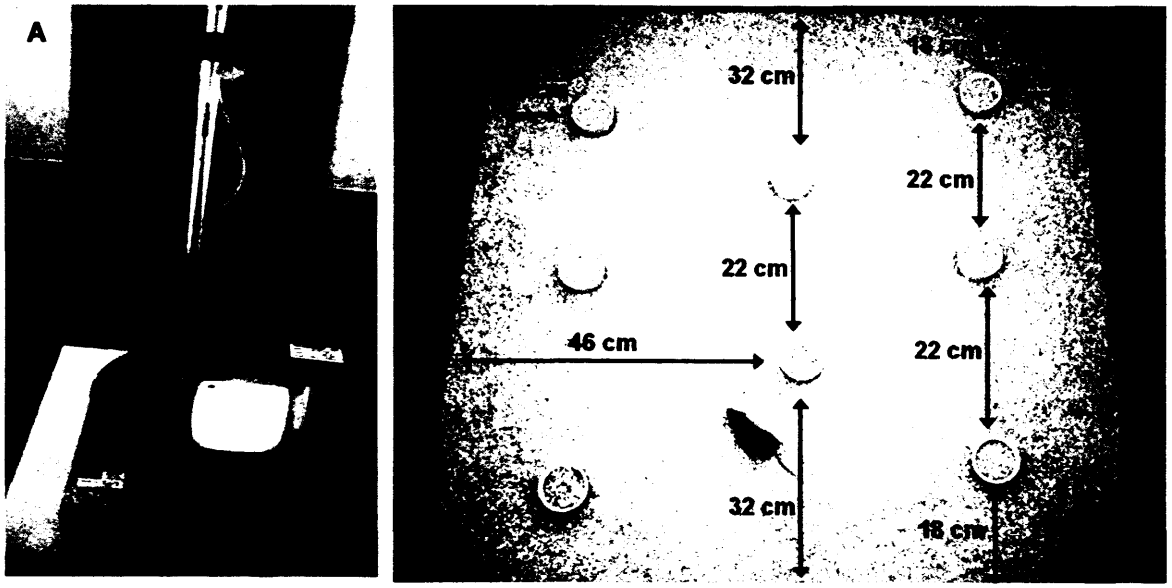


**Figure 3.1.** Open field foraging task equipment. Monitoring system (A) and arena containing 8 pots (B).

### 3.2.1.3 Procedure

This foraging test requires the mouse to find eight palatable rewards (pieces of the cereal coco-pops) buried individually in separate locations in white pots, at approximately 1 cm depth under sawdust, in an open field arena.

Mice were habituated, pre-trained and tested in the afternoon, during the lights-on period. During habituation and pre-training, the mice were food deprived to 85% of *ad libitum* body weight by feeding them 2 to 4 grams of their specific diet, once a day, at least 2 hours after the last training. Mice were maintained at this weight during the testing period using the same feeding protocol. The animals were weighed daily to ensure that the food restriction was not affecting their general health.

Mice were handled, habituated to transportation and pre-trained to forage for coco-pops for 6 days before starting the testing. The details of the procedure are given below:

- On day 1, in the morning, food was removed from the hopper and the mice were fed 2 to 4 grams of their special diet in the afternoon.
- On day 2, mice were trained to forage for coco-pops in their home cage. For the first exposure to coco-pops, a white ceramic pot full of sawdust was placed in the cage with four half coco-pops placed on the surface of the sawdust. The pot was left in the cage for about half an hour. Then, mice were trained to forage for

coco-pops by placing a pot full of sawdust in the cage with two half coco-pops on the surface and just covered by a sprinkle of sawdust on top. The pot was left in the cage for about half an hour and the operation was repeated. One to two hours interval was left between each training phase.

- On day 3, mice were trained to forage for coco-pops in their home cage by placing a pot full of sawdust in the cage with two half coco-pops on the surface and just covered by a sprinkle of sawdust on top. Then the mice were trained three times by placing a pot full of sawdust in the cage with two half coco-pops buried in the sawdust at approximately 1 cm below the surface. Each pot was left in the cage for about half an hour and training was spread over six hours.
- On day 4, mice were trained three times by placing a pot full of sawdust in the home cage with two half coco-pops buried in the sawdust at approximately 1 cm below the surface. Each pot was left in the cage for about half an hour and the training was spread over approximately two hours.
- On day 5, mice were trained three times by placing a pot full of sawdust in the home cage with one half coco-pop buried in the sawdust at approximately 1 cm below the surface. Each pot was left in the cage for about half an hour and training was spread over about two hours during the morning session. In the afternoon, mice were placed for 10 min. into the empty foraging arena for habituation to the testing environment.
- On day 6, mice were trained in their home cage two times by placing a pot full of sawdust in the home cage with one half coco-pop buried in the sawdust at approximately 1 cm below the surface. Each pot was left in the cage for about half an hour and training was spread over about two hours during the morning session. In the afternoon, mice were placed for 10 min. in the arena with two pots each baited with one half coco pop, buried in the sawdust at approximately 1 cm below the surface.
- On day 7, mice were trained on one occasion in their home cage by placing a pot full of sawdust in the home cage with one half coco-pop buried in the sawdust at approximately 1 cm below the surface. The pot was left in the cage for about half an hour during the morning session. In the afternoon, mice were placed 10 min. in the arena with two pots each baited with one half coco pop buried in the sawdust at approximately 1 cm below the surface.

From day 2 to 7, mice were trained in their home cage to forage for coco-pops from the white ceramic pots so that all the mice ate the coco-pops within 5 min., by the end of the training period. At the end of training, one mouse (WT male on DHA-enriched diet) did not consume any of the rewards during the training period and so was excluded from further testing.

- From day 8 to 13, the main testing sessions were carried out. Each mouse underwent one trial per day for six consecutive days. For each trial, the mouse started in the centre of the arena containing eight pots; each baited with a half coco-pop buried in the sawdust at approximately 1 cm below the surface. The mice remained in the arena until they ate all the rewards or for a maximum of 10 min., after which, they were returned to their home cage.
- On day 13, after the end of the trial, the mice were placed back with *ad libitum* access to food.

Foraging efficiency was assessed by measuring three variables: the time to complete the task (collect all eight rewards), total number of errors (return visits to a depleted pot), and number of repeated errors (consecutive return visits to the same depleted pot).

#### 3.2.1.4 Data analysis

The mean completion times, total number of errors and number of repeated errors of the 6 sessions were calculated. Statistical analysis was carried out using an analysis of variance (ANOVA) with diet (oil blend or DHA), genotype (Tg or WT) and gender (male or female) as between subject factors, and day as within subject factor. When $p < 0.05$, the effect was considered statistically significant.

## 3.2.2 Experiment 2: T-maze forced choice alternation task

### 3.2.2.1 Subjects

Experimentally naive mice were first tested, using the T-maze forced alternation task, at 8 months of age, before Aβ plaques are detected in Tg mice. The same group of mice was then tested at 12 months of age, when Aβ plaques can be detected and 16 months of age, when plaque pathology is readily evident. The group assignments were as follows: 10 WT males on oil blend diet, 10 Tg males on oil blend diet, 10 WT males on DHA diet and 10 Tg males on DHA diet, at the start of the experiment. By the age of 12 months, the number of WT mice in oil blend diet group dropped to 9 mice and by the age of 16 months, there were 9 WT on oil blend diet, 10 Tg on oil blend diet, 10 WT on DHA diet and 7 Tg on DHA diet. These mice were part of Cohort 2, as described in section 2.4. A full description of the breeding, genotyping and maintenance of the mice is presented in Chapter 2.

### 3.2.2.2 Apparatus

The T-maze was composed of three 9 cm wide arms constructed from clear 13 cm high Perspex walls and a brown melamine floor. The start arm was 52 cm long and the goal arms were each 26 cm long. Guillotine doors, used to block the start and goal arms, were made from opaque Perspex. The T-maze was elevated 92 cm from the floor and was situated in a quiet and illuminated room with numerous visual cues, such as posters on the walls, benching, and air conditioning ducts. Mice ran for a reward of 50 μl of 25% sucrose solution in distilled water, which was placed in a food cup recessed into the floor of the apparatus at the end of each goal arm.

### 3.2.2.3 Procedure

All the behavioural experiments using the T-maze procedure were carried out by a research assistant (Victoria Staal). The data analysis was carried by myself.

Mice were water deprived and were only given free access to water in their home cage for 2 hours after every day training session. The animals were weighed daily to ensure that water restriction was not affecting their general health.

Habituation, pre-training and testing sessions began at 9:00 am every day. All the mice first underwent four days of habituation to the T-maze and the sucrose reward. Each day, each mouse was placed in the maze for 5 min. with all arms of the maze open. During the first three days of habituation and pre-training, drops of the sucrose solution were placed in the food wells and on the floor of the arena to encourage exploratory activity and to habituate the mice to the novel sucrose solution. On the final day of habituation, sucrose was located only in the food wells at the end of each goal arms.

During testing, the mice received six pairs of runs per day for a total of 10 days. This was made up of two blocks of five consecutive days of training separated by a two day break. On the first run of each trial (the sample run), both goal arms were baited. The mouse was released from the start box and allowed to enter the goal box selected by the experimenter. The location of the sample arm (left or right) was varied pseudorandomly across the session such that the mice received three left and three right sample presentations, with no more than two consecutive trials with the same sample location in each session. A (removable) opaque Perspex door blocked access to the remaining goal-arm during the sample trial. The door was placed at the access point to the goal arm. After entering the selected goal box, the mouse was allowed up to 30 seconds to consume the sucrose reward and was then placed in the start box. The experimenter then removed any residual sucrose reward from the sample arm and cleaned the floor of the maze with a damp cloth to obscure any odour cues left by the mouse. Approximately 30 seconds later, the mouse received its second (choice) run. On the choice run, both of the goal arms were accessible. The mouse was rewarded, however, for choosing the arm not visited on the sample run. Following a correct choice, the mouse was allowed up to 30 seconds to consume the sucrose reward. If an incorrect choice was made, then the guillotine door was lowered restricting the mouse to the non-rewarded arm for 15 seconds. The mouse was then placed in a cage for the duration of the inter-trial interval. The mice were run in groups of six to maintain an inter-trial interval of approximately 8-10 min. Where possible, equal numbers of Tg and WT from each condition were run in each batch.

### 3.2.2.4 Data analysis

Successful performance (alternation) is expressed as a percentage of the six trials conducted in each session. Statistical analyses were carried out using an analysis of variance (ANOVA) with diet (oil blend or DHA) and genotype (Tg or WT) as between subject factors, and day as within subject factor. When $p < 0.05$, the effect was considered significant.

Attrition affected the number of mice at 12 and 16 months. A separate ANOVA was carried out on the data from each age point. In addition, further analysis was carried out to determine whether terminal performance (accuracy on the last training day) varied systematically with age in the WT and Tg2576 mice across the diet conditions. Only the data from the mice that reached the final stage of testing were included in this analysis (Tg oil, n = 10; Tg DHA, n = 7; WT oil, n = 9; WT DHA, n = 10).

## 3.3 Results

### 3.3.1 Experiment 1: Open field foraging task

12 month-old Tg and WT mice were tested once a day for six consecutive days in the open field foraging task. The mouse cohort of 43 mice including males and females was composed of 11 Tg mice on DHA diet, 12 Tg mice on oil blend diet, 10 WT mice on DHA diet and 10 WT mice oil blend diet. Time of completion as well as total number of errors and repeated errors were measured, and analysis of variance was carried out using genotype, diet, gender and day as factors.

#### 3.3.1.1 Completion time

The time to complete the task on each of the six test sessions for WT and Tg mice in each dietary condition is presented in Figure 3.2. Figure 3.3 represents the performance of each of the four mouse groups averaged across the six test sessions.

The ANOVA, carried out using day as a within-subject factor and, genotype, diet and gender as between-subject factors, showed a significant main effect of day ($F(5,175) = 21.751$, $p < 0.001$; see Figure 3.2) with a reduction in completion time across testing, observed in Figure 3.2. However, the statistical analysis showed no significant main effect of genotype ($F(1,35) = 0.200$, $p = 0.658$), diet ($F(1,35) = 3.643$, $p = 0.065$; see Figure 3.3) or gender ($F(1,35) = 0.305$, $p = 0.584$) (data not shown) and no significant interaction of genotype by diet ($F(1,35) = 0.121$, $p = 0.730$), genotype by gender ($F(1,35) = 0.093$, $p = 0.762$) or diet by gender ($F(1,35) = 3.119$, $p = 0.086$). The statistical analysis also revealed no significant interaction of day by genotype ($F(5,175) = 1.393$, $p = 0.229$), day by diet ($F(5,175) = 0.786$, $p = 0.561$) or day by gender ($F(5,175) = 0.257$, $p = 0.936$) but there was a significant three-way interaction between, day, genotype and diet ($F(5,175) = 2.810$, $p = 0.018$). Subsequent analyses using tests of simple main effects indicated that the three way interaction was caused by the WT mice on DHA diet showing significantly shorter latencies than WT mice on oil blend diet ($F(1,35) = 6.145$, $p = 0.018$) but only on day 5, and the completion time of Tg mice on DHA diet did not differ significantly from the three other groups on any day of the experiment.

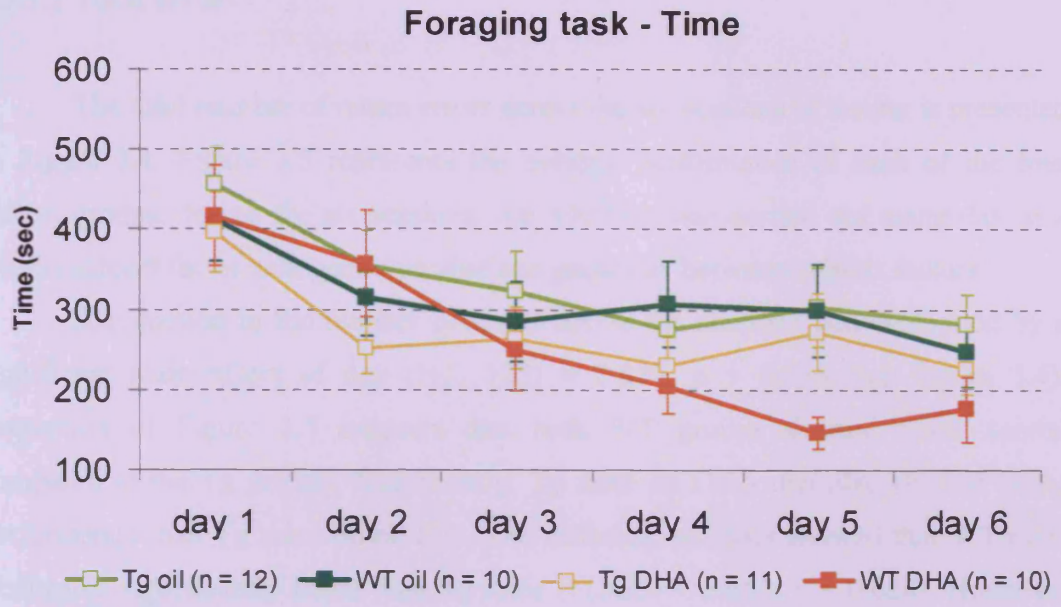


**Figure 3.2.** Open field foraging task. Mean completion time of the task by 12 month-old WT and Tg mice fed with the DHA diet or the oil blend diet, from day 1 to day 6 of the trial period. Values are mean ± SEM.

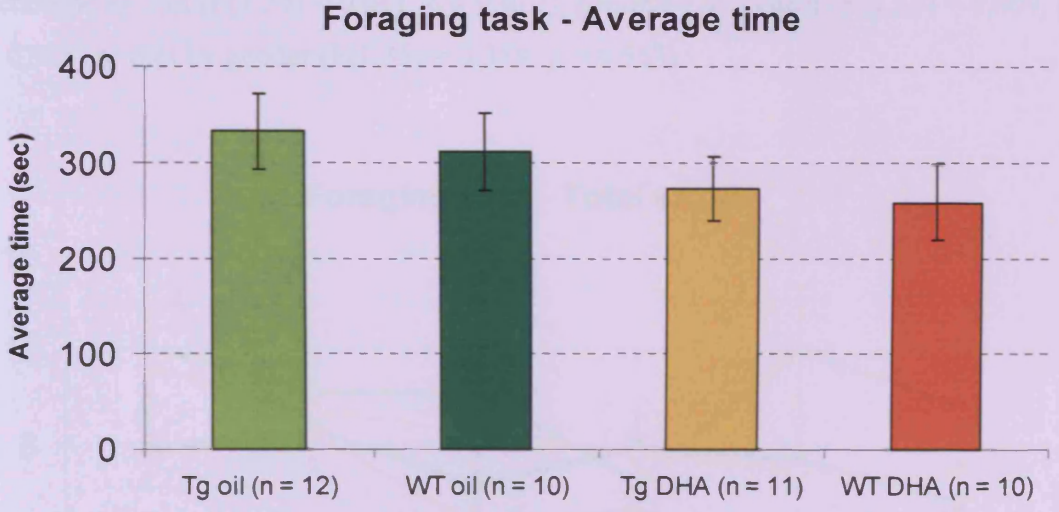


**Figure 3.3.** Open field foraging task. Average completion time of the task by 12 month-old WT and Tg mice fed with the DHA diet or the oil blend diet, over the 6 days of trial. Values are mean ± SEM.

### 3.3.1.2 Total errors

The total number of return errors across the six sessions of testing is presented in Figure 3.4. Figure 3.5 represents the average performance of each of the four mouse groups, during the six sessions. An ANOVA was carried out using day as a within-subject factor and, genotype, diet and gender as between-subject factors.

A reduction in the number of errors across the sessions was confirmed by a significant main effect of day ($F(5, 175) = 2.870$, $p = 0.016$; see Figure 3.4). Inspection of Figure 3.5 suggests that both WT groups showed fewer errors, compared to the Tg groups. Numerically, Tg mice on DHA diet also showed better performance than Tg mice on oil diet. The statistical analysis showed that WT mice performed significantly better than Tg mice ($F(1,35) = 5.603$, $p = 0.024$). However, there was no significant main effect of the diet ($F(1,35) = 2.451$, $p = 0.126$) or gender ($F(1,35) = 0.107$, $p = 0.746$) (data not shown). The statistical analysis also revealed no significant interaction of day by genotype ($F(5,175) = 1.326$, $p = 0.255$), day by diet ($F(5,175) = 1.742$, $p = 0.127$, day by gender ($F(5,175) = 1.331$, $p = 0.253$), genotype by diet ($F(1,35) = 0.011$, $p = 0.917$), genotype by gender ($F(1,35) = 0.009$, $p = 0.924$) or diet by gender ($F(1,35) = 0.359$, $p = 0.553$).

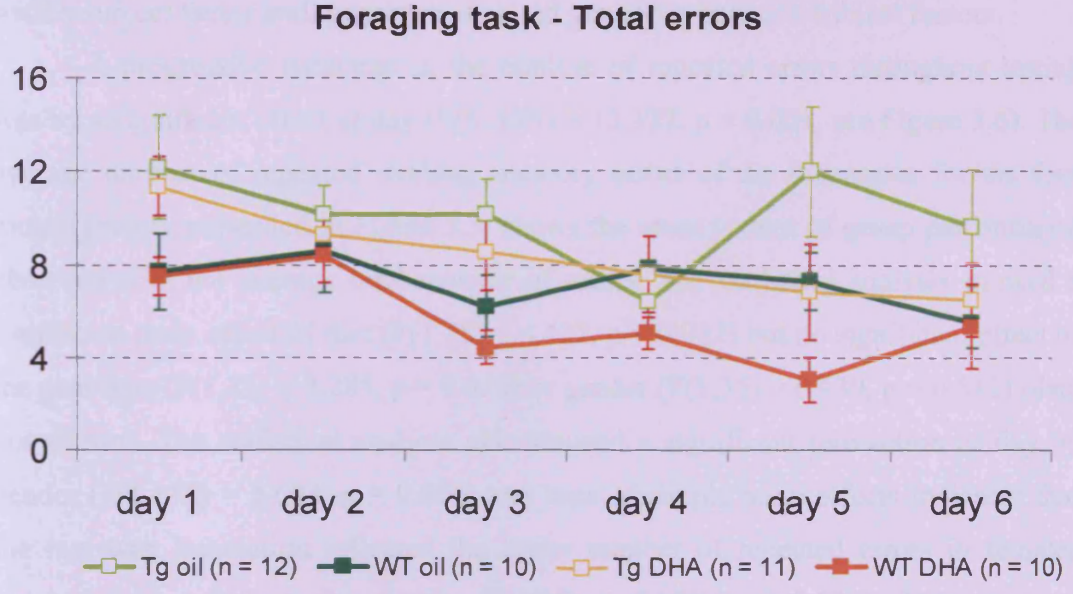


**Figure 3.4.** Open field foraging task. Mean total number of working memory errors during the completion of the task by 12 month-old WT and Tg mice fed with the DHA diet or the oil blend diet, from day 1 to day 6 of the trial period. Values are mean ± SEM.

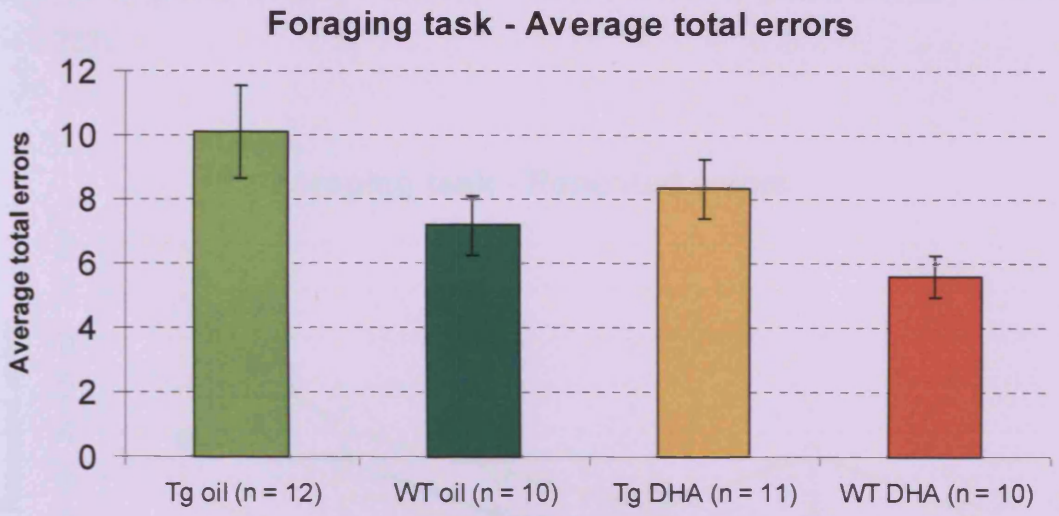


**Figure 3.5.** Open field foraging task. Average number of total working memory errors during the completion of the task by 12 month-old WT and Tg mice fed with the DHA diet or the oil blend diet, over the 6 days of trial. Values are mean ± SEM.

### 3.3.1.3 Consecutive return errors

The total number of repeated errors across the 6 sessions of testing is presented in Figure 3.6. Figure 3.7 represents the average performance of each of the four mouse groups, during the 6 sessions. An ANOVA was carried out using day as a within-subject factor and, genotype, diet and gender as between-subject factors.

A progressive reduction in the number of repeated errors throughout testing was by a significant effect of day ($F(5, 175) = 13.327$, $p < 0.001$, see Figure 3.6). The average number of repeated working memory errors of the 6 sessions for the four mouse groups, presented in Figure 3.7, shows the same pattern of group performance observed with the average total number of errors. The statistical analysis showed a significant main effect of diet ($F(1,35) = 4.437$, $p = 0.042$) but no significant effect of the genotype ($F(1,35) = 3.285$, $p = 0.078$) or gender ($F(1,35) = 0.439$, $p = 0.512$) (data not shown). The statistical analysis also showed a significant interaction of day by gender ($F(1,175) = 3.634$, $p = 0.004$) and tests of simple main effects indicated that the two way interaction reflected the lower number of repeated errors in females compared to males on day 1 only ($F(1,35) = 5.652$, $p = 0.023$). There was no significant interaction of day by genotype ($F(5,175) = 1.154$, $p = 0.334$), day by diet ($F(5,175) = 0.956$, $p = 0.446$), genotype by diet ($F(1,35) = 0.052$, $p = 0.820$),

genotype by gender ($F(1,35) = 0.039$, $p = 0.844$), or diet by gender ($F(1,35) = 1.188$, $p = 0.283$).

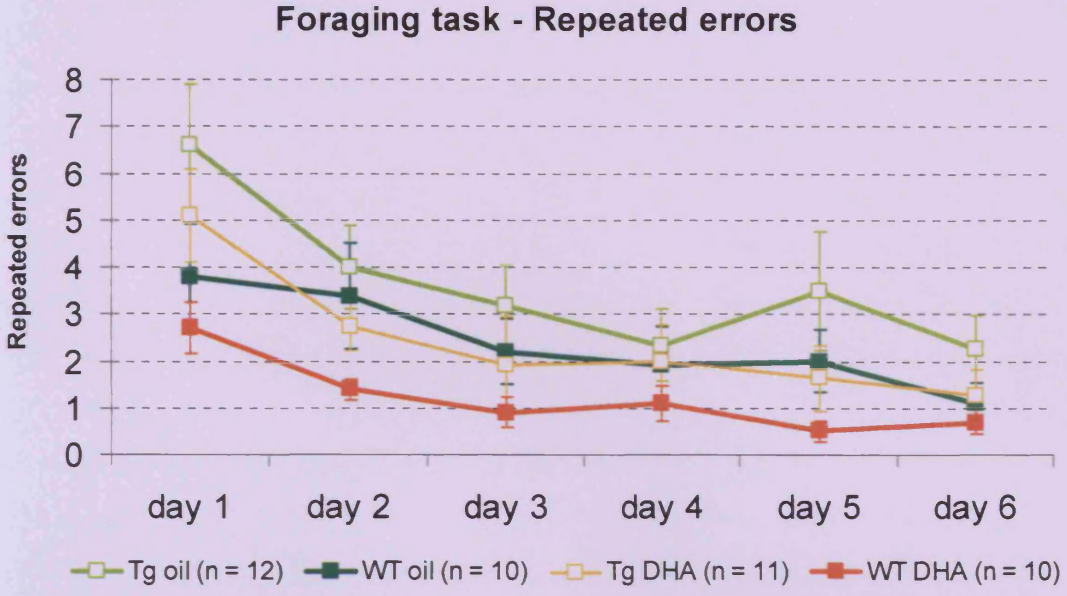


**Figure 3.6.** Open field foraging task. Mean number of repeated working memory errors during the completion of the task by 12 month-old WT and Tg mice fed with the DHA diet or the oil blend diet, from day 1 to day 6 of the trial period. Values are mean ± SEM.

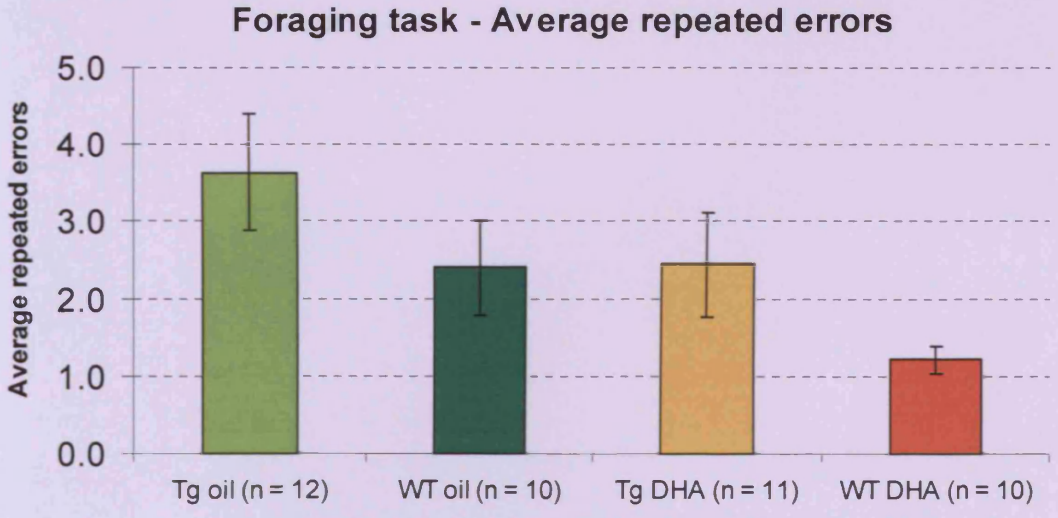


**Figure 3.7.** Open field foraging task. Average number of repeated working memory errors during the completion of the task by 12 month-old WT and Tg mice fed with the DHA diet or the oil blend diet, over the 6 days of trial. Values are mean ± SEM.

In summary, the results show that Tg mice were less accurate than WT mice in foraging efficiently for food rewards. In addition, although DHA did not ameliorate this impairment, there was evidence that DHA improved performance for both WT and Tg in terms of the number of consecutive return errors.

### 3.3.2 Experiment 2: T-maze forced choice alternation task

Figure 3.8 shows the percentage of correct choices for both Tg and WT mice on DHA or oil blend diet during acquisition of the T-maze forced choice alternation task at 8 months of age. Analysis of variance with day, genotype and diet as factors revealed a significant effect of genotype ($F(1,36) = 6.418$, $p = 0.016$) but no significant effect of diet ($F(1,36) = 1.445$, $p = 0.237$) and no significant interaction of genotype by diet ($F(1,36) = 0.053$, $p = 0.820$). This indicates that WT mice performed significantly better than Tg mice with no significant beneficial effect of DHA supplementation. Statistical analysis also revealed a significant effect of day ($F(9,324) = 11.125$, $p < 0.001$) and significant interaction of day by genotype ($F(9,324) = 3.502$, $p < 0.001$) and day by diet ($F(9,324) = 3.455$, $p < 0.001$). Subsequent tests of simple main effects revealed that WT mice performed significantly better than Tg mice on day 4 ($F(1,36) = 4.621$, $p = 0.038$), day 7 ($F(1,36) = 7.542$, $p = 0.009$) and day 9 ($F(1,36) = 22.877$, $p < 0.001$) and that mice on DHA diet performed significantly worse on day 2 ($F(1,36) = 5.853$, $p = 0.021$) and significantly better than mice on oil blend diet on day 5 ($F(1,36) = 4.825$, $p = 0.035$), day 6 ($F(1,36) = 5.924$, $p = 0.020$) and day 7 ($F(1,36) = 6.058$, $p = 0.019$).

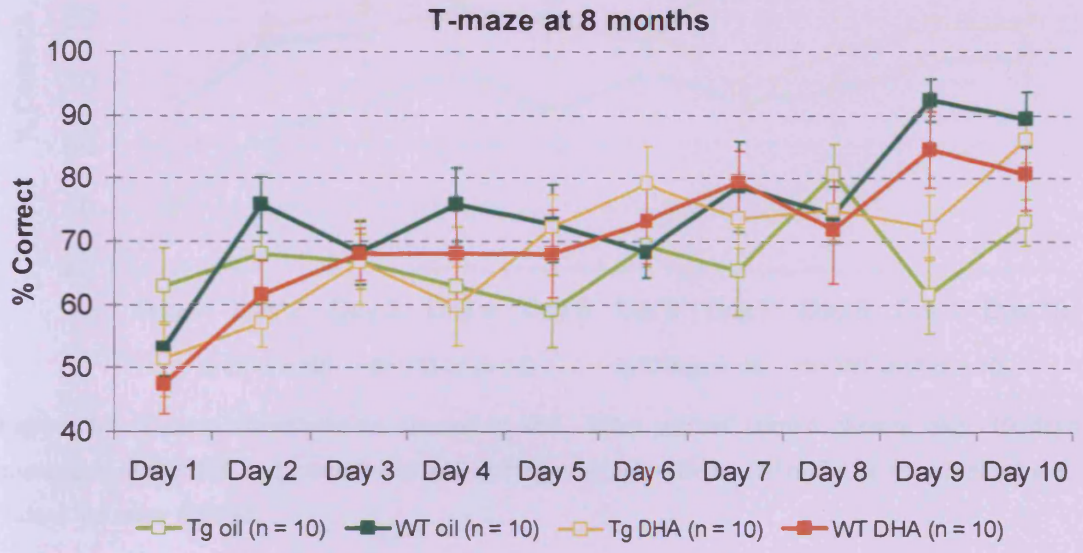


**Figure 3.8.** T-maze forced-choice alternation task. Mean percent correct choices over 10 days acquisition of the task in 8 month-old WT and Tg mice fed with the DHA diet or the oil blend diet. Values are mean ± SEM.

Figure 3.9 shows the percentage of correct choices for both Tg and WT mice on DHA or oil blend diet during acquisition of the T-maze forced choice alternation task at 12 months of age. Analysis of variance with day, genotype and diet as factors revealed a significant effect of genotype ($F(1,35) = 16.897$, $p < 0.001$) but no significant effect of diet ($F(1,35) = 2.815$, $p = 0.102$) and no significant interaction of genotype by diet ($F(1,35) = 0.558$, $p = 0.460$). This indicates that WT mice performed significantly better than Tg mice with no significant beneficial effect of DHA supplementation. Statistical analysis also revealed a significant effect of day ($F(9,315) = 10.968$, $p < 0.001$) and significant interaction of day by genotype ($F(9,315) = 1.916$, $p = 0.049$) but no significant interaction of day by diet ($F(9,315) = 0.705$, $p = 0.704$). Subsequent tests of simple main effects revealed that WT mice performed significantly better than Tg mice on day 2 ($F(1,35) = 4.862$, $p = 0.034$), day 5 ($F(1,35) = 5.797$, $p = 0.021$), day 7 ($F(1,35) = 20.570$, $p < 0.001$), day 8 ($F(1,35) = 10.817$, $p = 0.002$), day 9 ($F(1,35) = 29.961$, $p < 0.001$) and day 10 ($F(1,35) = 46.044$, $p < 0.001$).

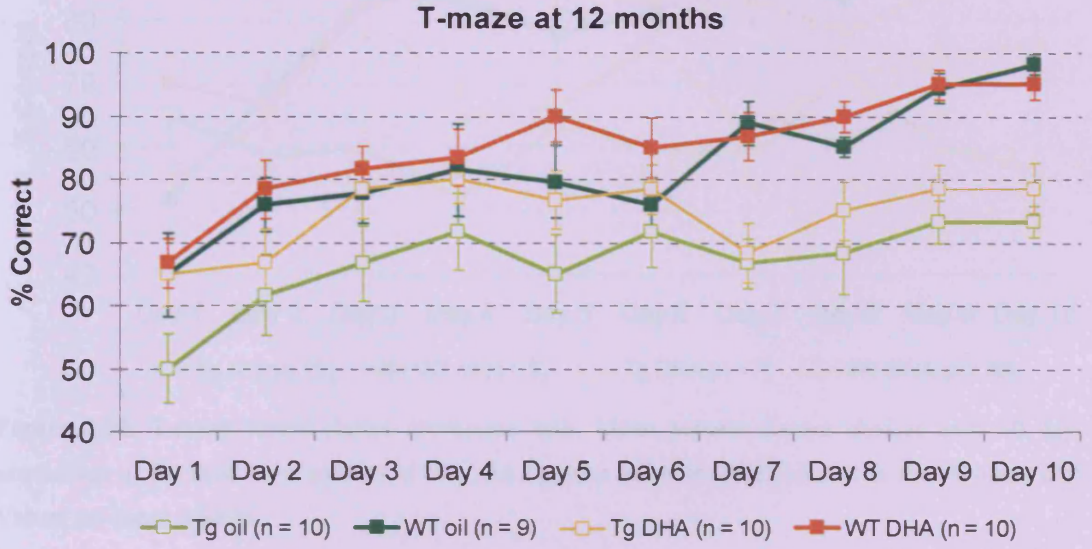


**Figure 3.9.** T-maze forced-choice alternation task. Mean percent correct choices over 10 days acquisition of the task in 12 month-old WT and Tg mice fed with the DHA diet or the oil blend diet. Values are mean ± SEM.

Figure 3.10 shows the percentage of correct choices for both Tg and WT mice on DHA or oil blend diet during acquisition of the T-maze forced choice alternation task at 16 months of age. Analysis of variance with day, genotype and diet as factors

revealed a significant effect of genotype ($F(1,32) = 22.118$, $p < 0.001$) but no significant effect of diet ($F(1,32) = 2.418$, $p = 0.130$) and no significant interaction of genotype by diet ($F(1,32) = 1.334$, $p = 0.257$). This indicates that WT mice performed significantly better than Tg mice with no significant beneficial effect of DHA supplementation. Statistical analysis also revealed a significant effect of day ($F(9,288) = 6.129$, $p < 0.001$) and significant interaction of day by genotype ($F(9,288) = 3.384$, $p = 0.001$) but no significant interaction of day by diet ($F(9,315) = 0.956$, $p = 0.477$). Subsequent tests of simple main effects revealed that WT mice performed significantly better than Tg mice on day 4 ($F(1,32) = 22.249$, $p < 0.001$), day 5 ($F(1,32) = 8.927$, $p = 0.005$), day 6 ($F(1,32) = 7.162$, $p = 0.012$), day 7 ($F(1,32) = 21.619$, $p < 0.001$), day 9 ($F(1,32) = 13.050$, $p = 0.001$) and day 10 ($F(1,32) = 23.145$, $p < 0.001$).

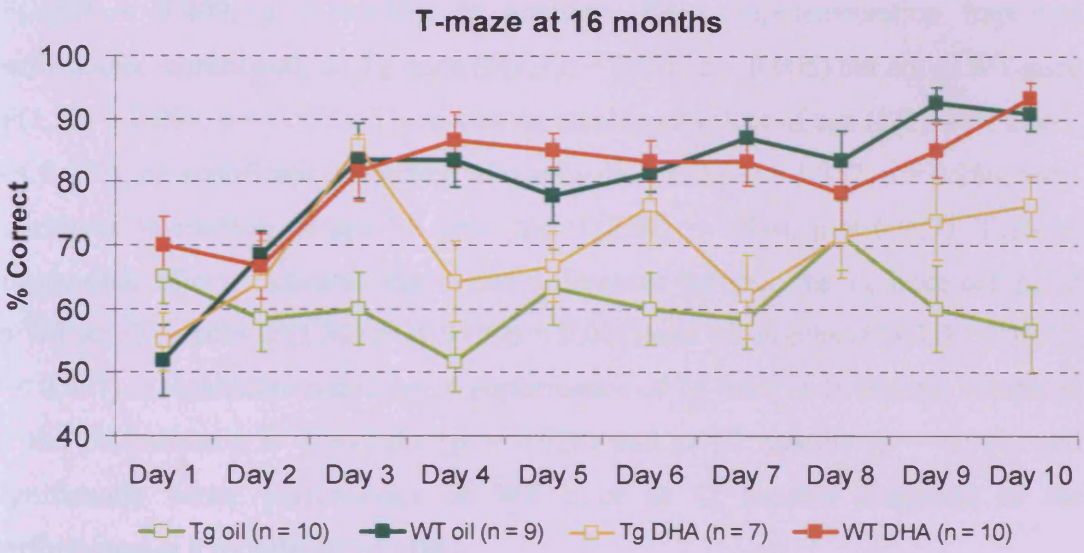


**Figure 3.10.** T-maze forced-choice alternation task. Mean percent correct choices over 10 days acquisition of the task in 16 month-old WT and Tg mice fed with the DHA diet or the oil blend diet. Values are mean ± SEM.

A significant three way interaction of day by genotype and diet ($F(9,288) = 2.474$, $p = 0.010$) was revealed and test of simple main effect showed that WT mice on DHA diet performed better than WT mice on oil blend diet on day 1 ($F(1,32) = 5.829$, $p = 0.022$), and Tg mice on DHA diet performed better than Tg mice on oil blend diet on day 3 ($F(1,32) = 9.166$, $p = 0.005$), day 6 ($F(1,32) = 4.288$, $p = 0.047$) and day 10 ($F(1,32) = 6.297$, $p = 0.017$).

Figure 3.11 shows the percentage of correct choices for both Tg and WT mice on DHA or oil blend diet on the last day of acquisition of the T-maze forced choice alternation task at 8, 12 and 16 months of age. It allows the comparison of the effect of genotype and diet as well as aging on the capacity of learning at different stages of the Aβ pathology. It was decided to compare the last day of testing because it corresponds to the time the mice had most exposures and trainings to the task and may correspond to the best performance the mice can reach, as a plateau effect can be observed after a few days of testing, especially in 16 month-old mice (Figure 3.10).

Analysis of variance with age, genotype and diet as factors revealed a significant main effect of genotype ($F(1,32) = 37.151$, $p < 0.001$), diet ($F(1,32) = 4.820$, $p = 0.036$) and significant interaction of genotype by diet ($F(1,32) = 4.467$, $p = 0.042$). Subsequent tests of simple main effects revealed that WT mice performed better than Tg mice on oil blend diet ($F(1,32) = 36.223$, $p < 0.001$) or DHA diet ($F(1,32) = 7.409$, $p = 0.010$). In addition, DHA supplementation improved performance significantly in Tg mice ($F(1,32) = 8.676$, $p = 0.006$) but not in WT mice ($F(1,32) = 0.004$, $p = 0.952$). There was no significant effect of age ($F(2,64) = 2.663$, $p = 0.077$), no significant interaction of age by diet ($F(2,64) = 1.352$, $p = 0.266$) but a significant interaction of age by genotype ($F(2,64) = 3.840$, $p = 0.027$). Tests of simple main effects indicated significant differences between the Tg mice compared to WT at 12 months ($F(1,32) = 39.938$, $p < 0.001$) and 16 months ($F(1,32) = 23.145$, $p < 0.001$), a significant reduction in performance of Tg mice at 16 months compared to the performance at 8 months ($p = 0.028$) and at 12 months ($p = 0.011$) and significantly better performance of WT mice at 12 months compared to the performance at 8 months ($p = 0.038$).

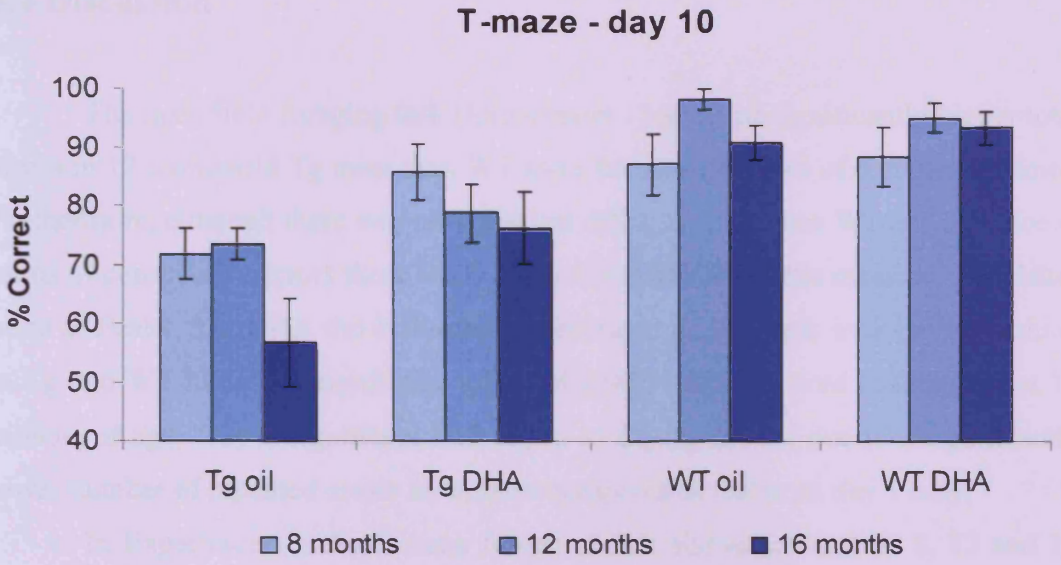


**Figure 3.11.** T-maze forced-choice alternation task. Percent correct choices on the last day of acquisition of the task (day 10) in 8, 12 and 16 month-old WT and Tg mice fed with the DHA diet or the oil blend diet. Values are mean ± SEM (Tg oil, n = 10; Tg DHA, n = 7; WT oil, n = 9; WT DHA, n = 10).

In summary the results of the T-maze study show that Tg2576 mice show a robust impairment on this task by 12 months of age. Furthermore, although DHA did not have a marked effect on performance during acquisition of the T-maze task at each age, analysis of terminal levels of performance indicated that DHA may have protected Tg2576 mice from an age-related decline in asymptotic levels of performance at 16 months of age.

## 3.4 Discussion

The open field foraging task (Experiment 1) revealed significantly higher total errors in 12 month-old Tg mice than WT mice but no difference of completion times. Furthermore, although there was no statistical difference between WT and Tg mice in terms of consecutive errors there was a main effect of diet on this measure. This latter point indicates that DHA did influence performance on this task in a similar fashion in Tg and WT mice. No significant effect of gender was observed in this task, at 12 months of age; only a significant interaction of day by gender due to a significantly lower number of repeated errors in females compared to males on day 1 only.

In Experiment 2, the T-maze forced choice alternation task at 8, 12 and 16 months of age, Tg mice were significantly impaired relative to their WT littermates. These results accord with previous findings where Tg2576 mice were significantly impaired in T-maze or Y-maze alternation tasks (Barnes et al., 2004; Chapman et al., 1999; Corcoran et al., 2002; Hsiao et al., 1996; King and Arendash, 2002; Lalonde et al., 2003; Ognibene et al., 2005). At 8 months of age as well as 12 and 16 months of age, the mice also showed significantly increased percentage of correct choice throughout the 10 days of the trial, suggesting that the mice learned the task. In addition, there was a significant interaction of day by genotype with increasing number of days the difference between WT and Tg was significant: WT mice performed better than Tg mice, on three days at 8 months of age, and on 6 days at 12 and 16 months, suggesting that the severity of learning and memory impairments increased with aging and with the progression of the Aβ pathology. DHA supplementation did not have a statistically robust effect on acquisition at any age. However, there was a significant interaction of day by diet at 8 months of age caused by a significantly better performance of oil blend-fed mice on day 2, and DHA-fed mice on day 5, 6 and 7. There was also a three ways interaction of day, genotype and diet at 16 months of age caused by significantly better performance of WT DHA-fed mice than WT oil blend-fed mice on day 1 and Tg DHA-fed mice than Tg oil blend-fed mice on day 3, 6 and 10, suggesting that DHA has a beneficial effect on spatial learning and memory. In addition, it is interesting to note that the proportion of percentage of correct choices being higher in DHA-fed mice than oil blend-fed mice, during the 10 days of trial, was 20% for WT mice and 50% for Tg mice at 8 months

of age, 80% for WT mice and 100% for Tg mice at 12 months of age, and 50% for WT mice and 80% for Tg mice at 16 months of age. This suggests that DHA diet did not improve performance at an early stage of the Aβ pathology but was more beneficial at 12 months for both WT and Tg, and at 16 months for Tg mice, when the Aβ pathology was at a more advanced stage.

The comparison of the performance of the four mouse groups on the last day of each of the three periods of T-maze testing showed a significant learning impairment of Tg mice compared to WT mice and a significant beneficial effect of DHA supplementation, suggesting that the APPswe mutation has a detrimental effect on learning and that DHA supplementation, by contrast, improves terminal levels of performance. The performance of the DHA-fed Tg mice compared to oil blend-fed Tg mice appeared significantly better, suggesting that DHA supplementation alleviates learning impairments caused by the Aβ pathology. However, as the performance of Tg mice on the DHA diet appeared significantly poorer than WT mice on the same diet, the impairment caused by the transgene was not completely alleviated by DHA supplementation. The performance of WT mice on either diet remained around 90% correct choice across the three trials with no significant effect of diet, and with a first significant increase of the mean from 8 to 12 months and a non-significant decrease from 12 to 16 months, suggesting that non naïve mice carried on learning the task from 8 to 12 months and might experience slight learning decline due to aging after the age of 12 months. Although there was no significant effect of age on learning, Tg mice appeared significantly impaired from 12 months of age compared to WT mice, and the performance of Tg mice appeared significantly lower at 16 months compared to 8 and 12 months, especially for oil blend-fed Tg mice but not for DHA-fed Tg mice. This suggests that DHA supplementation alleviated the age-related decline in learning presumably caused by the Aβ pathology.

Taken together, the data suggests that DHA supplementation mildly alleviates spatial learning and memory impairments caused by the Aβ pathology with a greater benefit as the mice age and the Aβ pathology increases. According to the literature, the plaque formation in hippocampal and cortical regions starts around 12 months of age (Kawarabayashi et al., 2001; Westerman et al., 2002), so it is interesting to note that Tg2576 mice presented spatial learning and working memory deficits before the appearance of plaques and that DHA supplementation seemed to have a greater impact after the appearance of plaques. However, brain Aβ levels had to be measured

in our mice before we were able to draw a conclusion, and this is the subject of Chapter 6 which presents an analysis of different forms of Aβ in cortex and hippocampus by immunohistochemistry and enzyme-linked immunosorbent assay.

The T-maze task is a robust test, widely used to assess the spatial learning and memory status in rodents. In our study, it allowed us to clearly discriminate Tg mice from WT mice which is necessary to test the effect of dietary DHA on the Aβ pathology carried by the Tg mice only. In fact, between the two tasks, the T-maze task was the most consistent in showing an impairment of the Tg2576 mice and a significant effect of dietary DHA. Although the open field foraging task is similar in principle to another widely used task, the radial-arm maze, the data collected from this task may not be sufficiently sensitive to drug effects in Tg2576 mice. In addition, impairments in olfactory discrimination and odour recognition memory are amongst early symptoms of AD (Gilbert et al., 2004). Moreover, it was shown that olfactory senses are also affected in Tg2576 mice (Young et al., 2009) and may also be affected by dietary DHA (Fedorova and Salem, 2006). Therefore, the outcome of the two tasks might have been affected by the olfactory abilities of the mice. For instance, in the open field foraging task, although the food rewards are buried under saw dust, WT mice may be able to perceive the odour of the rewards and therefore might use their olfactory sense to go from one pot to the other, instead of memorising the location of the visited pots, while Tg mice may have olfactory impairments and therefore may perform poorly on the task. In order to confirm our findings, future work may have to be carried out using additional tasks such as the Morris water maze, radial arm water maze or the an appetitive version of the radial-arm maze to assess the generality of the beneficial effects of DHA on performance across different motor and sensory domains.

# CHAPTER 4

# Effect of dietary docosahexaenoic acid on the plasma fatty acid composition in Tg2576 mice

## 4.1 Introduction

The aim of the work presented in this chapter was to investigate the effect of dietary DHA supplementation on the blood plasma fatty acid composition of both Tg and WT mice. Analyses were carried out from mice of different ages (12, 16 and 21 month-old mice) in order to investigate the effect of the development of the Aβ pathology, the dietary DHA supplementation and the duration of the feeding period on the fatty acid composition of mouse plasma. After a brief introduction regarding blood plasma and Alzheimer's disease or dietary DHA, the method used to analyse fatty acids in plasma will be presented. The results of these experiments will then be reported and discussed.

In blood plasma, fatty acids are present as free fatty acids and as constituents of phospholipids, cholesterol esters and triacylglycerols (TAG). Fatty acids provided by the diet are absorbed across the intestinal mucosa. The shorter chain fatty acids, with less than 10 or 12 carbons, are transported from the mucosal cells to the liver via portal blood. The longer chain fatty acids, with more than 10 or 12 carbons, are esterified into TAG and cholesterol esters in the intestinal mucosal cells. TAG and cholesterol esters then, together with proteins, phospholipids and cholesterol, form chylomicrons and are transported via the lymph system into the bloodstream. Together with very low density lipoproteins (VLDL) formed predominantly by the liver, the chylomicrons provide fatty acids for various tissues. Non-esterified fatty acids are transported while bound to various sites on serum albumin (Gurr et al., 2002).

There is some evidence that low blood levels of DHA or n-3 PUFA are associated with Alzheimer's disease. For example, a study of 1,188 elderly American subjects showed a correlation between serum phosphatidylcholine-DHA and the occurrence of AD (Kyle et al., 1999). Participants whose serum phosphatidylcholine-DHA was in the lower half of the distribution, but who had no symptoms of AD at the time the blood samples were taken, had a 67% greater risk of developing AD in the subsequent 10 years of life. Conquer et al. (2000) also found differences in the plasma phospholipid fatty acid composition in patients with AD compared to a control group. Fatty acid analyses were carried out on plasma total phospholipid, phosphatidylcholine (PC), phosphatidylethanolamine (PE) and lysophosphatidylcholine (lysoPC), from 84 participants including 19 cases of AD. The analyses revealed lower levels of EPA, DHA and combined n-3 PUFA in total phospholipid, PE and PC from AD patients compared to controls, with no significant changes in lysoPC fatty acid levels. In plasma total phospholipid, the proportion of EPA was decreased by approximately 42%, DHA by about 32% and total n-3 fatty acids by about 28%. Conversely, the relative concentration of total n-6 fatty acid was significantly higher in the AD group while total saturated, mono-unsaturated and poly-unsaturated fatty acids as well as total phospholipid did not differ significantly from the control group. In a case-control study, on 148 subjects with dementia and 45 controls, serum cholesteryl ester-EPA and DHA levels were significantly lower in AD patients compared with controls ($p < 0.05$ and $p < 0.001$ respectively) with a progressive decrease of DHA but not EPA found with the severity of dementia (Tully et al., 2003). In the Framingham Heart Study, plasma PC fatty acid levels were measured on 899 participants of 76 years median age and free of dementia (Schaefer et al., 2006). During the mean 9.1 years follow-up, 99 subjects developed dementia including 71 cases of AD. Subjects in the upper quartile of plasma PC DHA levels had a significant 47% reduction of the risk of developing dementia (95% confidence interval, 0.29-0.97, $p = 0.04$) and a relative risk of 0.61 of developing AD (95% CI, 0.31-1.18, $p = 0.14$). In addition, high levels of DHA in plasma PC were associated with high DHA and fish intake (18 g per day of DHA or 3 servings of fish per week). In the InCHIANTI study, carried out on 935 people over 65 years of age, participant with dementia had significantly lower n-3 PUFA levels (2.9% versus 3.2%, $p < 0.05$) and particularly 18:3n-3 levels (0.34% versus 0.39%, $p < 0.05$) than did participants with normal cognitive function (Cherubini et al., 2007). In the Atherosclerosis Risk in

Communities Study, fatty acid analysis was carried out on plasma cholesteryl esters and phospholipids from 2,251 subject aged 50-65 years (Beydoun et al., 2007). It revealed that elevated 16:0 in both fractions, high 20:4n-6 and low 18:2n-6 in cholesteryl esters were associated to a global cognitive decline in the 8 years follow up while higher levels of DHA + EPA were associated with a lower risk of word fluency decline. Although most studies show that higher blood levels of DHA are associated with a lower risk of AD, not all studies showed this inverse relationship between the level of DHA or n-3 PUFA and the risk of AD. One study showed a direct relationship between plasma levels of n-3 PUFA and dementia with increased levels of DHA in plasma phospholipids of demented patients (Laurin et al., 2003). In a study on 1,214 non-demented participants, 65 developed dementia during the following 4 years and showed that higher plasma EPA concentration was associated with a lower incidence of dementia whereas higher ratios of AA to DHA and of n-6 to n-3 fatty acids were related to an increased risk of dementia but the relations between plasma DHA, total n-3 PUFA, and incidence of dementia were not significant (Samieri et al., 2008). In a recent study, no significant associations were found between erythrocyte membrane total n-3 PUFA, DHA or EPA and AD or other forms of dementia (Kröger et al., 2009).

In addition to showing that higher levels of DHA in plasma is associated with a lower risk of AD, the study of Schaefer at al. (2006) also showed it was associated with the fish intake, the main source of DHA in the human diet. Other studies on humans also showed that diet influences blood fatty acid composition (Nikkari et al., 1983; Philibert et al., 2006) and dietary intake of DHA increases the blood level of DHA (Cao et al., 2006; Luukkainen et al., 1996; Meyer et al., 2007; Van De Rest et al., 2008). It is hypothesised that dietary DHA supplementation may compensate the deficiency in DHA by maintaining high blood levels of DHA and alleviate AD. Although previous studies showed that dietary n-3 PUFA supplementation (Higuchi et al., 2008; Lim and Suzuki, 2000; Maldonado et al., 2002) or depletion (Peltier et al., 2008) has a significant effect on the fatty acid composition of mouse plasma, there is no published work showing the effect of dietary DHA supplementation on plasma fatty acid composition in combination with the development of the Aβ pathology in a mouse model of AD. To date, there is only one published study on the 3xTg-AD mouse model in which red blood cells fatty acids were analysed after the mice were fed with different diets containing 1.27% DHA or a control diet (Green et

al., 2007). The analysis revealed that the percentage of DHA in red blood cells was more than double the control percentage after 3, 6 or 9 months on the DHA containing diets. However, this study did not include wild type mice that would allow the examination of the effect of the AD pathology and the red blood cell fatty acid profile. Hashimoto at al. also studied the effect of dietary DHA pre-administration prior to Aβ1-40 injection into the cerebral ventricle of Wistar rats and showed that plasma DHA levels were 23% lower in the Aβ rats than in the control rats and, conversely, 25.7% and 36% higher, respectively, in the DHA + Aβ and DHA rats (Hashimoto et al., 2002). In addition, the level of DHA was lower in the plasma of Aβ rats than control rats, suggesting that Aβ had a lowering effect on the plasma DHA level. In a following study, dietary DHA administration following Aβ1-40 injection increased significantly the level of plasma DHA in both the DHA + Aβ and the DHA groups compared to the control and Aβ groups, with a conversely significant decrease in arachidonic acid (Hashimoto et al., 2005b).

The purpose of the work presented in this chapter is to assess the effect of diet and evolution of Aβ pathology in Tg2576 mice on their plasma total fatty acid composition. In this study, Tg and WT mice were fed two different diets, a DHA-enriched diet containing approximately 1.8% of DHA (equivalent to a DHA content of 27.5% of total fatty acids), and a control oil blend diet containing 18:3n-3 as the main source of n-3 fatty acids. The dietary essential fatty acids 18:3n-3 and 18:2n-6 were present in both diets with respectively 2.7% and 28.0% of total fatty acids in the oil blend diet and, 2.5% and 23.6% of total fatty acid in the DHA diet. According to the studies cited earlier, mice fed with the DHA diet may show significantly higher levels of DHA in plasma compared to mice fed with the oil blend diet. Due to the development of the Aβ pathology, it was also hypothesised that Tg mice on oil blend diet might also present decreased plasma levels of n-3 PUFA compared to WT mice on the same diet, while these changes might be prevented by DHA supplementation. As the mice grew older (from 12 to 21 months of age) and as the symptoms of AD caused by the accumulation of Aβ became more and more severe, changes of plasma fatty acid composition in Tg mice may also become increasingly evident.

## 4.2 Materials and methods

### 4.2.1 Subjects

Fatty acid analyses were carried out on blood plasma from 12, 16 and 21 month-old Tg2576 and WT mice which had been on experimental diets from the age of 4 months. A full description of the breeding, genotyping and maintenance of the mice is presented in Chapter 2. The origin of the plasma samples is presented in Table 4.1.

**Table 4.1.** Tg and WT mice on which plasma fatty acid analyses were carried out: 12, 16 and 21 month-old Tg mice on oil blend diet (Tg oil), WT mice on oil blend diet (WT oil), Tg mice on DHA diet (Tg DHA) and WT mice on DHA diet (WT DHA).

| | Cohort 1 12 months | | Cohort 2 16 months | | Cohort 3 21 months | |
|---|---|---|---|---|---|---|
| Gender | male | female | male | female | male | female |
| Tg oil | 2 | 2 | 3 | - | - | 3 |
| WT oil | 2 | 2 | 3 | - | - | 2 |
| Tg DHA | 2 | 2 | 3 | - | - | 3 |
| WT DHA | 2 | 2 | 3 | - | - | 3 |

### 4.2.2 Fatty acid analysis by gas liquid chromatography

In order to analyse plasma total fatty acid composition by gas liquid chromatography (GLC), mouse blood was collected at the time of their sacrifice. Following decapitation, trunk blood was collected in tubes containing 10 µl EDTA 0.5 M as an anti-coagulant. Samples were placed on ice before being centrifuged at 1,500 r.p.m. for 15 min. in order to separate plasma from red blood cells. Plasma was transferred to clean tubes and samples were stored at -80°C until use.

For the extraction of plasma lipids, MilliQ$^{TM}$ water (Millipore, Bedford, MA, USA) was added to each sample up to 1 g. Then, lipids were extracted using the method of Garbus et al. (1963) as described in section 5.2.4 and resuspended in 300 µl of chloroform-methanol (2:1 by volume) with 0.1% (w/v) BHT. The amount of lipid extract necessary for each fatty acid analysis was equivalent to approximately 50 µl of plasma. For this analysis, 20 µg of pentadecanoic fatty acid (15:0) (Nu-Chek

Prep Inc., Elysian, MN, USA) were added to each sample. Then, samples were treated and analysed by GLC, as described in sections 5.2.6, 5.2.7 and 5.2.8.

### 4.2.3 Data analysis

For each fatty acid, the result was expressed as a percentage of total fatty acids. The mean and standard error of the mean were calculated for the four groups (Tg oil, WT oil, Tg DHA and WT DHA). Statistical analysis of DHA relative concentration was carried across the three cohorts using analysis of variance (ANOVA) with genotype, diet, age and gender as factors. Statistical analyses of plasma types of fatty acids (saturated, monounsaturated, polyunsaturated, n-3 and n-6 fatty acids) were carried out individually, on each type of fatty acid of each cohort, by ANOVA with genotype, diet and gender as factors. Statistical analysis of major plasma fatty acids were carried on individual cohorts by ANOVA with genotype, diet and gender as between subject factors and fatty acid as a within subject factor, followed by tests of simple main effect when the interaction between factors was significant.

## 4.3 Results

Fatty acid analyses were carried out on plasma from 12 month-old, 16 month-old and 21 month-old Tg2576 mice on oil blend or DHA diet since the age of 4 months. The results are presented in three sections divided by the cohort age: 12, 16 and 21 months. A table showing percentages of total fatty acids of the different classes and a graph showing percentages of the main individual fatty acids are presented in each of the three sections. Complete plasma fatty acid compositions at 12, 16 and 21 months of age are presented in Appendix 1, 2 and 3.

At the three different time points, the main fatty acids present in plasma were palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1n-9), linoleic acid (18:2n-6), arachidonic acid (20:4n-6) and DHA (Figure 4.1, 4.2 and 4.3). Polyunsaturated fatty acids represented the largest fraction of fatty acids in plasma, with 42.9% ± 2.3% to 63.0% ± 0.2% of total fatty acids (Table 4.2, 4.3 and 4.4). Total saturated fatty acids and total monounsaturated represented 24.6% ± 0.3% to 32.8% ± 1.4% and 12.4% ± 0.4 to 25.4% ± 2.8% of total fatty acids respectively (Table 4.2, 4.3 and 4.4).

The comparison of the plasma fatty acid composition with the fatty acid composition of the diets (Figure 2.4) revealed that the main long chain fatty acids remained the same in both diet and plasma. These fatty acids were 16:0, 18:0, 18:1n-9 and 18:2n-6 (Figure 4.1, 4.2 and 4.3). However, major differences were observed in the very long chain PUFA. Arachidonic acid (20:4n-6) was not detected in either of the two diets while it represented 8.5% ± 1.6% to 17.2% ± 3.2% of total fatty acids in plasma from mice on oil blend diet and 0.4% ± 0.1% to 1.4% ± 0.2% of total fatty acids in plasma from mice on DHA diet. DHA was also present and represented 2.5% ± 0.4% to 5.1% ± 0.7% of total fatty acids in plasma from oil blend-fed mice despite not being detected in the oil blend diet. It was present at a much higher level in plasma from DHA-fed mice compared to oil fed mice ($F(1,23) = 547.936$, $p < 0.001$), with 13.9% ± 1.2% to 28.2% ± 2.2% of total fatty acids across the three age groups. The proportion of DHA was also significantly different between the three age groups ($F(2,23) = 23.045$, $p < 0.001$) with a significant interaction of diet and age ($F(2,23) = 25.232$, $p < 0.001$) caused by significant difference of DHA level between oil blend

and DHA-fed mice at the three time points ($p < 0.001$), and by significant differences between DHA-fed mice at 12,16 and 21 months of age ($p < 0.005$) but not between age groups of oil blend-fed mice ($p > 0.7$). There was no significant effect of genotype ($F(1,23) = 0.169$, $p = 0.685$) or gender ($F(1,23) = 1.313$, $p = 0.264$) (data not shown) on the level of DHA in plasma. Interestingly, the proportion of arachidonic acid and the proportion of DHA appeared counterbalanced, arachidonic acid replacing DHA when DHA was not provided by the diet.

### 4.3.1 Twelve months of age

Table 4.2 shows that at 12 months of age, diet or genotype did not have a significant effect on the proportion of total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) or polyunsaturated fatty acids (PUFA) (max. $F(1,8) = 3.325$, $p > 0.05$). However, the proportion of total n-3 fatty acids was significantly higher ($F(1,8) = 130.531$, $p < 0.001$) while the proportion of total n-6 fatty acids was significantly lower ($F(1,8) = 47.029$, $p < 0.001$) in plasma from DHA-fed mice than oil blend-fed mice with no significant effect of genotype ($p > 0.05$). The n-3 to n-6 ratio was also significantly higher in plasma from mice on the DHA diet than mice on the oil blend diet ($F(1,8) = 48.416$, $p < 0.001$). The effect of gender was also tested and the levels of total saturated fatty acids were significantly higher in females than males with respectively 32.72% ± 0.64% and 30.55% ± 0.64% of total fatty acids ($F(1,8) = 5.611$, $p = 0.045$) (data not shown).

**Table 4.2.** Fatty acid composition of plasma from 12 month-old WT and Tg mice on oil blend or DHA diet. Values represent mean percentages of total fatty acids ± SEM.

| Fatty acids | Tg oil (n = 4) | WT oil (n = 4) | Tg DHA (n = 4) | WT DHA (n = 4) |
|---|---|---|---|---|
| Total SFA | 31.6 ± 1.1 | 31.0 ± 0.7 | 32.8 ± 1.4 | 31.1 ± 1.0 |
| Total MUFA | 25.4 ± 2.8 | 23.9 ± 1.5 | 24.3 ± 2.7 | 21.8 ± 1.0 |
| Total PUFA | 42.9 ± 2.3 | 45.1 ± 1.0 | 42.9 ± 1.3 | 47.0 ± 1.0 |
| Total n-3 FA *** | 5.5 ± 0.7 | 5.2 ± 0.2 | 17.8 ± 1.3 | 19.3 ± 1.6 |
| Total n-6 FA*** | 37.2 ± 1.7 | 39.8 ± 0.9 | 24.9 ± 2.5 | 27.5 ± 1.1 |
| n-3/n-6 ratio *** | 0.15 ± 0.01 | 0.13 ± 0.01 | 0.72 ± 0.14 | 0.70 ± 0.08 |

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. Significant effect of diet, *** $p < 0.001$.

The main fatty acids present in plasma at 12 months are presented in Figure 4.1. Levels of 16:0 ($F(1,8) = 5.689$, $p = 0.044$) and DHA ($F(1,8) = 102.149$, $p <$

0.001) were significantly higher in plasma from DHA-fed mice than oil blend-fed mice while the level of 20:4n-6 was significantly higher in plasma from oil blend-fed mice ($F(1,8) = 49.663$, $p < 0.001$).

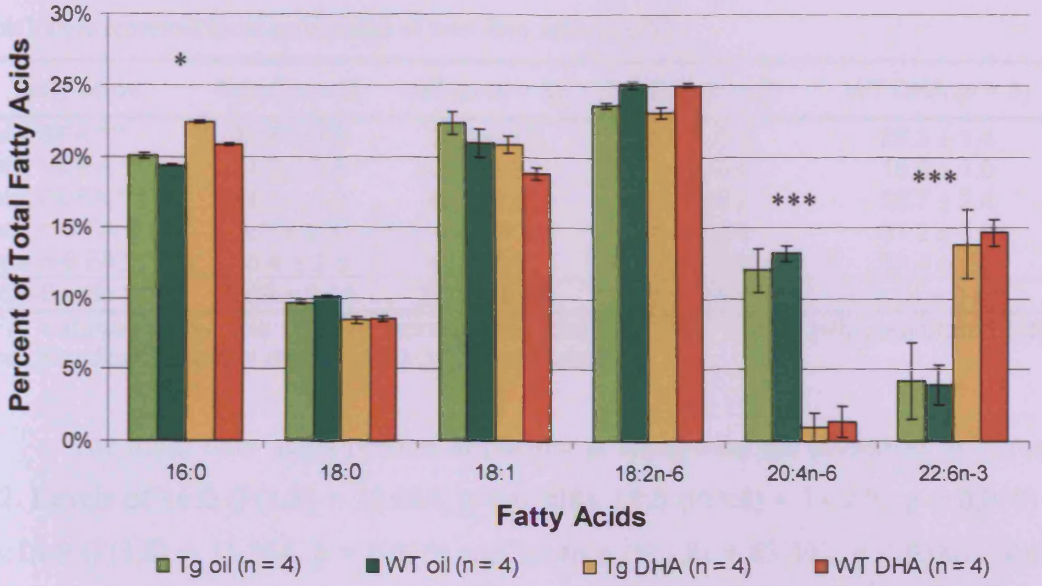


**Figure 4.1.** Main fatty acids in plasma from 12 month-old WT and Tg mice on oil blend or DHA diet. Results are represented as mean percentages of total fatty acids ± SEM. 18:1 includes 18:1n-9 and 18:1n-7. Significant effect of diet, * $p < 0.05$, *** $p < 0.001$.

### 4.3.2 Sixteen months of age

At 16 and 21 months of age, the proportion of total SFA, MUFA and PUFA seemed to reflect the different proportions of fatty acids present in the diets to a greater degree than at 12 months of age.

At 16 months of age, levels of total SFA, MUFA, PUFA, n-3 FA or n-6 FA in plasma, presented in Table 4.3, were not significantly different between WT and Tg mice (max. $F(1,8) = 0.512$, $p > 0.05$). However, the statistical analysis showed that the proportions of the different classes of fatty acids were significantly different with the two diets: levels of total SFA, total MUFA and total n-6 fatty acids were significantly higher (respectively $F(1,8) = 34.670$, $p < 0.001$, $F(1,8) = 17.269$, $p = 0.003$ and $F(1,8) = 34.781$, $p < 0.001$) in the plasma from oil blend-fed mice, while levels of total PUFA and total n-3 fatty acids were significantly higher ($F(1,8) =$

39.456, $p < 0.001$ and $F(1,8) = 241.255$, $p < 0.001$) in plasma from DHA-fed mice. The n-3 to n-6 ratio was also significantly higher in plasma from mice on the DHA diet than mice on the oil blend diet ($F(1,8) = 91.188$, $p < 0.001$).

**Table 4.3.** Fatty acid composition of plasma from 16 month-old WT and Tg mice on oil blend or DHA diet. Values represent mean percentages of total fatty acids ± SEM.

| Fatty acids | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
|---|---|---|---|---|
| Total SFA *** | 30.8 ± 0.9 | 30.3 ± 0.3 | 24.6 ± 0.3 | 26.3 ± 1.4 |
| Total MUFA ** | 24.9 ± 2.9 | 20.4 ± 3.0 | 12.4 ± 0.4 | 15.0 ± 1.0 |
| Total PUFA *** | 44.3 ± 2.0 | 49.3 ± 3.2 | 63.0 ± 0.2 | 58.7 ± 2.4 |
| Total n-3 FA *** | 3.7 ± 0.3 | 6.3 ± 0.7 | 32.5 ± 2.8 | 31.2 ± 1.9 |
| Total n-6 FA*** | 40.4 ± 2.0 | 42.9 ± 2.7 | 30.4 ± 2.6 | 27.4 ± 1.0 |
| n-3/n-6 ratio *** | 0.09 ± 0.01 | 0.15 ± 0.01 | 1.11 ± 0.20 | 1.14 ± 0.07 |

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. Significant effect of diet, ** $p < 0.01$, *** $p < 0.001$.

The main fatty acids present in plasma at 16 months are presented in Figure 4.2. Levels of 16:0 ($F(1,8) = 12.084$, $p = 0.008$), 18:0 ($F(1,8) = 11.279$, $p = 0.010$), 18:1n-9 ($F(1,8) = 11.364$, $p = 0.010$) and 20:4n-6 ($F(1,8) = 43.301$, $p < 0.001$) were significantly higher in plasma from oil blend-fed mice than DHA-fed mice while the level of DHA was higher in plasma from DHA-fed mice ($F(1,8) = 233.815$, $p < 0.001$).

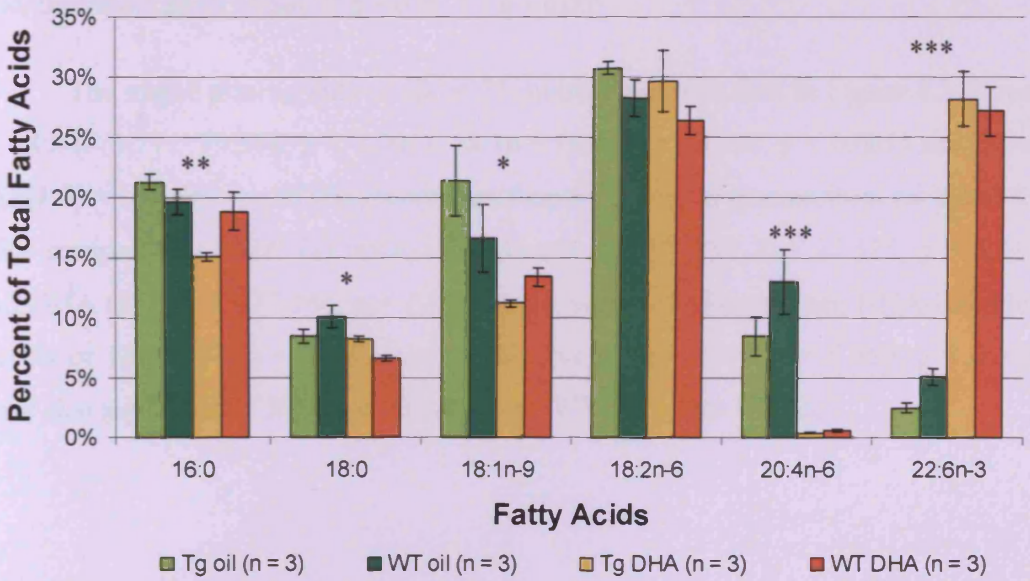


**Figure 4.2.** Main fatty acids in plasma from 16 month-old WT and Tg mice on oil blend or DHA diet. Results are represented as mean percentages of total fatty acids ± SEM. Significant effect of diet, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

### 4.3.3 Twenty one months of age

At 21 months of age, levels of total SFA, MUFA, PUFA, n-3 FA or n-6 FA in plasma, presented in Table 4.4, were not significantly different between WT and Tg mice (max. $F(1,7) = 2.081$, $p > 0.05$). However, levels of some of the different classes of fatty acids were significantly different with the two diets: levels of total MUFA and total n-6 fatty acids were significantly higher (respectively $F(1,7) = 29.476$, $p = 0.001$ and $F(1,7) = 215.363$, $p < 0.001$) in the plasma from oil blend-fed mice, while the levels of total PUFA and total n-3 fatty acids were significantly higher ($F(1,7) = 267.858$, $p < 0.001$ and $F(1,7) = 520.658$, $p < 0.001$) in plasma from DHA-fed mice. The n-3/n-6 ratio was also significantly higher in plasma from mice on DHA diet than mice on oil blend diet ($F(1,7) = 220.168$, $p < 0.001$).

**Table 4.4.** Fatty acid composition of plasma from 21 month-old WT and Tg mice on oil blend or DHA diet. Values represent mean percentages of total fatty acids ± SEM.

| Fatty acids | Tg oil (n = 3) | WT oil (n = 2) | Tg DHA (n = 3) | WT DHA (n = 3) |
|---|---|---|---|---|
| Total SFA | 31.7 ± 1.0 | 32.8 ± 0.3 | 31.2 ± 0.2 | 32.4 ± 1.0 |
| Total MUFA ** | 25.4 ± 1.5 | 22.5 ± 0.6 | 17.6 ± 0.3 | 19.2 ± 0.9 |
| Total PUFA *** | 43.0 ± 0.5 | 44.7 ± 0.2 | 51.2 ± 0.4 | 48.4 ± 0.2 |
| Total n-3 FA *** | 4.4 ± 0.5 | 4.8 ± 0.3 | 22.9 ± 0.7 | 21.7 ± 1.1 |
| Total n-6 FA*** | 38.4 ± 0.7 | 39.7 ± 0.5 | 28.3 ± 0.7 | 26.5 ± 0.9 |
| n-3/n-6 ratio *** | 0.11 ± 0.02 | 0.12 ± 0.01 | 0.81 ± 0.04 | 0.82 ± 0.07 |

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. Significant effect of diet, ** $p < 0.01$, *** $p < 0.001$.

The major plasma fatty acids at 21 months are presented in Figure 4.3. Levels of 18:0 ($F(1,7) = 39.518$, $p < 0.001$), 18:1n-9 ($F(1,7) = 50.292$, $p < 0.001$) and 20:4n-6 ($F(1,7) = 110.882$, $p < 0.001$) were significantly higher in plasma from oil blend-fed mice compared to DHA-fed mice, while levels of 16:0 ($F(1,7) = 11.525$, $p = 0.012$) and DHA ($F(1,7) = 677.744$, $p < 0.001$) were higher in plasma from DHA-fed mice. Levels of 18:0 ($F(1,7) = 6.420$, $p = 0.039$) and 20:4n-6 ($F(1,7) = 5.669$, $p = 0.049$) were also significantly higher in plasma from WT mice than Tg mice.

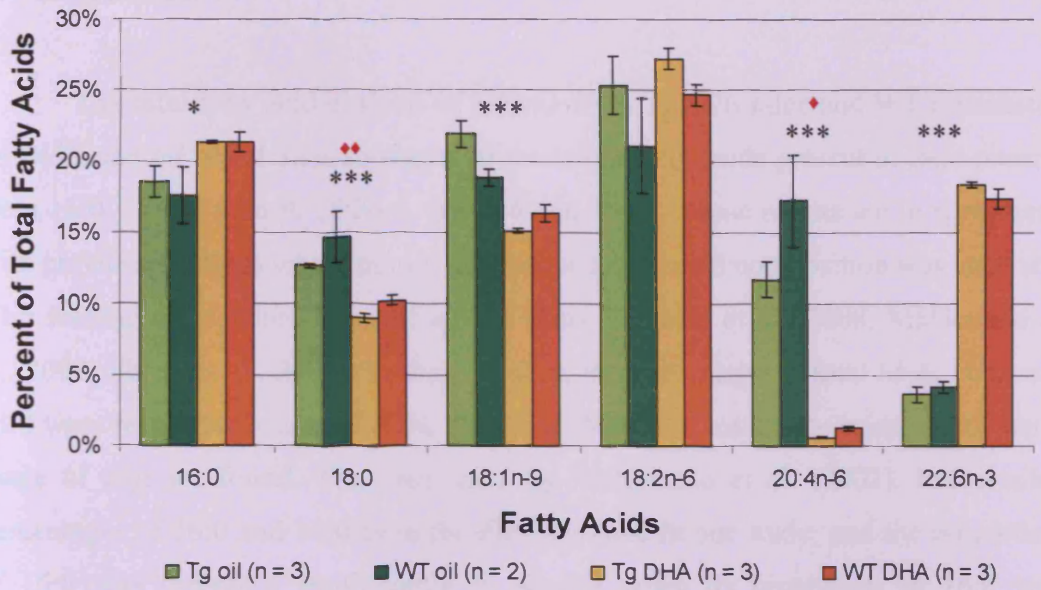


**Figure 4.3.** Main fatty acids in plasma from 21 month-old WT and Tg mice on oil blend or DHA diet. Results are represented as mean percentages of total fatty acids ± SEM. Significant effect of diet, * $p < 0.05$, *** $p < 0.001$, Significant effect of genotype, ♦ $p = 0.049$, ♦♦ $p = 0.039$.

## 4.4 Discussion

The total fatty acid analysis of plasma from Tg2576 mice and WT littermates on DHA and oil blend diets showed that the major fatty acids present in their plasma were 16:0, 18:0, 18:1n-9, 18:2n-6, 20:4n-6 and DHA. These results are in agreement with previous findings where mouse plasma total fatty acid composition was analysed after feeding on standard chow or special diets (Higuchi et al., 2008; Maldonado et al., 2002; Shirai et al., 2005). In these studies, the percentage of total SFA, 16:0 and 18:0 were respectively around 30%, 20% and 7%-9% of total fatty acids, in the same range of that we found. The diets used by Maldonado et al. (2002), had similar percentages of 16:0 and 18:0 as in the diets we used in our study; and the proportion of 16:0 was increased significantly in plasma when its proportion of 16:0 was increased in the diet. This is in agreement with our findings at 16 and 21 months where the proportions of 16:0 and 18:0 were higher in plasma from mice fed with the oil blend diet than mice fed with the DHA diet, suggesting that 16:0 and 18:0 present in plasma were absorbed from the diet. Our results also showed variations of the relative concentrations of total MUFA and 18:1n-9, with respectively 12% to 25% and 11% to 22% of total fatty acids, and with a significant effect of the diet associated with a lower proportion of 18:1n-9 in the DHA diet. The proportion of these fatty acids and 18:2n-6 also appeared variable in the studies of Maldonado et al. (2002) and Shirai et al. (2005), with around 15% to 20% MUFA, 13% to 24% 18:1n-9 and 13% to 32% 18:2n-6. In the study of Maladonado et al. (2002), the proportion of 18:2n-6 was significantly deceased when the mice were fed with a diet containing less 18:2n-6. The proportions of 18:1 and 18:2n-6 were also respectively lower and higher in the standard chow than in our oil blend diet, and these differences were reproduced in the plasma fatty acid composition, suggesting that the presence of mono- and di-unsaturated fatty acids in plasma is largely a result of absorption of these fatty acids from the diet.

Although the proportion of fatty acids containing 18 or less carbons in plasma seems to be in keeping with their levels in the diet, the presence of very long chain PUFA in plasma appeared less closely correlated to their levels in diet. Despite the absence of 20:4n-6 in both diets, the fatty acid was present in plasma from oil blend and DHA-fed mice with a significantly higher proportion in plasma from oil blend-

fed mice compared to DHA-fed mice ($p < 0.001$). DHA was also present in plasma from oil blend-fed mice despite not being detected in the oil blend diet. As expected, the proportion of DHA was significantly higher in plasma from DHA-fed mice compared to oil blend-fed mice ($p < 0.001$). These results are in agreement with the study of Maladonado et al. (2002). In their study, when the mice first received a standard chow containing only 0.2% 20:4n-6 and 0.1% DHA, the proportion of DHA was low but higher than in the diet and the proportion of 20:4n-6 represented around three times the proportion of DHA. When the diet was changed to a fish oil-supplemented diet with 9.9% DHA and 2% 20:4n-6, the plasma proportions were reversed with a significantly higher proportion of DHA and significantly lower proportion of 20:4n-6 compared to the proportions previously observed. These results also agree with the findings of Higuchi et al. (2008) where DHA was present in plasma, with or without DHA in the diet, and the proportion of DHA was increased as the level in the diet was increased, with a reverse effect on the proportion of 20:4n-6. 18:3n-3 and 18:2n-6, which are both present in both oil blend and DHA diets, are precursors for DHA and 20:4n-6 respectively, so in the absence of dietary DHA and 20:4n-6, both may be synthesized from their respective precursors. In the presence of dietary DHA, the level of 20:4n-6 was low but still present, suggesting that its metabolism was changed with either an increased turnover or a decreased conversion rate of 18:2n-6 than in absence of DHA, and DHA was present in a much higher proportion compared to plasma from oil blend-fed mice. These results suggest that when DHA is present in the diet, it is absorbed, but when DHA is not in the diet, only a small amount of DHA can be synthesised from 18:3n-3. Therefore, the reduced amount of DHA is replaced, to a large extent, by 20:4n-6, suggesting that the best source of DHA may be from the diet and that conversion of n-3 precursors into DHA may not be efficient enough to achieve the maximum levels of DHA in plasma. It has been shown that n-3 PUFA deprivation increases coefficients of conversion of 18:3n-3 to DHA by up-regulating elongases and desaturases expression in rat liver (Igarashi et al., 2007b). However, in human, it was found that only a small amount of 18:3n-3 was converted to DHA and increasing intake of 18:3n-3 only increased the proportion of EPA but not that of DHA in plasma (Burdge and Calder, 2005), underlining the importance of DHA intake *per se*. The relative concentrations of 20:4n-6 and DHA interchanged, suggesting that their biosynthesis and acyl transfer were in competition. Several studies have also shown a balance between n-3 and n-6 fatty acids. For

example, in monkeys, changing n-3 fatty acid-deficient diet to an n-3 fatty acid rich diet increased total plasma n-3 fatty acids, including EPA, 22:5n-3 and DHA, and a reciprocal decrease in n-6 fatty acids including, linoleic acid and 20:4n-6, was also observed (Connor et al., 1990). Another example was also shown in plasma as well as brain and other rat tissues, where DHA was replaced by 22:5n-6 only in the absence of dietary DHA, suggesting that n-6 fatty acids do not compete with DHA (Stark et al., 2007). These findings suggest that in oil blend-fed mice, the absence of dietary DHA may be compensated by higher levels of n-6 fatty acids in plasma. The conversion pathways of 18:3n-3 to DHA and 18:2n-6 to AA are using the same enzymes and these conversions occur principally in the liver, by sequential Δ-6 desaturation, elongation, and Δ-5 desaturation for the synthesis of AA and further conversions for the synthesis of DHA, with an additional two sequential elongations, Δ-6 desaturation and β-oxidation (Gurr et al., 2002). This suggests that there may be a competition between 18:2n-6 and 18:3n-3 for the enzymes which could also be a reason of the counterbalanced effect between DHA and AA. DHA synthesis is also more complex, especially via the Sprecher pathway, which could be another reason why AA is more abundant in absence of dietary DHA.

In addition to a decreased level of DHA in the plasma of oil blend-fed mice we have seen increased levels of n-6 fatty acids and especially 20:4n-6. AA can be metabolised to eicosanoids such as prostaglandins (Gurr et al., 2002) while DHA can give rise to neuroprotectins and resolvins (Bannenberg et al., 2007; Serhan et al., 2004), and possibly other eicosanoids. Previous work showed that AA metabolites are highly pro-inflammatory mediators whereas EPA and DHA metabolites are less inflammatory or even anti-inflammatory (Bannenberg et al., 2007; Gurr et al., 2002). Therefore increased levels of AA due to decreased levels of DHA could be a cause of neuroinflammation and trigger the development of AD.

The effect of diet appeared more and more prevalent as the mice were getting older. At 12 months of age (8 months on the diets), no significant differences were observed in the global fatty acid analysis. Differences were only observed in total n-3 and n-6 fatty acids and some individual fatty acids. Significant differences of total SAT, MUFA and PUFA appeared from the age of 16 months (12 months on the diets) with more differences in individual fatty acids. The same trends were observed in the 21 month-old cohort (17 months on the diets). These results suggest that mice might

need to be fed for a long period of time for the diet to have a maximum effect on the plasma fatty acid composition. However, the study of Maldonado et al. (2002) suggested that 10 days of fish oil supplementation were sufficient to reach maximum proportions of n-3 PUFA in mouse plasma. In the study of Connor et al. (1990), 10 weeks of fish oil supplementation appeared to be enough time to reach the maximum effect of dietary change in monkeys. This suggests that rather than the effect of time of administration of diets, the changes may be due to aging (from 12 to 21 months). In old mice, the regulation of plasma fatty acid composition may not be as tightly regulated as in young mice, due to deficiency of enzymes involved in this regulation. As a consequence, the fatty acid composition of plasma could reflect more closely the fatty acid composition of the diet.

A significant effect of genotype was only observed at the latest stage, in 21 month-old mice, with a lower relative proportion of 18:0 and 20:4n-6 in plasma from Tg mice compared to WT mice ($p < 0.05$). The lower percentage of 20:4n-6 in Tg mice plasma, might indicate a deficiency in converting the 18:2n-6 precursor to very long chain PUFA, caused by the development of the Aβ pathology. In the study of Tully et al. (2003), in addition to significantly lower plasma levels of cholesteryl ester-EPA and DHA in AD patients compared to controls, most AD patients had significantly lower plasma levels of 18:2n-6 and total n-6 PUFA compared to controls. In addition, it has previously been proposed that the metabolic capacity of fatty acid synthesis declines with age and in age-related diseases, which could contribute to a reduction of 20:4n-6 and DHA in plasma of AD patients (Babin et al., 1999). This suggests that old Tg2576 mice may have an alteration of Δ-5 and/or Δ-6 desaturase activity which may also be the cause of decreased levels of DHA in AD. This reduction in Δ-5 and/or Δ-6 desaturase activity might also cause an accumulation of the precursor 18:2n-6 in plasma (Duffin et al., 2001; Obukowicz et al., 1998). Although, 18:2n-6 was the only major fatty acid for which no significant effect of diet or genotype was observed, at 16 and 21 months, the average percentage of 18:2n-6 was always higher in Tg mice compared to the average of the matching WT mice. These findings are in agreement with the study of Tully et al. (2003) in which plasma cholesteryl ester levels of 18:2n-6 and total n-6 PUFA levels but not 20:4n-6 levels were significantly lower in the AD subjects with lowest scores at the mini mental state examination.

In addition to the effect of aging, gender may also be responsible for some effects. Burdge et al. (2005) suggested that due to a regulatory effect of oestrogen, women may have a greater ability to convert 18:3n-3 to DHA (with a possible up-regulation during pregnancy) than men. Gender differences in the plasma fatty acid composition were also reported in mice (Peltier et al., 2008). In our study, the analysis at 12 months of age was done on both males and females, and the only significant effect of gender was on total SFA where the proportion was higher in females compared to males. Thereafter, analyses were done on plasma from males at 16 months and plasma from females at 21 months so, in addition to the effect of aging, there may be some effects due to gender. For 16:0 which is a saturated fatty acid, the proportion was significantly higher in DHA-fed mice at 12 months when females had a significantly higher proportion of total SFA and at 21 months, when the analysis was done on female plasma only. Conversely, the proportion of 16:0 was significantly lower in DHA-fed mice compared to oil blend-fed mice at 16 months of age, when the analysis was done on plasma from males only. As the effect of gender was seen at 12 months on total SFA only, it is acceptable to compare the effect of aging in males between 12 and 16 months of age and in females between 12 and 21 months of age but the comparison between the analysis at 16 and 21 months may include effects of both gender and age. In DHA-fed mice, the relative concentration of DHA increased from the age of 12 months to the age of 16 months in males and to the age of 21 months in females. As some fatty acid levels seem to reflect more closely the diet than others, it might also be important to note the mice have not been fasted before sacrifice so some variations between cohorts might also be due to the time when the mice were sacrificed.

In conclusion, the results showed that dietary DHA was absorbed and detected in the plasma of both Tg2576 and WT mice. In the absence of dietary DHA, the proportion of DHA was essentially replaced by AA in plasma. Since AA was not present in the diets, this suggested that while AA could be synthesised from 18:2n-6, dietary DHA is the best source of DHA. Moreover, the increased levels of plasma AA may lead to increased inflammation which could trigger the development of AD or exacerbate the disease. The decreased percentage of AA in plasma of old Tg mice compared to WT mice suggested a reduction of desaturation and/or elongation enzymatic activity which may be caused by the trangene in Tg2576 mice. As these

enzymes are also involved in DHA synthesis from 18:3n-3, this may also affect DHA levels in AD.

# CHAPTER 5

# Effect of dietary docosahexaenoic acid on the lipid composition of cortex, hippocampus and cerebellum in Tg2576 mice

## 5.1. Introduction

The aim of the work presented in this chapter was to investigate the effect of dietary DHA supplementation on the fatty acid composition of brain total lipids and individual phospholipids in both Tg and WT mice. Analyses were carried out at two different time points, on brain lipid extracts from 12 and 16 month-old mice, in order to investigate the effect of the APPswe mutation and the dietary DHA supplementation on the lipid composition of mouse brain. After a brief introduction on the brain lipid composition in Alzheimer's disease and following dietary DHA, the methods used to analyse lipids in brain will be presented. Then, the results of these experiments will be reported and discussed.

Phospholipids are essential components of cell membranes, providing important structural and functional properties. They consist of two fatty acids joined to a polar head group. In the glycerophospholipids, the two fatty acids are bound to carbon atoms of a glycerol molecule and the third carbon atom of glycerol is bound to a phosphate group, which is usually esterified to another small polar molecule, such as ethanolamine, choline, serine or inositol. Sphingomyelin, the only significant non-glycerol phospholipid in cell membranes, is a sphingolipid. The sphingosine base linked through its amino group to an acyl chain and the terminal hydroxyl residue is attached to a phosphocholine (Gurr et al., 2002).

The structure of the most common class of phospholipids, the phosphoglycerides, is based on glycerol, a three-carbon alcohol with the formula

$CH_2OH$–CHOH–$CH_2OH$. Two fatty acid chains, each typically having an even number of carbon atoms between 14 and 24, are esterified to the first and second carbons of the glycerol molecule, denoted as the *sn*-1 and *sn*-2 positions, respectively. Since fatty acids can vary in length and degree of unsaturation, each phospholipid comprises numerous molecular species. The third hydroxyl group of glycerol, at position *sn*-3, reacts with phosphoric acid to form phosphatidate. Common phospholipids, widely distributed in nature, are produced by further reaction of the phosphate group in phosphatidate with an alcohol, such as serine, ethanolamine, choline, glycerol, or inositol. The resulting phospholipids include, for example, phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), and phosphatidylglyercol (PG), for example (Berg et al., 2006; Gurr et al., 2002; Voet et al., 2006). Depending on the type of bond present at the *sn*-1 position, PE can be either phosphatidylethanolamine (diacyl) as PE 36a:3 or alkyl ether PE as PE 36e:4 or plasmenyl PE as PE 38p:6. In the same way, PC can be phosphatidylcholine (diacyl) as PC 36a:4 or its ether (36e:4) or plasmenyl PC (36p:4). A typical phospholipid arrangement is the presence of a saturated fatty acid, such as palmitic or stearic acid, at the *sn*-1 position, and an unsaturated or polyunsaturated fatty acid, such as oleic or arachidonic acid, at *sn*-2 (Figure 5.1). For more information on the fatty acid composition, another nomenclature can be used. For example, PE 36a:3 can be written PE (18:1a/18:2), indicating that PE contains a 18 carbon fatty acid with a double bond, in the *sn*-1 position, and an 18 carbon fatty acid with two double bonds, in *sn*-2 position (Murphy, 2002).

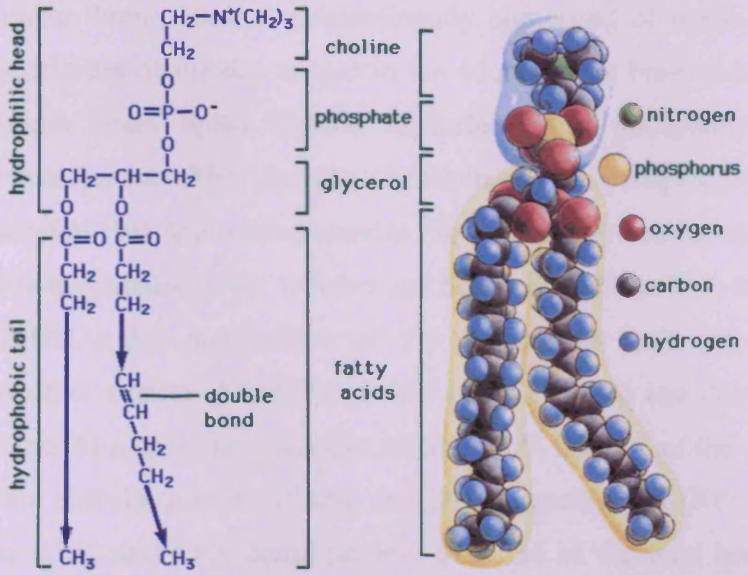


**Figure 5.1.** The structure of a phospholipid. Structures represent a classic glycerophospholipid, phosphatidylcholine (PC), composed of choline, phosphate, glycerol, and two fatty acids (Gould et al., 1996).

Another example of a phospholipid is sphingomyelin where the backbone of the sphingolipid is sphingosine, an amino alcohol (rather than glycerol). Although sphingomyelin only contains a single acyl chain, the long hydrocarbon nature of the sphingosine base gives it similar amphipathic properties to the phosphoglycerides. Sphingolipids, including sphingomyelin, occur in high amounts in nervous tissue. They can form cholesterol-rich domains (membrane rafts) within the lipid bilayer that may be important for the functions of some membrane proteins (Berg et al., 2006)

In phospholipids, DHA is mostly found in *sn*-2 position often paired with palmitic acid (16:0), as represented in Figure 5.2, stearic acid (18:0) or oleic acid (18:1) in *sn*-1 position, or in some cases with another long-chain PUFA.

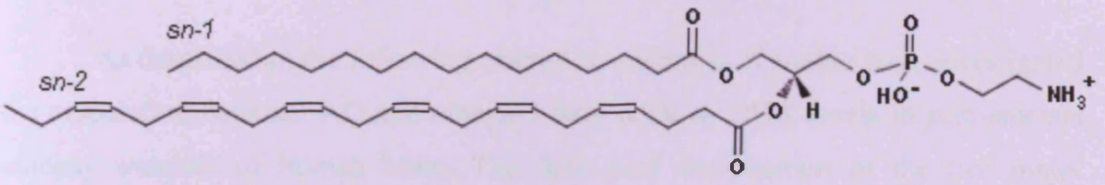


**Figure 5.2.** The structure of phosphatidylethanolamine (PE) with 16:0 in *sn*-1 position and DHA (22:6n-3) in *sn*-2 position.

Mammalian brain tissue is predominantly composed of lipids, representing approximately 50-60% of the dry weight in the adult human brain (Lauritzen et al., 2001). The main brain lipids include cholesterol and phospholipids such as phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylinositol (PI) and sphingomyelin (Sph) which are major constituents of cellular membranes (Ansell, 1973; O'Brien and Sampson, 1965). With its particularly high level of DHA, the composition of the mammalian brain phospholipids is different from other tissues. Arachidonic acid (AA, 20:4n-6) and docosahexaenoic acid (DHA, 22:6n-3) respectively account for about 6% and 8% of the dry weight of the human brain (Muskieta et al., 2006), and DHA represents 10-20% of total fatty acids, whereas ALA and EPA comprise less than 1% of the total brain fatty acid composition (McNamara and Carlson, 2006). The DHA content of different phospholipid species varies considerably and DHA is especially abundant in PE and PS (Svennerholm, 1968).

Although, the brain fatty acid composition varies between species, the general overall pattern is the same in other mammalian species (Ansell, 1973; Farkas et al., 2000; Norton et al., 1975). Studies demonstrated that DHA concentration also differs between brain regions and cell types (Ansell, 1973; Norton et al., 1975; O'Brien and Sampson, 1965; Svennerholm, 1968). In rodents, DHA is most concentrated in the frontal cortex and hippocampus (around 16-22% of total fatty acids) (McNamara and Carlson, 2006). Among subcellular fractions of brain tissue, the highest levels of DHA are found in synaptosomal membranes, synaptic vesicles, astrocytes, and growth cones (Horrocks and Farooqui, 2004; McNamara and Carlson, 2006), where its presence is critical for maintaining normal membrane integrity, electrical insulation, vesicular trafficking, and synaptic transmission.

As described in the following examples, a number of studies have investigated the association between AD and omega-3 fatty acids or DHA levels in *post-mortem* autopsy samples of human brain. The fatty acid composition of the two major phospholipid classes in brain, PE and PC was analysed in frontal gray matter, frontal white matter, hippocampus and pons from AD patients compared to controls. The relative amount of DHA appeared significantly decreased in PE from the four brain regions ($p < 0.05$). In PC, the relative amount of DHA was much lower than in PE and was only significantly decreased in the frontal gray matter ($p < 0.05$) of AD

patients compared to controls. Interestingly these changes appeared rather specific to AD as there were no significant changes in the fatty acid pattern of PE and PC during aging (Prasad et al., 1998; Soderberg et al., 1991). The fatty acid analysis of cardiolipin, which represents 1-3% of total phospholipids in brain, in frontal, temporal and occipital cortex of 6 AD cases also revealed a significant decrease of DHA in temporal cortex from AD cases ($p < 0.05$) compared to 6 controls, and this was the only fatty acid for which a significant change was observed (Guan et al., 1994). The fatty acid analysis of PC, PE, PI and free fatty acids from the hippocampus and parahippocampus gyrus (HPG), superior and middle temporal gyri (SMTG), inferior parietal lobule (IPL) and cerebellum of 9 AD patients compared to 9 control subjects revealed a significant decrease of PE total fatty acids in the HPG (by 36%, $p < 0.05$) and the IPL (by 32%, $p < 0.05$), PI total fatty acids in the HPG (by 36%, $p < 0.05$) and a significant increase of total free fatty acids in cerebellum (by 18%, $p < 0.05$) in AD patients compared to controls (Prasad et al., 1998). This was accompanied by decreased levels of DHA in the PE fraction of the HPG (by 45%, $p < 0.05$) and in the PC fraction of the cerebellum (by 24%, $p < 0.05$). No major changes were observed in the SMTG but significant deceased levels of 18:0, 18:1n-9 and 20:4n-6 were observed in PE from the HPG and the IPL of AD patients.

Two additional investigations did not report significant changes in DHA concentrations in the grey and white matter of the frontal, parietal and parahippocampal regions of 15 AD patients except for a higher proportion of DHA in the parietal white matter in the AD patients (Skinner et al., 1993) or in the parahippocampal cortex of 8 AD patients (Corrigan et al., 1998). Skinner et al. (1993) observed other highly significant and specific differences between AD patients and controls. Adrenic acid (22:4n-6) was three to four times higher in the grey matter but lower in the white matter of the frontal, parietal and parahippocampal regions in the AD brains than in the control group. These alterations were compensated by reciprocal changes in 18:0 in the grey matter and 16:1 in the white matter. In the study of Corrigan et al. (1998), PE and PS from AD brains showed a deficit in adrenic acid (22:4n-6) and PE also contained less arachidonic acid (20:4n-6). In AD subjects, the cholesterol esters contained significantly less n-3 PUFA caused by a reduction in alpha-linolenic acid (18:3n-3). In another *post-mortem* analysis, PE molecular species were analysed by mass spectrometry in different brain regions from AD patients and controls (Han et al., 2001). The level of total PE was significantly

lower in the cortex from AD subjects than controls, mainly caused by lower levels of plasmenyl PE including DHA containing species such as 18:0/22:6 and 18:1/22:6 and other molecular species such as 18:1/18:1, 16:0/22:4 or 18:0/20:4, and 18:0/22:4.

Although there is no data on the effect of DHA supplementation on brain lipids in AD patients, studies have been carried out using animal models. Studies in rats have established that dietary deficiency of n-3 fatty acids results in decreases in brain phospholipid DHA, with concomitant increases in n-6 fatty acids (Galli et al., 1971; Ikemoto et al., 2001; Murthy et al., 2002). Moreover, it was shown, in monkeys (Connor et al., 1990), in rats (Gamoh et al., 1999; Marteinsdottir et al., 1998) and in mice (Carrie et al., 2000; Lim and Suzuki, 2000; Suzuki et al., 1998), that n-3 PUFA supplementation is associated with increased levels of DHA in brain phospholipids and can improve learning and memory (Carrie et al., 2000; Gamoh et al., 1999; Lim and Suzuki, 2000; Suzuki et al., 1998). Other n-3 PUFA supplementation studies have been carried out in rodent models of AD. Pre-administration of 300 mg dietary DHA/kg of body weight per day for 12 weeks, to 20 weeks old Wistar rats prior to Aβ1-40 injection into the cerebral ventricle resulted in a significant increase in the DHA levels in the cortex and the hippocampus (Hashimoto et al., 2006; Hashimoto et al., 2002). In a more recent study where DHA was replaced by EPA, similar results were observed with increased levels of EPA and DHA in cortex and hippocampus (Hashimoto et al., 2008). In the study of Oksman et al. (2006), ten month-old APPswe/PS1dE6 male mice were fed with a soy oil supplemented diet (with a n-6/n-3 ratio of 8), a "lipid neutral diet" (with a n-6/n-3 ratio of 23), a "typical Western diet" (with a n-6/n-3 ratio of 23 and 1% cholesterol) or a DHA-enriched diet (with a n-6/n-3 ratio of 3 and 0.5% DHA) from 6 months of age (Oksman et al., 2006). Analysis of Aβ levels by ELISA revealed that the DHA group had significant lower levels of hippocampal total Aβ1-40 and total Aβ1-42 than the "typical Western diet" group, with a significantly decreased membrane n-6/n-3 ratio in cerebellum compared to the other diet groups and significantly decreased plasma cholesterol levels relative to the "lipid neutral diet" and the "typical Western diet" groups. There was a significant positive correlation between cerebellar n-6/n-3 FA ratio and hippocampal Aβ1-40 levels. In another study using the same animal model, the mice received a "typical Western diet", a DHA diet (0.4% DHA) or a standard diet from the age of 6 months. At 18 months of age DHA and AA levels in brain phospholipids were not significantly affected in Tg mice compared to WT mice on the standard diet.

However, the DHA diet increased significantly the level of DHA and decreased significantly the level of AA in brain phospholipids of Tg mice compared to Tg mice on the standard diet (Hooijmans et al., 2007). In a following study from the same lab, the mice received the experimental diets from the age of 2 months. Again, DHA levels were significantly increased and AA levels were significantly decreased in the brain of mice that received the DHA diet compared to mice on the standard diet or the "typical Western diet" (Hooijmans et al., 2009). Two month-old APPswe/PS1dE6 mice as well as WT littermates were fed a high omega-3 or a standard diet (Arendash et al., 2007). At 6-9 months of age, the high omega-3 diet increased the levels of n-3 PUFA in the frontal cortex of WT mice ($p < 0.01$) but not Tg mice and decreased levels of n-6 PUFA in both Tg and WT frontal cortex. The high omega-3 diet also increased the level of DHA by 92% and decreased the level of AA by 10% in the frontal cortex compared to the standard diet in WT mice, but did not have a significant effect on the level of these two fatty acids in Tg mice. This was accompanied by no significant diet effect on cognitive performance or on levels of Aβ in hippocampus. In the study of Green et al. (2007), triple mutant 3xTg-AD mice were fed with different DHA-containing diets (n-6/n-3 = 1:1, DHA, DHA-DPA, DHA-ARA) or a control diet (n-6/n-3 = 10:1) from the age of 3 months. Fatty acid analysis of whole brain as well as brain PC, PE and PS showed increased levels of DHA and also decreased levels of AA with increased dietary DHA, along with significantly reduced the levels of soluble Aβ1-40 and Aβ1-42 in whole brain (Green et al., 2007).

Only the research group of Greg Cole studied brain lipids in the Tg2576 mouse model in the context of DHA supplementation. In their studies, 17 month-old WT and Tg mice were fed with a control diet, a safflower oil-based n-3 PUFA depleted diet or a safflower oil-based n-3 PUFA-depleted + 0.6% DHA diet (Calon et al., 2004). After about 103 days of diets, DHA levels were significantly decreased in the frontal cortex of n-3 PUFA-depleted Tg mice compared to WT mice on the same diet ($p < 0.05$) and Tg mice on the control diet ($p < 0.01$), suggesting that the Aβ pathology has a lowering effect on the levels of DHA in brain. By adding DHA to the n-3 PUFA depleted diet, cortical levels of AA were significantly decreased ($p < 0.01$) while cortical levels of DHA were significantly increased ($p < 0.01$), compared with the mice on the n-3 PUFA-depleted diet. However, there was no significant difference of DHA or AA levels between mice on the low n-3 PUFA + DHA diet and

mice on the control diet. The same effects were also observed after 3 to 5 months on the experimental diets (Calon et al., 2005). Interestingly, an earlier study showed that the levels of PE molecular species 16:0p/22:4 or 18:0p/20:4, 18:0p/22:6 or 18:1/22:5, 16:0a/22:6 and 18:0a/22:6 or 18:1a/22:5 were significantly lower in the cortex of 18 month-old Tg2576 mice compared to controls (Han et al., 2001). However, the composition of PE was not significantly affected in the cortex of 9 month-old Tg mice and in the cerebellum at either age. In the context of our study, this suggests that old Tg2576 mice may show decreased levels of DHA in specific brain regions and that the DHA supplementation may restore the deficiency. According to the previous findings, the increase in DHA caused by dietary DHA may also be accompanied by a decrease of n-6 PUFA levels.

In the present chapter, traditional analysis methods of phospholipids including separation by thin-layer chromatography (TLC) and fatty acid analysis by gas liquid chromatography (GLC) after transmethylation were used to analyse changes of the phospholipid and fatty acid distribution as a function of the transgene and the DHA diet, in total brain lipids and individual phospholipids PE, PC, PS, PI and sphingomyelin. In addition, the distribution of individual molecular species were analysed in the individual phospholipids PE, PC, PS and PI. The latter analysis was carried out using reverse phase liquid chromatography separation followed by electrospray ionization tandem mass spectrometry (ESI-MS-MS).

## 5.2. Materials and methods

### 5.2.1 Subjects

Analyses of brain phospholipids and fatty acids were carried out on brain lipid extracts from 12 and 16 month-old Tg2576 and WT mice on special diets from the age of 4 months. A full description of the breeding, genotyping and maintenance of the mice is presented in Chapter 2. The origin of the lipid samples is presented in Table 5.1.

**Table 5.1.** Tg and WT mice on which brain phospholipids and fatty acid analyses were carried out: 12 and 16 month-old Tg mice on the oil blend diet (Tg oil), WT mice on the oil blend diet (WT oil), Tg mice on the DHA diet (Tg DHA) and WT mice on the DHA diet (WT DHA).

| | Cohort 1 12 months | | Cohort 2 16 months | |
|---|---|---|---|---|
| Gender | male | female | male | female |
| Tg oil | 3 | 3 | 3 | - |
| WT oil | 3 | 3 | 3 | - |
| Tg DHA | 3 | 3 | 3 | - |
| WT DHA | 3 | 3 | 3 | - |

### 5.2.2 Chemicals

Standard chemicals and solvents of analytical or HPLC grade were purchased from Fisher Scientific (Loughborough, UK) or Sigma (Poole, UK). The dimyristoyl phospholipid standards, dimyristoyl-phosphatidylethanolamine (DMPE), dimyristoyl-phosphatidylcholine (DMPC) and dimyristoyl-phosphatidylserine (DMPS), were obtained from Avanti Polar Lipids Inc. (Alabaster, AL, USA). Other phospholipid standards used for identification were obtained from Sigma (Poole, UK). Fatty acid standards used for GLC analysis were obtained from Nu-Chek Prep Inc. (Elysian, MN, USA).

### 5.2.3 Tissue preparation

The mice were sacrificed by neck dislocation and the brain was removed straight away. Cortex, cerebellum and hippocampus were dissected, snap-frozen in liquid nitrogen and stored at -80°C until use. The experiments were completed in full compliance with Home Office (United Kingdom) guidelines.

### 5.2.4 Lipid extraction

Lipids were extracted from mouse brain tissue (as well as mouse diets and plasma) using a modified protocol from Bligh and Dyer (1959), the method of Garbus et al. (1963). This extraction procedure consists in using chloroform:methanol (1:2, by volume) followed by the addition of chloroform and 2 M potassium chloride (KCl) in phosphate buffer to give two phases where even strongly polar lipids partition into the organic phase.

MilliQ$^{TM}$ water was added to the tissue up to a total of 1 g and it was homogenised in 3.75 ml chloroform:methanol (1:2, by volume), using a pestle and mortar. The tissue was further disrupted by sonication for 15 min. at room temperature. 1.25 ml chloroform, 1.25 ml Garbus solution (2 M KCl in 0.5 M potassium phosphate buffer, pH 7.4) and phospholipid standards (DMPE, DMPC and DMPS) were then added. After thorough mixing, the sample was centrifuged at 1,500 r.p.m. for 5 min. (Baird & Tatlock Auto Bench Centrifuge Mark IV). The upper phase was removed and discarded. The lower lipid-containing phase was washed using 4.45 ml of fresh upper phase. After further thorough mixing and centrifugation, the upper phase was removed and discarded. The lower phase was transferred to a clean tube and remaining lipids were extracted from the tissue residue by addition of 3 ml of water:chloroform:methanol:Garbus solution (4:10:10:5, by volume). After thorough mixing and centrifugation at 1,500 r.p.m. for 5 min., the upper phase was removed and discarded, and the lower phase was combined to the first extract. The combined lower phase was centrifuged at 1,500 r.p.m. for 5 min., transferred to a clean tube and dried down under nitrogen. Lipids were re-suspended in 300 µl chloroform:methanol (2:1, by volume) with 0.1 mg/ml of butylated hydroxytoluene (BHT) (BDH Chemicals, Poole, UK) to reduce oxidation. Samples were stored under nitrogen, in glass vials at -20°C.

### 5.2.5 Separation of phospholipids by thin layer chromatography

Individual phospholipids were separated by two-dimensional thin layer chromatography (TLC) on 10 × 10 cm silica gel G 60 TLC glass plates (Merck, Darmstadt, Germany). In order to separate phosphatidylinositol (PI) from phosphatidylserine (PS), the TLC plates had to be impregnated with boric acid. The plates were dipped for 2 min. in a solution of 1.2% (w/v) boric acid in ethanol:water (1:1, by volume) (Hamilton and Hamilton, 1992) and activated by heating them at 60°C for two days. The lipid extract was then applied as a spot on the TLC plate and chromatographed in two dimensions using the following solvent system adapted from the method of Katyal et al. (1985):

- Solvent system 1: chloroform:methanol:ammonium hydroxide (65:35:10, by volume),
- Solvent system 2: n-butanol:acetic acid:water (90:20:20, by volume).

When the solvent front had reached the top of the plate, plates were allowed to dry for 1 hr. after running solvent system 1, and for 2hrs., after running solvent system 2. Plates were then sprayed with 0.2% (w/v) ANSA (8-anilino-1-naphthalene sulphonic acid) in dry methanol and phospholipids were visualized under UV light (2UV Transilluminator UVP) (Hamilton and Hamilton, 1992). The phospholipids were identified by comparison with phospholipid standards and using specific colour reagents such as Dittmer for phospholipids, Dragendorff for quaternary ammonium compounds and ninhydrin for amino-lipids (Kates, 1986).

### 5.2.6 Preparation of fatty acid methyl esters

Total fatty acids from lipid extracts and individual phospholipids separated by TLC were analysed by gas liquid chromatography (GLC), but before fatty acids could be analysed by GLC, they had to be converted into fatty acid methyl esters (FAME) by acid-catalysed transmethylation. Pentadecanoic acid (15:0) was added to each sample, as an internal standard. Then, 3 ml 2.5% (v/v) sulphuric acid in dry methanol:toluene (2:1, by volume) were added and, after incubation at 70°C for 2 hrs., FAME were extracted using 2 ml 5% (w/v) sodium chloride aqueous solution and 3 ml analytical grade petroleum ether. Each sample was vigorously mixed and the top FAME-containing layer was transferred to a clean tube. Another 3 ml analytical grade petroleum ether were added and the operation was repeated. To neutralize any remaining acidity, the combined petroleum ether fractions were washed with 3 ml of a 2% (w/v) potassium bicarbonate aqueous solution. Anhydrous sodium sulphate was used to remove residual water and petroleum ether was evaporated under nitrogen. FAME samples were re-suspended in HPLC grade hexane and transferred to glass GLC injection vials (Chromacol Ltd., UK). Samples were analysed or stored at -20°C until use.

### 5.2.7 Fatty acid analysis by gas liquid chromatography

Fatty acid methyl esters were analysed by gas liquid chromatography (GLC) using a Clarus 500 gas chromatograph (Perkin Elmer, Norwalk, Connecticut) fitted with a Perkin Elmer 8500 flame ionisation detector (FID) (Perkin Elmer, Nowalk, Connecticut) and a 30 m × 0.25 mm internal diameter Elite 225 polar capillary column (Perkin Elmer, Norwalk, Connecticut). The temperature programme used was as follows: initial temperature of 170°C for 3 min., followed by heating up to 220°C at 4°C.min.$^{-1}$ and held at 220°C for 30 min. Samples were injected at a flow rate of 20 ml.min.$^{-1}$ with a split ratio of 20:1 and nitrogen was used as the carrier gas.

### 5.2.8 Gas liquid chromatography data analysis

Peaks corresponding to the different fatty acids represented in the chromatograms were routinely identified by comparing retention times with those obtained from fatty acid standards. The percentage of each identified fatty acid was expressed as a percentage of total fatty acids using the areas under the peaks. The proportion of each phospholipid among the total phospholipids analysed was calculated for each brain region using their fatty acid content. The main fatty acids were defined as fatty acids representing around 2% of the total fatty acids or over.

Statistical analysis of brain total fatty acids as well as the main fatty acids of the most abundant phospholipid classes were carried out individually by univariate ANOVA with genotype, diet and gender as factors. When $p < 0.05$, the effect was considered statistically significant.

### 5.2.9 Phospholipid analysis by electrospray ionisation mass spectrometry

Individual molecular species of the different phospholipid classes PE, PC, PS and PI, were analysed in mouse brain lipid extracts using online reverse phase high performance liquid chromatography separation followed by electrospray ionization tandem mass spectrometry analysis (Postle et al., 2007; Wang et al., 2005a).

Cortex, cerebellum and hippocampus lipid extracts were diluted 1:1000 in methanol and injected into the HPLC system with an autosampler. Reverse phase separation of phospholipids was carried out using a Luna 3 µm C18 150 ×2 mm column (Phenomenex Ltd.) with a gradient of 0 to 100% solvent B over 30 min. (solvent A: acetonitrile : methanol, 35:65; solvent B: acetonitrile : methanol : triethylamine, 35:65:1.5, by volume) at a flow rate of 200 $\mu l.min.^{-1}$. The effluent from the column was passed directly to the ion spray source connected to the 4000 Q-Trap mass spectrometer from Applied Biosystems/MDS Sciex, Concord, ON, Canada. ESI-MS-MS spectra were acquired in positive or negative ion mode, depending on the phospholipid class, using specific parent to daughter transitions to detect individual molecular species of the different phospholipid classes, as indicated in Table 5.2 for PE, Table 5.3 for PC, Table 5.4 for PS and Table 5.5 for PI, and parent scans were used for quantification. PE, PS and PI were analysed in negative ion mode using different parent to daughter transitions corresponding to their different

constituent fatty acids with ionisation parameters of 0.3 psi (pressure) and 1.38 kV. PC was analysed in positive ion mode using *m/z = +184* as daughter ion, corresponding to the protonated phosphocholine head group with ionisation parameters of 0.3 psi (pressure) and 1.38 kV. In order to identify the composition of PC molecular species, PC was also analysed in negative ion mode. Spectra were obtained from 550 to 1000 *m/z* over 12 seconds with 10 to 20 spectra acquired and averaged for each analysis. The experiments were carried out using collision energy of -20 V. The peak area for each transition was determined by integration of the peaks using the software Analyst 1.4.1 (Applied Biosystems/MDS Sciex, Concord, ON, Canada) and percentages of phospholipid species were expressed for each phospholipid class, as percentages of their respective totals of phospholipid species. The compositions are reported for the species that were identified and individually contributed > 1 % to the total phospholipid signal of each respective phospholipid class.

Tools from the LIPID MAPS data base (http://www.lipidmaps.org) and the book Spectrometry of phospholipids: tables of molecular and product ions (Murphy, 2002) were used to identify the phospholipid molecular species corresponding to the parent ion and daughter ion molecular weights.

**Table 5.2.** PE molecular species

| $[M-H]^-$ | Transitions | Identity | Possible *sn*-1/*sn*-2 | RT |
|---|---|---|---|---|
| 634.4 | 634.4/227.2 | 28a:0 | DMPE 14:0a/14:0 | 27.10 |
| 700.6 | 700.6/281.0 | 34p:1 | 16:0p/18:1 | 34.48 |
| 716.7 | 716.7/281.0 | 34a:1 | 16:0a/18:1 | 32.85 |
| 722.6 | 722.6/303.0 | 36p:4 | 16:0p/20:4 | 31.34 |
| 726.6 | 726.6/281.0 | 36p:2 | 18:1p/18:1 | 34.88 |
| 728.6 | 728.6/281.0 | 36p:1 | 18:0p/18:1 | 38.81 |
| 738.6 | 738.6/303.0 | 36a:4 | 16:0a/20:4 | 30.24 |
| 742.6 | 742.6/281.0 | 36a:2 | 18:1a/18:1 | 33.24 |
| 744.6 | 744.6/281.0 | 36a:1 | 18:0a/18:1 | 36.67 |
| 746.6 | 746.6/283.0 | 36a:0 | 18:0a/18:0 | 30.69 |
| 746.6 | 746.6/327.0 | 38p:6 | 16:0p/22:6 | 30.70 |
| 748.6 | 748.6/329.0 | 38p:5 | 16:0p/22:5 | 31.61 |
| 750.6 | 750.6/303.0 | 38p:4 | 18:0p/20:4 | 34.76 |
| 750.6 | 750.6/331.0 | 38p:4 | 16:0p/22:4 | 33.86 |
| 752.6 | 752.6/307.0 | 38p:3 | 18:1p/20:2 | 35.92 |
| 752.6 | 752.6/303.0 | 38e:4 | 18:0e/20:4 | 34.76 |
| 754.6 | 754.6/309.0 | 38p:2 | 18:1p/20:1 | 39.15 |
| 762.6 | 762.6/327.0 | 38a:6 | 16:0a/22:6 | 29.63 |
| 764.6 | 764.6/255.0 | 38a:5 | 22:5a/16:0 | 29.60 |
| 764.6 | 764.6/281.0 | 38a:5 | 18:1a/20:4 | 30.58 |
| 766.6 | 766.6/303.0 | 38a:4 | 18:0a/20:4 | 33.18 |
| 768.6 | 768.6/305.0 | 38a:3 | 18:0a/20:3 | 34.82 |
| 772.6 | 772.6/283.0 | 38a:1 | 20:1a/18:0 | 30.96 |
| 772.6 | 772.6/327.0 | 40e:8/40p:7 | 18:2e/22:6 or 18:1p/22:6 | 31.04 |
| 774.6 | 774.6/327.0 | 40p:6 | 18:0p/22:6 | 33.86 |
| 776.6 | 776.6/329.0 | 40p:5 | 18:0p/22:5 | 35.18 |
| 778.6 | 778.6/331.0 | 40e:5/40p:4 | 18:1e/22:4 or 18:0p/22:4 | 38.06 |
| 788.6 | 788.6/327.0 | 40a:7 | 18:1a/22:6 | 29.95 |
| 790.6 | 790.6/327.0 | 40a:6 | 18:0a/22:6 | 32.36 |
| 794.6 | 794.6/331.0 | 40a:4 | 18:0a/22:4 | 35.99 |

The identity of each PE species indicates the total number of carbons (first number), the liaison in *sn*-1 position (a, diacyl; p, plasmenyl; e, ether) and the number of double bonds (last number). The possible *sn*-1/*sn*-2 combinations are the possible fatty acids constituting the different PE species, i.e. the two fatty acids at position *sn*-1 and *sn*-2. The saturated fatty acid such as 16:0 and 18:0 are usually in *sn*-1 position and unsaturated fatty acids such as 18:1 and 22:6 are usually in *sn*-2 position.

**Table 5.3.** PC molecular species

| $[M+H]^+$ | RT | $[M+H]^-$ | Transitions | Identity | Possible *sn*-1/*sn*-2 |
|---|---|---|---|---|---|
| 678.8 | 26.83 | 662.8/227.2 | 678.8/184.0 | 28a:0 | DMPC 14:0a/14:0 |
| 730.9 | 30.09 | Not confirmed | 730.8/184.0 | 32a:2 | 16:1a/16:1 |
| 732.9 | 30.16 | 716.9/253.0 | 732.9/184.0 | 32a:1 | 16:0a/16:1 |
| 734.9 | 32.98 | 718.9/255.0 | 734.9/184.0 | 32a:0 | 16:0a/16:0 |
| 758.8 | 30.82 | 742.8/279.0 | 758.8/184.0 | 34a:2 | 16:0a/18:2 |
| 760.9 | 33.37 | 744.9/281.0 | 760.9/184.0 | 34a:1 | 16:0a/18:1 |
| 762.9 | 37.53 | 746.9/283.0 | 762.9/184.0 | 34a:0 | 16:0a/18:0 |
| 774.7 | 35.70 | 758.7/255.0 | 774.7/184.0 | 36p:0/36e:1 | 16:0p/20:0 or 16:0e/20:1 |
| 782.9 | 30.19 | 766.9/255.0 | 782.9/184.0 | 36a:4 | 16:0a/20:4 |
| 784.7 | 31.72 | 768.7/255.0 | 784.7/184.0 | 36a:3 | 16:0a/20:3 |
| 786.9 | 34.00 | 770.9/281.0 | 786.9/184.0 | 36a:2 | 18:0a/18:2 |
| 788.9 | 38.17 | 772.9/281.0 | 788.9/184.0 | 36a:1 | 18:0a/18:1 |
| 790.7 | 38.16 | Not confirmed | 790.7/184.0 | 36a:0/38p:6 | 18:0a/18:0, 16:0p/22:6 |
| 806.9 | 29.55 | Not confirmed | 806.9/184.0 | 38a:6 | 16:0a/22:6 |
| 808.8 | 30.55 | Not confirmed | 808.8/184.0 | 38a:5 | 18:1a/20:4 |
| 810.9 | 33.73 | 794.9/303.0 | 810.9/184.0 | 38a:4 | 18:0a/20:4 |
| 832.8 | 29.92 | 816.8/327.0 | 832.8/184.0 | 40a:7 | 18:1a/22:6 |
| 834.9 | 32.85 | 818.9/327.0 | 834.9/184.0 | 40a:6 | 18:0a/22:6 |

The identity of each PC species indicates the total number of carbons (first number), the liaison in *sn*-1 position (a, diacyl; p, plasmenyl; e, ether) and the number of double bonds (last number). The possible *sn*-1/*sn*-2 combinations are the possible fatty acids constituting the different PC species, i.e. the two fatty acids at position *sn*-1 and *sn*-2. The saturated fatty acid such as 16:0 and 18:0 are usually in *sn*-1 position and unsaturated fatty acids such as 18:1 and 22:6 are usually in *sn*-2 position.

**Table 5.4.** PS molecular species

| $[M-H]^-$ | Transitions | Identity | Possible *sn*-1-*sn*-2 | RT |
|---|---|---|---|---|
| 678.5 | 678.5/227.0 | DMPS | 14:0/14:0 | 24.65 |
| 760.5 | 760.5/281.0 | 34:1 | 16:0/18:1 | 28.53 |
| 788.7 | 788.7/281.0 | 36:1 | 18:0/18:1 | 31.17 |
| 790.5 | 790.5/283.0 | 36:0 | 18:0/18:0 | 32.38 |
| 810.6 | 810.6/303.0 | 38:4 | 18:0/20:4 | 28.95 |
| 818.7 | 818.7/283.0 | 38:0 | 18:0/20:0 | 36.03 |
| 834.6 | 834.6/327.0 | 40:6 | 18:0/22:6 | 28.56 |

**Table 5.5.** PI molecular species

| $[M-H]^-$ | Transitions | Identity | Possible *sn*-1-*sn*-2 | RT |
|---|---|---|---|---|
| 835.5 | 835.5/281.0 | 34:1 | 16:0/18:1 | 27.80 |
| 857.6 | 857.6/303.0 | 36:4 | 16:0/20:4 | 26.21 |
| 859.6 | 859.6/305.0 | 36:3 | 16:0/20:3 | 26.74 |
| 863.8 | 863.8/283.0 | 36:1 | 18:0/18:1 | 30.01 |
| 881.7 | 881.7/327.0 | 38:6 | 16:0/22:6 | 25.97 |
| 885.7 | 885.7/283.0 | 38:4 | 18:0/20:4 | 28.16 |
| 909.8 | 909.8/283.0 | 40:6 | 18:0/22:6 | 27.72 |

## 5.3 Results

### 5.3.1 Fatty acid analysis of brain lipids by gas liquid chromatography

Fatty acid analyses were carried out on brain lipid extracts from 12 month-old and 16 month-old Tg mice and WT mice fed on the oil blend diet or on the DHA diet from the age of 4 months. The results are presented in three sections presenting the total fatty acid analysis of cortex, hippocampus and cerebellum, the phospholipid distribution in cortex, hippocampus and cerebellum, and the fatty acid analysis of the individual phospholipids in the three brain regions. The results of the different fatty acid analysis are presented as mean percentages of total fatty acids. Only the main fatty acids are presented in the result section. Complete fatty acid compositions are presented in Appendix 4 to Appendix 39.

#### 5.3.1.1 Fatty acid analysis of cortex, hippocampus and cerebellum

Percentages of the different fatty acid classes in cortex, hippocampus and cerebellum at 12 and 16 months of age are shown in Tables 5.6 and 5.7. Total saturated, monounsaturated and polyunsaturated fatty acids showed relatively constant and balanced proportions, from 19.8% ± 0.3% to 44.6% ± 0.6% of total fatty acids across the four mouse groups and the three brain regions, with 37.9% ± 0.2% to 44.6% ± 0.6% total saturated fatty acids (SFA), 19.8 ± 0.3% to 35.0% ± 1.5% total monounsaturated fatty acids (MUFA), and 23.4% ± 1.1% to 35.5% ±1.2% polyunsaturated fatty acids (PUFA). There were no obvious differences between the percentages observed at 12 months and 16 months of age. However, the percentages of these three fatty acid classes appeared similar in cortex and hippocampus, with SFA > PUFA > MUFA, but slightly different in cerebellum, with SFA > MUFA > PUFA. Major changes were observed within total n-3 fatty acids, total n-6 fatty acids and in the n-3 to n-6 ratio. Percentages of total n-3 fatty acids and the n-3 to n-6 ratio values were significantly higher in the brain of mice on the DHA diet than in oil blend-fed mice, with 13.9% ± 0.9% to 21.1% ± 0.8% total n-3 fatty acids in brain from oil blend-fed mice and 18.6% ± 1.2% to 26.2% ± tr. total n-3 fatty acids in the brain of DHA-fed mice, a maximum n-3 to n-6 ratio value of 1.73 ± 0.04 in the brain

of oil blend-fed mice and a minimum n-3 to n-6 ratio value of 2.00 ± 0.16 in the brain of DHA-fed mice. Percentages of total n-6 fatty acids were conversely higher in the brain of oil blend-fed mice than in the brain of DHA-fed mice, with 9.0% ± 0.4% to 16.5% ± 0.5% total n-6 fatty acids in the brain of oil blend-fed mice and 4.2% ± 0.3% to 11.6% ± 0.5% total n-6 fatty acids in the brain of DHA-fed mice.

The main fatty acids present in cortex, hippocampus and cerebellum at 12 months of age (Figure 5.3, 5.4, 5.5) and 16 months of age (Figure 5.6, 5.7, 5.8) were palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1n-9), 18:1n-7, 20:1, arachidonic acid (20:4n-6), 22:4n-6, DHA (22:6n-3) and 24:1. DHA represented one of the most abundant fatty acids in the three brain regions, with 19.7% ± 0.3% to 25.5% ± 0.1% of total fatty acids in cortex, 16.8% ± 0.4% to 22.8% ± 0.1% of total fatty acids in hippocampus and 13.9% ± 0.9% to 19.9% ± 0.8% of total fatty acids in cerebellum. Major changes in the fatty acid distribution were observed among n-3 and n-6 polyunsaturated fatty acids with higher percentages of DHA in the brain of DHA-fed mice and conversely, higher percentages of 20:4n-6 and 22:4n-6 in the brain of oil blend-fed mice.

#### *5.3.1.1.1 Fatty acid analysis of cortex, hippocampus and cerebellum at 12 months of age*

Table 5.6 shows the fatty acid composition, divided into fatty acid classes, of cortex, hippocampus and cerebellum of 12 month-old Tg and WT mice on the oil blend or on the DHA diet. In cortex as well as in hippocampus and cerebellum, percentages of total SFA were higher than percentages of total MUFA or total PUFA. The percentage of total MUFA was lower than the percentage of total PUFA, in cortex and hippocampus but in cerebellum, the percentage of total MUFA was higher than the percentage of total PUFA. Percentages of total n-3 fatty acids appeared generally higher than percentages of total n-6 fatty acids and, as expected, percentages of both fatty acid classes were mainly affected by the diet, reflected by the n-3 to n-6 ratio values, from 1.06 ± 0.03 to 1.65 ± 0.03 in the different brain regions of mice on the oil blend diet and from 2.00 ± 0.16 to 3.68 ± 0.19 in the brain regions of DHA-fed mice.

Statistical analysis revealed that in cortex as well as in hippocampus and cerebellum, the percentage of total SFA was not affected by the diet or the genotype ($p > 0.05$). The percentage of total MUFA was higher in cortex of DHA-fed mice than in cortex of oil blend-fed mice ($F(1,16) = 6.283$, $p = 0.023$) but was not significantly affected by diet or genotype in hippocampus or cerebellum ($p > 0.05$). The percentage of total PUFA was higher in cerebellum of Tg mice than in cerebellum of WT mice ($F(1,16) = 15.556$, $p = 0.01$) and was not significantly affected by diet or genotype in cortex or hippocampus ($p > 0.05$). Despite no significant changes of total PUFA percentages due to the diet, the percentage of total n-3 fatty acids was significantly higher (cortex, $F(1,16) = 47.326$, $p < 0.001$; hippocampus, $F(1,16) = 130.285$, $p < 0.001$; cerebellum, $F(1,16) = 46.796$, $p < 0.001$) and the percentage of total n-6 fatty acids was significantly lower (cortex, $F(1,16) = 574.439$, $p < 0.001$; hippocampus, $F(1,16) = 553.732$, $p < 0.001$; cerebellum, $F(1,16) = 542.205$, $p < 0.001$) in the three brain regions from DHA-fed mice than oil blend-fed mice with no significant effect of genotype ($p > 0.05$). n-3 to n-6 ratio values were also significantly higher in the three brain regions from mice on the DHA diet than mice on the oil blend diet (cortex, $F(1,16) = 264.152$, $p < 0.001$; hippocampus, $F(1,16) = 375.740$, $p < 0.001$; cerebellum, $F(1,16) = 212.600$, $p < 0.001$). The effect

of gender was also tested but did not show any significant effect on the fatty acid composition of the three brain regions ($p > 0.05$) (data not shown).

**Table 5.6.** Fatty acid composition of cortex, hippocampus and cerebellum of WT and Tg mice on the oil blend or on the DHA diet, at 12 months of age. Values represent mean percentages of total fatty acids ± SEM.

| | Cortex | | | |
|---|---|---|---|---|
| | Tg oil (n = 6) | WT oil (n = 6) | Tg DHA (n = 6) | WT DHA (n = 6) |
| Total SFA | 42.7 ± 0.3 | 42.5 ± 0.3 | 42.0 ± 0.4 | 42.9 ± 0.8 |
| Total MUFA * | 20.8 ± 0.3 | 21.1 ± 0.3 | 21.4 ± 0.3 | 22.0 ± 0.2 |
| Total PUFA | 34.8 ± 0.4 | 34.7 ± 0.4 | 34.7 ± 0.5 | 33.5 ± 1.0 |
| Total n-3 FA *** | 19.9 ± 0.4 | 19.9 ± 0.3 | 24.5 ± 0.5 | 23.8 ± 1.0 |
| Total n-6 FA *** | 14.7 ± 0.1 | 14.6 ± 0.3 | 10.0 ± 0.2 | 9.5 ± 0.2 |
| n-3/n-6 ratio *** | 1.35 ± 0.02 | 1.37 ± 0.04 | 2.45 ± 0.06 | 2.52 ± 0.10 |
| | Hippocampus | | | |
| | Tg oil (n = 6) | WT oil (n = 6) | Tg DHA (n = 6) | WT DHA (n = 6) |
| Total SFA | 39.4 ± 0.7 | 39.5 ± 0.9 | 39.7 ± 0.8 | 39.3 ± 0.8 |
| Total MUFA | 21.8 ± 1.4 | 22.0 ± 1.4 | 22.9 ± 1.1 | 23.1 ± 1.2 |
| Total PUFA | 34.4 ± 0.8 | 35.2 ± 1.3 | 35.0 ± 1.5 | 34.2 ± 0.8 |
| Total n-3 FA *** | 17.6 ± 0.4 | 18.7 ± 0.9 | 23.1 ± 1.5 | 23.0 ± 0.9 |
| Total n-6 FA *** | 16.6 ± 0.5 | 16.3 ± 0.6 | 11.6 ± 0.5 | 10.9 ± 0.5 |
| n-3/n-6 ratio *** | 1.06 ± 0.03 | 1.14 ± 0.05 | 2.00 ± 0.16 | 2.11 ± 0.15 |
| | Cerebellum | | | |
| | Tg oil (n = 6) | WT oil (n = 6) | Tg DHA (n = 6) | WT DHA (n = 6) |
| Total SFA | 38.3 ± 0.2 | 38.3 ± 0.1 | 37.9 ± 0.2 | 38.2 ± 0.4 |
| Total MUFA | 32.2 ± 0.4 | 32.6 ± 0.4 | 32.8 ± 0.4 | 34.0 ± 0.5 |
| Total PUFA ** | 27.0 ± 0.6 * | 26.1 ± 0.4 | 29.1 ± 0.7 *** | 25.1 ± 0.7 |
| Total n-3 FA *** | 16.7 ± 0.5 | 16.1 ± 0.3 | 20.7 ± 0.5 | 19.6 ± 0.7 |
| Total n-6 FA*** • | 10.1 ± 0.1 | 9.8 ± 0.1 | 5.8 ± 0.3 | 5.4 ± 0.2 |
| n-3/n-6 ratio *** | 1.65 ± 0.02 | 1.65 ± 0.03 | 3.57 ± 0.15 | 3.68 ± 0.19 |

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. Significant effect of diet, * $p < 0.05$, *** $p < 0.001$; significant effect of genotype • $p < 0.05$, •• $p < 0.01$, ••• $p < 0.001$.

Percentages of the main fatty acids present in cortex at 12 months are presented in Figure 5.3. Statistical analysis revealed higher percentages of 18:1n-9, ($F(1,16) = 28.949$, $p < 0.001$) and 22:6n-3 ($F(1,16) = 25.605$, $p < 0.001$), and lower percentages of 20:4n-6 ($F(1,16) = 914.396$, $p < 0.001$) and 22:4n-6 ($F(1,16) = 384.268$, $p < 0.001$) in cortex from DHA-fed mice than in cortex from oil blend-fed mice.

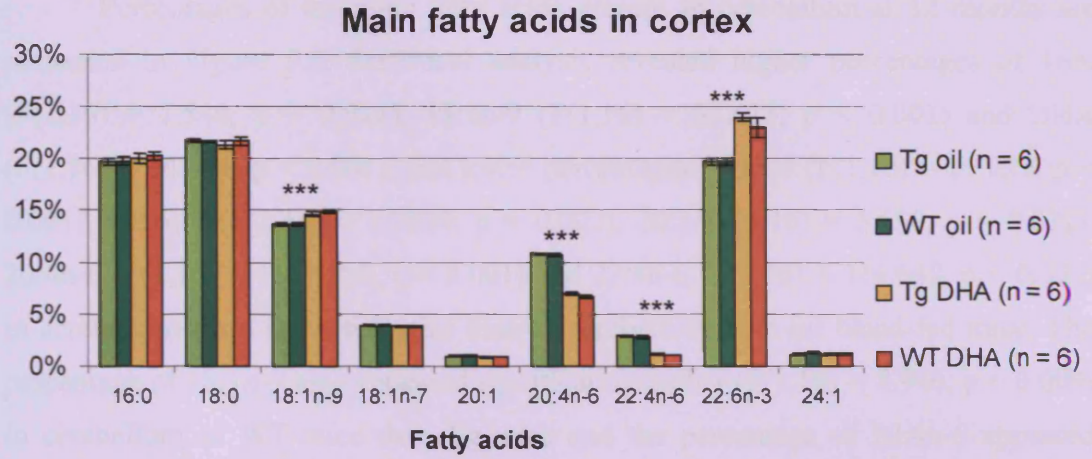


**Figure 5.3.** Main fatty acids in cortex from 12 month-old WT and Tg mice on the oil blend or on the DHA diet. Results are represented as mean percentages of total fatty acids ± SEM. Significant effect of diet, *** $p < 0.001$.

Percentages of the main fatty acids present in hippocampus at 12 months are presented in Figure 5.4. Statistical analysis revealed higher percentages of 18:1n-9 ($F(1,16) = 21.075$, $p < 0.001$) and DHA ($F(1,16) = 105.209$, $p < 0.001$), and lower percentages of 18:1n-7 ($F(1,16) = 8.351$, $p = 0.011$), 20:4n-6 ($F(1,16) = 542.526$, $p < 0.001$) and 22:4n-6 ($F(1,16) = 615.407$, $p < 0.001$) in hippocampus from DHA-fed mice than in hippocampus from oil blend-fed mice.

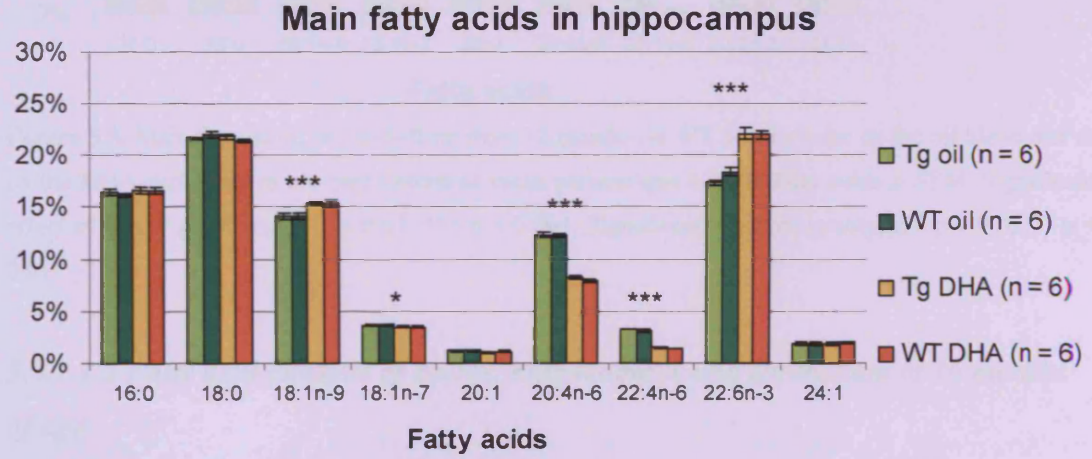


**Figure 5.4.** Main fatty acids in hippocampus from 12 month-old WT and Tg mice on the oil blend diet or on the DHA diet. Results are represented as mean percentages of total fatty acids ± SEM. Significant effect of diet, * $p < 0.05$, *** $p < 0.001$.

Percentages of the main fatty acids present in cerebellum at 12 months are presented in Figure 5.5. Statistical analysis revealed higher percentages of 16:0 ($F(1,16) = 7.544$, $p = 0.014$), 18:1n-9 ($F(1,16) = 33.615$, $p < 0.001$) and DHA ($F(1,16) = 34.491$, $p < 0.001$), and lower percentages of 18:0 ($F(1,16) = 29.039$, $p < 0.001$), 18:1n-7 ($F(1,16) = 14.054$, $p = 0.002$), 20:1 ($F(1,16) = 5.550$, $p = 0.032$), 20:4n-6 ($F(1,16) = 1389.956$, $p < 0.001$) and 22:4n-6 ($F(1,16) = 314.640$, $p < 0.001$) in cerebellum from DHA-fed mice than in cerebellum from oil blend-fed mice. The percentage of 18:1n-7 also appeared significantly higher ($F(1,16) = 8.940$, $p = 0.009$) in cerebellum of WT mice than Tg mice and the percentage of 20:4n-6 appeared significantly lower ($F(1,16) = 6.048$, $p = 0.026$) in cerebellum of WT mice than Tg mice. Gender only had a significant effect on the percentage of 16:0 ($F(1,16) = 4.617$, $p = 0.047$) (data not shown).

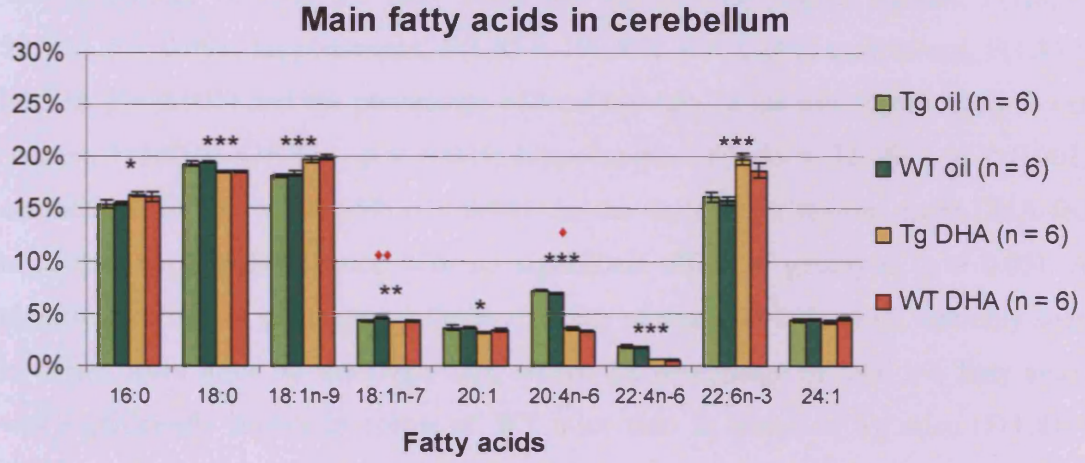


**Figure 5.5.** Main fatty acids in cerebellum from 12 month-old WT and Tg mice on the oil blend diet or on the DHA diet. Results are represented as mean percentages of total fatty acids ± SEM. Significant effect of diet, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Significant effect of genotype, ♦ $p < 0.05$, ♦♦ $p < 0.01$.

### *5.3.1.1.2 Fatty acid analysis of cortex, hippocampus and cerebellum at 16 months of age*

Table 5.7 shows the fatty acid composition, divided into fatty acids classes, of cortex, hippocampus and cerebellum of 16 month-old Tg and WT mice on the oil blend diet or on the DHA diet. In cortex as well as in hippocampus and cerebellum, and as previously observed at 12 months, percentages of total SFA were higher than

the percentages of total MUFA or total PUFA. The percentage of total MUFA was lower than the percentage of total PUFA, in cortex and hippocampus but in cerebellum, the percentage of total MUFA was higher than the percentage of total PUFA. The percentage of total n-3 fatty acids also appeared higher than the percentage of total n-6 fatty acids and, as expected, percentages of both fatty acid classes were mainly affected by the diet, as reflected by the values of the n-3 to n-6 ratio, from 1.02 ± 0.02 to 1.73 ± 0.04 in brain regions of mice on the oil blend diet and from 2.26 ± 0.08 to 4.93 ± 0.16 in brain regions of DHA-fed mice.

Statistical analysis revealed that in cortex as well as in hippocampus and cerebellum, percentages of total SFA and total PUFA were not affected by the diet or the genotype ($p > 0.05$). The percentage of total MUFA was higher in cortex of DHA-fed mice than in cortex of oil blend-fed mice ($F(1,8) = 8.425$, $p = 0.020$) and was not significantly affected by diet or genotype in hippocampus or cerebellum ($p > 0.05$). The percentage of total n-3 fatty acids was significantly higher (cortex, $F(1,8) = 92.510$, $p < 0.001$; hippocampus, $F(1,8) = 190.875$, $p < 0.001$; cerebellum, $F(1,8) = 30.028$, $p = 0.001$) and the percentage of total n-6 fatty acids was significantly lower (cortex, $F(1,8) = 436.922$, $p < 0.001$; hippocampus, $F(1,8) = 323.649$, $p < 0.001$; cerebellum, $F(1,8) = 388.557$, $p < 0.001$) in the three brain regions from DHA-fed mice than oil blend-fed mice with no significant effect of genotype ($p > 0.05$). A significant effect of genotype on the percentage of total n-6 fatty acids was only seen in cortex from mice on the DHA diet, where the percentage of total n-6 fatty acids was significantly higher in cortex of WT mice than in cortex of Tg mice ($F(1,8) = 5.389$, $p = 0.049$). n-3 to n-6 ratio values were also significantly higher in the three brain regions from mice on the DHA diet than mice on the oil blend diet (cortex, $F(1,8) = 352.696$, $p < 0.001$; hippocampus, $F(1,8) = 796.644$, $p < 0.001$; cerebellum, $F(1,8) = 395.501$, $p < 0.001$). In the three brain regions, n-3 to n-6 ratio values were higher in Tg mice on the DHA diet than in WT mice on the same diet (cortex, $F(1,8) = 9.213$, $p = 0.016$; hippocampus, $F(1,8) = 7.755$, $p = 0.024$; cerebellum, $F(1,8) = 23.875$, $p = 0.001$). The effect of gender was also tested but did not show any significant effect on the fatty acid composition of the three brain regions ($p > 0.05$) (data not shown).

A comparison of the fatty acid composition of cortex, hippocampus and cerebellum between 12 and 16 months (Table 5.6 and Table 5.7) also suggest some changes of the proportions of the fatty acid classes in hippocampus, with an increased

percentage of total SFA and decreased percentage of total PUFA at 16 months compared to 12 months.

**Table 5.7.** Fatty acid composition of cortex, hippocampus and cerebellum of WT and Tg mice on the oil blend diet or on the DHA diet, at 16 months of age. Values represent mean percentages of total fatty acids ± SEM.

| | Cortex | | | |
|---|---|---|---|---|
| | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
| Total SFA | 43.9 ± 0.5 | 44.3 ± 0.7 | 44.6 ± 0.6 | 43.2 ± 0.2 |
| Total MUFA * | 20.3 ± 0.3 | 19.8 ± 0.3 | 20.8 ± 0.5 | 21.3 ± 0.3 |
| Total PUFA | 35.4 ± 0.7 | 35.5 ± 1.2 | 34.3 ± 0.3 | 35.0 ± 0.2 |
| Total n-3 FA *** | 20.5 ± 0.7 | 21.1 ± 0.8 | 26.2 ± tr. | 26.0 ± 0.4 |
| Total n-6 FA *** | 14.8 ± 0.1 | 14.3 ± 0.4 | 7.9 ± 0.3 ♦ | 8.9 ± 0.2 |
| n-3/n-6 ratio *** | 1.38 ± 0.04 | 1.48 ± 0.04 | 3.32 ± 0.13 ♦ | 2.93 ± 0.11 |
| | Hippocampus | | | |
| | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
| Total SFA | 43.0 ± 0.3 | 43.9 ± 0.9 | 42.3 ± 0.6 | 43.1 ± 0.5 |
| Total MUFA | 21.9 ± 0.3 | 22.1 ± 0.6 | 22.5 ± 0.5 | 22.0 ± 0.3 |
| Total PUFA | 33.3 ± 0.4 | 32.7 ± 1.3 | 33.5 ± 0.1 | 33.0 ± 0.3 |
| Total n-3 FA *** | 16.8 v 0.4 | 17.0 ± 0.6 | 23.7% ± tr. | 22.6 ± 0.6 |
| Total n-6 FA *** | 16.4 ± 0.1 | 15.6 ± 0.6 | 9.7 ± 0.1 | 10.0 ± 0.2 |
| n-3/n-6 ratio *** | 1.02 ± 0.02 | 1.09 ± 0.02 | 2.44 ± 0.02 ♦ | 2.26 ± 0.08 |
| | Cerebellum | | | |
| | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
| Total SFA | 39.9 ± 0.5 | 41.4 ± 0.7 | 41.1 ± 0.7 | 41.0 ± tr. |
| Total MUFA | 35.0 ± 1.5 | 32.1 ± 0.2 | 33.1 ± 0.8 | 33.9 ± 1.5 |
| Total PUFA | 23.4 ± 1.1 | 24.8 ± 0.9 | 25.0 ± 1.0 | 23.4 ± 1.2 |
| Total n-3 FA *** | 13.9 ± 0.9 | 15.6 ± 0.6 | 20.7 ± 0.8 | 18.6 ± 1.2 |
| Total n-6 FA *** | 9.3 ± 0.1 | 9.0 ± 0.4 | 4.2 ± 0.3 | 4.7 ± 0.1 |
| n-3/n-6 ratio *** ♦ | 1.50 ± 0.08 | 1.73 ± 0.04 | 4.93 ± 0.16 ♦♦ | 3.95 ± 0.22 |

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; tr., trace (less than 0.05). Significant effect of diet, * $p < 0.05$, *** $p < 0.001$; significant effect of genotype, ♦ $p < 0.05$, ♦♦ $p < 0.01$.

Percentages of the main fatty acids present in cortex at 16 months are presented in Figure 5.6. Statistical analysis revealed higher percentages of 18:1n-9, ($F(1,8) = 26.969$, $p = 0.001$) and DHA ($F(1,8) = 67.714$, $p < 0.001$), and lower percentages of 18:1n-7 ($F(1,8) = 12.566$, $p = 0.008$), 20:4n-6 ($F(1,8) = 358.385$, $p < 0.001$) and 22:4n-6 ($F(1,8) = 3201.066$, $p < 0.001$) in cortex from DHA-fed mice than in cortex from oil blend-fed mice.

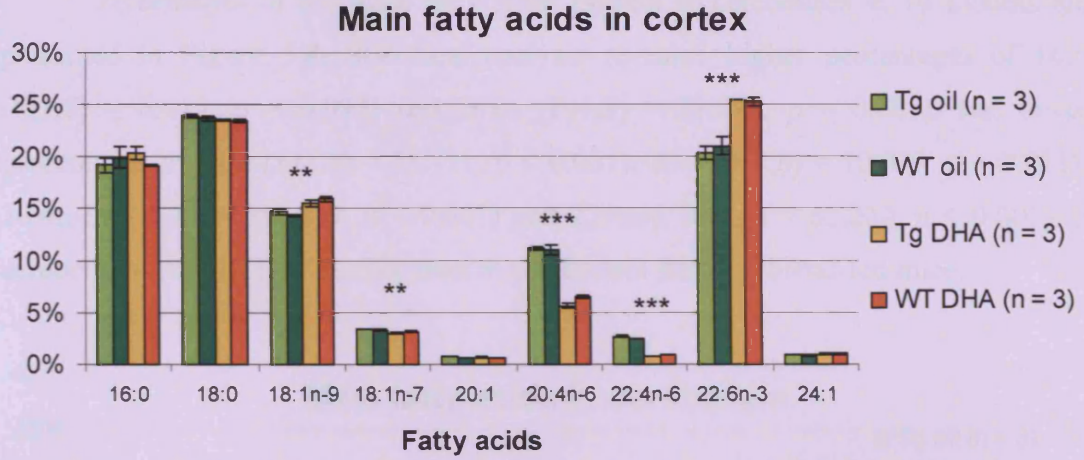


**Figure 5.6.** Main fatty acids in cortex from 16 month-old WT and Tg mice on the oil blend diet or on the DHA diet. Results are represented as mean percentages of total fatty acids ± SEM. Significant effect of diet, ** $p < 0.01$, *** $p < 0.001$.

Percentages of the main fatty acids present in hippocampus at 16 months are presented in Figure 5.7. Statistical analysis revealed higher percentages of 18:1n-9, ($(F(1,8) = 15.798$, $p = 0.004$) and DHA ($F(1,8) = 152.776$, $p < 0.001$), and lower percentages of 18:1n-7 ($F(1,8) = 22.084$, $p = 0.002$), 20:4n-6 ($F(1,8) = 255.419$, $p < 0.001$) and 22:4n-6 ($F(1,8) = 713.972$, $p < 0.001$) in hippocampus from DHA-fed mice than in hippocampus from oil blend-fed mice.

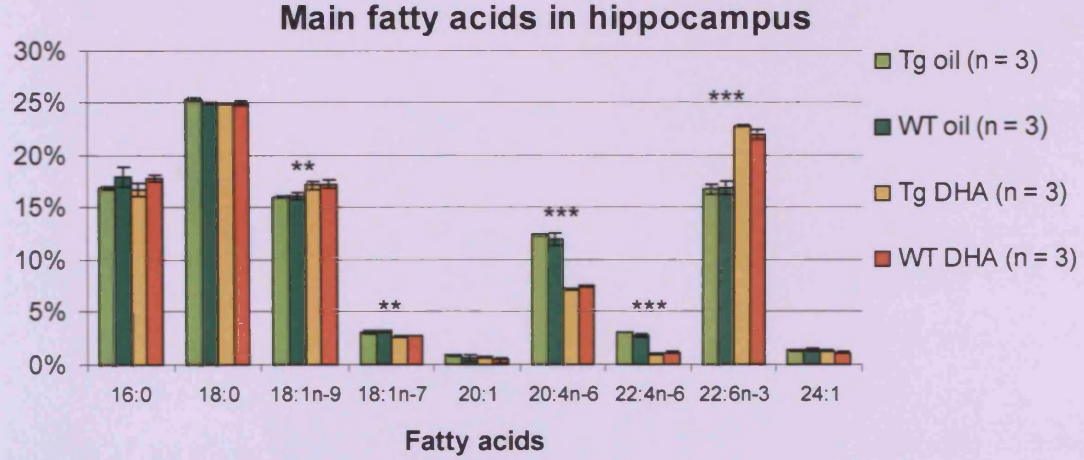


**Figure 5.7.** Main fatty acids in hippocampus from 16 month-old WT and Tg mice on the oil blend diet or on the DHA diet. Results are represented as mean percentages of total fatty acids ± SEM. Significant effect of diet, ** $p < 0.01$, *** $p < 0.001$.

Percentages of the main fatty acids present in cerebellum at 16 months are presented in Figure 5.8. Statistical analysis revealed higher percentages of 16:0 ($F(1,8) = 6.449$, $p = 0.035$) and DHA ($F(1,8) = 23.697$, $p = 0.001$), and lower percentages of 18:0 ($F(1,8) = 35.771$, $p < 0.001$), 20:1 ($F(1,8) = 10.899$, $p = 0.011$), 20:4n-6 ($F(1,8) = 254.237$, $p < 0.001$) and 22:4n-6 ($F(1,8) = 62.200$, $p < 0.001$) in cerebellum from DHA-fed mice than in cerebellum from oil blend-fed mice.

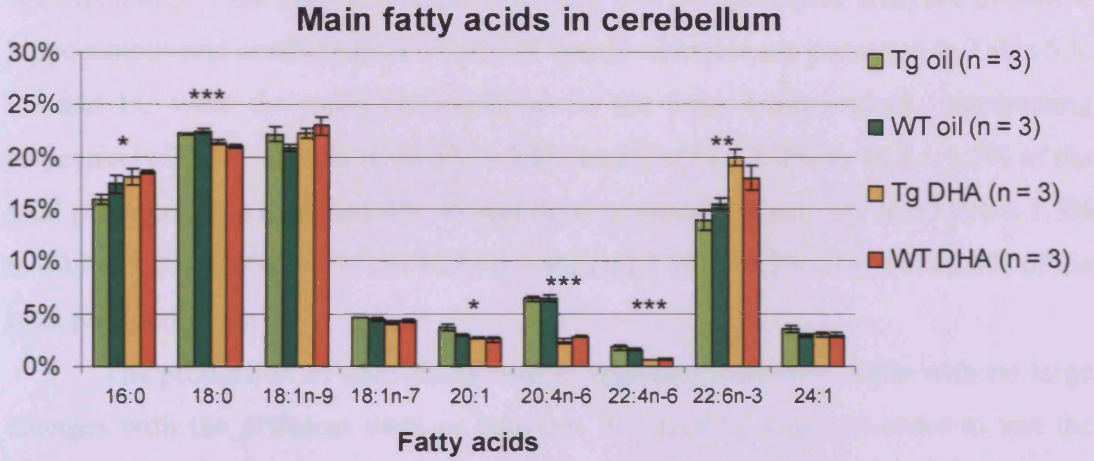


**Figure 5.8.** Main fatty acids in cerebellum from 16 month-old WT and Tg mice on the oil blend diet or on the DHA diet. Results are represented as mean percentages of total fatty acids ± SEM. Significant effect of diet, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

### 5.3.1.2 Phospholipids in cortex, hippocampus and cerebellum

The main phospholipids present in the three brain regions, cortex, hippocampus and cerebellum were phosphatidylethanolamine (PE), phosphatidylcholine (PC) and phosphatidylserine (PS). Phosphatidylinositol (PI) was also present at much lower percentages and minor percentages of sphingomyelin (Sph) were also detected in each of the three brain regions. The proportions corresponding to the fatty acid content of these five phospholipids analysed in cortex, hippocampus and cerebellum of 12 and 16 month-old mice are presented in Table 5.8. PE and PC were the main phospholipids in the three brain regions, representing respectively 32.3% ± 1.3% to 40.6% ± 1.6% and 33.5% ± 2.2% to 42.2 ± 1.7% of the total phospholipids analysed. PS, PI and Sph consisted respectively in 13.0% ± 1.3% to 20.9 ± 1.7%, 3.9% ± 0.7% to 8.2% ± 0.4% and 2.1% ± 0.3% to 8.0% ± 2.8% of the total phospholipids.

The proportion of each phospholipid appeared relatively stable with no large changes with the different diets or between WT and Tg mice. In order to test the effect of genotype and diet on the proportion of each phospholipid, univariate ANOVA was carried out for each phospholipid analysed in cortex, hippocampus and cerebellum at 12 months and 16 months of age, with genotype, diet and gender (only at 12 months of age) as factors. At 12 months of age, the statistical analysis revealed no significant effect of genotype, diet or gender (data not shown) on the percentage of the phospholipids analysed in cortex and cerebellum. In hippocampus, univariate ANOVA of Sph revealed a significant effect of gender ($F(1,8) = 8.430$, $p = 0.02$) (data not shown) and significant interactions of genotype by diet ($F(1,8) = 7.436$, $p = 0.026$) and genotype by diet and gender ($F(1,8) = 16.952$, $p = 0.003$) (data not shown) but univariate ANOVA of PE, PC, PS and PI revealed no significant effect of any of the three factors. At 16 months of age, when the analysis was carried out on males only, the statistical analysis revealed no significant effect of genotype or diet on the proportion of PE, PC, PS, PI and Sph analysed in cortex, hippocampus and cerebellum ($p > 0.05$).

**Table 5.8.** Phospholipids analysed in cortex, hippocampus and cerebellum of 12 month-old and 16 month-old Tg and WT mice, on the oil blend diet or on the DHA diet. Results are represented as mean percentages of the total fatty acids of phospholipids analysed ± SEM.

| | | n | PE | PC | PS | PI | Sph |
|---|---|---|---|---|---|---|---|
| | | | | 12 months | | | |
| Cortex | Tg oil | 4 | 40.2 ± 1.5 | 39.4 ± 0.5 | 13.0 ± 1.3 | 5.3 ± 1.2 | 2.1 ± 0.3 |
| | WT oil | 4 | 36.3 ± 0.9 | 41.4 ± 2.9 | 13.3 ± 2.0 | 6.4 ± 1.1 | 2.6 ± 0.4 |
| | Tg DHA | 4 | 34.9 ± 4.3 | 38.8 ± 2.9 | 17.8 ± 2.8 | 4.8 ± 1.0 | 3.6 ± 0.8 |
| | WT DHA | 4 | 33.1 ± 2.8 | 41.8 ± 2.0 | 17.4 ± 5.0 | 3.9 ± 0.7 | 3.8 ± 0.7 |
| Hippocampus | Tg oil | 4 | 34.9 ± 2.6 | 39.8 ± 1.2 | 16.1 ± 1.5 | 4.4 ± 0.5 | 4.9 ± 1.7 |
| | WT oil | 4 | 32.5 ± 0.4 | 37.7 ± 0.5 | 19.9 ± 1.0 | 5.8 ± 0.8 | 4.0 ± 0.2 |
| | Tg DHA | 4 | 38.7 ± 2.0 | 33.5 ± 2.2 | 17.8 ± 1.9 | 6.1 ± 1.6 | 3.9 ± 0.5 |
| | WT DHA | 4 | 33.1 ± 3.3 | 39.2 ± 3.8 | 15.4 ± 1.3 | 4.4 ± 0.8 | 8.0 ± 2.8 |
| Cerebellum | Tg oil | 4 | 35.3 ± 2.0 | 34.9 ± 1.4 | 18.1 ± 0.9 | 7.9 ± 0.9 | 3.8 ± 0.6 |
| | WT oil | 3 | 33.0 ± 1.8 | 34.5 ± 1.1 | 20.9 ± 1.7 | 7.2 ± 0.3 | 4.3 ± 1.1 |
| | Tg DHA | 3 | 32.3 ± 1.3 | 39.8 ± 2.2 | 19.7 ± 0.8 | 8.2 ± 0.4 | 4.1 ± 0.2 |
| | WT DHA | 4 | 34.2 ± 1.8 | 38.5 ± 2.0 | 19.3 ± 2.9 | 7.1 ± 0.7 | 5.9 ± 1.1 |
| | | | | 16 months | | | |
| Cortex | Tg oil | 3 | 34.3 ± 0.9 | 39.6 ± 0.8 | 16.6 ± 1.7 | 4.8 ± 0.3 | 4.7 ± 0.5 |
| | WT oil | 3 | 34.9 ± 2.2 | 39.4 ± 1.0 | 15.4 ± 1.4 | 5.7 ± 0.8 | 4.6 ± 0.7 |
| | Tg DHA | 3 | 36.5 ± 1.1 | 37.0 ± 2.9 | 15.3 ± 1.8 | 5.7 ± 0.6 | 5.6 ± 0.7 |
| | WT DHA | 3 | 34.2 ± 1.9 | 39.9 ± 1.7 | 15.4 ± 1.6 | 5.3 ± 0.7 | 5.3 ± 1.1 |
| Hippocampus | Tg oil | 3 | 35.1 ± 1.3 | 39.0 ± 1.8 | 16.8 ± 2.5 | 4.8 ± 0.4 | 4.3 ± 0.6 |
| | WT oil | 3 | 33.4 ± 1.9 | 40.2 ± 0.9 | 16.8 ± 2.2 | 5.0 ± 0.6 | 4.6 ± 0.4 |
| | Tg DHA | 3 | 34.1 ± 0.9 | 39.8 ± 1.3 | 16.2 ± 1.5 | 4.5 ± 0.2 | 5.4 ± 0.6 |
| | WT DHA | 3 | 34.0 ± 0.9 | 42.2 ± 1.7 | 13.5 ± 2.7 | 5.1 ± 0.7 | 5.2 ± 0.6 |
| Cerebellum | Tg oil | 3 | 39.6 ± 1.1 | 36.8 ± 0.6 | 15.7 ± 0.3 | 4.2 ± 0.3 | 3.7 ± 0.4 |
| | WT oil | 3 | 39.6 ± 1.4 | 37.9 ± 0.5 | 14.7 ± 0.7 | 4.2 ± 0.1 | 3.6 ± 0.6 |
| | Tg DHA | 3 | 39.1 ± 1.6 | 39.0 ± 0.4 | 14.4 ± 1.5 | 4.3 ± 0.1 | 3.2 ± 0.3 |
| | WT DHA | 3 | 40.6 ± 1.6 | 36.9 ± tr. | 14.2 ± 1.1 | 4.8 ± 0.2 | 3.4 ± 0.3 |

n, number of mice; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; Sph, sphingomyelin; tr., trace (less than 0.05).

### 5.3.1.3 Fatty acid analysis of individual phospholipids

#### *5.3.1.3.1 Fatty acid distribution in phosphatidylethanolamine*

The main fatty acids present in phosphatidylethanolamine (PE) from cortex, hippocampus and cerebellum were 18:0, 20:4n-6 and DHA (Tables 5.9, 5.10 and 5.11). Significant amounts of 16:0, 18:1n-9, 18:1n-7, 20:1, 22:4n-6 and three unidentified fatty acids, labelled X1, X2 and X3 were also found. 18:1n-9 was more prevalent in cerebellum. DHA was a major fatty acid in PE with 25% ± 1.2% to 37.9% ± 0.7% of total fatty acids in cortex, 22.2% ± 1.0% to 32.6% ± 1.4% of total fatty acids in hippocampus and 19.1% ± 1.3% to 28.5% ± 1.6% of total fatty acids in cerebellum. Notable differences in the fatty acid composition of PE were most apparent among n-3 and n-6 polyunsaturated fatty acids, where percentages of DHA were higher in PE from DHA-fed mice and the percentages of 20:4n-6 and 22:4n- 6 were higher in PE from oil blend-fed mice.

The comparison of the composition of PE from cortex, hippocampus and cerebellum between 12 months and 16 months of age suggests an age-related decrease of the proportion of very long chain fatty acids such as 20:4n-6, 22:4n-6 and DHA and conversely, an increased proportion of saturated fatty acids such as 16:0 and 18:0. This was especially found in cortex and hippocampus.

##### *5.3.1.3.1.1 Fatty acid distribution in phosphatidylethanolamine from cortex*

The distribution of the main fatty acids in PE from cortex of 12 and 16 month-old WT and Tg mice on the oil blend diet or on the DHA diet is presented in Table 5.9. At 12 months of age, percentages of 18:1n-7 ($F(1,8) = 6.000$, $p = 0.031$), 20:4n-6 ($F(1,8) = 287.386$, $p < 0.001$) and 22:4n-6 ($F(1,8) = 367.737$, $p < 0.001$) were significantly higher in PE from cortex of oil blend-fed mice than PE from cortex of DHA-fed mice, and percentages of 18:1n-9 ($F(1,8) = 28.342$, $p < 0.001$) and DHA ($F(1,8) = 113.933$, $p < 0.001$) were significantly higher in PE from cortex of DHA-fed mice compared to PE from oil blend-fed mice. The proportion of 20:1 also appeared significantly higher in PE from cortex of WT mice than in PE from cortex of Tg mice ($F(1,8) = 8.963$, $p = 0.011$). At 16 months of age, percentages of 18:0 ($F(1,8) = 18.894$, $p = 0.002$), 20:4n-6 ($F(1,8) = 373.254$, $p < 0.001$) and 22:4n-6

($F(1,8) = 1147.259$, $p < 0.001$) were significantly higher in PE from cortex of oil blend-fed mice than in PE from cortex of DHA-fed mice, while percentages of X2 ($F(1,8) = 6.149$, $p = 0.038$), 18:1n-9 ($F(1,8) = 19.546$, $p = 0.002$) and DHA ($F(1,8) = 35.867$, $p < 0.001$) were significantly higher in PE from cortex of the DHA-fed mice than in PE from cortex of the oil blend-fed mice.

**Table 5.9.** Main fatty acids in phosphatidylethanolamine from cortex of WT and Tg mice on the oil blend diet or on the DHA diet, at 12 months and at 16 months of age. Results are represented as mean percentages of total fatty acids ± SEM.

| 12 months | | | | |
|---|---|---|---|---|
| Fatty acid | Tg oil (n = 4) | WT oil (n = 4) | Tg DHA (n = 4) | WT DHA (n = 4) |
| X1 | 2.1 ± 0.4 | 2.1 ± 0.3 | 1.5 ± 0.5 | 2.0 ± 0.3 |
| 16:0 | 4.6 ± 0.2 | 5.4 ± 0.6 | 5.5 ± 0.8 | 5.1 ± 0.3 |
| X2 | 5.4 ± 0.5 | 5.0 ± 1.2 | 4.6 ± 1.0 | 5.5 ± 0.5 |
| X3 | 2.2 ± 0.2 | 2.1 ± 0.5 | 2.0 ± 0.5 | 2.4 ± 0.1 |
| 18:0 | 22.1 ± 1.0 | 21.7 ± 0.5 | 21.9 ± 0.7 | 21.6 ± 0.5 |
| 18:1n-9 *** | 8.6 ± 0.2 | 8.7 ± 0.2 | 9.6 ± 0.2 | 10.1 ± 0.3 |
| 18:1n-7 * | 1.8 ± 0.1 | 1.8 ± tr. | 1.6 ± tr. | 1.7 ± 0.1 |
| 20:1 ♦ | 1.5 ± 0.1 | 1.9 ± 0.1 | 1.5 ± 0.1 | 1.7 ± 0.1 |
| 20:4n-6 *** | 13.3 ± 0.4 | 13.0 ± 0.4 | 7.7± 0.2 | 7.4 ± 0.2 |
| 22:4n-6 *** | 5.6 ± 0.2 | 5.6 ± 0.3 | 2.0 ± tr. | 1.9 ± 0.1 |
| 22:6n-3 *** | 30.1 ± 0.8 | 30.1 ± 0.6 | 37.9 ± 0.7 | 37.1 ± 0.6 |
| 16 months | | | | |
| Fatty acid | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
| X1 | 2.7 ± 0.4 | 3.1 ± 0.4 | 3.3 ± 0.2 | 3.7 ± 0.3 |
| 16:0 | 7.1 ± 0.5 | 7.1 ± 0.5 | 7.0 ± 0.5 | 7.6 ± 0.7 |
| X2 * | 4.4 ± 0.7 | 5.3 ± 0.2 | 5.9 ± 0.3 | 5.7 ± tr. |
| X3 | 2.2 ± 0.2 | 2.0 ± 0.1 | 2.2 ± 0.3 | 2.0 ± 0.2 |
| 18:0 ** | 27.8 ± 0.3 | 26.9 ± 0.3 | 25.7 ± 0.4 | 26.3 ± 0.2 |
| 18:1n-9 ** | 9.6 ± 0.3 | 9.2 ± 0.3 | 10.6 ± 0.4 | 11.0 ± 0.2 |
| 18:1n-7 | 2.2 ± 0.2 | 2.0 ± 0.2 | 1.7 ± 0.4 | 1.9 ± 0.2 |
| 20:1 | 0.8 ± 0.4 | 0.8 ± 0.4 | 0.7 ± 0.4 | 0.8 ± 0.4 |
| 20:4n-6 *** | 12.1 ± 0.1 | 11.9 ± 0.5 | 5.1 ± 0.3 | 6.1 ± 0.2 |
| 22:4n-6 *** | 4.1 ± 0.1 | 3.8 ± 0.1 | 0.9 ± 0.1 | 1.2 ± 0.1 |
| 22:6n-3 *** | 25.0 ± 1.2 | 26.1 ± 1.0 | 34.1 ± 1.4 | 30.9 ± 0.9 |

tr., trace (less than 0.05); significant effect of diet, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; significant effect of genotype, ♦ $p < 0.05$. Fatty acids, including the unidentified fatty acids X1, X2 and X3 are listed in the order that they eluted from the GLC column.

#### *5.3.1.3.1.2 Fatty acid distribution in phosphatidylethanolamine from hippocampus*

The distribution of the main fatty acids in PE from hippocampus of 12 and 16 month-old WT and Tg mice on the oil blend diet or on the DHA diet is presented in Table 5.10. At 12 months of age, the percentages of 20:4n-6 ($F(1,8) = 69.165$, $p < 0.001$) and 22:4n-6 ($F(1,8) = 32.720$, $p < 0.001$) were significantly higher in PE from hippocampus of oil blend-fed mice than in PE from hippocampus of DHA-fed mice, and percentages of DHA ($F(1,8) = 35.909$, $p < 0.001$) were significantly higher in PE from hippocampus of DHA-fed mice compared to PE from oil blend-fed mice. At 16 months of age, percentages of 20:4n-6 ($F(1,8) = 260.015$, $p < 0.001$) and 22:4n-6 ($F(1,8) = 606.391$, $p < 0.001$) were significantly higher in PE from hippocampus of oil blend-fed mice than in PE from hippocampus of DHA-fed mice, while percentages of 18:1n-9 ($F(1,8) = 21.010$, $p = 0.002$) and DHA ($F(1,8) = 130.822$, $p < 0.001$) were significantly higher in PE from hippocampus of mice on the DHA diet than in PE from hippocampus of the oil blend-fed mice.

**Table 5.10.** Main fatty acids in phosphatidylethanolamine from hippocampus of WT and Tg mice on the oil blend diet or on the DHA diet, at 12 months and at 16 months of age. Results are represented as mean percentages of total fatty acids ± SEM.

| | 12 months | | | |
|---|---|---|---|---|
| Fatty acid | Tg oil (n = 4) | WT oil (n = 4) | Tg DHA (n = 4) | WT DHA (n = 4) |
| X1 | 1.7 ± 0.2 | 1.4 ± 0.6 | 0.8 ± 0.5 | 1.3 ± 0.4 |
| 16:0 | 6.4 ± 0.5 | 7.1 ± 0.3 | 6.5 ± 0.7 | 6.6 ± 0.8 |
| X2 | 2.0 ± 0.2 | 2.8 ± 1.3 | 2.5 ± 0.6 | 1.7 ± 0.2 |
| X3 | 1.1 ± 0.2 | 1.8 ± 0.4 | 1.2 ± 0.5 | 0.7 ± 0.1 |
| 18:0 | 23.6 ± 0.5 | 22.8 ± 0.6 | 23.1 ± 0.9 | 22.3 ± 0.3 |
| 18:1n-9 | 11.3 ± 0.8 | 11.7 ± 1.5 | 13.7 ± 0.9 | 13.2 ± 1.0 |
| 18:1n-7 | 1.7 ± 0.2 | 2.1 ± 0.1 | 1.9 ± 0.1 | 1.9 ± 0.1 |
| 20:1 | 1.9 ± 0.5 | 2.4 ± 0.3 | 1.9 ± 0.5 | 2.0 ± 0.4 |
| 20:4n-6 *** | 15.9 ± 0.3 | 14.5 ± 0.4 | 9.5 ± 0.1 | 10.8 ± 1.2 |
| 22:4n-6 *** | 6.7 ± 0.2 | 6.0 ± 0.5 | 2.5 ± 0.1 | 3.5 ± 1.1 |
| 22:6n-3 *** | 25.6 ± 0.5 | 25.7 ± 0.7 | 32.6 ± 1.4 | 32.3 ± 1.9 |
| | 16 months | | | |
| Fatty acid | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
| X1 | 3.2 ± 0.1 | 3.0 ± 0.3 | 2.4 ± 0.4 | 2.1 ± 0.6 |
| 16:0 | 6.2 ± 0.3 | 6.7 ± 0.2 | 6.9 ± 0.2 | 7.0 ± 0.3 |
| X2 | 5.3 ± 0.3 | 5.2 ± 0.4 | 4.5 ± 0.4 | 4.6 ± 0.8 |
| X3 | 3.0 ± 0.2 | 3.2 ± 0.3 | 2.7 ± 0.2 | 2.7 ± 0.1 |
| 18:0 | 26.6 ± 1.8 | 24.4 ± 0.4 | 25.8 ± 0.7 | 25.6 ± 0.8 |
| 18:1n-9 ** | 10.3 ± 0.1 | 10.5 ± 0.4 | 12.5 ± 0.4 | 12.2 ± 0.6 |
| 18:1n-7 | 1.5 ± tr. | 1.8 ± 0.2 | 1.4 ± 0.1 | 1.4 ± tr. |
| 20:1 | 1.5 ± tr. | 1.4 ± 0.3 | 1.3 ± 0.3 | 1.0 ± 0.1 |
| 20:4n-6 *** | 13.8 ± 0.4 | 13.8 ± 0.5 | 6.7 ± 0.4 | 7.9 ± 0.3 |
| 22:4n-6 *** | 4.7 ± 0.2 | 4.4 ± tr. | 1.1 ± 0.1 | 1.4 ± 0.1 |
| 22:6n-3 *** | 22.2 ± 1.0 | 24.1 ± 0.5 | 31.6 ± 0.6 | 31.2 ± 0.7 |

tr., trace (less than 0.05); significant effect of diet, ** $p < 0.01$, *** $p < 0.001$. Fatty acids, including the unidentified fatty acids X1, X2 and X3 are listed in the order that they eluted from the GLC column.

### *5.3.1.3.1.3 Fatty acid distribution in phosphatidylethanolamine from cerebellum*

The distribution of the main fatty acids in PE from cerebellum of 12 and 16 month-old WT and Tg mice on the oil blend diet or on the DHA diet is presented in Table 5.11. At 12 months of age, percentages of 20:1 ($F(1,8) = 11.521$, $p = 0.009$), 20:4n-6 ($F(1,8) = 588.295$, $p < 0.001$) and 22:4n-6 ($F(1,8) = 329.704$, $p < 0.001$) were significantly higher in PE from the cerebellum of oil blend-fed mice than in PE from cerebellum of DHA-fed mice, and percentages of 18:1n-9 ($F(1,8) = 8.719$, $p = 0.018$) and DHA ($F(1,8) = 68.334$, $p < 0.001$) were significantly higher in PE from the cerebellum of DHA-fed mice compared to PE from oil blend-fed mice. At 16 months of age, percentages of 18:0 ($F(1,8) = 5.941$, $p = 0.041$), 20:4n-6 ($F(1,8) = 308.666$, p

< 0.001) and 22:4n-6 ($F(1,8) = 1711.125$, $p < 0.001$) were significantly higher in PE from the cerebellum of oil blend-fed mice than in PE from cerebellum of DHA-fed mice, while percentages of DHA ($F(1,8) = 22.124$, $p = 0.002$) were significantly higher in PE from cerebellum of the DHA-fed mice than in PE from cerebellum of the oil blend-fed mice. Percentages of 22:4n-6 also appeared significantly higher in PE from cerebellum of Tg mice than in PE from cerebellum of WT mice ($F(1,8) = 10.125$, $p = 0.013$) with a significant interaction of genotype by diet caused by a significantly higher proportion of 22:4n-6 in PE from Tg mice than in PE from WT mice on the oil blend diet ($F(1,8) = 30.250$, $p = 0.001$) but not in PE from mice on the DHA diet ($p > 0.05$).

**Table 5.11**. Main fatty acids in phosphatidylethanolamine from cerebellum of WT and Tg mice on the oil blend diet or on the DHA diet, at 12 months and at 16 months of age. Results are represented as mean percentages of total fatty acids ± SEM.

| | | | 12 months | |
|---|---|---|---|---|
| Fatty acid | Tg oil (n = 4) | WT oil (n = 4) | Tg DHA (n = 4) | WT DHA (n = 4) |
| X1 | 2.8 ± 1.0 | 3.7 ± 0.4 | 2.5 ± 0.7 | 3.1 ± 0.5 |
| 16:0 | 5.1 ± 0.4 | 5.2 ± 0.2 | 5.4 ± 0.5 | 5.9 ± 0.5 |
| X2 | 7.1 ± 1.1 | 8.3 ± 0.6 | 6.6 ± 0.9 | 5.9 ± 1.2 |
| X3 | 4.5 ± 0.2 | 5.4 ± 0.2 | 4.6 ± 0.2 | 3.7 ± 0.9 |
| 18:0 | 19.2 ± 0.7 | 17.8 ± 1.0 | 18.8 ± 1.3 | 17.7 ± 0.7 |
| 18:1n-9 * | 17.3 ± 0.4 | 17.8 ± 0.5 | 19.3 ± 0.4 | 19.9 ± 0.9 |
| 18:1n-7 | 2.0 ± 0.1 | 2.6 ± 0.5 | 2.0 ± 0.1 | 2.3 ± tr. |
| 20:1 ** | 5.5 ± 0.4 | 6.0 ± tr. | 4.4 ± 0.1 | 5.6 ± 0.2 |
| 20:4n-6 *** | 8.4 ± 0.6 | 8.2 ± 0.1 | 3.3 ± 0.3 | 3.2 ± 0.2 |
| 22:4n-6 *** | 2.6 ± 0.2 | 2.6 ± 0.1 | 0.7 ± 0.1 | 0.7 ± 0.1 |
| 22:6n-3 *** | 22.5 ± 1.8 | 19.4 ± 1.1 | 28.5 ± 1.6 | 27.7 ± 0.3 |
| | | | 16 months | |
| Fatty acid | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
| X1 | 4.0 ± 0.2 | 2.8 ± 0.4 | 3.3 ± 0.1 | 3.6 ± 0.6 |
| 16:0 | 5.2 ± 0.1 | 5.4 ± 0.3 | 5.3 ± 0.2 | 5.7 ± 0.4 |
| X2 | 7.4 ± 0.3 | 6.6 ± 0.5 | 7.4 ± 0.3 | 7.1 ± tr. |
| X3 | 5.1 ± 0.4 | 4.6 ± 0.1 | 4.9 ± 0.5 | 4.7 ± 0.1 |
| 18:0 * | 18.5 ± 0.1 | 19.2 ± 0.5 | 17.5 ± 0.6 | 18.2 ± 0.2 |
| 18:1n-9 | 20.1 ± 1.2 | 18.8 ± 0.9 | 21.7 ± 1.1 | 21.4 ± 0.5 |
| 18:1n-7 | 2.5 ± 0.1 | 2.8 ± 0.4 | 2.4 ± 0.2 | 2.7 ± 0.5 |
| 20:1 | 6.0 ± 0.4 | 5.0 ± 0.2 | 5.3 ± 0.3 | 4.8 ± 0.4 |
| 20:4n-6 *** | 7.5 ± 0.4 | 8.0 ± 0.3 | 2.1 ± 0.2 | 2.7 ± 0.1 |
| 22:4n-6 *** * | 2.6 ± tr. ** | 2.2 ± tr. | 0.4 ± tr. | 0.5 ± tr. |
| 22:6n-3 *** | 19.1 ± 1.3 | 22.9 ± 0.8 | 27.2 ± 1.1 | 26.5 ± 1.1 |

tr., trace (less than 0.05); significant effect of diet, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; significant effect of genotype, * $p < 0.05$, ** $p < 0.01$. Fatty acids, including the unidentified fatty acids X1, X2 and X3 are listed in the order that they eluted from the GLC column.

#### *5.3.1.3.2 Fatty acid distribution in phosphatidylcholine*

The main fatty acids present in phosphatidylcholine (PC) from cortex, hippocampus and cerebellum were 16:0, 18:0 and 18:1n-9 (Tables 5.12, 5.13 and 5.14). Vaccenic (18:1n-7), arachidonic (20:4n-6) and docosahexaenoic (22:6n-3) acids were also present in significant amounts. In PC, DHA represented less than 10% of fatty acids with 2.7% ± 0.2% to 7.5% ± 0.9% of total fatty acids in cortex, 1.7% ± 0.1% to 4.0% ± 0.2% of total fatty acids in hippocampus and 4.6% ± 0.5% to 8.5% ± 0.3% of total fatty acids in cerebellum. Only minor differences in the fatty acid composition of PC were apparent among n-3 and n-6 polyunsaturated fatty acids, where percentages of DHA were higher in PC from DHA-fed mice and the percentages of 20:4n-6 were higher in PC from oil blend-fed mice.

The comparison of the composition of PC from cortex, hippocampus and cerebellum between 12 months and 16 months of age suggests an age related decrease of the proportion of 20:4n-6 and DHA and a conversely increased proportion of saturated 18:0. As previously noted in PE, this was observed more strongly in cortex and hippocampus.

##### *5.3.1.3.2.1 Fatty acid distribution in phosphatidylcholine from cortex*

The distribution of the main fatty acids in PC from cortex of 12 and 16 month-old WT and Tg mice on the oil blend diet or on the DHA diet is presented in Table 5.12. At 12 months of age, percentages of 18:1n-7 ($F(1,8) = 6.900$, $p = 0.030$) and 20:4n-6 ($F(1,8) = 73.958$, $p < 0.001$) were significantly higher in PC from cortex of oil blend-fed mice than PC from cortex of DHA-fed mice but percentages of DHA were not significantly higher in PC from cortex of DHA-fed mice compared to PC from oil blend-fed mice ($F(1,8) = 5.154$, $p = 0.053$). At 16 months of age, percentages of 20:4n-6 were significantly higher in PC from cortex of oil blend-fed mice than in PC from cortex of DHA-fed mice ($F(1,8) = 112.862$, $p < 0.001$), while percentages of DHA were significantly higher in PC from cortex of the DHA-fed mice than in PC from cortex of the oil blend-fed mice ($F(1,8) = 25.399$, $p = 0.001$).

**Table 5.12.** Main fatty acids in phosphatidylcholine from cortex of WT and Tg mice on the oil blend diet or on the DHA diet, at 12 months and at 16 months of age. Results are represented as mean percentages of total fatty acids ± SEM.

| | 12 months | | | |
|---|---|---|---|---|
| Fatty acid | Tg oil (n = 4) | WT oil (n = 4) | Tg DHA (n = 4) | WT DHA (n = 4) |
| 16:0 | 40.3 ± 1.6 | 39.1 ± 2.3 | 39.9 ± 2.8 | 40.2 ± 1.8 |
| 18:0 | 13.7 ± 0.6 | 13.6 ± 0.8 | 13.6 ± 0.9 | 13.9 ± 0.5 |
| 18:1n-9 | 22.2 ± 0.3 | 22.5 ± 0.7 | 23.6 ± 1.1 | 23.9 ± 0.6 |
| 18:1n-7 * | 6.4 ± 0.2 | 6.6 ± 0.2 | 5.9 ± 0.2 | 5.8 ± 0.2 |
| 20:4n-6 *** | 7.2 ± 0.4 | 7.5 ± 0.5 | 4.4 ± 0.2 | 3.9 ± 0.2 |
| 22:6n-3 | 5.3 ± 0.6 | 5.7 ± 0.5 | 7.5 ± 0.9 | 6.8 ± 0.5 |
| | 16 months | | | |
| Fatty acid | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
| 16:0 | 50.7 ± 1.1 | 52.5 ± 0.7 | 52.0 ± 1.2 | 52.2 ± 1.4 |
| 18:0 | 13.2 ± 0.4 | 12.6 ± 0.2 | 13.1 ± 0.3 | 12.2 ± 0.3 |
| 18:1n-9 | 20.4 ± 0.5 | 19.7 ± 0.5 | 21.2 ± 0.6 | 21.6 ± 0.9 |
| 18:1n-7 | 5.7 ± 0.1 | 5.6 ± 0.3 | 4.9 ± 0.4 | 5.2 ± 0.4 |
| 20:4n-6 *** | 4.9 ± 0.3 | 4.6 ± 0.3 | 1.9 ± 0.1 | 2.4 ± 0.3 |
| 22:6n-3 ** | 2.7 ± 0.2 | 2.7 ± 0.1 | 4.4 ± 0.4 | 4.0 ± 0.4 |

Significant effect of diet, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

### *5.3.1.3.2.2 Fatty acid distribution in phosphatidylcholine from hippocampus*

The distribution of the main fatty acids in PC from the hippocampus of 12 and 16 month-old WT and Tg mice on the oil blend diet or on the DHA diet is presented in Table 5.13. At 12 months age as well as 16 months of age, percentages of 20:4n-6 were significantly higher in PC from hippocampus of oil blend-fed mice than PC from hippocampus of DHA-fed mice (respectively, $F(1,8) = 113.203$, $p < 0.001$ and $F(1,8) = 96.604$, $p < 0.001$), and percentages of 18:1n-9 (respectively, $F(1,8) = 28.190$, $p = 0.001$ and $F(1,8) = 34.897$, $p < 0.001$) and DHA (respectively, $F(1,8) = 42.285$, $p < 0.001$ and $F(1,8) = 55.078$, $p < 0.001$) were significantly higher in PC from hippocampus of DHA-fed mice compared to PC from oil blend-fed mice.

**Table 5.13.** Main fatty acids in phosphatidylcholine from hippocampus of WT and Tg mice on the oil blend diet or on the DHA diet, at 12 months and at 16 months of age. Results are represented as mean percentages of total fatty acids ± SEM.

| | | | 12 months | |
|---|---|---|---|---|
| Fatty acid | Tg oil (n = 4) | WT oil (n = 4) | Tg DHA (n = 4) | WT DHA (n = 4) |
| 16:0 | 44.0 ± 0.8 | 46.0 ± 1.2 | 43.7 ± 1.0 | 44.6 ± 1.2 |
| 18:0 | 13.5 ± 0.4 | 13.1 ± 0.3 | 13.3 ± 0.1 | 14.0 ± 0.4 |
| 18:1n-9 ** | 20.1 ± 0.3 | 19.3 ± 0.1 | 21.9 ± 0.2 | 21.8 ± 0.7 |
| 18:1n-7 | 9.1 ± 0.3 | 8.5 ± 0.7 | 8.5 ± 0.3 | 8.3 ± 1.4 |
| 20:4n-6 *** | 7.3 ± 0.3 | 6.7 ± 0.2 | 4.2 ± 0.2 | 3.9 ± 0.3 |
| 22:6n-3 *** | 2.7 ± 0.1 | 2.8 ± 0.3 | 3.9 ± 0.1 | 3.7 ± 0.2 |
| | | | 16 months | |
| Fatty acid | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
| 16:0 | 50.8 ± 0.6 | 51.9 ± 0.6 | 51.8 ± 1.4 | 52.3 ± 0.7 |
| 18:0 | 14.4 ± 0.5 | 13.9 ± 0.4 | 14.0 ± 0.4 | 13.4 ± 0.3 |
| 18:1n-9 *** | 19.8 ± 0.1 | 19.9 ± 0.2 | 22.1 ± 0.5 | 21.6 ± 0.4 |
| 18:1n-7 | 5.7 ± 0.5 | 5.4 ± 0.5 | 4.5 ± 0.5 | 5.1 ± 0.4 |
| 20:4n-6 *** | 5.4 ± 0.3 | 5.1 ± 0.3 | 2.5 ± 0.3 | 2.8 ± 0.1 |
| 22:6n-3 *** | 1.7 ± 0.1 | 1.8 ± 0.1 | 2.8 ± 0.2 | 2.5 ± 0.1 |

Significant effect of diet, ** $p < 0.01$, *** $p < 0.001$.

#### *5.3.1.3.2.3 Fatty acid distribution in phosphatidylcholine from cerebellum*

The distribution of the main fatty acids in PC from cerebellum of 12 and 16 month-old WT and Tg mice on the oil blend diet or on the DHA diet is presented in Table 5.14. At 12 months age, percentages of 20:4n-6 were significantly higher in PC from cerebellum of oil blend-fed mice than PC from cerebellum of DHA-fed mice ($F(1,8) = 124.298$, $p < 0.001$), and percentages of DHA were significantly higher in PC from cerebellum of DHA-fed mice compared to PC from oil blend-fed mice ($F(1,8) = 19.282$, $p = 0.002$). At 16 months age, percentages of 18:0 ($F(1,8) = 38.355$, $p < 0.001$), 18:1n-7 ($F(1,8) = 7.753$, $p = 0.024$ and 20:4n-6 ($F(1,8) = 70.778$, $p < 0.001$) were significantly higher in PC from cerebellum of oil blend-fed mice than PC from cerebellum of DHA-fed mice, and percentages of 18:1n-9 ($F(1,8) = 6.547$, $p = 0.034$) and DHA ($F(1,8) = 13.460$, $p = 0.006$) were significantly higher in PC from cerebellum of DHA-fed mice compared to PC from oil blend-fed mice.

**Table 5.14.** Main fatty acids in phosphatidylcholine from cerebellum of WT and Tg mice on the oil blend diet or on the DHA diet, at 12 months and at 16 months of age. Results are represented as mean percentages of total fatty acids ± SEM.

| 12 months | | | | |
|---|---|---|---|---|
| Fatty acid | Tg oil (n = 4) | WT oil (n = 4) | Tg DHA (n = 4) | WT DHA (n = 4) |
| 16:0 | 44.4 ± 0.9 | 44.0 ± 1.8 | 46.2 ± 2.0 | 43.5 ± 0.2 |
| 18:0 | 16.4 ± 0.3 | 16.8 ± 0.2 | 15.8 ± 0.7 | 15.4 ± 0.3 |
| 18:1n-9 | 19.8 ± 0.4 | 19.6 ± 0.5 | 19.3 ± 0.9 | 20.5 ± 0.4 |
| 18:1n-7 | 5.9 ± 0.2 | 6.5 ± 0.8 | 5.1 ± 0.4 | 5.7 ± 0.2 |
| 20:4n-6 *** | 2.6 ± 0.2 | 2.5 ± 0.2 | 0.8 ± 0.1 | 0.8 ± tr. |
| 22:6n-3 ** | 6.1 ± 0.4 | 5.8 ± 0.7 | 8.1 ± 0.8 | 8.5 ± 0.3 |
| 16 months | | | | |
| Fatty acid | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
| 16:0 | 43.2 ± 0.3 | 44.2 ± 1.2 | 45.2 ± 0.3 | 45.1 ± 0.8 |
| 18:0 *** | 17.5 ± 0.3 | 16.8 ± 0.4 | 15.2 ± 0.2 | 15.6 ± 0.1 |
| 18:1n-9 * | 21.6 ± 0.5 | 20.2 ± 0.2 | 22.3 ± 0.5 | 21.8 ± 0.6 |
| 18:1n-7 * | 7.2 ± 0.2 | 7.7 ± 0.4 | 5.9 ± 0.5 | 6.7 ± 0.4 |
| 20:4n-6 *** | 1.8 ± 0.2 | 1.9 ± 0.3 | 0.3 ± tr. | 0.5 ± tr. |
| 22:6n-3 ** | 4.6 ± 0.5 | 5.5 ± 0.9 | 7.7 ± 0.4 | 6.8 ± 0.4 |

tr., trace (less than 0.05); significant effect of diet, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

#### *5.3.1.3.3 Fatty acid distribution in phosphatidylserine*

The main fatty acids present in phosphatidylserine (PS) from cortex, hippocampus and cerebellum were stearic acid (18:0), oleic acid (18:1n-9) and DHA (Tables 5.15, 5.16 and 5.17). 16:0, 20:4n-6 and 22:4n-6 were also present at significant levels. In PS, DHA represented over 10% of fatty acids with 26.9% ± 1.5% to 34.7% ± 1.7% of total fatty acids in cortex, 20.1% ± 0.2% to 28.0% ± 1.1% of total fatty acids in hippocampus and 11.1% ± 2.1% to 21.6% ± 0.9% of total fatty acids in cerebellum. Major differences in the fatty acid composition of PS were apparent among n-6 polyunsaturated fatty acids, where percentages of 20:4n-6 and 22:4n-6 were higher in PS from oil blend-fed mice. In some case percentages of DHA were also higher in PS from DHA-fed mice.

The comparison of the composition of PS from cortex, hippocampus and cerebellum between 12 months and 16 months of age suggests an age related decrease of the proportion of DHA and a conversely increased proportion of saturated 18:0.

##### *5.3.1.3.3.1 Fatty acid distribution in phosphatidylserine from cortex*

The distribution of the main fatty acids in PS from cortex of 12 and 16 month-old WT and Tg mice on the oil blend diet or on the DHA diet is presented in Table 5.15. At 12 months of age, percentages of 16:0 ($F(1,8) = 5.493$, $p = 0.047$), 20:4n-6 ($F(1,8) = 34.215$, $p < 0.001$) and 22:4n-6 ($F(1,8) = 49.920$, $p < 0.001$) were significantly higher in PS from cortex of oil blend-fed mice than PS from cortex of DHA-fed mice, but percentages of DHA were not significantly higher in PS from cortex of DHA-fed mice compared to PS from oil blend-fed mice ($F(1,8) = 0.445$, $p = 0.533$). At 16 months of age, percentages of 20:4n-6 ($F(1,8) = 58.132$, $p < 0.001$) and 22:4n-6 ($F(1,8) = 574.083$, $p < 0.001$) were significantly higher in PS from cortex of oil blend-fed mice than in PS from cortex of DHA-fed mice and, as observed at 12 months of age, percentages of DHA were not significantly affected by the diet ($F(1,8) = 2.031$, $p = 0.192$).

**Table 5.15.** Main fatty acids in phosphatidylserine from cortex of WT and Tg mice on the oil blend diet or on the DHA diet, at 12 months and at 16 months of age. Results are represented as mean percentages of total fatty acids ± SEM.

| | | | 12 months | |
|---|---|---|---|---|
| Fatty acid | Tg oil (n = 4) | WT oil (n = 4) | Tg DHA (n = 4) | WT DHA (n = 4) |
| 16:0 * | 1.3 ± 0.1 | 1.3 ± tr. | 0.9 ± 0.1 | 0.9 ± 0.2 |
| 18:0 | 44.6 ± 2.0 | 42.8 ± 1.9 | 44.3 ± 1.4 | 45.2 ± 0.3 |
| 18:1n-9 | 12.6 ± 1.0 | 12.2 ± 0.7 | 12.5 ± 0.5 | 12.5 ± 0.5 |
| 20:4n-6 *** | 2.1 ± 0.1 | 2.1 ± 0.3 | 1.3 ± 0.1 | 1.3 ± 0.1 |
| 22:4n-6 *** | 2.8 ± 0.1 | 2.2 ± 0.4 | 1.0 ± tr. | 0.9 ± tr. |
| 22:6n-3 | 30.8 ± 2.9 | 33.8 ± 3.1 | 34.7 ± 1.7 | 33.4 ± 0.8 |
| | | | 16 months | |
| Fatty acid | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
| 16:0 | 1.6 ± tr. | 1.9 ± 0.1 | 1.9 ± 0.1 | 1.6 ± tr. |
| 18:0 | 54.1 ± 1.1 | 52.9 ± 1.5 | 53.4 ± 2.0 | 52.6 ± 1.3 |
| 18:1n-9 | 12.0 ± 0.5 | 12.5 ± 0.3 | 12.3 ± 0.6 | 13.6 ± 0.4 |
| 20:4n-6 *** | 1.4 ± 0.1 | 1.5 ± 0.1 | 0.6 ± tr. | 0.8 ± tr. |
| 22:4n-6 *** | 1.9 ± tr. | 1.8 ± 0.1 | 0.4 ± tr. | 0.5 ± 0.1 |
| 22:6n-3 | 26.9 ± 1.5 | 27.3 ± 1.1 | 29.7 ± 2.0 | 28.8 ± 1.1 |

tr., trace (less than 0.05); significant effect of diet, * $p < 0.05$, *** $p < 0.001$.

### *5.3.1.3.3.2 Fatty acid distribution in phosphatidylserine from hippocampus*

The distribution of the main fatty acids in PS from hippocampus of 12 and 16 month-old WT and Tg mice on the oil blend diet or on the DHA diet is presented in Table 5.16. At 12 months of age, percentages of 20:4n-6 ($F(1,8) = 35.328$, $p < 0.001$) and 22:4n-6 ($F(1,8) = 17.907$, $p = 0.003$) were significantly higher in PS from hippocampus of oil blend-fed mice than PS from hippocampus of DHA-fed mice, but percentages of DHA were not significantly affected by the diet ($F(1,8) = 4.507$, $p = 0.067$). At 16 months of age, percentages of 20:4n-6 ($F(1,8) = 30.817$, $p = 0.001$) and 22:4n-6 ($F(1,8) = 181.289$, $p < 0.001$) were significantly higher in PS from hippocampus of oil blend-fed mice than in PS from hippocampus of DHA-fed mice and, percentages of DHA were significantly higher in hippocampus from DHA-fed mice than from mice on the oil blend diet ($F(1,8) = 13.134$, $p = 0.007$).

**Table 5.16.** Main fatty acids in phosphatidylserine from hippocampus of WT and Tg mice on the oil blend diet or on the DHA diet, at 12 months and at 16 months of age. Results are represented as mean percentages of total fatty acids ± SEM.

| | | | 12 months | |
|---|---|---|---|---|
| Fatty acid | Tg oil (n = 4) | WT oil (n = 4) | Tg DHA (n = 4) | WT DHA (n = 4) |
| 16:0 | 2.0 ± 0.4 | 2.0 ± tr. | 2.0 ± 0.3 | 1.8 ± 0.2 |
| 18:0 | 43.8 ± 1.3 | 45.2 ± 1.9 | 42.9 ± 0.9 | 43.2 ± 1.0 |
| 18:1n-9 | 20.2 ± 1.6 | 19.4 ± 1.7 | 20.6 ± 1.2 | 20.3 ± 2.0 |
| 20:4n-6 *** | 2.8 ± 0.2 | 2.7 ± 0.3 | 1.8 ± 0.2 | 1.7 ± 0.1 |
| 22:4n-6 ** | 2.0 ± 0.4 | 2.0 ± 0.2 | 0.8 ± 0.1 | 0.9 ± 0.3 |
| 22:6n-3 | 25.2 ± 1.8 | 25.3 ± 0.4 | 27.4 ± 1.7 | 28.0 ± 1.1 |
| | | | 16 months | |
| Fatty acid | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
| 16:0 | 2.2 ± 0.4 | 2.3 ± 0.3 | 2.8 ± 0.3 | 2.3 ± 0.5 |
| 18:0 | 54.6 ± 0.8 | 54.2 ± 0.2 | 55.0 ± 0.3 | 53.2 ± 1.0 |
| 18:1n-9 | 16.5 ± 0.3 | 17.4 ± 0.5 | 15.9 ± 0.9 | 16.8 ± 1.0 |
| 20:4n-6 ** | 1.7 ± 0.1 | 1.7 ± tr. | 0.8 ± 0.1 | 1.0 ± 0.2 |
| 22:4n-6 *** | 2.0 ± 0.1 | 1.7 ± 0.1 | 0.5 ± tr. | 0.5 ± 0.1 |
| 22:6n-3 ** | 20.1 ± 0.2 | 20.8 ± 0.5 | 22.4 ± 1.0 | 23.4 ± 0.7 |

tr., trace (less than 0.05); significant effect of diet, ** $p < 0.01$, *** $p < 0.001$.

### *5.3.1.3.3.3 Fatty acid distribution in phosphatidylserine from cerebellum*

The distribution of the main fatty acids in PS from cerebellum of 12 and 16 month-old WT and Tg mice on the oil blend diet or on the DHA diet is presented in Table 5.17. At 12 months of age, percentages of 20:4n-6 ($F(1,8) = 51.598$, $p < 0.001$) and 22:4n-6 ($F(1,8) = 160.000$, $p < 0.001$) were significantly higher in PS from cerebellum of oil blend-fed mice than PS from cerebellum of DHA-fed mice, and percentages of DHA were significantly higher in PS from cerebellum of DHA-fed mice than PS from cerebellum of mice on the oil blend diet ($F(1,8) = 22.825$, $p = 0.001$). DHA percentages were also significantly higher in PS from cerebellum of Tg mice than WT mice ($F(1,8) = 7.066$, $p = 0.029$). Gender also had a significant effect on the percentages of 22:4n-6 ($F(1,8) = 6.400$, $p = 0.035$) and 22:6n-3 ($F(1,8) = 14.226$, $p = 0.005$) with lower percentages in females than males (data not shown). At 16 months of age, percentages of 20:4n-6 ($F(1,8) = 31.500$, $p = 0.001$) and 22:4n-6 ($F(1,8) = 38.111$, $p < 0.001$) were significantly higher in PS from cerebellum of oil blend-fed mice than in PS from cerebellum of DHA-fed mice and, percentages of DHA were significantly higher in cerebellum from DHA-fed mice than from mice on the oil blend diet ($F(1,8) = 5.429$, $p = 0.048$).

**Table 5.17.** Main fatty acids in phosphatidylserine from cerebellum of WT and Tg mice on the oil blend diet or on the DHA diet, at 12 months and at 16 months of age. Results are represented as mean percentages of total fatty acids ± SEM.

| | 12 months | | | |
|---|---|---|---|---|
| Fatty acid | Tg oil (n = 4) | WT oil (n = 4) | Tg DHA (n = 4) | WT DHA (n = 4) |
| 16:0 | 2.1 ± 0.3 | 2.0 ± 0.3 | 1.9 ± 0.3 | 2.3 ± 0.3 |
| 18:0 | 45.3 ± 2.3 | 44.2 ± 1.5 | 41.2 ± 1.4 | 41.9 ± 1.4 |
| 18:1n-9 | 24.9 ± 0.7 | 26.1 ± 1.3 | 25.8 ± 1.1 | 26.3 ± 1.2 |
| 20:4n-6 *** | 2.0 ± 0.2 | 1.9 ± 0.2 | 1.2 ± 0.2 | 1.0 ± 0.1 |
| 22:4n-6 *** | 1.6 ± 0.1 | 1.5 ± 0.1 | 0.6 ± tr. | 0.5 ± 0.1 |
| 22:6n-3 ** ♦ | 17.3 ± 1.3 | 16.5 ± 0.9 | 21.6 ± 0.9 | 18.8 ± 0.7 |
| | 16 months | | | |
| Fatty acid | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
| 16:0 | 2.9 ± 0.5 | 2.7 ± 0.7 | 2.6 ± 0.2 | 2.4 ± 0.1 |
| 18:0 | 45.4 ± 1.3 | 44.3 ± 1.1 | 42.8 ± 0.5 | 42.8 ± 1.2 |
| 18:1n-9 | 32.3 ± 0.7 | 31.2 ± 0.5 | 31.8 ± 1.2 | 32.5 ± 0.9 |
| 20:4n-6 ** | 1.3 ± 0.2 | 1.5 ± 0.1 | 0.6 ± 0.1 | 0.8 ± 0.1 |
| 22:4n-6 *** | 0.9 ± 0.2 | 1.1 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 |
| 22:6n-3 * | 11.1 ± 2.1 | 13.8 ± 1.9 | 16.5 ± 1.3 | 15.9 ± 0.7 |

tr., trace (less than 0.05); significant effect of diet, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; significant effect of genotype, ♦ $p < 0.05$.

#### *5.3.1.3.4 Fatty acid distribution in phosphatidylinositol*

The main fatty acids present in phosphatidylinositol (PI) from cortex, hippocampus and cerebellum were, 16:0, 18:0, 18:1n-9 and 20:4n-6 (Table 5.18, 5.19 and 5.20). Vaccenic acid (18:1n-7), EPA and DHA were also found at significant levels. In PI, DHA represented less than 10% of fatty acids with 1.3% ± 0.1% to 3.5% ± 0.2% of total fatty acids in cortex, 0.1% ± 0.1% to 2.2% ± 0.4% of total fatty acids in hippocampus and 2.2% ± 1.1% to 7.2% ± 0.4% of total fatty acids in cerebellum. Major differences in the fatty acid composition of PI were apparent among n-3 and n-6 polyunsaturated fatty acids, where, in some cases, percentages of 20:4n-6 and 22:4n-6 were higher in PI from oil blend-fed mice, and EPA and DHA were higher in PI from DHA-fed mice.

The comparison of the composition of PI from cortex, hippocampus and cerebellum between 12 months and 16 months of age suggests an age-related decrease of the proportion of PUFA and conversely increased proportions of 16:0 and 18:0.

##### *5.3.1.3.4.1 Fatty acid distribution in phosphatidylinositol from cortex*

The distribution of the main fatty acids in PI from the cortex of 12 and 16 month-old WT and Tg mice on the oil blend diet or on the DHA diet is presented in Table 5.18. At 12 months of age, percentages of 20:4n-6 ($F(1,8) = 11.786$, $p = 0.009$) were significantly higher in PI from cortex of oil blend-fed mice than PI from cortex of DHA-fed mice, and percentages of 20:5n-3 ($F(1,8) = 53.772$, $p < 0.001$) but not percentages of DHA ($F(1,8) = 2.730$, $p = 0.137$) were significantly higher in PI from cortex of DHA-fed mice compared to PI from oil blend-fed mice. At 16 months of age, percentages of 20:4n-6 ($F(1,8) = 6.683$, $p = 0.032$) were again significantly higher in PI from cortex of oil blend-fed mice than in PI from cortex of DHA-fed mice, and percentages of 18:1n-9 ($F(1,8) = 18.570$, $p = 0.003$), 20:5n-3 ($F(1,8) = 121.000$, $p < 0.001$) and DHA ($F(1,8) = 120.714$, $p < 0.001$) were significantly higher in PI from cortex of DHA-fed mice. A significant interaction of diet by genotype ($F(1,8) = 6.429$, $p = 0.035$) also revealed that percentages of DHA were higher in PI from cortex of WT mice on the oil blend diet than in PI from the cortex of Tg mice on the same diet ($F(1,8) = 8.229$, $p = 0.021$) but there was no significant difference

between DHA percentages in PI from Tg mice and WT mice, on the DHA diet ($F(1,8) = 0.514$, $p = 0.494$).

**Table 5.18.** Main fatty acids in phosphatidylinositol from cortex of WT and Tg mice on the oil blend diet or on the DHA diet, at 12 months and at 16 months of age. Results are represented as mean percentages of total fatty acids ± SEM.

| | | | 12 months | |
|---|---|---|---|---|
| Fatty acid | Tg oil (n = 4) | WT oil (n = 4) | Tg DHA (n = 4) | WT DHA (n = 4) |
| 16:0 | 6.0 ± 1.1 | 5.4 ± 0.9 | 6.3 ± 0.7 | 7.5 ± 0.7 |
| 18:0 | 35.3 ± 1.8 | 34.4 ± 1.8 | 34.5 ± 1.5 | 37.0 ± 0.7 |
| 18:1n-9 | 5.8 ± 0.2 | 5.4 ± 0.3 | 6.1 ± 0.2 | 6.0 ± 0.2 |
| 18:1n-7 | 2.5 ± tr. | 2.4 ± 0.1 | 2.6 ± 0.1 | 2.0 ± 0.7 |
| 20:4n-6 ** | 38.7 ± 2.3 | 41.6 ± 2.8 | 33.5 ± 2.5 | 32.2 ± 0.9 |
| 20:5n-3 *** | N.D. | 0.1 ± tr. | 2.0 ± 0.4 | 1.8 ± 0.1 |
| 22:6n-3 | 2.6 ± 0.5 | 3.0 ± 0.4 | 3.4 ± 0.5 | 3.5 ± 0.2 |
| | | | 16 months | |
| Fatty acid | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
| 16:0 | 10.1 ± 1.0 | 8.5 ± 0.6 | 9.1 ± 0.5 | 9.6 ± 0.3 |
| 18:0 | 45.4 ± 1.2 | 45.8 ± 1.6 | 46.7 ± 1.6 | 45.0 ± 1.8 |
| 18:1n-9 ** | 5.9 ± 0.4 | 5.7 ± tr. | 7.1 ± 0.2 | 6.5 ± 0.1 |
| 18:1n-7 | 1.6 ± 0.1 | 1.6 ± tr. | 1.6 ± 0.1 | 1.7 ± 0.2 |
| 20:4n-6 * | 33.0 ± 1.9 | 35.2 ± 0.8 | 28.1 ± 2.4 | 30.9 ± 1.6 |
| 20:5n-3 *** | N.D. | N.D. | 1.7 ± 0.2 | 1.2 ± 0.1 |
| 22:6n-3 *** | 1.3 ± 0.1 ♦ | 1.7 ± 0.1 | 2.6 ± 0.1 | 2.5 ± tr. |

N.D., not detected; tr., trace (less than 0.05); significant effect of diet, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; significant effect of genotype, ♦ $p < 0.05$.

### *5.3.1.3.4.2 Fatty acid distribution in phosphatidylinositol from hippocampus*

The distribution of the main fatty acids in PI from hippocampus of 12 and 16 month-old WT and Tg mice on the oil blend diet or on the DHA diet is presented in Table 5.19. At 12 months of age, percentages of 20:5n-3 ($F(1,8) = 74.690$, $p < 0.001$) but not percentages of DHA ($F(1,8) = 4.072$, $p = 0.078$) were significantly higher in PI from hippocampus of DHA-fed mice compared to PI from oil blend-fed mice. At 16 months of age, percentages of 20:4n-6 ($F(1,8) = 14.884$, $p = 0.005$) were significantly higher in PI from hippocampus of oil blend-fed mice than in PI from hippocampus of DHA-fed mice, and percentages of 18:1n-9 ($F(1,8) = 5.938$, $p = 0.041$), 20:5n-3 ($F(1,8) = 340.071$, $p < 0.001$) but not percentages of DHA ($F(1,8) = 0.498$, $p = 0.500$) were significantly higher in PI from hippocampus of DHA-fed mice. Percentages of

20:5n-3 were also significantly higher in PI from hippocampus of Tg mice than from WT mice ($F(1,8) = 8.643$, $p = 0.019$).

**Table 5.19.** Main fatty acids in phosphatidylinositol from hippocampus of WT and Tg mice on the oil blend diet or on the DHA diet, at 12 months and at 16 months of age. Results are represented as mean percentages of total fatty acids ± SEM.

| | 12 months | | | |
|---|---|---|---|---|
| Fatty acid | Tg oil (n = 4) | WT oil (n = 4) | Tg DHA (n = 4) | WT DHA (n = 4) |
| 16:0 | 7.9 ± 1.2 | 9.1 ± 1.1 | 7.0 ± 1.7 | 6.7 ± 1.5 |
| 18:0 | 40.0 ± 1.2 | 40.7 ± 2.7 | 41.0 ± 1.1 | 40.6 ± 1.3 |
| 18:1n-9 | 9.7 ± 0.6 | 8.6 ± 0.7 | 10.4 ± 0.9 | 9.3 ± 1.2 |
| 18:1n-7 | 3.8 ± 0.7 | 3.5 ± 0.7 | 3.5 ± 0.8 | 3.0 ± 1.2 |
| 20:4n-6 | 32.4 ± 1.7 | 33.1 ± 1.8 | 29.2 ± 1.3 | 30.5 ± 1.2 |
| 20:5n-3 *** | N.D. | N.D. | 1.4 ± 0.2 | 1.5 ± 0.2 |
| 22:6n-3 | 1.0 ± 0.3 | 1.2 ± 0.2 | 1.5 ± 0.5 | 2.2 ± 0.4 |
| | 16 months | | | |
| Fatty acid | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
| 16:0 | 8.9 ± 1.1 | 9.7 ± 1.4 | 10.7 ± 0.9 | 10.4 ± 1.2 |
| 18:0 | 51.0 ± 2.4 | 48.3 ± 1.8 | 51.0 ± 1.8 | 48.1 ± 2.6 |
| 18:1n-9 * | 6.4 ± 0.9 | 6.6 ± 1.2 | 9.1 ± 0.5 | 8.6 ± 1.1 |
| 18:1n-7 | 0.5 ± 0.5 | 0.9 ± 0.5 | 1.4 ± tr. | 1.3 ± 0.1 |
| 20:4n-6 ** | 28.2 ± 1.1 | 28.7 ± 2.0 | 22.1 ± 1.3 | 24.2 ± 1.0 |
| 20:5n-3 *** ♦ | N.D. | N.D. | 1.3 ± 0.1 | 1.0 ± 0.1 |
| 22:6n-3 | 0.1 ± 0.1 | 0.2 ± 0.2 | 0.4 ± 0.4 | 0.3 ± 0.3 |

N.D., not detected; tr., trace (less than 0.05); significant effect of diet, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; significant effect of genotype, ♦ $p < 0.05$.

### *5.3.1.3.4.3 Fatty acid distribution in phosphatidylinositol from cerebellum*

The distribution of the main fatty acids in PI from cerebellum of 12 and 16 month-old WT and Tg mice on the oil blend diet or on the DHA diet is presented in Table 5.20. At 12 months of age, percentages of 18:0 ($F(1,8) = 11.888$, $p = 0.009$) and 20:4n-6 ($F(1,8) = 20.707$, $p = 0.002$) were significantly higher in PI from cerebellum of oil blend-fed mice than PI from cerebellum of DHA-fed mice, and percentages of 18:1n-9 ($F(1,8) = 8.342$, $p = 0.020$), 20:5n-3 ($F(1,8) = 5.946$, $p < 0.041$) and DHA ($F(1,8) = 51.705$, $p < 0.001$) were significantly higher in PI from cerebellum of DHA-fed mice compared to PI from oil blend-fed mice. At 16 months of age, percentages of 20:5n-3 ($F(1,8) = 508$, $p < 0.001$) and DHA ($F(1,8) = 41.921$, $p < 0.001$) were significantly higher in PI from cortex of DHA-fed mice.

**Table 5.20.** Main fatty acids in phosphatidylinositol from cerebellum of WT and Tg mice on the oil blend diet or on the DHA diet, at 12 months and at 16 months of age. Results are represented as mean percentages of total fatty acids ± SEM.

| 12 months | | | | |
|---|---|---|---|---|
| Fatty acid | Tg oil (n = 4) | WT oil (n = 4) | Tg DHA (n = 4) | WT DHA (n = 4) |
| 16:0 | 7.3 ± 0.9 | 6.1 ± 0.6 | 6.5 ± 1.0 | 8.7 ± 1.3 |
| 18:0 ** | 40.6 ± 2.5 | 36.2 ± 0.6 | 34.4 ± 0.3 | 33.2 ± 1.2 |
| 18:1n-9 * | 7.4 ± 0.5 | 6.3 ± 0.7 | 8.4 ± 1.0 | 10.0 ± 0.5 |
| 18:1n-7 | 1.9 ± 0.2 | 1.5 ± 0.6 | 1.8 ± 0.3 | 2.4 ± 0.2 |
| 20:4n-6 ** | 32.3 ± 2.7 | 31.4 ± 1.7 | 26.8 ± 1.2 | 23.1 ± 0.9 |
| 20:5n-3 * | 0.5 ± 0.3 | 2.1 ± 0.7 | 2.4 ± 0.5 | 2.3 ± 0.3 |
| 22:6n-3 *** | 3.4 ± 0.4 | 3.6 ± 0.7 | 7.2 ± 0.4 | 6.7 ± 0.2 |
| 16 months | | | | |
| Fatty acid | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
| 16:0 | 11.3 ± 1.4 | 10.6 ± 2.0 | 11.1 ± 0.5 | 10.1 ± 0.2 |
| 18:0 | 44.2 ± 1.1 | 43.6 ± 0.1 | 41.6 ± 0.8 | 43.0 ± 1.6 |
| 18:1n-9 | 10.1 ± 1.6 | 9.8 ± 1.5 | 12.1 ± 0.9 | 11.7 ± 0.9 |
| 18:1n-7 | 1.8 ± 0.2 | 1.8 ± 0.2 | 2.3 ± 0.3 | 2.3 ± 0.2 |
| 20:4n-6 | 25.0 ± 3.6 | 27.3 ± 4.4 | 19.6 ± 1.8 | 21.5 ± 1.2 |
| 20:5n-3 *** | N.D. | N.D. | 2.1 ± 0.1 | 1.8 ± 0.1 |
| 22:6n-3 *** | 2.4 ± 0.4 | 2.2 ± 1.1 | 6.9 ± 0.2 | 5.8 ± 0.4 |

N.D., not detected; tr., trace (less than 0.05); significant effect of diet, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

#### *5.3.1.3.5 Fatty acid distribution in sphingomyelin*

The predominant fatty acid detected in sphingomyelin (Sph) from cortex, hippocampus and cerebellum was stearic acid (18:0) (Tables 5.21, 5.22, 5.23). 16:0, 24:1, 16:1n-7, 18:1n-9, 20:0 and 22:0 were other significant fatty acids. In Sph, DHA represented less than 2% of fatty acids. No major changes due to diet or genotype were apparent in the fatty acid composition of Sph.

A comparison of the composition of Sph from cortex, hippocampus and cerebellum between 12 months and 16 months of age suggests an age-related decrease of the proportion of 22:0 and 24:1, and conversely increased proportions of 16:0 and 18:0.

##### *5.3.1.3.5.1 Fatty acid distribution in sphingomyelin from cortex*

The distribution of the main fatty acids in Sph from cortex of 12 and 16 month-old WT and Tg mice on the oil blend diet or on the DHA diet is presented in Table 5.21. At 12 months of age, there was only a significant effect of gender on the percentage of 16:1n-7 ($F(1,8) = 6.107$, $p = 0.039$), 18:0 ($F(1,8) = 8.604$, p 0.019), 18:1n-9 ($F(1,8) = 13.407$, $p = 0.006$) and 22:0 ($F(1,8) = 2.730$, $p = 0.137$), with higher percentages of 18:0 in females and higher percentages of the other fatty acids in males (data not shown). No significant differences due to diet or genotype were found.

**Table 5.21.** Main fatty acids in sphingomyelin from cortex of WT and Tg mice on the oil blend diet or on the DHA diet, at 12 months and at 16 months of age. Results are represented as mean percentages of total fatty acids ± SEM.

| | | | 12 months | |
|---|---|---|---|---|
| Fatty acid | Tg oil (n = 4) | WT oil (n = 4) | Tg DHA (n = 4) | WT DHA (n = 4) |
| 16:0 | 4.5 ± 0.7 | 4.6 ± 0.9 | 4.0 ± 0.7 | 3.9 ± 0.7 |
| 16:1n-7 | 2.8 ± 0.5 | 2.2 ± 0.3 | 2.3 ± 0.4 | 2.5 ± 0.5 |
| 18:0 | 68.5 ± 3.7 | 70.5 ± 1.9 | 60.7 ± 7.7 | 69.6 ± 4.1 |
| 18:1n-9 | 3.8 ± 0.4 | 3.7 ± 0.7 | 3.8 ± 0.6 | 4.1 ± 1.0 |
| 20:0 | 2.0 ± 0.2 | 2.0 ± 0.1 | 2.0 ± 0.3 | 2.3 ± 0.1 |
| 22:0 | 2.1 ± 0.4 | 2.3 ± 0.1 | 3.8 ± 1.0 | 2.4 ± 0.4 |
| 24:1 | 5.4 ± 1.4 | 5.5 ± 1.4 | 6.0 ± 1.3 | 5.0 ± 1.2 |
| | | | 16 months | |
| Fatty acid | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
| 16:0 | 6.9 ± 0.8 | 7.5 ± 1.0 | 6.8 ± 0.9 | 6.7 ± 1.0 |
| 16:1n-7 | 0.5 ± 0.5 | 0.6 ± 0.6 | 0.8 ± 0.8 | 0.8 ± 0.4 |
| 18:0 | 85.6 ± 1.0 | 84.2 ± 1.9 | 84.2 ± 2.2 | 85.0 ± 1.2 |
| 18:1n-9 | 3.7 ± 0.5 | 3.9 ± 0.6 | 4.1 ± 0.7 | 3.9 ± 0.5 |
| 20:0 | 1.5 ± tr. | 1.6 ± 0.1 | 1.6 ± tr. | 1.6 ± 0.1 |
| 22:0 | 0.3 ± 0.3 | 0.6 ± 0.3 | 0.7 ± 0.4 | 0.5 ± 0.3 |
| 24:1 | N.D. | N.D. | 0.2 ± 0.2 | N.D. |

N.D., not detected; tr., trace (less than 0.05).

### *5.3.1.3.5.2 Fatty acid distribution in sphingomyelin from hippocampus*

The distribution of the main fatty acids in Sph from hippocampus of 12 and 16 month-old WT and Tg mice on the oil blend diet or on the DHA diet is presented in Table 5.22. At 12 months of age, percentages of 18:0 were significantly higher in Sph from hippocampus of WT mice than Tg mice ($F(1,8) = 7.869$, $p = 0.023$). At 16 months of age, no significant differences due to diet or genotype were found.

**Table 5.22.** Main fatty acids in sphingomyelin from hippocampus of WT and Tg mice on the oil blend diet or on the DHA diet, at 12 months and at 16 months of age. Results are represented as mean percentages of total fatty acids ± SEM.

| | 12 months | | | |
|---|---|---|---|---|
| Fatty acid | Tg oil (n = 4) | WT oil (n = 4) | Tg DHA (n = 4) | WT DHA (n = 4) |
| 16:0 | 7.6 ±1.3 | 7.3 ± 0.6 | 8.7 ± 2.2 | 4.4 ± 0.9 |
| 16:1n-7 | 1.6 ± 0.2 | 1.6 ± 0.1 | 1.9 ± 0.1 | 1.7 ± 0.3 |
| 18:0 * | 71.5 ± 1.0 | 75.4 ± 1.8 | 70.8 ± 2.6 | 74.9 ± 0.7 |
| 18:1n-9 | 9.9 ± 0.3 | 6.8 ± 2.2 | 9.3 ± 0.7 | 8.1 ± 1.8 |
| 20:0 | 1.3 ± 0.1 | 1.3 ± 0.1 | 1.2 ± 0.1 | 1.6 ± 0.3 |
| 22:0 | 1.3 ± 0.2 | 1.0 ± 0.4 | 0.8 ± 0.4 | 1.4 ± 0.5 |
| 24:1 | 1.8 ± 0.7 | 2.2 ± 0.8 | 2.0 ± 0.8 | 2.5 ± 1.1 |
| | 16 months | | | |
| Fatty acid | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
| 16:0 | 10.7 ± 1.2 | 11.3 ± 2.6 | 10.5 ± 2.6 | 9.1 ± 0.8 |
| 16:1n-7 | 4.0 ± 1.1 | 2.6 ± 0.5 | 3.3 ± 0.7 | 3.0 ± 1.2 |
| 18:0 | 75.3 ± 1.4 | 74.9 ± 5.0 | 74.7 ± 5.1 | 78.7 ± 1.9 |
| 18:1n-9 | 5.6 ± 0.6 | 5.8 ± 0.8 | 6.3 ± 0.7 | 6.4 ± 0.2 |
| 20:0 | 1.1 ± 0.1 | 1.1 ± 0.1 | 1.1 ± tr. | 0.7 ± 0.4 |
| 22:0 | 0.1 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 | N.D. |
| 24:1 | N.D. | N.D. | N.D. | N.D. |

N.D., not detected; tr., trace (less than 0.05); significant effect of genotype, * $p < 0.05$.

### *5.3.1.3.5.3 Fatty acid distribution in sphingomyelin from cerebellum*

The distribution of the main fatty acids in Sph from cerebellum of 12 and 16 month-old WT and Tg mice on the oil blend diet or on the DHA diet is presented in Table 5.23. At 12 months of age, statistical analysis of 18:0 percentages revealed a significant interaction of genotype by gender ($F(1,8) = 7.355$, $p = 0.027$) with higher percentages of 18:0 in Sph from WT mice than Tg mice, in females only ($F(1,8) = 7.182$, $p = 0.028$) and higher percentages of 18:0 in Sph from males than females, for Tg mice only (data not shown). At 16 months of age, no significant differences due to diet or genotype were found.

**Table 5.23**. Main fatty acids in sphingomyelin from cerebellum of WT and Tg mice on the oil blend diet or on the DHA diet, at 12 months and at 16 months of age. Results are represented as mean percentages of total fatty acids ± SEM.

| 12 months | | | | |
|---|---|---|---|---|
| Fatty acid | Tg oil (n = 4) | WT oil (n = 4) | Tg DHA (n = 4) | WT DHA (n = 4) |
| 16:0 | 10.3 ± 1.0 | 9.7 ± 1.5 | 9.7 ± 2.1 | 8.5 ± 1.3 |
| 16:1n-7 | 1.5 ± 0.4 | 1.9 ± 0.2 | 1.7 ± 0.6 | 1.9 ± 0.3 |
| 18:0 | 55.5 ± 4.4 | 54.4 ± 3.9 | 49.8 ± 5.9 | 58.8 ± 2.9 |
| 18:1n-9 | 5.2 ± 1.4 | 4.0 ± 0.1 | 3.2 ± 1.4 | 6.3 ± 0.8 |
| 20:0 | 3.3 ± 0.7 | 5.3 ± 1.5 | 4.0 ± 2.0 | 3.8 ± 0.5 |
| 22:0 | 0.5 ± 0.3 | 0.7 ± 0.2 | 2.8 ± 1.3 | 1.5 ± 0.8 |
| 24:1 | 2.8 ± 1.7 | 0.8 ± 0.8 | 5.7 ± 3.6 | 1.8 ± 1.8 |
| 16 months | | | | |
| Fatty acid | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
| 16:0 | 17.1 ± 1.9 | 14.7 ± 1.6 | 14.3 ± 1.4 | 12.4 ± 0.9 |
| 16:1n-7 | 3.6 ± 0.8 | 3.3 ± 1.0 | 2.4 ± 0.5 | 3.0 ± 0.8 |
| 18:0 | 59.9 ± 3.1 | 64.7 ± 3.1 | 64.8 ± 1.0 | 69.1 ± 2.5 |
| 18:1n-9 | 11.1 ± 2.5 | 8.6 ± 2.6 | 6.9 ± 0.9 | 6.5 ± 0.2 |
| 20:0 | 1.4 ± tr. | 1.3 ± 0.1 | 1.5 ± 0.1 | 1.4 ± tr. |
| 22:0 | 0.9 ± 0.5 | 0.8 ± 0.4 | 1.4 ± 0.5 | 1.5 ± 0.1 |
| 24:1 | 1.3 ± 0.7 | 2.3 ± 1.2 | 3.4 ± 0.4 | 2.2 ± 0.3 |

tr., trace (less than 0.05).

## 5.3.2 Phospholipid analysis of brain lipids by mass spectrometry

### 5.3.2.1 Analysis of phosphatidylethanolamine

Negative-ion ESI-MS-MS analysis of PE in cortex, hippocampus and cerebellum of 12 and 16 month-old WT and Tg mice on the oil blend diet or on the DHA diet demonstrated predominant peaks corresponding to its most abundant molecular species 18:1p/18:1, 18:0a/20:4 and 18:0a/22:6 in cortex and hippocampus, and 16:0p/18:1, 18:0p/18:1, 18:1a/18:1, 18:1p/18:1 and 18:1p/20:1 in cerebellum (Table 5.24 to Table 5.29).

#### *5.3.2.1.1 Analysis of phosphatidylethanolamine in cortex*

The molecular species composition of PE from cortex of 12 and 16 month-old WT and Tg mice on the oil blend diet or on the DHA diet are presented in Table 5.24 and Table 5.25.

At 12 months of age, as well as 16 months of age, the percentages of most phospholipid species containing only the fatty acids 16:0, 18:0 or 18:1 were significantly higher in PE from cortex of DHA-fed mice than PE from the cortex of oil blend-fed mice ($p < 0.05$). The percentages of DHA, 22:5 or 20:3 containing phospholipid species mostly appeared significantly higher in the cortex of DHA-fed mice than of oil blend-fed mice, while the percentages of 20:4 and 22:4 containing phospholipid species mostly appeared significantly higher in the cortex of oil blend-fed mice than of DHA-fed mice.

At 12 months of age, the proportion of 18:1p/20:1 was significantly higher in cortex PE from WT mice ($F(1,16) = 5.006$, $p = 0.040$), while the proportion of 16:0p/22:4 was significantly higher in cortex PE from Tg mice ($F(1,16) = 4.991$, $p = 0.040$). At 16 months of age, the proportion of 18:0a/20:3 was significantly higher in cortex PE from Tg mice ($F(1,8) = 27.769$, $p = 0.001$), while the proportion of 16:0a/20:4 was significantly higher in cortex PE from WT mice ($F(1,8) = 13.000$, $p = 0.007$). A significant effect of gender was also observed at 12 months of age, on the percentages of the phospholipid species 18:1/20:1, 18:0a/20:3, 18:0a/20:4 and 16:0p/22:5 ($p < 0.05$) (data not shown).

In cortex, the molecular species of PE containing DHA represented 15.8% to 19.2% of total cortex PE species from oil blend-fed mice and 19.7% to 26.3% of total cortex PE species from DHA-fed mice, while the molecular species of PE containing arachidonic acid represented 23.6% to 27.3% of total cortex PE from oil blend-fed mice and 14.1% to 16.1% of total cortex PE from DHA-fed mice.

**Table 5.24.** Phosphatidylethanolamine molecular species composition of the cortex of WT and Tg mice on the oil blend diet or on the DHA diet, at 12 months of age. Results are represented as mean percentages of total molecular species analysed ± SEM.

| Molecular species | Tg oil (n = 6) | WT oil (n = 6) | Tg DHA (n = 6) | WT DHA (n = 6) |
|---|---|---|---|---|
| 16:0a/18:1 *** | 2.6 ± 0.1 | 2.7 ± 0.1 | 3.0 ± 0.1 | 3.1 ± 0.1 |
| 16:0p/18:1 * | 4.1 ± 0.1 | 3.9 ± 0.1 | 4.5 ± 0.3 | 4.5 ± 0.1 |
| 18:0a/18:0 *** | 2.2 ± 0.1 | 2.1 ± 0.1 | 2.9 ± 0.1 | 2.7 ± 0.1 |
| 18:0a/18:1 | 6.1 ± 0.2 | 5.7 ± 0.2 | 6.1 ± 0.3 | 5.9 ± 0.2 |
| 18:0p/18:1 *** | 7.4 ± 0.2 | 7.5 ± 0.2 | 8.5 ± 0.4 | 8.4 ± 0.2 |
| 18:1a/18:1 *** | 5.8 ± 0.1 | 5.7 ± 0.2 | 7.9 ± 0.3 | 8.6 ± 0.2 |
| 18:1p/18:1 *** | 14.7 ± 0.6 | 15.0 ± 0.5 | 17.6 ± 0.6 | 18.4 ± 0.6 |
| 18:0a/20:1 *** | 0.7 ± tr. | 0.7 ± 0.1 | 1.0 ± 0.1 | 1.0 ± tr. |
| 18:1p/20:1 ♦ | 4.9 ± 0.3 | 5.4 ± 0.3 | 5.2 ± 0.2 | 5.7 ± 0.2 |
| 18:1p/20:2 | 0.4 ± tr. | 0.4 ± tr. | 0.4 ± tr. | 0.4 ± tr. |
| 18:0a/20:3 *** | 0.6 ± tr. | 0.5 ± tr. | 2.8 ± 0.2 | 2.5 ± 0.1 |
| 16:0a/20:4 *** | 1.6 ± 0.1 | 1.7 ± 0.1 | 1.0 ± tr. | 1.0 ± 0.1 |
| 16:0p/20:4 *** | 2.4 ± 0.1 | 2.4 ± 0.1 | 1.4 ± 0.1 | 1.3 ± 0.1 |
| 18:0a/20:4 *** | 12.1 ± 0.5 | 11.9 ± 0.3 | 8.3 ± 0.3 | 7.7 ± 0.3 |
| 18:0p/20:4 *** | 5.6 ± 0.2 | 6.1 ± 0.2 | 3.1 ± 0.2 | 2.9 ± 0.1 |
| 18:0e/20:4 *** | 0.4 ± tr. | 0.4 ± tr. | 0.2 ± tr. | 0.2 ± tr. |
| 18:1a/20:4 *** | 1.5 ± tr. | 1.5 ± 0.1 | 0.8 ± tr. | 0.8 ± tr. |
| 16:0p/22:4 *** ♦ | 3.2 ± 0.1 | 2.9 ± 0.1 | 1.2 ± 0.1 | 1.0 ± 0.1 |
| 18:0a/22:4 *** | 2.8 ± 0.1 | 2.8 ± 0.1 | 1.1 ± tr. | 0.9 ± 0.1 |
| 18:0p/22:4 or 18:1e/22:4 *** | 4.0 ± 0.1 | 3.9 ± 0.2 | 1.6 ± 0.1 | 1.5 ± 0.1 |
| 16:0a/22:5 | 0.5 ± tr. | 0.5 ± tr. | 0.5 ± tr. | 0.5 ± tr. |
| 16:0p/22:5 *** | 0.2 ± tr. | 0.2 ± tr. | 0.4 ± tr. | 0.4 ± tr. |
| 18:0p/22:5 *** | 0.2 ± tr. | 0.2 ± tr. | 0.7 ± 0.1 | 0.7 ± 0.1 |
| 16:0a/22:6 * | 1.8 ± 0.1 | 2.0 ± 0.1 | 2.2 ± 0.2 | 2.3 ± 0.1 |
| 16:0p/22:6 ** | 2.1 ± 0.1 | 2.0 ± 0.1 | 2.8 ± 0.1 | 2.5 ± 0.2 |
| 18:0a/22:6 ** | 6.3 ± 0.3 | 6.0 ± 0.3 | 7.1 ± 0.1 | 7.4 ± 0.2 |
| 18:0p/22:6 *** | 3.7 ± 0.1 | 4.1 ± 0.2 | 5.0 ± 0.2 | 5.2 ± 0.3 |
| 18:1a/22:6 | 0.5 ± tr. | 0.5 ± tr. | 0.6 ± tr. | 0.6 ± tr. |
| 18:1p/22:6 or 18:2e/22:6 *** | 1.4 ± 0.1 | 1.4 ± 0.1 | 2.0 ± 0.1 | 2.0 ± 0.1 |

tr., trace (less than 0.05); significant effect of diet, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; significant effect of genotype, ♦ $p < 0.05$.

**Table 5.25.** Phosphatidylethanolamine molecular species composition of the cortex of WT and Tg mice on the oil blend diet or on the DHA diet, at 16 months of age. Results are represented as mean percentages of total molecular species analysed ± SEM.

| Molecular species | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
|---|---|---|---|---|
| 16:0a/18:1 *** | 3.3 ± 0.1 | 3.5 ± tr. | 4.2 ± 0.3 | 4.4 ± 0.1 |
| 16:0p/18:1 | 3.4 ± 0.2 | 3.0 ± 0.2 | 4.0 ± 0.4 | 3.4 ± 0.2 |
| 18:0a/18:0 ** | 2.0 ± tr. | 2.0 ± 0.1 | 2.7 ± 0.2 | 2.6 ± 0.1 |
| 18:0a/18:1 ** | 5.2 ± 0.1 | 5.2 ± 0.2 | 5.6 ± 0.1 | 5.7 ± 0.1 |
| 18:0p/18:1 ** | 4.9 ± 0.2 | 4.5 ± 0.3 | 6.4 ± 0.4 | 5.7 ± 0.3 |
| 18:1a/18:1 *** | 6.2 ± tr. | 6.4 ± 0.1 | 9.0 ± 0.5 | 9.2 ± 0.2 |
| 18:1p/18:1 * | 12.6 ± 0.6 | 12.5 ± 1.8 | 14.6 ± 0.8 | 16.2 ± 0.5 |
| 18:0a/20:1 | 1.0 ± tr. | 1.1 ± tr. | 1.5 ± tr. | 1.5 ± tr. |
| 18:1p/20:1 | 4.0 ± 0.1 | 4.0 ± 0.7 | 4.2 ± 0.4 | 4.3 ± 0.2 |
| 18:1p/20:2 | 0.4 ± tr. | 0.3 ± 0.1 | 0.5 ± 0.2 | 0.3 ± tr. |
| 18:0a/20:3 *** •• | 0.4 ± tr. | 0.3 ± tr. | 2.3 ± 0.1 | 1.7 ± 0.1 |
| 16:0a/20:4 *** •• | 1.7 ± tr. | 1.8 ± tr. | 0.8 ± 0.1 | 1.1 ± tr. |
| 16:0p/20:4 ** | 1.7 ± tr. | 1.5 ± 0.2 | 0.8 ± 0.1 | 1.0 ± 0.1 |
| 18:0a/20:4 *** | 16.2 ± 0.3 | 16.7 ± 0.4 | 8.8 ± 0.4 | 10.1 ± 0.4 |
| 18:0p/20:4 *** | 5.3 ± 0.1 | 5.5 ± 0.5 | 2.6 ± 0.3 | 2.9 ± 0.1 |
| 18:0e/20:4 | 0.3 ± tr. | 0.3 ± 0.1 | 0.2 ± tr. | 0.1 ± tr. |
| 18:1a/20:4 *** | 1.6 ± tr. | 1.5 ± tr. | 0.6 ± tr. | 0.7 ± tr. |
| 16:0p/22:4 *** | 3.3 ± 0.1 | 3.1 ± 0.3 | 0.9 ± 0.1 | 1.1 ± tr. |
| 18:0a/22:4 *** | 3.1 ± 0.1 | 2.9 ± 0.1 | 0.9 ± tr. | 1.1 ± 0.1 |
| 18:0p/22:4 or *** 18:1e/22:4 | 3.7 ± 0.1 | 3.6 ± tr. | 1.2 ± 0.1 | 1.3 ± 0.1 |
| 16:0a/22:5 | 0.7 ± tr. | 0.7 ± tr. | 0.8 ± 0.1 | 0.8 ± tr. |
| 16:0p/22:5 ** | 0.1 ± tr. | 0.1 ± tr. | 0.3 ± 0.1 | 0.2 ± tr. |
| 18:0p/22:5 ** | 0.1 ± tr. | 0.1 ± tr. | 0.7 ± 0.2 | 0.4 ± tr. |
| 16:0a/22:6 *** | 2.3 ± 0.1 | 2.4 ± 0.2 | 3.1 ± 0.2 | 3.1 ± tr. |
| 16:0p/22:6 *** | 2.1 ± tr. | 2.2 ± 0.1 | 3.1 ± tr. | 2.7 ± 0.1 |
| 18:0a/22:6 ** | 7.4 ± 0.4 | 7.5 ± 0.9 | 10.3 ± 0.5 | 9.4 ± 0.1 |
| 18:0p/22:6 *** | 4.6 ± 0.2 | 4.8 ± 0.2 | 6.7 ± 0.2 | 5.9 ± 0.1 |
| 18:1a/22:6 ** | 0.7 ± tr. | 0.7 ± 0.1 | 1.0 ± tr. | 1.0 ± tr. |
| 18:1p/22:6 or *** 18:2e/22:6 | 1.4 ± tr. | 1.6 ± tr. | 2.1 ± tr. | 2.1 ± 0.1 |

tr., trace (less than 0.05); significant effect of diet, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; significant effect of genotype, •• $p < 0.01$.

### *5.3.2.1.2 Analysis of phosphatidylethanolamine in hippocampus*

The molecular species compositions of PE from hippocampus of 12 and 16 month-old WT and Tg mice on the oil blend diet or on the DHA diet are presented in Table 5.26 and Table 5.27.

At 12 months of age, as well as 16 months of age and as previously observed in cortex, the percentages of most phospholipid species containing only the fatty acids 16:0, 18:0 or 18:1 were significantly higher in PE from hippocampus of DHA-fed mice than PE from hippocampus of oil blend-fed mice ($p < 0.05$). The percentages of DHA, 22:5 or 20:3 containing phospholipid species mostly appeared significantly higher in the hippocampus of DHA-fed mice than of oil blend-fed mice ($p < 0.05$), while the percentages of 20:4 and 22:4 containing phospholipid species mostly appeared significantly higher in the hippocampus of oil blend-fed mice than of DHA-fed mice ($p < 0.05$).

At 12 months of age, the proportion of 18:1a/18:1 was significantly higher in hippocampus PE from WT mice ($F(1,16) = 6.867$, $p = 0.019$), while the proportion of 18:0a/20:3 was significantly higher in hippocampus PE from Tg mice ($F(1,16) = 7.603$, $p = 0.014$). At 16 months of age, the proportions of 18:1a/18:1 and 18:0p/22:6 were significantly higher in hippocampus PE from WT mice (respectively, $F(1,8) = 7.143$, $p = 0.028$, and $F(1,8) = 7.606$, $p = 0.025$), while the proportion of 18:0a/18:1 was significantly higher in hippocampus PE from Tg mice ($F(1,8) = 5.885$, $p = 0.041$). A significant effect of gender was also observed at 12 months of age, on the percentage of the phospholipid species 16:0a/22:5 ($p < 0.05$) (data not shown).

In hippocampus, the molecular species of PE containing DHA represented 13.6% to 17.9% of total hippocampus PE species from oil blend-fed mice and 16.7% to 23.2% of total hippocampus PE species from DHA-fed mice, while the molecular species of PE containing arachidonic acid represented 24.1% to 28.4% of total hippocampus PE from oil blend-fed mice and 15.6% to 19.5% of total hippocampus PE from DHA-fed mice.

**Table 5.26.** Phosphatidylethanolamine molecular species composition of the hippocampus of WT and Tg mice on the oil blend diet or on the DHA diet, at 12 months of age. Results are represented as mean percentages of total molecular species analysed ± SEM.

| Molecular species | Tg oil (n = 6) | WT oil (n = 6) | Tg DHA (n = 6) | WT DHA (n = 6) |
|---|---|---|---|---|
| 16:0a/18:1 *** | 2.7 ± 0.1 | 2.5 ± 0.1 | 3.1 ± 0.2 | 3.3 ± 0.1 |
| 16:0p/18:1 | 4.3 ± 0.2 | 4.3 ± 0.2 | 4.5 ± 0.4 | 4.8 ± 0.2 |
| 18:0a/18:0 ** | 2.1 ± tr. | 2.0 ± 0.1 | 2.6 ± 0.1 | 2.4 ± 0.1 |
| 18:0a/18:1 | 6.8 ± 0.1 | 6.6 ± 0.2 | 6.7 ± 0.2 | 6.5 ± 0.1 |
| 18:0p/18:1 ** | 6.7 ± 0.1 | 6.7 ± 0.1 | 7.6 ± 0.4 | 7.7 ± 0.3 |
| 18:1a/18:1 *** * | 4.8 ± 0.1 | 4.9 ± 0.1 | 7.1 ± 0.3 | 8.0 ± 0.3 |
| 18:1p/18:1 *** | 16.1 ± 0.7 | 16.9 ± 0.8 | 19.7 ± 0.8 | 20.6 ± 0.7 |
| 18:0a/20:1 *** | 0.6 ± tr. | 0.7 ± tr. | 1.0 ± tr. | 0.9 ± tr. |
| 18:1p/20:1 | 5.2 ± 0.4 | 5.6 ± 0.4 | 5.2 ± 0.4 | 6.1 ± 0.2 |
| 18:1p/20:2 | 0.5 ± tr. | 0.5 ± tr. | 0.5 ± 0.1 | 0.5 ± tr. |
| 18:0a/20:3 *** * | 0.5 ± tr. | 0.5 ± tr. | 2.0 ± 0.1 | 1.7 ± 0.1 |
| 16:0a/20:4 ** | 1.9 ± 0.1 | 1.7 ± 0.2 | 1.3 ± 0.1 | 1.1 ± 0.1 |
| 16:0p/20:4 *** | 1.7 ± 0.1 | 1.6 ± 0.1 | 1.0 ± 0.1 | 1.0 ± 0.1 |
| 18:0a/20:4 *** | 15.0 ± 0.4 | 13.6 ± 0.8 | 10.4 ± 0.7 | 9.5 ± 0.4 |
| 18:0p/20:4 *** | 4.7 ± 0.2 | 5.0 ± 0.2 | 2.5 ± 0.1 | 2.6 ± 0.2 |
| 18:0e/20:4 *** | 0.4 ± tr. | 0.4 ± tr. | 0.2 ± tr. | 0.2 ± tr. |
| 18:1a/20:4 *** | 1.7 ± 0.1 | 1.7 ± 0.1 | 1.0 ± 0.1 | 0.9 ± tr. |
| 16:0p/22:4 *** | 3.3 ± 0.1 | 3.1 ± 0.1 | 1.3 ± 0.1 | 1.2 ± 0.1 |
| 18:0a/22:4 *** | 2.5 ± 0.1 | 2.6 ± 0.1 | 1.1 ± tr. | 1.0 ± 0.1 |
| 18:0p/22:4 or 18:1e/22:4 *** | 4.2 ± 0.1 | 4.3 ± 0.2 | 1.7 ± 0.1 | 1.6 ± 0.1 |
| 16:0a/22:5 | 0.4 ± tr. | 0.4 ± tr. | 0.4 ± tr. | 0.4 ± tr. |
| 16:0p/22:5 *** | 0.2 ± tr. | 0.2 ± tr. | 0.4 ± tr. | 0.4 ± tr. |
| 18:0p/22:5 *** | 0.2 ± tr. | 0.2 ± tr. | 0.7 ± 0.1 | 0.7 ± 0.1 |
| 16:0a/22:6 | 1.5 ± 0.1 | 1.6 ± 0.2 | 1.8 ± 0.1 | 1.7 ± 0.1 |
| 16:0p/22:6 *** | 2.0 ± tr. | 1.9 ± 0.1 | 2.6 ± 0.1 | 2.3 ± 0.1 |
| 18:0a/22:6 ** | 4.7 ± 0.2 | 5.0 ± 0.4 | 6.2 ± 0.2 | 5.5 ± 0.2 |
| 18:0p/22:6 *** | 3.6 ± 0.1 | 3.9 ± 0.2 | 4.8 ± 0.2 | 4.8 ± 0.2 |
| 18:1a/22:6 ** | 0.4 ± tr. | 0.5 ± tr. | 0.5 ± tr. | 0.5 ± tr. |
| 18:1p/22:6 or 18:2e/22:6 *** | 1.4 ± tr. | 1.4 ± 0.1 | 2.0 ± 0.1 | 1.9 ± 0.1 |

tr., trace (less than 0.05); significant effect of diet, ** $p < 0.01$, *** $p < 0.001$; significant effect of genotype, * $p < 0.05$.

**Table 5.27.** Phosphatidylethanolamine molecular species composition of the hippocampus of WT and Tg mice on the oil blend diet or on the DHA diet, at 16 months of age. Results are represented as mean percentages of total molecular species analysed ± SEM.

| Molecular species | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
|---|---|---|---|---|
| 16:0a/18:1 ** | 3.3 ± 0.1 | 3.5 ± tr. | 4.2 ± 0.2 | 4.2 ± 0.2 |
| 16:0p/18:1 | 3.3 ± 0.1 | 3.1 ± 0.1 | 3.6 ± 0.3 | 3.5 ± 0.3 |
| 18:0a/18:0 ** | 1.9 ± tr. | 2.0 ± 0.1 | 2.3 ± 0.1 | 2.4 ± 0.1 |
| 18:0a/18:1 ♦ | 5.9 ± 0.2 | 5.4 ± 0.3 | 6.1 ± 0.1 | 5.7 ± 0.2 |
| 18:0p/18:1 | 4.5 ± 0.2 | 4.4 ± 0.5 | 5.6 ± 0.4 | 4.6 ± 0.3 |
| 18:1a/18:1 *** ♦ | 5.2 ± 0.1 | 5.8 ± 0.5 | 7.7 ± tr. | 8.8 ± 0.4 |
| 18:1p/18:1 ** | 14.9 ± 0.6 | 13.5 ± 0.3 | 16.8 ± 1.2 | 17.0 ± 0.6 |
| 18:0a/20:1 *** | 1.1 ± tr. | 1.1 ± 0.1 | 1.5 ± 0.1 | 1.5 ± tr. |
| 18:1p/20:1 | 4.3 ± 0.1 | 4.1 ± 0.2 | 4.2 ± 0.3 | 4.1 ± 0.2 |
| 18:1p/20:2 | 0.3 ± 0.1 | 0.3 ± tr. | 0.4 ± 0.1 | 0.4 ± tr. |
| 18:0a/20:3 *** | 0.3 ± tr. | 0.4 ± 0.1 | 1.6 ± 0.1 | 1.4 ± 0.1 |
| 16:0a/20:4 *** | 1.7 ± 0.1 | 1.8 ± 0.1 | 1.1 ± 0.1 | 1.2 ± tr. |
| 16:0p/20:4 ** | 1.2 ± 0.1 | 1.4 ± 0.1 | 0.7 ± 0.2 | 0.8 ± 0.1 |
| 18:0a/20:4 ** | 19.3 ± 0.4 | 17.9 ± 1.7 | 12.9 ± 0.5 | 13.8 ± 0.6 |
| 18:0p/20:4 *** | 4.5 ± 0.2 | 5.0 ± 0.4 | 2.3 ± 0.1 | 2.5 ± 0.1 |
| 18:0e/20:4 ** | 0.3 ± 0.1 | 0.4 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 18:1a/20:4 *** | 1.4 ± tr. | 1.5 ± 0.1 | 0.7 ± tr. | 0.8 ± tr. |
| 16:0p/22:4 *** | 3.4 ± 0.1 | 3.1 ± 0.2 | 1.1 ± 0.1 | 1.1 ± 0.1 |
| 18:0a/22:4 *** | 2.9 ± 0.1 | 2.9 ± tr. | 1.0 ± tr. | 0.9 ± tr. |
| 18:0p/22:4 or 18:1e/22:4 *** | 4.0 ± 0.1 | 3.7 ± 0.1 | 1.3 ± 0.1 | 1.4 ± tr. |
| 16:0a/22:5a * | 0.6 ± 0.1 | 0.6 ± 0.1 | 0.7 ± tr. | 0.8 ± tr. |
| 16:0p/22:5 ** | 0.1 ± tr. | 0.1 ± tr. | 0.3 ± tr. | 0.4 ± 0.1 |
| 18:0p/22:5 *** | 0.1 ± tr. | 0.1 ± tr. | 0.5 ± tr. | 0.4 ± 0.1 |
| 16:0a/22:6 ** | 1.8 ± tr. | 2.1 ± 0.2 | 2.6 ± 0.1 | 2.5 ± 0.1 |
| 16:0p/22:6 ** | 1.9 ± 0.1 | 2.0 ± 0.1 | 2.6 ± 0.1 | 2.4 ± 0.1 |
| 18:0a/22:6 ** | 5.9 ± 0.1 | 6.7 ± 0.5 | 9.0 ± 0.8 | 8.3 ± 0.1 |
| 18:0p/22:6 *** ♦ | 3.9 ± 0.1 | 4.9 ± 0.2 | 6.1 ± 0.3 | 6.1 ± 0.2 |
| 18:1a/22:6 ** | 0.6 ± tr. | 0.7 ± tr. | 0.9 ± 0.1 | 0.9 ± tr. |
| 18:1p/22:6 or 18:2e/22:6 *** | 1.5 ± tr. | 1.5 ± 0.1 | 2.0 ± 0.1 | 2.1 ± 0.1 |

tr., trace (less than 0.05); significant effect of diet, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; significant effect of genotype, ♦ $p < 0.05$.

### *5.3.2.1.3 Analysis of phosphatidylethanolamine in cerebellum*

The molecular species compositions of PE from cerebellum of 12 and 16 month-old WT and Tg mice on the oil blend diet or on the DHA diet are presented in Table 5.28 and Table 5.29.

At 12 months of age, as well as 16 months of age and as previously observed in cortex and hippocampus, the percentages of most molecular species containing only the fatty acids 16:0, 18:0 or 18:1 were significantly higher in PE from cerebellum of DHA-fed mice than PE from cerebellum of oil blend-fed mice ($p < 0.05$). The percentages of DHA, 22:5 or 20:3 containing phospholipid species mostly appeared significantly higher in the cerebellum of DHA-fed mice than of oil blend-fed mice ($p < 0.05$), while the percentages of 20:4 and 22:4 containing phospholipid species mostly appeared significantly higher in the cerebellum of oil blend-fed mice than of DHA-fed mice ($p < 0.05$).

At 12 months of age, the proportion of 18:1p/20:1 was significantly higher in cerebellum PE from WT mice ($F(1,16) = 10.244$, $p = 0.006$), while the proportion of 18:0a/20:4 was significantly higher in cerebellum PE from Tg mice ($F(1,16) = 8.800$, $p = 0.009$). At 16 months of age, the proportions of 18:0p/18;1 and 16:0p/22:4 were significantly higher in cerebellum PE from Tg mice (respectively, $F(1,8) = 21.491$, $p = 0.002$, and $F(1,8) = 9.000$, $p = 0.017$), while the proportions of 18:1a/22:6 and 18:1p/22:6 or 18:2e/22:6 were significantly higher in cerebellum PE from WT mice (respectively, $F(1,8) = 6.250$, $p = 0.037$, and $F(1,8) = 9.000$, $p = 0.017$). No significant effect of gender was observed at 12 months of age ($p > 0.05$) (data not shown).

In cerebellum, the molecular species of PE containing DHA represented 9.4% to 10.8% of total cerebellum PE species from oil blend-fed mice and 10.4% to 15.2% of total cerebellum PE species from DHA-fed mice, while the molecular species of PE containing arachidonic acid represented 11.4% to 13.3% of total cerebellum PE from oil blend-fed mice and 4.6% to 5.2% of total cerebellum PE from DHA-fed mice.

**Table 5.28**. Phosphatidylethanolamine molecular species composition of the cerebellum of WT and Tg mice on the oil blend diet or on the DHA diet, at 12 months of age. Results are represented as mean percentages of total molecular species analysed ± SEM.

| Molecular species | Tg oil (n = 6) | WT oil (n = 6) | Tg DHA (n = 6) | WT DHA (n = 6) |
|---|---|---|---|---|
| 16:0a/18:1 *** | 2.5 ± 0.1 | 2.3 ± tr. | 2.9 ± 0.1 | 2.9 ± 0.1 |
| 16:0p/18:1 *** | 9.3 ± 0.2 | 9.1 ± 0.2 | 10.9 ± 0.5 | 10.7 ± 0.3 |
| 18:0a/18:0 *** | 0.9 ± tr. | 0.9 ± 0.1 | 1.2 ± 0.1 | 1.1 ± 0.1 |
| 18:0a/18:1 * | 5.6 ± 0.1 | 5.5 ± 0.1 | 5.3 ± 0.2 | 5.2 ± 0.1 |
| 18:0p/18:1 | 13.1 ± 0.2 | 13.2 ± 0.2 | 13.6 ± 0.3 | 13.4 ± 0.1 |
| 18:1a/18:1 *** | 7.6 ± 0.1 | 7.3 ± 0.2 | 10.2 ± 0.3 | 10.0 ± 0.3 |
| 18:1p/18:1 *** | 24.7 ± 0.3 | 25.6 ± 0.6 | 28.1 ± 0.5 | 28.7 ± 0.6 |
| 18:0a/20:1 *** | 0.3 ± tr. | 0.3 ± tr. | 0.4 ± tr. | 0.4 ± tr. |
| 18:1p/20:1 * ** | 10.1 ± 0.2 | 10.4 ± 0.3 | 9.1 ± 0.2 | 10.3 ± 0.3 |
| 18:1p/20:2 *** | 0.5 ± tr. | 0.5 ± tr. | 0.3 ± tr. | 0.3 ± tr. |
| 18:0a/20:3 *** | 0.3 ± tr. | 0.3 ± tr. | 0.7 ± 0.1 | 0.6 ± tr. |
| 16:0a/20:4 *** | 0.7 ± 0.1 | 0.6 ± 0.1 | 0.4 ± tr. | 0.4 ± tr. |
| 16:0p/20:4 *** | 1.0 ± tr. | 0.9 ± tr. | 0.4 ± tr. | 0.4 ± tr. |
| 18:0a/20:4 *** ** | 5.0 ± 0.1 | 4.6 ± 0.1 | 2.2 ± 0.1 | 1.9 ± 0.1 |
| 18:0p/20:4 *** | 3.5 ± 0.1 | 3.4 ± 0.1 | 1.2 ± tr. | 1.0 ± 0.1 |
| 18:0e/20:4 *** | 0.3 ± tr. | 0.3 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 18:1a/20:4 *** | 1.4 ± 0.1 | 1.4 ± tr. | 0.6 ± tr. | 0.6 ± tr. |
| 16:0p/22:4 *** | 0.9 ± tr. | 0.9 ± tr. | 0.3 ± tr. | 0.3 ± tr. |
| 18:0a/22:4 *** | 0.7 ± tr. | 0.7 ± tr. | 0.2 ± tr. | 0.2 ± tr. |
| 18:0p/22:4 or *** 18:1e/22:4 | 1.7 ± 0.1 | 1.8 ± 0.1 | 0.5 ± tr. | 0.5 ± tr. |
| 16:0a/22:5 | 0.2 ± tr. | 0.2 ± tr. | 0.3 ± tr. | 0.2 ± tr. |
| 16:0p/22:5 * | 0.1 ± tr. | 0.1 ± tr. | 0.2 ± tr. | 0.2 ± tr. |
| 18:0p/22:5 *** | 0.1 ± tr. | 0.1 ± tr. | 0.3 ± tr. | 0.4 ± tr. |
| 16:0a/22:6 | 0.9 ± 0.1 | 0.9 ± 0.1 | 1.0 ± 0.1 | 1.0 ± 0.1 |
| 16:0p/22:6 ** | 0.9 ± tr. | 0.8 ± tr. | 1.2 ± 0.1 | 1.0 ± 0.1 |
| 18:0a/22:6 | 3.9 ± 0.2 | 3.9 ± 0.3 | 4.2 ± 0.2 | 4.2 ± 0.3 |
| 18:0p/22:6 | 2.8 ± 0.1 | 2.9 ± 0.1 | 3.1 ± 0.1 | 3.0 ± 0.1 |
| 18:1a/22:6 | 0.4 ± tr. | 0.4 ± tr. | 0.4 ± tr. | 0.5 ± tr. |
| 18:1p/22:6 or *** 18:2e/22:6 | 0.5 ± tr. | 0.5 ± tr. | 0.8 ± tr. | 0.7 ± tr. |

tr., trace (less than 0.05); significant effect of diet, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; significant effect of genotype, ** $p < 0.01$.

**Table 5.29.** Phosphatidylethanolamine molecular species composition of the cerebellum of WT and Tg mice on the oil blend diet or on the DHA diet, at 16 months of age. Results are represented as mean percentages of total molecular species analysed ± SEM.

| Molecular species | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
|---|---|---|---|---|
| 16:0a/18:1 *** | 3.2 ± 0.1 | 3.6 ± 0.1 | 4.5 ± 0.1 | 4.2 ± 0.3 |
| 16:0p/18:1 * | 9.1 ± 0.4 | 7.6 ± 0.5 | 9.8 ± tr. | 9.4 ± 0.7 |
| 18:0a/18:0 ** | 0.8 ± 0.1 | 0.9 ± 0.1 | 1.2 ± tr. | 1.2 ± tr. |
| 18:0a/18:1 * | 5.5 ± 0.1 | 5.2 ± 0.1 | 5.0 ± 0.1 | 4.8 ± 0.2 |
| 18:0p/18:1 ** ♦♦ | 10.5 ± 0.2 | 9.6 ± 0.3 | 11.5 ± 0.1 | 10.3 ± 0.3 |
| 18:1a/18:1 *** | 8.3 ± 0.3 | 8.8 ± 0.1 | 11.4 ± 0.1 | 11.7 ± 0.4 |
| 18:1p/18:1 * | 23.8 ± 1.2 | 24.0 ± 0.3 | 24.8 ± 0.3 | 27.6 ± 1.1 |
| 18:0a/20:1 ** | 0.4 ± tr. | 0.4 ± tr. | 0.6 ± tr. | 0.6 ± tr. |
| 18:1p/20:1 | 10.4 ± 0.5 | 9.3 ± 0.5 | 8.6 ± 0.2 | 8.9 ± 0.4 |
| 18:1p/20:2 ** | 0.5 ± tr. | 0.4 ± tr. | 0.3 ± tr. | 0.3 ± tr. |
| 18:0a/20:3 *** | 0.2 ± tr. | 0.2 ± tr. | 0.5 ± tr. | 0.5 ± 0.1 |
| 16:0a/20:4 *** | 0.7 ± tr. | 0.8 ± 0.1 | 0.4 ± 0.1 | 0.4 ± tr. |
| 16:0p/20:4 *** | 0.7 ± tr. | 0.8 ± 0.1 | 0.3 ± 0.1 | 0.3 ± tr. |
| 18:0a/20:4 *** | 6.0 ± 0.7 | 6.6 ± 0.3 | 2.4 ± 0.1 | 2.6 ± 0.2 |
| 18:0p/20:4 *** | 3.3 ± 0.2 | 3.4 ± 0.2 | 1.1 ± 0.2 | 1.1 ± tr. |
| 18:0e/20:4 * | 0.2 ± 0.1 | 0.2 ± 0.1 | N.D. | 0.1 ± tr. |
| 18:1a/20:4 *** | 1.3 ± 0.1 | 1.3 ± tr. | 0.5 ± tr. | 0.5 ± tr. |
| 16:0p/22:4 *** ♦ | 1.1 ± 0.1 | 0.9 ± tr. | 0.3 ± tr. | 0.2 ± tr. |
| 18:0a/22:4 *** | 0.9 ± 0.1 | 0.8 ± tr. | 0.2 ± tr. | 0.2 ± tr. |
| 18:0p/22:4 or 18:1e/22:4 *** | 1.8 ± 0.1 | 1.7 ± tr. | 0.5 ± tr. | 0.4 ± tr. |
| 16:0a/22:5 | 0.4 ± tr. | 0.4 ± tr. | 0.4 ± tr. | 0.4 ± tr. |
| 16:0p/22:5 * | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 18:0p/22:5 ** | 0.1 ± tr. | 0.1 ± tr. | 0.4 ± 0.1 | 0.2 ± tr. |
| 16:0a/22:6 * | 1.1 ± 0.1 | 1.4 ± 0.1 | 1.6 ± tr. | 1.5 ± 0.1 |
| 16:0p/22:6 *** | 0.8 ± 0.1 | 0.9 ± tr. | 1.2 ± tr. | 1.2 ± tr. |
| 18:0a/22:6 * | 5.0 ± 0.4 | 5.7 ± 0.1 | 6.8 ± 0.3 | 6.0 ± 0.4 |
| 18:0p/22:6 * | 2.8 ± 0.3 | 3.6 ± tr. | 4.0 ± 0.2 | 3.9 ± 0.3 |
| 18:1a/22:6 ** ♦ | 0.6 ± tr. | 0.7 ± tr. | 0.8 ± tr. | 0.7 ± tr. |
| 18:1p/22:6 or 18:2e/22:6 *** ♦ | 0.5 ± tr. | 0.6 ± tr. | 0.8 ± tr. | 0.8 ± tr. |

tr., trace (less than 0.05); significant effect of diet, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; significant effect of genotype, ♦ $p < 0.05$, ♦♦ $p < 0.01$.

### 5.3.2.2 Analysis of phosphatidylcholine

Positive-ion ESI-MS-MS analysis of PC in cortex, hippocampus and cerebellum of 12 and 16 month-old WT and Tg mice on the oil blend diet or on the DHA diet demonstrated predominant peaks corresponding to its most abundant molecular species 16:0a/16:0 (at 12 months of age), 16:0a/18:1. 18:0a/18:1, 18:0a/20:4, 18:1a/20:4 and 16:0a/22:6 (Table 5.30 to Table 5.35).

#### *5.3.2.2.1 Analysis of phosphatidylcholine in cortex*

The molecular species compositions of PC from cortex of 12 and 16 month-old WT and Tg mice on the oil blend diet or on the DHA diet are presented in Table 5.30 and Table 5.31.

At 12 months of age, as well as 16 months of age, the percentages of most phospholipid species containing only the fatty acids 16:0, 16:1, 18:0, 18:1 or 18:2 did not vary significantly with the diet ($p > 0.05$) but significant changes were observed with genotype ($p < 0.05$). At 12 months of age, the proportions of 16:0a/16:1 and 16:0a/18:2 were significantly higher in cortex PC from Tg mice (respectively, $F(1,16) = 9.207$, $p = 0.008$, and $F(1,16) = 10.782$, $p = 0.005$), while the proportion of 18:0a/18:1 was significantly higher in cortex PC from WT mice ($F(1,16) = 4.930$, $p = 0.041$). At 16 months of age, the proportion of 16:0a/18:2 was significantly higher in cortex PC from Tg mice ($F(1,8) = 11.256$, $p = 0.010$). A significant effect of gender was also observed at 12 months of age ($p < 0.05$), on the percentages of the phospholipid species 18:0a/18:1 and 18:0a/18:0 or 16:0p/22:6 (data not shown). The percentages of DHA or 20:3 containing phospholipid species mostly appeared significantly higher in the cortex of DHA-fed mice than of oil blend-fed mice ($p < 0.05$), while the percentage of 20:4 containing phospholipid species mostly appeared significantly higher in the cortex of oil blend-fed mice than of DHA-fed mice ($p < 0.05$).

In cortex, at 12 months of age, the molecular species of PC containing DHA represented 7.9% of total cortex PC of Tg mice on the oil blend diet, 8.7% of total cortex PC of WT mice on the oil blend diet, 11.2% of total cortex PC of Tg mice on the DHA diet, and 10.6% of total cortex PC of WT mice on the DHA diet. The molecular species of PC containing arachidonic acid represented 14.0% of total

cortex PC of Tg mice on the oil blend diet, 14.1% of total cortex PC of WT mice on the oil blend diet, 9.1% of total cortex PC of Tg mice on the DHA diet, and 8.2% of total cortex PC of WT mice on the DHA diet. At 16 months of age, the molecular species of PC containing DHA represented 17.1% of total cortex PC of Tg mice on the oil blend diet, 17.4% of total cortex PC of WT mice on the oil blend diet, 27.4% of total cortex PC of Tg mice on the DHA diet, and 24.2% of total cortex PC of WT mice on the DHA diet. The molecular species of PC containing arachidonic acid represented 25.6% of total cortex PC of Tg mice on the oil blend diet, 25.5% of total cortex PC of WT mice on the oil blend diet, 13.1% of total cortex PC of Tg mice on the DHA diet, and 16.7% of total cortex PC of WT mice the on DHA diet.

**Table 5.30.** Phosphatidylcholine molecular species composition of the cortex of WT and Tg mice on the oil blend diet or on the DHA diet, at 12 months of age. Results are represented as mean percentages of total molecular species analysed ± SEM.

| Molecular species | Tg oil (n = 6) | WT oil (n = 6) | Tg DHA (n = 6) | WT DHA (n = 6) |
|---|---|---|---|---|
| 16:0a/16:0 | 18.2 ± 0.6 | 18.4 ± 0.1 | 18.1 ± 0.5 | 19.5 ± 0.4 |
| 16:0a/16:1 $^{\bullet\bullet}$ | 2.6 ± 0.1 | 1.9 ± 0.2 | 2.5 ± 0.2 | 2.2 ± 0.2 |
| 16:1a/16:1 | 1.7 ± 0.1 | 1.6 ± 0.1 | 1.6 ± 0.1 | 1.9 ± 0.1 |
| 16:0a/18:0 | 4.8 ± 0.2 | 4.6 ± 0.1 | 4.2 ± 0.2 | 4.6 ± 0.2 |
| 16:0a/18:1 | 34.3 ± 0.8 | 33.8 ± 0.9 | 35.8 ± 1.1 | 35.3 ± 0.9 |
| 18:0a/18:1 $^{\bullet}$ | 6.6 ± 0.3 | 7.2 ± 0.4 | 6.4 ± 0.3 | 6.9 ± 0.2 |
| 16:0a/18:2 * $^{\bullet\bullet}$ | 2.1 ± tr. | 1.8 ± tr. | 2.2 ± 0.1 | 2.1 ± 0.1 |
| 18:0a/18:2 | 2.9 ± 0.2 | 2.8 ± 0.1 | 3.0 ± 0.1 | 3.0 ± 0.1 |
| 16:0p/20:0 or 16:0e/20:1 | 0.7 ± tr. | 0.7 ± tr. | 0.6 ± tr. | 0.7 ± tr. |
| 16:0a/20:3 ** | 2.7 ± 0.1 | 2.7 ± 0.1 | 3.7 ± 0.3 | 3.4 ± 0.3 |
| 16:0a/20:4 *** | 7.1 ± 0.4 | 7.2 ± 0.4 | 4.4 ± 0.4 | 4.0 ± 0.3 |
| 18:0a/20:4 *** | 3.8 ± 0.2 | 3.9 ± 0.1 | 2.1 ± 0.1 | 1.9 ± 0.1 |
| 18:1a/20:4 ** | 3.1 ± 0.2 | 3.0 ± 0.1 | 2.6 ± 0.1 | 2.3 ± 0.1 |
| 18:0a/18:0 or 16:0p/22:6 | 1.6 ± 0.1 | 1.7 ± 0.1 | 1.6 ± 0.1 | 1.7 ± 0.1 |
| 16:0a/22:6 ** | 5.5 ± 0.5 | 6.0 ± 0.2 | 7.7 ± 0.4 | 7.4 ± 0.6 |
| 18:0a/22:6 *** | 1.0 ± tr. | 1.3 ± 0.1 | 1.5 ± 0.1 | 1.5 ± 0.1 |
| 18:1a/22:6 * | 1.4 ± 0.1 | 1.4 ± 0.1 | 2.0 ± 0.2 | 1.7 ± 0.1 |

tr., trace (less than 0.05); significant effect of diet, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; significant effect of genotype, $^{\bullet} p < 0.05$, $^{\bullet\bullet} p < 0.01$.

**Table 5.31.** Phosphatidylcholine molecular species composition of the cortex of WT and Tg mice on the oil blend diet or on the DHA diet, at 16 months of age. Results are represented as mean percentages of total molecular species analysed ± SEM.

| Molecular species | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
|---|---|---|---|---|
| 16:0a/16:0 | 5.0 ± 0.1 | 5.6 ± 0.1 | 6.8 ± 1.3 | 5.4 ± 0.1 |
| 16:0a/16:1 | 0.3 ± tr. | 0.2 ± 0.1 | 0.5 ± 0.1 | 0.4 ± 0.1 |
| 16:1a/16:1 | 0.1 ± tr. | 0.1 ± tr. | 0.2 ± tr. | 0.1 ± tr. |
| 16:0a/18:0 | 4.2 ± 0.1 | 3.4 ± 0.4 | 4.0 ± 0.2 | 3.8 ± 0.1 |
| 16:0a/18:1 | 19.4 ± 0.3 | 21.3 ± 1.2 | 19.8 ± 2.3 | 20.5 ± 0.5 |
| 18:0a/18:1 | 16.8 ± 0.3 | 16.6 ± 0.2 | 14.0 ± 2.0 | 15.6 ± 0.8 |
| 16:0a/18:2 ♦ | 1.9 ± tr. | 1.6 ± tr. | 2.1 ± 0.2 | 1.7 ± 0.1 |
| 18:0a/18:2 | 3.5 ± 0.1 | 3.3 ± 0.2 | 3.7 ± 0.1 | 3.8 ± 0.2 |
| 16:0p/20:0 or 16:0e/20:1 | 1.0 ± 0.1 | 0.8 ± tr. | 0.9 ± 0.1 | 1.0 ± tr. |
| 16:0a/20:3 *** | 1.5 ± 0.1 | 1.1 ± 0.1 | 4.7 ± 0.9 | 3.6 ± 0.5 |
| 16:0a/20:4 *** | 5.8 ± 0.2 | 6.4 ± 0.3 | 3.0 ± 0.1 | 3.1 ± 0.1 |
| 18:0a/20:4 *** | 8.9 ± 0.1 | 9.6 ± 0.6 | 3.9 ± 0.5 | 4.0 ± 0.5 |
| 18:1a/20:4 * | 10.9 ± 0.1 | 9.5 ± 1.5 | 6.2 ± 0.8 | 9.6 ± 0.3 |
| 18:0a/18:0 or 16:0p/22:6 | 3.5 ± 0.1 | 3.1 ± 0.1 | 2.9 ± 0.2 | 3.2 ± 0.2 |
| 16:0a/22:6 ** | 10.4 ± 0.6 | 10.3 ± 0.3 | 16.8 ± 2.0 | 14.6 ± 0.1 |
| 18:0a/22:6 ** | 3.5 ± 0.1 | 3.7 ± 0.1 | 5.2 ± 0.5 | 4.5 ± 0.1 |
| 18:1a/22:6 ** | 3.2 ± 0.1 | 3.4 ± 0.3 | 5.4 ± 1.0 | 5.1 ± 0.1 |

tr., trace (less than 0.05); significant effect of diet, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; significant effect of genotype, ♦ $p < 0.05$.

### *5.3.2.2.2 Analysis of phosphatidylcholine in hippocampus*

The molecular species compositions of PC from hippocampus of 12 and 16 month-old WT and Tg mice on the oil blend diet or on the DHA diet are presented in Table 5.32 and Table 5.33.

At 12 months of age, as well as 16 months of age, the percentages of some phospholipid species containing only the fatty acids 16:0, 16:1, 18:0, 18:1 or 18:2 varied significantly with the diet ($p < 0.05$) and some significant changes were also observed with genotype ($p < 0.05$). At 12 months of age, the proportion of 16:0a/18:2 was significantly higher in hippocampus PC from Tg mice ($F(1,16) = 5.841$, $p = 0.028$). At 16 months of age, the proportion of 16:0a/18:2 was significantly higher in hippocampus PC from Tg mice ($F(1,8) = 29.389$, $p = 0.001$), while the proportion of 16:0a/18:0 was significantly higher in hippocampus PC from WT mice ($F(1,8) = 5.885$, $p = 0.041$). No significant effect of gender was observed at 12 months of age ($p > 0.05$) (data not shown). The percentages of DHA or 20:3-containing phospholipid species mostly appeared significantly higher in the hippocampus of DHA-fed mice than of oil blend-fed mice ($p < 0.05$), while the percentages of 20:4

containing phospholipid species mostly appeared significantly higher in the hippocampus of oil blend-fed mice than of DHA-fed mice ($p < 0.05$).

In hippocampus, at 12 months of age, the molecular species of PC containing DHA represented 5.3% of total hippocampus PC of Tg mice on the oil blend diet, 6.3% of total hippocampus PC of WT mice on the oil blend diet, 7.6% of total hippocampus PC of Tg mice on the DHA diet, and 7.9% of total hippocampus PC of WT mice on the DHA diet. The molecular species of PC containing arachidonic acid represented 14.0% of total hippocampus PC of Tg mice on the oil blend diet, 13.9% of total hippocampus PC of WT mice on the oil blend diet, 9.1% of total hippocampus PC of Tg mice on the DHA diet, and 8.8% of total hippocampus PC of WT on the DHA diet. At 16 months of age, the molecular species of PC containing DHA represented 14.0% of total hippocampus PC of Tg mice on the oil blend diet, 15.7% of total hippocampus PC of WT mice on the oil blend diet, 23.3% of total hippocampus PC of Tg mice on the DHA diet, and 21.5% of total hippocampus PC of WT mice on the DHA diet. The molecular species of PC containing arachidonic acid represented 27.9% of total hippocampus PC of Tg mice on the oil blend diet, 25.8% of total hippocampus PC of WT mice on the oil blend diet, 14.4% of total hippocampus PC of Tg mice on the DHA diet, and 17.2% of total hippocampus PC of WT mice on the DHA diet.

**Table 5.32.** Phosphatidylcholine molecular species composition of the hippocampus of WT and Tg mice on the oil blend diet or on the DHA diet, at 12 months of age. Results are represented as mean percentages of total molecular species analysed ± SEM.

| Molecular species | Tg oil (n = 6) | WT oil (n = 6) | Tg DHA (n = 6) | WT DHA (n = 6) |
|---|---|---|---|---|
| 16:0a/16:0 | 19.5 ± 0.4 | 20.2 ± 0.5 | 19.8 ± 0.4 | 19.2 ± 0.5 |
| 16:0a/16:1 | 1.7 ± 0.1 | 2.0 ± 0.2 | 2.2 ± 0.1 | 1.7 ± 0.1 |
| 16:1a/16:1 | 2.3 ± 0.1 | 2.5 ± 0.2 | 2.3 ± 0.1 | 2.4 ± 0.1 |
| 16:0a/18:0 | 3.7 ± 0.1 | 3.6 ± 0.2 | 3.4 ± 0.1 | 3.6 ± 0.1 |
| 16:0a/18:1 ** | 38.2 ± 0.4 | 36.1 ± 0.7 | 39.1 ± 0.9 | 39.8 ± 0.4 |
| 18:0a/18:1 | 6.6 ± 0.2 | 6.9 ± 0.4 | 6.6 ± 0.2 | 6.9 ± 0.3 |
| 16:0a/18:2 ** $^{\bullet}$ | 1.6 ± tr. | 1.5 ± 0.1 | 2.0 ± 0.1 | 1.7 ± 0.1 |
| 18:0a/18:2 *** | 2.2 ± 0.1 | 2.3 ± 0.1 | 2.6 ± 0.1 | 2.6 ± 0.1 |
| 16:0p/20:0 or 16:0e/20:1 | 0.6 ± tr. | 0.6 ± tr. | 0.6 ± tr. | 0.6 ± tr. |
| 16:0a/20:3 ** | 2.6 ± tr. | 2.4 ± 0.1 | 3.1 ± 0.2 | 2.9 ± 0.2 |
| 16:0a/20:4 *** | 7.3 ± 0.4 | 7.1 ± 0.1 | 4.3 ± 0.3 | 4.2 ± 0.3 |
| 18:0a/20:4 *** | 3.6 ± 0.1 | 3.6 ± 0.2 | 2.2 ± 0.1 | 2.1 ± 0.1 |
| 18:1a/20:4 *** | 3.1 ± 0.1 | 3.2 ± 0.1 | 2.6 ± 0.1 | 2.5 ± 0.1 |
| 18:0a/18:0 or 16:0p/22:6 | 1.7 ± tr. | 1.7 ± 0.1 | 1.7 ± 0.1 | 1.8 ± 0.1 |
| 16:0a/22:6 *** | 3.9 ± 0.3 | 4.3 ± 0.3 | 5.3 ± 0.3 | 5.7 ± 0.4 |
| 18:0a/22:6 ** | 0.7 ± tr. | 0.9 ± 0.1 | 1.0 ± 0.1 | 1.0 ± 0.1 |
| 18:1a/22:6 ** | 0.7 ± 0.1 | 1.1 ± 0.1 | 1.3 ± 0.1 | 1.2 ± 0.1 |

tr., trace (less than 0.05); significant effect of diet, ** $p < 0.01$, *** $p < 0.001$; significant effect of genotype, $^{\bullet}p < 0.05$.

**Table 5.33.** Phosphatidylcholine molecular species composition of the hippocampus of WT and Tg mice on the oil blend diet or on the DHA diet, at 16 months of age. Results are represented as mean percentages of total molecular species analysed ± SEM.

| Molecular species | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
|---|---|---|---|---|
| 16:0a/16:0 | 4.9 ± 0.1 | 5.4 ± 0.4 | 4.9 ± 0.1 | 5.1 ± 0.2 |
| 16:0a/16:1 | 0.4 ± 0.1 | 0.4 ± 0.1 | 0.6 ± tr. | 0.3 ± 0.1 |
| 16:1a/16:1 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.2 ± tr. |
| 16:0a/18:0 $^{\bullet}$ | 2.5 ± 0.2 | 3.5 ± 0.3 | 2.7 ± 0.1 | 2.8 ± 0.2 |
| 16:0a/18:1 * | 21.9 ± 0.1 | 21.4 ± 1.0 | 23.7 ± 0.8 | 24.0 ± 1.1 |
| 18:0a/18:1 | 17.0 ± 0.4 | 17.4 ± 0.8 | 17.4 ± 0.7 | 16.8 ± 0.4 |
| 16:0a/18:2 ** $^{\bullet\bullet}$ | 1.7 ± 0.1 | 1.5 ± 0.1 | 2.2 ± 0.1 | 1.6 ± 0.1 |
| 18:0a/18:2 ** | 3.4 ± 0.1 | 3.3 ± 0.2 | 4.2 ± 0.1 | 4.1 ± 0.3 |
| 16:0p/20:0 or 16:0e/20:1 | 0.8 ± tr. | 0.8 ± tr. | 0.7 ± 0.1 | 0.9 ± tr. |
| 16:0a/20:3 | 2.1 ± 1.2 | 1.0 ± 0.2 | 2.3 ± 0.2 | 2.2 ± 0.2 |
| 16:0a/20:4 *** | 7.6 ± 0.3 | 6.4 ± 0.8 | 3.9 ± 0.2 | 4.4 ± 0.2 |
| 18:0a/20:4 *** | 12.4 ± 0.4 | 11.1 ± 1.4 | 6.5 ± 0.4 | 7.7 ± 0.2 |
| 18:1a/20:4 *** | 7.9 ± 0.1 | 8.3 ± 1.1 | 4.0 ± 0.3 | 5.1 ± 0.3 |
| 18:0a/18:0 or 16:0p/22:6 | 3.4 ± 0.1 | 3.5 ± 0.2 | 3.4 ± 0.1 | 3.3 ± 0.3 |
| 16:0a/22:6 ** | 8.4 ± 0.4 | 9.5 ± 0.9 | 14.0 ± 0.3 | 12.6 ± 1.2 |
| 18:0a/22:6 *** | 3.1 ± 0.2 | 3.4 ± 0.1 | 4.7 ± tr. | 4.6 ± tr. |
| 18:1a/22:6 *** | 2.5 ± 0.3 | 2.8 ± 0.3 | 4.6 ± 0.3 | 4.3 ± 0.2 |

tr., trace (less than 0.05); significant effect of diet, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; significant effect of genotype, $^{\bullet}p < 0.05$, $^{\bullet\bullet}p < 0.01$.

### *5.3.2.2.3 Analysis of phosphatidylcholine in cerebellum*

The molecular species compositions of PC from cerebellum of 12 and 16 month-old WT and Tg mice on the oil blend diet or on the DHA diet are presented in Table 5.34 and Table 5.35.

At 12 months of age, as well as 16 months of age, the percentages of some molecular species containing only the fatty acids 16:0, 16:1, 18:0, 18:1 or 18:2 varied significantly with the diet ($p < 0.05$) but no significant changes were observed with genotype ($p > 0.05$). Not significant effect of gender was observed at 12 months of age ($p > 0.05$) (data not shown). The percentages of DHA or 20:3 containing molecular species mostly appeared significantly higher in the cerebellum of DHA-fed mice than of oil blend-fed mice ($p < 0.05$), while the percentages of 20:4 containing molecular species mostly appeared significantly higher in the cerebellum of oil blend-fed mice than of DHA-fed mice ($p < 0.05$).

In cerebellum, at 12 months of age, the molecular species of PC containing DHA represented 11.9% of total cerebellum PC of Tg mice on the oil blend diet, 13.4% of total cerebellum PC of WT mice on the oil blend diet, 16.1% of total cerebellum PC of Tg mice on the DHA diet, and 15.5% of total cerebellum PC of WT mice on the DHA diet. The molecular species of PC containing arachidonic acid represented 7.2% of total cerebellum PC of Tg mice on the oil blend diet, 7.1% of total cerebellum PC of WT mice on the oil blend diet, 3.6% of total cerebellum PC of Tg mice on the DHA diet, and 3.2% of total cerebellum PC of WT mice on the DHA diet. At 16 months of age, the molecular species of PC containing DHA represented 28.2% of total cerebellum PC of Tg mice on the oil blend diet, 30.8% of total cerebellum PC of WT mice on the oil blend diet, 41.2% of total cerebellum PC of Tg mice on the DHA diet, and 37.4% of total cerebellum PC of WT mice on the DHA diet. The molecular species of PC containing arachidonic acid represented 9.3% of total cerebellum PC of Tg mice on the oil blend diet, 9.0% of total cerebellum PC of WT mice on the oil blend diet, 3.4% of total cerebellum PC of Tg mice on the DHA diet, and 5.1% of total cerebellum PC of WT mice on the DHA diet.

**Table 5.34.** Phosphatidylcholine molecular species composition of the cerebellum of WT and Tg mice on the oil blend diet or on the DHA diet, at 12 months of age. Results are represented as mean percentages of total molecular species analysed ± SEM.

| Molecular species | Tg oil (n = 6) | WT oil (n = 6) | Tg DHA (n = 6) | WT DHA (n = 6) |
|---|---|---|---|---|
| 16:0a/16:0 * | 14.6 ± 0.4 | 14.0 ± 0.5 | 14.6 ± 0.4 | 16.1 ± 0.4 |
| 16:0a/16:1 ** | 1.3 ± 0.1 | 1.5 ± 0.2 | 1.8 ± 0.1 | 1.9 ± 0.2 |
| 16:1a/16:1 | 2.1 ± 0.1 | 2.1 ± 0.1 | 1.9 ± 0.1 | 2.2 ± 0.1 |
| 16:0a/18:0 | 5.0 ± 0.2 | 4.9 ± 0.3 | 4.8 ± 0.1 | 4.5 ± 0.3 |
| 16:0a/18:1 | 39.1 ± 0.6 | 37.9 ± 0.8 | 39.3 ± 0.9 | 38.7 ± 0.8 |
| 18:0a/18:1 * | 9.3 ± 0.3 | 9.5 ± 0.4 | 8.3 ± 0.5 | 8.4 ± 0.3 |
| 16:0a/18:2 * | 1.7 ± tr. | 1.6 ± 0.1 | 2.0 ± 0.1 | 1.9 ± 0.1 |
| 18:0a/18:2 | 2.9 ± 0.1 | 3.0 ± 0.1 | 3.1 ± 0.1 | 3.0 ± tr. |
| 16:0p/20:0 or * 16:0e/20:1 | 0.9 ± tr. | 1.0 ± tr. | 0.9 ± tr. | 0.9 ± tr. |
| 16:0a/20:3 | 1.6 ± tr. | 1.5 ± 0.1 | 1.6 ± 0.1 | 1.4 ± 0.1 |
| 16:0a/20:4 *** | 3.5 ± 0.3 | 3.3 ± 0.2 | 1.2 ± 0.2 | 1.0 ± 0.1 |
| 18:0a/20:4 *** | 1.7 ± tr. | 1.7 ± 0.1 | 0.5 ± tr. | 0.5 ± tr. |
| 18:1a/20:4 * | 2.0 ± 0.1 | 2.1 ± 0.1 | 1.9 ± 0.1 | 1.7 ± 0.1 |
| 18:0a/18:0 or * 16:0p/22:6 | 2.3 ± 0.1 | 2.4 ± 0.1 | 2.1 ± 0.1 | 2.2 ± 0.1 |
| 16:0a/22:6 *** | 7.0 ± 0.4 | 7.5 ± 0.6 | 9.8 ± 0.3 | 9.3 ± 0.7 |
| 18:0a/22:6 ** | 2.8 ± 0.1 | 3.1 ± 0.1 | 3.4 ± 0.1 | 3.2 ± 0.2 |
| 18:1a/22:6 | 2.1 ± 0.2 | 2.8 ± 0.2 | 2.9 ± 0.2 | 3.0 ± 0.3 |

tr., trace (less than 0.05); significant effect of diet, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

**Table 5.35.** Phosphatidylcholine molecular species composition of the cerebellum of WT and Tg mice on the oil blend diet or on the DHA diet, at 16 months of age. Results are represented as mean percentages of total molecular species analysed ± SEM.

| Molecular species | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
|---|---|---|---|---|
| 16:0a/16:0 | 3.0 ± 0.2 | 3.6 ± 0.2 | 3.6 ± 0.3 | 3.4 ± 0.2 |
| 16:0a/16:1 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.2 ± tr. | 0.2 ± 0.1 |
| 16:1a/16:1 | N.D. | N.D. | N.D. | N.D. |
| 16:0a/18:0 | 3.6 ± 0.2 | 3.6 ± 0.3 | 3.7 ± 0.4 | 3.2 ± 0.3 |
| 16:0a/18:1 | 20.9 ± 0.7 | 20.3 ± 0.6 | 18.6 ± 1.1 | 19.9 ± 0.6 |
| 18:0a/18:1 *** | 23.0 ± 0.3 | 21.1 ± 0.2 | 17.5 ± 1.0 | 18.5 ± 0.4 |
| 16:0a/18:2 ** | 1.3 ± 0.2 | 1.3 ± tr. | 1.7 ± 0.1 | 1.6 ± tr. |
| 18:0a/18:2 | 4.1 ± 0.2 | 4.1 ± tr. | 4.1 ± 0.2 | 4.2 ± 0.1 |
| 16:0p/20:0 or 16:0e/20:1 | 1.1 ± tr. | 1.1 ± 0.1 | 1.0 ± 0.1 | 1.1 ± 0.1 |
| 16:0a/20:3 *** | 0.7 ± tr. | 0.8 ± 0.1 | 1.5 ± tr. | 1.5 ± 0.2 |
| 16:0a/20:4 *** | 2.5 ± 0.2 | 2.5 ± 0.2 | 0.7 ± 0.1 | 0.9 ± tr. |
| 18:0a/20:4 *** | 4.7 ± 0.2 | 4.6 ± 0.3 | 0.7 ± tr. | 1.0 ± 0.1 |
| 18:1a/20:4 | 2.1 ± 0.1 | 1.9 ± 0.1 | 2.0 ± 0.4 | 3.2 ± 2.0 |
| 18:0a/18:0 or * 16:0p/22:6 | 4.5 ± 0.3 | 3.9 ± 0.1 | 3.6 ± 0.1 | 3.8 ± 0.1 |
| 16:0a/22:6 *** | 13.7 ± 0.4 | 14.1 ± 0.7 | 21.2 ± 0.3 | 18.9 ± 0.9 |
| 18:0a/22:6 ** | 9.8 ± 0.5 | 11.1 ± 0.9 | 13.0 ± 0.2 | 11.8 ± 0.5 |
| 18:1a/22:6 ** | 4.7 ± 0.2 | 5.6 ± 0.4 | 7.0 ± 0.7 | 6.7 ± 0.1 |

tr., trace (less than 0.05); significant effect of diet, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

### 5.3.2.3 Analysis of phosphatidylserine

Negative-ion ESI-MS-MS analysis of PS in cortex, hippocampus and cerebellum of 12 and 16 month-old WT and Tg mice on the oil blend diet or on the DHA diet demonstrated a predominant peak corresponding to its most abundant molecular species, 18:0/18:0 (Tables 5.36, 5.37 and 5.38).

#### *5.3.2.3.1 Analysis of phosphatidylserine in cortex*

The molecular species compositions of PS from cortex of 12 and 16 month-old WT and Tg mice on the oil blend diet or on the DHA diet are presented in Table 5.36.

At 12 months of age, the percentage of PS 18:0/18:0 was significantly higher in cortex PS from DHA-fed mice ($F(1,16) = 5.075$, $p = 0.039$). The percentage of the DHA containing molecular species, 18:0/22:6, representing 6.2% to 7.6% of total cortex PS at 12 months and 11.6% to 13.6% at 16 months, was not significantly affected by diet or genotype ($p > 0.05$), while the percentage of the 20:4 containing molecular species, 18:0/20:4, was significantly higher in the cortex of oil blend-fed mice than of DHA-fed mice at 12 months and 16 months of age (respectively, $F(1,16) = 70.560$, $p < 0.001$, and $F(1,8) = 39.963$, $p < 0.001$). At 12 months of age, the percentage of 18:0/20:4 was also higher in the cortex of WT mice than of Tg mice ($F(1,16) = 4.840$, $p = 0.043$). No significant effect of gender was observed at 12 months of age ($p > 0.05$) (data not shown).

**Table 5.36.** Phosphatidylserine molecular species composition of the cortex of WT and Tg mice on the oil blend diet or on the DHA diet, at 12 months and 16 months of age. Results are represented as mean percentages of total molecular species analysed ± SEM.

| | | | 12 months | |
|---|---|---|---|---|
| Molecular species | Tg oil (n = 6) | WT oil (n = 6) | Tg DHA (n = 6) | WT DHA (n = 6) |
| 16:0/18:1 | 0.8 ± tr. | 0.8 ± 0.1 | 0.6 ± 0.1 | 0.7 ± 0.1 |
| 18:0/18:0 * | 82.0 ± 0.5 | 79.6 ± 0.5 | 82.7 ± 0.7 | 82.1 ± 0.8 |
| 18:0/18:1 | 8.9 ± 0.4 | 9.8 ± 0.2 | 8.9 ± 0.3 | 8.9 ± 0.3 |
| 18:0/20:0 | 0.9 ± 0.1 | 0.7 ± 0.1 | 0.7 ± tr. | 0.8 ± 0.1 |
| 18:0/20:4 *** • | 1.2 ± 0.1 | 1.5 ± 0.1 | 0.7 ± 0.1 | 0.7 ± 0.1 |
| 18:0/22:6 | 6.2 ± 0.2 | 7.6 ± 0.4 | 6.4 ± 0.4 | 6.9 ± 0.5 |
| | | | 16 months | |
| Molecular species | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
| 16:0/18:1 | 0.5 ± 0.1 | 0.7 ± tr. | 0.5 ± tr. | 0.6 ± 0.1 |
| 18:0/18:0 | 72.9 ± 0.5 | 68.8 ± 4.6 | 75.3 ± 0.5 | 72.6 ± 0.9 |
| 18:0/18:1 | 12.2 ± 0.1 | 14.0 ± 2.0 | 9.7 ± 0.1 | 12.1 ± 1.1 |
| 18:0/20:0 | 1.0 ± 0.2 | 1.0 ± 0.1 | 1.0 ± 0.1 | 0.9 ± 0.2 |
| 18:0/20:4 *** | 1.8 ± 0.1 | 1.9 ± 0.3 | 0.7 ± 0.1 | 0.9 ± tr. |
| 18:0/22:6 | 11.6 ± 0.6 | 13.6 ± 2.5 | 12.9 ± 0.6 | 12.9 ± 1.6 |

tr., trace (less than 0.05); significant effect of diet, * $p < 0.05$, *** $p < 0.001$; significant effect of genotype, • $p < 0.05$.

### *5.3.2.3.2 Analysis of phosphatidylserine in hippocampus*

The molecular species compositions of PS from hippocampus of 12 and 16 month-old WT and Tg mice on the oil blend diet or on the DHA diet are presented in Table 5.37.

At 12 months of age, the percentage of PS 18:0/18:0 was significantly higher in hippocampus PS from DHA-fed mice ($F(1,16) = 5.571$, $p = 0.031$). The percentage of the DHA containing molecular species, 18:0/22:6, represented 11.6% to 12.7% of total hippocampus PS at 12 months and 13.6% to 16.2% at 16 months and was not significantly affected by diet or genotype ($p > 0.05$), while the percentage of the 20:4 containing molecular species, 18:0/20:4, was significantly higher in the hippocampus of oil blend-fed mice than of the DHA-fed mice at 12 months and 16 months of age (respectively, $F(1,16) = 14.025$, $p = 0.002$, and $F(1,8) = 13.290$. $p = 0.007$). No significant effect of gender was observed at 12 months ($p > 0.05$) (data not shown).

**Table 5.37.** Phosphatidylserine molecular species composition of the hippocampus of WT and Tg mice on the oil blend diet or on the DHA diet, at 12 months and 16 months of age. Results are represented as mean percentages of total molecular species analysed ± SEM.

| | | | 12 months | |
|---|---|---|---|---|
| Molecular species | Tg oil (n = 6) | WT oil (n = 6) | Tg DHA (n = 6) | WT DHA (n = 6) |
| 16:0/18:1 | 2.4 ± 0.4 | 1.8 ± 0.2 | 2.0 ± 0.1 | 1.7 ± 0.2 |
| 18:0/18:0 * | 62.4 ± 1.9 | 63.3 ± 1.9 | 66.8 ± 1.1 | 66.4 ± 1.2 |
| 18:0/18:1 | 18.9 ± 1.9 | 18.3 ± 2.0 | 16.8 ± 1.2 | 17.0 ± 1.0 |
| 18:0/20:0 | 1.1 ± 0.1 | 1.1 ± tr. | 1.2 ± 0.2 | 1.2 ± 0.1 |
| 18:0/20:4 ** | 2.6 ± 0.2 | 3.1 ± 0.6 | 1.5 ± 0.2 | 1.5 ± 0.2 |
| 18:0/22:6 | 12.7 ± 0.6 | 12.4 ± 0.5 | 11.6 ± 0.8 | 12.2 ± 0.8 |
| | | | 16 months | |
| Molecular species | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
| 16:0/18:1 | 1.2 ± 0.1 | 1.2 ± 0.4 | 0.8 ± 0.1 | 1.1 ± 0.2 |
| 18:0/18:0 | 62.9 ± 0.6 | 64.5 ± 4.1 | 66.5 ± 1.5 | 67.2 ± 0.5 |
| 18:0/18:1 | 16.2 ± 0.3 | 16.9 ± 3.0 | 13.5 ± 1.5 | 13.5 ± 0.6 |
| 18:0/20:0 | 1.3 ± 0.1 | 1.2 ± 0.1 | 1.7 ± 0.1 | 1.4 ± 0.3 |
| 18:0/20:4 ** | 3.8 ± 0.5 | 2.5 ± 0.4 | 1.3 ± tr. | 1.9 ± 0.5 |
| 18:0/22:6 | 14.5 ± 1.1 | 13.6 ± 1.1 | 16.2 ± 0.1 | 14.9 ± 0.6 |

tr., trace (less than 0.05); significant effect of diet, * $p < 0.05$, ** $p < 0.01$.

### *5.3.2.3.3 Analysis of phosphatidylserine in cerebellum*

The molecular species compositions of PS from cerebellum of 12 and 16 month-old WT and Tg mice on the oil blend diet or on the DHA diet are presented in Table 5.38.

The percentage of PS 18:0/18:0 was significantly higher in cerebellum PS from DHA-fed mice, at 16 months of age ($F(1,8) = 10.970$, $p = 0.011$). The percentage of the DHA containing molecular species, 18:0/22:6, representing 1.2% to 1.3% of total cerebellum PS at 12 months and 2.0% to 2.4% at 16 months, was not significantly affected by diet or genotype ($p > 0.05$), while the percentage of the 20:4 containing molecular species, 18:0/20:4, was significantly higher in the cerebellum of oil blend-fed mice than of DHA-fed mice at 12 months and 16 months of age (respectively, $F(1,16) = 42.885$, $p < 0.001$, and $F(1,8) = 23.287$, $p = 0.001$). No significant effect of gender was observed at 12 months ($p > 0.05$) (data not shown).

**Table 5.38.** Phosphatidylserine molecular species composition of the cerebellum of WT and Tg mice on the oil blend diet or on the DHA diet, at 12 months and 16 months of age. Results are represented as mean percentages of total molecular species analysed ± SEM.

| | | | 12 months | |
|---|---|---|---|---|
| Molecular species | Tg oil (n = 6) | WT oil (n = 6) | Tg DHA (n = 6) | WT DHA (n = 6) |
| 16:0/18:1 | 1.9 ± 0.4 | 1.8 ± 0.2 | 1.3 ± 0.1 | 1.7 ± 0.1 |
| 18:0/18:0 | 61.4 ± 1.5 | 62.3 ± 1.5 | 65.0 ± 1.1 | 64.8 ± 3.1 |
| 18:0/18:1 | 25.4 ± 1.4 | 24.8 ± 1.0 | 23.3 ± 1.2 | 23.6 ± 2.7 |
| 18:0/20:0 | 8.3 ± 0.5 | 7.9 ± 0.3 | 8.3 ± 0.5 | 7.7 ± 0.5 |
| 18:0/20:4 *** | 1.7 ± 0.1 | 1.9 ± 0.2 | 0.9 ± 0.1 | 0.9 ± 0.1 |
| 18:0/22:6 | 1.3 ± 0.1 | 1.3 ± 0.2 | 1.2 ± 0.1 | 1.2 ± tr. |
| | | | 16 months | |
| Molecular species | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
| 16:0/18:1 | 1.1 ± tr. | 1.3 ± 0.3 | 0.8 ± 0.1 | 1.0 ± 0.3 |
| 18:0/18:0 * | 58.3 ± 2.4 | 59.7 ± 1.3 | 66.0 ± 1.7 | 63.0 ± 0.7 |
| 18:0/18:1 | 25.9 ± 2.5 | 23.5 ± 0.5 | 21.5 ± 1.6 | 22.1 ± 0.9 |
| 18:0/20:0 | 10.0 ± 0.9 | 10.9 ± 1.1 | 9.2 ± 0.9 | 10.6 ± 0.8 |
| 18:0/20:4 ** | 2.4 ± 0.2 | 2.2 ± 0.4 | 0.6 ± 0.3 | 1.3 ± 0.1 |
| 18:0/22:6 | 2.4 ± 0.4 | 2.4 ± 0.4 | 2.0 ± 0.1 | 2.0 ± 0.1 |

tr., trace (less than 0.05); significant effect of diet, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

### 5.3.2.4 Analysis of phosphatidylinositol

Negative-ion ESI-MS-MS analysis of PI in cortex, hippocampus and cerebellum of 12 and 16 month-old WT and Tg mice on the oil blend diet or on the DHA diet demonstrated predominant peaks corresponding to its most abundant molecular species 16:0/20:4 and18:0/20:4 (Tables 5.39, 5.40 and 5.41).

#### *5.3.2.4.1 Analysis of phosphatidylinositol in cortex*

The molecular species compositions of PI from cortex of 12 and 16 month-old WT and Tg mice on the oil blend diet or on the DHA diet are presented in Table 5.39.

The percentage of PI 18:0/18:1 and 16:0/20:3 at 12 months of age and the percentage of PI 16:0/18:1 and 18:0/18:1 at 16 months of age were significantly higher in cortex PI from DHA-fed mice ($p < 0.05$). The percentage of the DHA containing molecular species, 16:0/22:6 and 18:0/22:6, were significantly higher in cortex PI from DHA-fed mice, at 16 months only (respectively, $F(1,8) = 47.212$, $p < 0.001$, and $F(1,8) = 15.337$, $p = 0.004$). In cortex, the molecular species of PI containing DHA represented 5.9% of total cortex PI of Tg mice on the oil blend diet, 5.4% of total cortex PI of WT mice on the oil blend diet, 7.4% of total cortex PI of Tg mice on the DHA diet, and 9.4% of total cortex PI of WT mice on the DHA diet, at 12 months of age; and 7.3% of total cortex PI of Tg mice on the oil blend diet, 6.9% of total cortex PI of WT mice on the oil blend diet, 12.4% of total cortex PI of Tg mice on the DHA diet, and 12.7% of total cortex PI of WT mice on the DHA diet, at 16 months of age. The molecular species of PI containing 20:4 represented 86.3% of total cortex PI of Tg mice on the oil blend diet, 86.9% of total cortex PI of WT mice on the oil blend diet, 81.2% of total cortex PI of Tg mice on the DHA diet, and 78.2% of total cortex PI of WT mice on the DHA diet, at 12 months of age; and 89.4% of total cortex PI of Tg mice on the oil blend diet, 88.1% of total cortex PI of WT mice on the oil blend diet, 81.1% of total cortex PI of Tg mice on the DHA diet, and 82.2% of total cortex PI of WT mice on the DHA diet, at 16 months of age. No significant effect of diet or genotype was observed on the percentage of 20:4 containing molecular species ($p > 0.05$). No significant effect of gender was observed at 12 months ($p > 0.05$) (data not shown).

**Table 5.39.** Phosphatidylinositol molecular species composition of the cortex of WT and Tg mice on the oil blend diet or on the DHA diet, at 12 months and 16 months of age. Results are represented as mean percentages of total molecular species analysed ± SEM.

| | 12 months | | | |
|---|---|---|---|---|
| Molecular species | Tg oil (n = 6) | WT oil (n = 6) | Tg DHA (n = 6) | WT DHA (n = 6) |
| 16:0/18:1 | 2.1 ± 0.3 | 2.0 ± 0.3 | 2.6 ± 0.4 | 2.9 ± 0.7 |
| 18:0/18:1 ** | 5.2 ± 0.8 | 5.0 ± 0.6 | 7.6 ± 0.7 | 8.0 ± 0.8 |
| 16:0/20:3 ** | 1.5 ± 0.2 | 1.7 ± 0.2 | 2.6 ± 0.4 | 2.9 ± 0.6 |
| 16:0/20:4 | 45.4 ± 6.7 | 51.6 ± 7.7 | 39.9 ± 7.6 | 40.7 ± 7.4 |
| 18:0/20:4 | 40.9 ± 7.4 | 35.3 ± 7.5 | 41.3 ± 8.1 | 37.5 ± 7.7 |
| 16:0/22:6 | 4.4 ± 1.0 | 3.7 ± 1.0 | 4.7 ± 1.4 | 6.6 ± 1.5 |
| 18:0/22:6 | 1.5 ± 0.4 | 1.7 ± 0.3 | 2.7 ± 0.5 | 2.8 ± 0.7 |
| | 16 months | | | |
| Molecular species | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
| 16:0/18:1 * | 1.5 ± 0.1 | 1.1 ± 0.2 | 2.9 ± 0.7 | 2.0 ± 0.5 |
| 18:0/18:1 ** | 0.9 ± 0.2 | 1.9 ± 0.3 | 2.8 ± 0.4 | 2.2 ± 0.3 |
| 16:0/20:3 | 0.8 ± 0.1 | 1.1 ± 0.2 | 0.8 ± 0.1 | 0.7 ± 0.1 |
| 16:0/20:4 | 43.7 ± 1.4 | 39.0 ± 3.6 | 36.5 ± 0.6 | 38.9 ± 1.4 |
| 18:0/20:4 | 45.7 ± 1.3 | 49.1 ± 3.4 | 44.6 ± 1.0 | 43.3 ± 0.4 |
| 16:0/22:6 *** | 4.9 ± 0.3 | 4.4 ± 0.4 | 8.5 ± tr. | 9.3 ± 1.1 |
| 18:0/22:6 ** | 2.4 ± 0.2 | 2.5 ± 0.3 | 3.9 ± 0.3 | 3.4 ± 0.4 |

tr., trace (less than 0.05); significant effect of diet, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

### *5.3.2.4.2 Analysis of phosphatidylinositol in hippocampus*

The molecular species compositions of PI from hippocampus of 12 and 16 month-old WT and Tg mice on the oil blend diet or on the DHA diet are presented in Table 5.40.

Only the percentage of PI 18:0/18:1 and 16:0/20:3 at 12 months of age were significantly higher in cortex PI from DHA-fed mice ($p < 0.05$). No significant effect of diet or genotype was observed on the percentage of DHA containing phospholipid species and 20:4 containing phospholipid species ($p > 0.05$). The molecular species of PI containing DHA represented 3.6% of total hippocampus PI of Tg mice on the oil blend diet, 3.4% of total hippocampus PI of WT mice on the oil blend diet, 5.8% of total hippocampus PI of Tg mice on the DHA diet, 5.7% of total hippocampus PI of WT mice on the DHA diet, at 12 months of age; and 5.3% of total hippocampus PI of Tg mice on the oil blend diet, 4.8% of total hippocampus PI of WT mice on the oil blend diet, 6.9% of total hippocampus PI of Tg mice on the DHA diet, and 7.0% of total hippocampus PI of WT mice on the DHA diet, at 16 months of age. The molecular species of PI containing 20:4 represented 86.9% of total hippocampus PI of

Tg mice on the oil blend diet, 86.5% of total hippocampus PI of WT on the oil blend diet, 78.6% of total hippocampus PI of Tg mice on the DHA diet, and 80.5% of total hippocampus PI of WT on the DHA diet, at 12 months of age; and 89.8% of total hippocampus PI of Tg mice on the oil blend diet, 90.4% of total hippocampus PI of WT mice on the oil blend diet, 85.7% of total hippocampus PI of Tg on the DHA diet, and 87.1% of total hippocampus PI of WT mice on the DHA diet, at 16 months of age. No significant effect of gender was observed at 12 months ($p > 0.05$) (data not shown).

**Table 5.40.** Phosphatidylinositol molecular species composition of the hippocampus of WT and Tg mice on the oil blend diet or on the DHA diet, at 12 months and 16 months of age. Results are represented as mean percentages of total molecular species analysed ± SEM.

| | | | 12 months | |
|---|---|---|---|---|
| Molecular species | Tg oil (n = 6) | WT oil (n = 6) | Tg DHA (n = 6) | WT DHA (n = 6) |
| 16:0/18:1 | 2.2 ± 0.5 | 2.4 ± 0.5 | 2.9 ± 0.4 | 2.6 ± 0.3 |
| 18:0/18:1 * | 6.1 ± 0.9 | 6.1 ± 0.5 | 10.3 ± 1.4 | 8.3 ± 1.4 |
| 16:0/20:3 * | 1.2 ± 0.3 | 1.5 ± 0.5 | 2.5 ± 0.5 | 2.9 ± 0.5 |
| 16:0/20:4 | 34.2 ± 6.3 | 36.6 ± 8.0 | 32.2 ± 7.3 | 34.3 ± 5.8 |
| 18:0/20:4 | 52.7 ± 7.1 | 49.9 ± 8.3 | 46.4 ± 7.7 | 46.2 ± 7.1 |
| 16:0/22:6 | 2.2 ± 0.4 | 1.7 ± 0.4 | 3.5 ± 0.7 | 3.2 ± 1.0 |
| 18:0/22:6 | 1.4 ± 0.2 | 1.7 ± 0.4 | 2.3 ± 0.6 | 2.5 ± 0.4 |
| | | | 16 months | |
| Molecular species | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
| 16:0/18:1 | 1.5 ± 0.1 | 3.2 ± 1.9 | 2.6 ± 1.0 | 1.9 ± 0.1 |
| 18:0/18:1 | 2.8 ± 0.3 | 2.5 ± 1.0 | 4.0 ± 0.2 | 2.6 ± 1.1 |
| 16:0/20:3 | 0.4 ± tr. | 0.5 ± 0.1 | 0.3 ± 0.2 | 0.8 ± 0.4 |
| 16:0/20:4 | 30.7 ± 0.9 | 36.5 ± 3.4 | 31.0 ± 0.7 | 31.2 ± 1.2 |
| 18:0/20:4 | 59.1 ± 0.8 | 53.9 ± 4.0 | 54.7 ± 0.8 | 55.9 ± 0.5 |
| 16:0/22:6 | 2.9 ± 0.5 | 3.1 ± 1.1 | 4.4 ± 0.8 | 3.4 ± 1.0 |
| 18:0/22:6 | 2.4 ± 0.5 | 1.7 ± 0.2 | 2.5 ± 0.4 | 3.6 ± 0.7 |

tr., trace (less than 0.05); significant effect of diet, * $p < 0.05$.

### *5.3.2.4.3 Analysis of phosphatidylinositol in cerebellum*

The molecular species compositions of PI from cerebellum of 12 and 16 month-old WT and Tg mice on the oil blend diet or on the DHA diet are presented in Table 5.41.

At 16 months of age, the percentages of PI 16:0/18:1 and 18:0/18:1 were significantly higher in cerebellum PI from DHA-fed mice ($p < 0.05$). The percentages of the DHA containing molecular species, 16:0/22:6 and 18:0/22:6, were significantly

higher in cerebellum PI from DHA-fed mice at 12 months (respectively, $F(1,16) = 7.763$, $p = 0.013$, and $F(1,16) = 7.071$, $p = 0.017$) and at 16 months of age (respectively, $F(1,8) = 49.973$, $p < 0.001$, and $F(1,8) = 52.926$, $p < 0.001$). In cerebellum, the molecular species of PI containing DHA represented 11.5% of total cerebellum PI of Tg mice on the oil blend diet, 11.6% of total cerebellum PI of WT mice on the oil blend diet, 21.5% of total cerebellum PI of Tg mice on the DHA diet, and 21.2% of total cerebellum PI of WT mice on the DHA diet, at 12 months of age; and 13.4% of total cerebellum PI of Tg mice on the oil blend diet, 13.4% of total cerebellum PI of WT mice on the oil blend diet, 30.5% of total cerebellum PI of Tg mice on the DHA diet, and 24.4% of total cerebellum PI of WT mice on the DHA diet, at 16 months of age. The percentage of the 20:4 containing molecular species, 16:0/20:4 and 18:0/20:4, were significantly higher in cerebellum PI from oil blend-fed mice at 16 months of age only (respectively, $F(1,8) = 46.143$, $p < 0.001$, and $F(1,8) = 38.822$, $p < 0.001$). The molecular species containing 20:4 represented 75.1% of total cerebellum PI of Tg mice on the oil blend diet, 76.3% of total cerebellum PI of WT mice on the oil blend diet, 62.3% of total cerebellum PI of Tg mice on the DHA diet, 62.2% of total cerebellum PI of WT mice on the DHA diet, at 12 months of age; and 79.9% of total cerebellum PI of Tg mice on the oil blend diet, 79.7% of total cerebellum PI of WT mice on the oil blend diet, 57.3% of total cerebellum PI of Tg mice on the DHA diet, and 64.7% of total cerebellum PI of WT mice on the DHA diet, at 16 months of age. No significant effect of gender was observed at 12 months ($p > 0.05$) (data not shown).

**Table 5.41**. Phosphatidylinositol molecular species composition of the cerebellum of WT and Tg mice on the oil blend diet or on the DHA diet, at 12 months and 16 months of age. Results are represented as mean percentages of total molecular species analysed ± SEM.

| | | 12 months | | |
|---|---|---|---|---|
| Molecular species | Tg oil (n = 6) | WT oil (n = 6) | Tg DHA (n = 6) | WT DHA (n = 6) |
| 16:0/18:1 | 4.0 ± 0.6 | 3.2 ± 0.8 | 4.4 ± 0.6 | 5.8 ± 1.0 |
| 18:0/18:1 | 8.3 ± 1.4 | 7.7 ± 1.3 | 10.9 ± 1.2 | 9.0 ± 0.8 |
| 16:0/20:3 | 1.0 ± 0.3 | 1.2 ± 0.3 | 1.5 ± 0.3 | 1.8 ± 0.4 |
| 16:0/20:4 | 24.3 ± 6.4 | 26.0 ± 5.5 | 20.8 ± 4.2 | 17.5 ± 4.9 |
| 18:0/20:4 | 50.8 ± 7.2 | 50.3 ± 6.0 | 41.0 ± 5.6 | 44.7 ± 5.0 |
| 16:0/22:6 * | 5.1 ± 0.8 | 5.7 ± 1.6 | 13.5 ± 2.4 | 9.3 ± 2.6 |
| 18:0/22:6 * | 6.4 ± 1.0 | 5.9 ± 0.9 | 8.0 ± 1.8 | 11.9 ± 1.8 |
| | | 16 months | | |
| Molecular species | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
| 16:0/18:1 * | 2.9 ± 0.7 | 3.2 ± 0.4 | 7.9 ± 2.3 | 6.4 ± 1.2 |
| 18:0/18:1 * | 2.2 ± 0.1 | 2.5 ± 1.3 | 6.2 ± 1.0 | 5.2 ± 1.4 |
| 16:0/20:3 | 0.8 ± 0.2 | 0.2 ± 0.2 | 0.3 ± 0.1 | 0.3 ± 0.2 |
| 16:0/20:4 *** | 29.4 ± 1.8 | 30.2 ± 0.3 | 19.9 ± 1.1 | 21.8 ± 1.6 |
| 18:0/20:4 *** | 50.5 ± 1.6 | 49.5 ± 1.5 | 37.4 ± 1.0 | 42.9 ± 2.1 |
| 16:0/22:6 *** ♦ | 9.1 ± 0.1 | 6.7 ± 1.3 | 15.7 ± 0.7 | 12.9 ± 1.0 |
| 18:0/22:6 *** | 4.3 ± 1.5 | 6.7 ± 0.7 | 14.8 ± 1.0 | 11.5 ± 0.9 |

tr., trace (less than 0.05); significant effect of diet, * $p < 0.05$, *** $p < 0.001$; significant effect of genotype, ♦ $p < 0.05$.

## 5.4. Discussion

The analysis of total fatty acids in brain revealed major effects of diet, variations between brain regions but very few significant changes related to the genotype. SFA appeared as the main fatty acid class in the three brain regions. The proportion of total PUFA was higher than the proportion of total MUFA in cortex and hippocampus, while the proportion of total MUFA was higher than the proportion of total PUFA in cerebellum. DHA was a major fatty acid in all the brain regions whether the mice were on the DHA or the oil blend diet (15.8% ± 0.3% to 25.5% ± 0.1% of total fatty acids). Although, the oil blend diet did not contain DHA, it was present as a major fatty acid in the three brain regions of mice that were on the oil blend diet. Although dietary DHA may be the best source of DHA, ALA (that was present in both diets) may be converted to DHA. Although ALA may not be converted to DHA in the brain, in significant amounts, it may be converted in the liver, transported to the brain and esterified to membrane glycerophospholipids (DeMar et al., 2008; Igarashi et al., 2007a; Igarashi et al., 2007b; Rapoport et al., 2007). The proportion of DHA appeared the highest in cortex (19.7% ± 0.3% to 25.5% ± 0.1% of total fatty acids), lower in hippocampus (16.8% ± 0.4% to 22.8% ± 0.1% of total fatty acids), and was lowest in cerebellum (13.9% ± 0.9% to 19.9% ± 0.8% of total fatty acids).

The percentage total n-3 PUFA was significantly increased in the three brain regions of DHA-fed mice ($p < 0.001$) while the percentage of total n-6 PUFA was significantly decreased ($p < 0.001$), and consequently the n-3/n-6 ratio was significantly increased ($p < 0.001$). The proportion of some individual fatty acids was also significantly affected by the diet. With the DHA diet, the proportions of DHA and 18:1n-9 were significantly increased in the three brain regions and the proportion of 16:0 was also increased in cerebellum. Conversely, the proportions of 20:4n-6 and 22:4n-6 were decreased in the three brain regions, the proportion of 20:1 was also decreased in cerebellum, and in some cases, the proportion of 18:1n-7 was also decreased. However, the diet had no significant effect on the levels of total SFA and total PUFA, and the proportion of total MUFA was only increased in the cortex of mice that were on the DHA diet ($p < 0.05$). The increase of DHA in brain fatty acids with the DHA diet and the converse decrease of AA and 22:4n-6 are in agreement

with previous findings (Calon et al., 2005; Hashimoto et al., 2006; Hashimoto et al., 2002; Hashimoto et al., 2005b; Hooijmans et al., 2007; Hooijmans et al., 2009; Lim et al., 2005).

The analysis of the fatty acid composition of individual phospholipids also revealed major effects of diet, and a few significant changes related to the genotype. In agreement with previous studies, PE and PC were the main phospholipids in mouse brain tissue with 32.3% ± 1.3% to 40.6% ± 1.6% and 33.5% ± 2.2% to 42.2% ± 1.7% of total fatty acids, respectively. PE and PS were also the main DHA containing phospholipids with 19.1% ± 1.3% to 37.9% ± 0.7% and 11.1% ± 2.1% to 34.7% ± 1.7% of total fatty acids, respectively, while DHA represented only 1.7% ± 0.1% to 8.5% ± 0.8% of total fatty acids in PC, 1.4% ± 0.2% to 14.8% ± 1.0% of total fatty acids in PI and was not detected in sphingomyelin.

The main changes in DHA levels, related to the diet, were observed in PE where the percentage of DHA was significantly increased in the three brain regions at both 12 and 16 months of age. The percentages of 20:4n-6 and 22:4n-6 were conversely decreased with the DHA diet. Interestingly, the two main DHA containing molecular species of PE were 18:0a/22:6 and 18:0p/22:6, and the two main AA containing molecular species of PE were 18:0a/20:4 and 18:0p/20:4, suggesting that with DHA supplementation, AA was replaced by DHA in theses two PE molecular species. It is interesting to note that a fish oil-enriched diet increased significantly the percentage of DHA in the brain of young rats and the shift between the two PE molecular species, 18:0a/22:6 and 18:0a/20:4, was also observed (Barceló-Coblijn et al., 2003). Although the level of 18:1n-9 was higher in the oil blend diet than in the DHA diet, it is interesting to note that the percentage of PE 18:1n-9 was increased in cortex, in hippocampus at 16 months and in cerebellum at 12 months of the mice that were on the DHA diet. In PC, the percentage of DHA was significantly increased with the DHA diet in cortex at 16 months only, in hippocampus and in cerebellum; and the percentage of AA was conversely decreased in the three brain regions at 12 and 16 months of age. Similarly to PE, in PC, DHA and AA were mainly associated to three other fatty acids 16:0, 18:0 and 18:1, suggesting that when DHA level is increased in the diet, DHA may replace AA in the three PC molecular species 16:0a/20:4, 18:0a/20:4 and 18:1a/20:4. Although PS is one of the main DHA containing phospholipid, the percentage of DHA was not significantly affected in cortex. However, DHA was significantly increased with the DHA diet, in

hippocampus PS at 16 months and cerebellum PS, and the percentages of 20:4n-6 and 22:4n-6 were significantly decreased in the three brain regions at 12 and 16 months of age. The percentage of PS 18:0/20:4 was significantly higher in the brain of oil blend-fed mice than DHA-fed mice, while the percentage of PS 18:0/22:6 was not significantly different. In PI, the percentage of EPA was significantly increased in the three brain regions of 12 and 16 month-old mice on DHA diet while the percentage of DHA was only increased in cortex at 16 months and cerebellum, and 20:4n-6 was decreased in cortex, hippocampus at 16 months and cerebellum at 12 months. The percentages of PI 18:0/22:6 and PI 16:0/22:6 were significantly increased with DHA diet in cortex at 16 months and cerebellum while the percentages of PI 18:0/20:4 and PI 16:0/20:4 were significantly decreased in cerebellum at 16 months. No significant effect of diet was observed in sphingomyelin.

Although the effect of diet appeared strong and sustained, significant effects of the transgene on the brain fatty acid composition appeared more random. At 12 months of age, significant differences between Tg and WT mice were only seen in the cerebellum, with higher levels of total n-6 PUFA and total PUFA in Tg mice than in WT mice caused by a significantly higher proportion of total PUFA in Tg mice on the DHA diet than for WT mice on the same diet, as well as an increased proportion of 18:1n-7 and a decreased proportion of 20:4n-6 in WT mice compared to Tg. It is rather surprising that changes related to the transgene were only observed in the cerebellum, as Aβ typically accumulates in the cortex and the hippocampus but not the cerebellum. At 16 months of age, surprisingly, the n-3/n-6 ratio was significantly higher in Tg mice on the DHA diet than in WT mice on the same diet, in the three brain regions. Total n-6 PUFA also appeared significantly lower in Tg mice on DHA diet than WT mice on the same diet, in the cortex only.

Although the effect of the transgene was not studied in most preceding studies of dietary DHA in mouse models of AD, Hooijmans et al. and Calon et al. respectively reported that APPswe/PS1dE6 and Tg2576 on normal diet did not show any significant changes in brain DHA or AA relative concentrations compared to WT mice (Calon et al., 2005; Calon et al., 2004; Hooijmans et al., 2007; Hooijmans et al., 2009). However, Hashimoto et al. showed significant variations that differed between their own studies. In two studies, Aβ infused rats presented decreased levels of AA and increased levels of DHA compared to controls, in cortex (Hashimoto et al., 2002; Hashimoto et al., 2005b), while levels of AA were increased and DHA decreased in a

third study (Hashimoto et al., 2006). Moreover, Calon et al. showed that Tg2576 mice on low n-3 PUFA diet presented decreased percentages of brain DHA compared to normal mice on the same diet (Calon et al., 2005; Calon et al., 2004). Interestingly, Han et al. (2001) showed that the levels of some PE molecular species were affected in the cortex of 18 month-old Tg2576 mice but not in the cortex of 12 month-old Tg2576 mice or in the cerebellum at either age, suggesting that changes appear only at a late stage of the Aβ pathology and only in related brain regions (Han et al., 2001). Levels of PE 16:0p/22:4 or 18:0p/20:4, 16:0a/22:6 and 18:0p/22:6 or 18:1p/22:5 were decreased in cortex of 18 month-old Tg2576 mice compared to controls.

In PE, we found that the percentage of 22:4n-6 was higher in Tg mice than WT mice on the oil blend diet (in cerebellum at 16 months), and that may be due to an increase of PE 16:0p/22:4 (in cerebellum at 16 months). PE 16:0p/22:4 (in cortex at 12 months of age) and PE 18:0a/20:4 (in cerebellum at 12 months of age) also appeared significantly higher in Tg mice than WT suggesting that the Aβ pathology increases levels of n-6 PUFA in PE of Tg mice. However, the percentage of 16:0a/20:4 (in cortex at 16 months of age) was higher in the brain of WT mice. The percentages of 18:0p/22:6 (in hippocampus at 16 months of age), 18:1a/22:6 and 18:1p/22:6 or 18:2e/22:6 (in cerebellum at 16 months of age) were also higher in the brain of WT mice compared to Tg mice, in agreement with the findings of Calon et al., suggesting that the Aβ pathology decreases levels of DHA in PE of Tg mice. It is also interesting to note that in AD patients, the main changes in the brain phospholipid composition were observed within PE molecular species (Corrigan et al., 1998; Ginsberg et al., 1995; Goodenowe et al., 2007; Han et al., 2001; Prasad et al., 1998; Soderberg et al., 1991; Wells et al., 1995). In PS, the percentage of DHA (in cerebellum at 12 months of age) was surprisingly higher in Tg mice than WT and the percentage of 18:0/20:4 (in cortex at 12 months) was higher in WT than Tg mice. In PI, the percentage of DHA (in cortex at 16 months) was higher in WT mice than Tg mice on the oil blend diet, but the percentage of 20:5n-3 (in hippocampus at 16 months of age) and 16:0/22:6 (in cerebellum at 16 months of age) were higher in Tg mice. The transgene had no significant effects on the levels of the main very long chain PUFA in PC and sphingomyelin.

In conclusion, PE appeared as the major phospholipid affected by either dietary DHA or the Aβ pathology, as it was the main DHA containing phospholipid and presented significant increase of DHA with the DHA diet and a decrease of some DHA containing molecular species in Tg mice, with a concomitant increase of AA; suggesting that, in the context of the Aβ pathology and Alzheimer's disease, PE may have a key role in the maintenance of brain DHA levels and in signalling through the action of fatty acid derived eicosanoids or protectins.

# CHAPTER 6

# Effect of dietary docosahexaenoic acid on amyloid-β accumulation in the brain of Tg2576 mice

## 6.1 Introduction

The aim of the work presented in this chapter was to investigate the effect of dietary DHA supplementation on the accumulation of Aβ in the brains of Tg2576 mice. The mice were fed the experimental diets from the age of 4 months and an analysis of Aβ, using an immunohistochemical technique and enzyme-linked immunosorbent assay (ELISA), was carried out to test the hypothesis that DHA supplementation would protect against the accumulation of the protein in Tg2576 mice. After a brief introduction on the nature of Aβ pathology in the Tg2576 mouse model, a summary of previous evidence regarding the effect of DHA on amyloid production and deposition will follow.

The formation of Aβ plaques is one of the main neuropathological abnormalities of AD, and according to the "amyloid cascade hypothesis" (Hardy and Selkoe, 2002), it is the key element in the development of the disease. Therefore, the study of the effect of DHA supplementation on the accumulation of Aβ proteins in the brain of the mice is necessary to test our hypothesis. When the APP is processed through the amyloidogenic pathway, the Aβ protein is produced (described in section 1.1.5.2.1). Depending on the location of the cleavage of the APP, Aβ1-40 and Aβ1-42 are generated. Secreted in a soluble form, these proteins form oligomers and then accumulate in the brain of AD patients in protein aggregates known as Aβ plaques.

Previous work has shown that adult Tg2576 mice display Aβ deposition in hippocampus, cerebral cortex and subiculum as well as Aβ angiopathy in the cerebellum (Harigaya et al., 2006; Hsiao et al., 1996; Kawarabayashi et al., 2001).

These studies also showed aged related increases in levels of Aβ1-40 and Aβ1-42 in the brain of Tg2576 mice. Changes in Aβ begin at 6-7 months with the appearance of insoluble Aβ1-40 and Aβ1-42, and then levels increase exponentially from 6 to 10 months of age. As insoluble Aβ appears, soluble Aβ levels decrease slightly, suggesting that it may be converted to an insoluble form (Kawarabayashi et al., 2001). Between 6 and 10 months, insoluble Aβ1-40 and Aβ1-42 are easily detected. Nevertheless, histopathology is minimal with only isolated Aβ cores. By 12 months, diffuse plaques are evident, and from 12 to 23 months, diffuse plaques, neuritic plaques with amyloid cores and biochemically extracted Aβ1-40 and Aβ1-42 increase to levels similar to those observed in the brain of AD patients.

Although some epidemiological studies have shown an association between low n-3 PUFA intake, low DHA levels in blood or brain and AD, and clinical trials suggested that DHA supplementation may slow down cognitive decline, there is currently no published report on the association of DHA and levels of Aβ in patients with AD. However, there is some evidence that dietary DHA may affect Aβ pathology in studies based on animal models (Green et al., 2007; Hashimoto et al., 2005a; Lim et al., 2005; Oksman et al., 2006) or cell cultures (Lukiw et al., 2005; Oksman et al., 2006). These different reports support the hypothesis that DHA may reduce the accumulation of Aβ proteins in the brain. However, as discussed in section 2.1.2.2, the design of some of these experiments, and especially the controls used to compare with DHA supplementation, may alter the outcome. Moreover, these studies often report mixed results and indeed others studies have indicated no effect of DHA on Aβ levels in the brain of the APPswe/PS1dE6 mouse model of AD (Arendash et al., 2007; Hooijmans et al., 2007). Therefore, whether DHA supplementation has a beneficial effect on Aβ accumulation in the brain is still a controversial issue.

The purpose of this work was to determine whether DHA supplementation affected the location and levels of Aβ proteins in the brain. To carry out this analysis, two different techniques were used, immunohistochemistry and an enzyme-linked immunosorbent assay. In both techniques, two different antibodies were used to detect specifically Aβ1-40 and Aβ1-42 proteins. The immunohistochemical analysis was used to describe the pattern of Aβ plaque formation in different brain regions of old Tg2576 mice on the DHA-enriched diet compared to those on the control diet. The ELISA analyses were used to study the effect of DHA supplementation on Aβ levels (including Aβ proteins from the soluble and the insoluble protein fractions) in

the two main brain regions affected by Aβ deposition, the cortex and the hippocampus. The analysis was carried out on three different age groups of mice, in order to observe the effect of DHA supplementation as the β-amyloid pathology progressed with age.

## 6.2 Material and methods

### 6.2.1 β-amyloid analysis by immunohistochemistry

#### 6.2.1.1 Subjects

Immunohistochemical analyses were carried out on brain tissue from 21 month-old Tg2576 mice of Cohort 3, as described in section 2.4. The cohort was composed of twelve females divided into four groups, three Tg mice on the oil blend diet, three WT mice on the oil blend diet, three Tg mice on the DHA diet and three WT mice on the DHA diet. All the mice were fed with the special diets from the age of 4 months and were sacrificed at 21 months of age. A full description of the breeding, genotyping and maintenance of the mice is presented in Chapter 2.

#### 6.2.1.2 Tissue preparation

The mice were sacrificed by a lethal injection of sodium pentobarbitone, followed by intra-cardiac perfusion of 60-80 ml 0.1 M PBS pH 7.4 and 300-400 ml 4% (w/v) paraformaldehyde in 0.1 M PBS, pH 7.4. The brains were extracted and fixed for 8 hours in 4% (w/v) paraformaldehyde in 0.1 M PBS pH 7.4, and then transferred to 25% (w/v) reagent grade sucrose solution (Fisher Scientific). The tissue remained in sucrose at room temperature until it sank and was then stored at 4°C until sectioned.

Each brain was sectioned at 40 μm thickness and stored in cryoprotectant (300 g analytical reagent grade sucrose (Fisher Scientific), 10 g molecular biology grade polyvinylpyrrolidone (BHD, Poole, UK), 300 ml ethylene glycol (VWR, Fontenay-sous-Bois, France) in 500 ml 1 M PBS buffer pH 7.4), at -20°C until use.

#### 6.2.1.3 Antibodies

The three following antibodies were used in the immunohistochemical procedures, a rabbit primary antibody anti-β-amyloid 1-40 (catalogue number AB5074P, Millipore), a rabbit primary antibody anti-β-amyloid 1-42 (catalogue

number AB5078P, Millipore) and a biotinylated secondary anti-rabbit antibody (Vectastain Elite ABC kit catalogue number PK-6101, Vector Laboratories).

#### 6.2.1.4 Immunohistochemistry procedure using free floating sections

Tissue sections were washed in 0.1 M TBS (1.2% (w/v) trizma base (Sigma), 0.9% (w/v) sodium chloride in distilled water, pH 7.4) in order to wash off the cryoprotectant. The sections were then incubated in 85% (v/v) formic acid in distilled water at 25°C for 10 min. as an antigen retrieval process. After a wash in 0.1 M TBS, the sections were incubated in the quench solution (10% (v/v) methanol, 10% (v/v) hydrogen peroxide in distilled water) for 5 min., in order to reduce the background caused by endogenous peroxidase activity. The tissue sections were washed in 0.1 M TBS three times for 10 min. and incubated in a blocking solution of 3% (v/v) normal goat serum in TXTBS (0.1% (v/v) Triton X-100 (Sigma) in 0.1 M TBS, pH 7.4) for one hour in order to block non-specific binding of the primary antibody. Then, the blocking solution was replaced by a solution of primary antibody, rabbit anti-β-amyloid 1-40 (catalogue number AB5074P, Millipore) or rabbit anti-β-amyloid 1-42 (catalogue number AB5078P, Millipore) at 0.1% (v/v) in TXTBS with 1% (v/v) normal goat serum and was incubated overnight at room temperature.

On the second day, the sections were washed three times in 0.1 M TBS for 10 min. and incubated in a solution of biotinylated secondary antibody (Vectastain Elite ABC kit catalogue number PK-6101, Vector Laboratories) at 0.5% (v/v) in 0.1 M TBS with 1% (v/v) normal goat serum, for two hours at room temperature. After three 10 min. washes in 0.1 M TBS, the sections were incubated in a streptavidin ABC complex solution 0.25% (v/v) reagent A (Avidin DH solution), 0.25% (v/v) solution B (biotinylated peroxidase) in 0.1 M TBS with 1% (v/v) normal goat serum (Vector Laboratories) for two hours at room temperature. Sections were washed three times for 10 min. in 0.1 M TBS and two times for 5 min. in 0.05 M TNS (0.6% (w/v) trizma base in distilled water, pH 7.4). The sections were then developed using the 3,3-diaminobenzidine (DAB) substrate kit for peroxidase (Vector Laboratories). Sections were incubated for approximately 10 min. in DAB solution with nickel (5 ml of distilled water, 1 drop of buffer stock solution, 2 drops of DAB stock solution, 1 drop of hydrogen peroxide solution, 1 drop of nickel solution) and washed in 0.1 M PBS, pH 7.4.

Sections were then mounted onto gelatine coated slides, air-dried, dehydrated by passing them through series of alcohols (2 min. in 50% (v/v) ethanol, 2 min. in 70% (v/v) ethanol, 2 min. in 90% (v/v) ethanol, 2 min. in 100% (v/v) ethanol, 2 min. in 100% (v/v) ethanol, 2 min. in xylene, 2 min. in xylene) and covered with glass cover slips using DPX mounting medium (Raymond A. Lamb, UK). After drying, the sections were observed under a light microscope.

#### 6.2.1.5 Image viewing

Sections were observed on a Leica DMRB microscope and digital photographs were taken using an Olympus DP70 camera. Deposition of Aβ1-40 and Aβ1-42 was observed in different areas of mouse brain: cortex, hippocampus and cerebellum, and the Aβ deposition was compared between brain areas from WT and Tg mice on DHA and oil blend diets.

### 6.2.2 β-amyloid analysis by enzyme-linked immunosorbent assay

#### 6.2.2.1 Subjects

Soluble and insoluble human Aβ1-40 and Aβ1-42, expressed from the APPswe transgene, were quantified by enzyme-linked immunosorbent assay (ELISA) in cortex and hippocampus from 12 and 21 month-old Tg2576 mice, and in cortex from 16 month-old Tg2576 mice, that were fed DHA or oil blend diets from the age of 4 months (Table 6.1). The levels of human Aβ were also measured in 16 and 21 month-old WT mice; at the same level of dilution used for samples from Tg mice. As expected, Aβ1-40 and Aβ1-42 were not detected in WT mice, as these mice do not carry the APPswe transgene. A full description of the breeding, genotyping and maintenance of the mice is presented in Chapter 2.

**Table 6.1.** Tg2576 mice used for the quantification of Aβ by ELISA: 12, 16 and 21 month-old Tg mice on oil blend diet (Tg oil) or DHA diet (Tg DHA).

| | Cohort1 12 months | | Cohort 2 16 months | | Cohort 3 21 months | |
|---|---|---|---|---|---|---|
| Gender | male | female | male | female | male | female |
| Tg oil | 3 | 3 | 3 | - | - | 3 |
| Tg DHA | 3 | 3 | 3 | - | - | 3 |

### 6.2.2.2 Tissue preparation

Mice were sacrificed by cervical dislocation. The brain was then dissected on ice into cortex and hippocampus. Tissue samples were immediately snap-frozen in liquid nitrogen and stored at -80°C for later analysis.

### 6.2.2.3 Protein extraction

Samples of cortex and hippocampus were homogenized in 2% (w/v) sodium dodecyl sulphate (SDS) in MilliQ™ water containing 1% (v/v) protease inhibitor cocktail (Protease Inhibitor Cocktail Set III, Calbiochem, San Diego, CA, USA). Homogenates were centrifuged at 21,000 g for 1 hour at 10°C. Supernatants containing soluble Aβ (SDS extracts) were collected and stored at -80°C until use. To further extract insoluble Aβ, the pellets were placed in ice-cold 5M guanidine hydrochloride in PBS (Dulbecco A) (Oxoid, Basingstoke, England) containing 1% (v/v) protease inhibitor cocktail, pH 7.4, and left at 4°C overnight. Samples were then centrifuged at 16,000 g for 20 min. at 4°C. The supernatant containing insoluble Aβ (guanidine extracts) was collected and stored at -80°C until use.

### 6.2.2.4 Protein assay

The BCA™ Protein Assay kit (Pierce, Rockfore, IL, USA) was used to measure the protein concentration of the samples extracted from cortex and hippocampus. This method combines the reduction of $Cu^{2+}$ and $Cu^{+}$ by proteins in an alkaline medium with a colorimetric detection of $Cu^{+}$ using a reagent containing bicinchoninic acid (BCA). The purple-coloured reaction product of this assay exhibits a strong absorbance at 562 nm with increasing protein concentrations over a working

range from 20 to 2,000 µg/ml. Standards and samples were prepared as indicated by the manufacturer. The microplate procedure was used with standards and samples in duplicate. After incubation, the absorbance was measured at 575 nm (FLUOstar Optima, BMG Labtech). The average absorbance of the blank replicates was subtracted from the absorbance obtained for all standards and samples. The standard curve was obtained by plotting the average blank-corrected absorbance of each bovine serum albumin (BSA) standard versus its concentration in µg/ml. The program Microsoft Excel was used to obtain the equation of the standard curve and calculate the protein concentration of each sample.

#### 6.2.2.5 Enzyme-linked immunosorbent assay

Aβ levels in cortex and hippocampus were measured by ELISA using commercial colorimetric ELISA kits (BioSource International Inc., Camarillo, CA, USA) for human Aβ1-40 (catalogue number KHB3482) and human Aβ1-42 (catalogue number KHB3442). Aβ1-40 and Aβ1-42 standards were reconstituted in 55 mM sodium bicarbonate, pH 9.0, as indicated by the manufacturer, diluted 1:20 in reaction buffer BSAT-DPBS (0.2 g/l KCl, 0.2 g/l $KH_2PO_4$, 8.0 g/l NaCl, 1.150 g/l $Na_2HPO_4$, 5% BSA, 0.03% Tween-20 in MilliQ$^{TM}$ water, pH 7.4) containing protease inhibitor cocktail, and further diluted in the Standard Diluent Buffer provided in the kits, from 500 pg/ml to 7.81 pg/ml for Aβ1-40 and from 1000 pg/ml to 15.63 pg/ml for Aβ1-42. Soluble and insoluble protein extracts were diluted 1:20 in reaction buffer BSAT-DPBS containing protease inhibitor cocktail and further diluted in the Standard Diluent Buffer as necessary. Aβ standards and diluted soluble (SDS extracts) and insoluble (guanidine extracts) protein extracts were incubated in duplicate with rabbit anti-human Aβ1-40 or Aβ1-42, in antibody-coated plates for 3 hours at room temperature, as indicated by the manufacturer. After washes, peroxidise-conjugated anti-rabbit IgG was added and incubated for 30 min. After washes, stabilized chromogen was added and incubated in the dark for another 30 min. The reaction was then stopped by adding a stop solution, and the absorbance at 450 nm was measured using a colorimetric plate reader (FLUOstar Optima, BMG Labtech). The Aβ concentrations in each sample were directly calculated using the software Optima 2.00, based on the value of the absorbance relative to the absorbance of the serial dilutions of the standard of a known concentration of Aβ.

Samples were analysed in duplicate using two different dilutions in order to ensure the concentration of at least one of the dilutions was in the range of the standards. When both values were in the range of the standards, they were averaged; otherwise the single value within the range was used. Aβ levels were expressed as pg of Aβ per μg of protein, using the protein concentration of the SDS extracts measured using the BCA™ Protein Assay.

#### 6.2.2.6 Statistical analysis

Statistical analyses of Aβ levels were carried out on each age group individually, by analysis of variance (ANOVA) with diet (oil blend or DHA) and gender (male or female, at 12 months of age only) as between subject factors, and brain region (cortex or hippocampus), extract (soluble or insoluble) and Aβ isoform (Aβ1-40 or Aβ1-42) as within subject factors. Statistically significant interactions were analysed using tests of simple main effect. Statistical analysis of Aβ levels were also carried out on each brain region individually (cortex and hippocampus), by ANOVA with age (12, 16 or 21 months of age), diet (oil blend or DHA) and gender (male or female, at 12 months of age only) as between subject factors, and Aβ form (soluble Aβ1-40, soluble Aβ1-42, insoluble Aβ1-40 and insoluble Aβ1-42) as within subject factor. When $p < 0.05$, the effect was considered significant.

## 6.3 Results

### 6.3.1 β-amyloid analysis by immunohistochemistry

In order to establish whether the DHA-enriched diet affected Aβ plaque load in various areas of the Tg2576 mouse brain, immunohistochemical analyses were performed on brain sections from 21 month-old female mice.

As WT mice do not carry the human APPswe gene, brain sections from WT animals were used as negative controls. As expected, no Aβ deposits were observed on the brain sections from WT animals on either the DHA or the oil blend diet. All areas of interest, frontal cortex, cerebral cortex, hippocampus as well as cerebellum were clear of Aβ deposits, as shown in Figure 6.1.

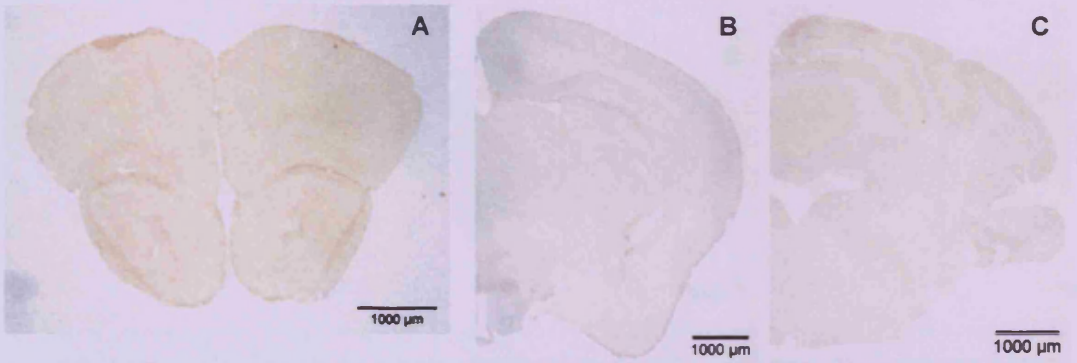


**Figure 6.1.** Representative example of brain sections without Aβ plaques. A, frontal cortex; B cerebral cortex and hippocampus; C, cerebellum. 40 µm brain sections from a WT 21 month-old female mouse were immunostained for human Aβ1-42 (AB5078P antibody at 0.1% in TXTBS with 3% normal goat serum) using the ABC method (Vectastain Elite PK-6101 antibody at 0.5% in 0.1 M TBS with 1% normal goat serum) with DAB and nickel staining. Scale bar, 1000 µm.

Representative immunohistochemical sections stained for Aβ1-40 from Tg mice on the oil blend and the DHA diets are shown in Figures 6.2, 6.3 and 6.4. In the frontal cortex (Figure 6.2), Aβ1-40 deposits appeared as compact spots and formed relatively large plaques. Aβ deposition was evident throughout the frontal cortex. However, plaque load was very variable and there were no overt differences in the density of staining between Tg mice on the oil blend diet and Tg mice on the DHA diet. In cerebral cortex and hippocampus (Figure 6.3), Aβ1-40 deposits were also

present as large scattered deposits. In hippocampus, plaques appeared concentrated in two areas, as a line along the outer molecular layer of the dentate gyrus, and along the hippocampal fissure. In some cases, plaques were observed in the subiculum. Again, there were no overt differences in plaque deposition as a function of diet. In cerebellum (Figure 6.4), no plaques were present but some staining was present along the preculminate fissure, probably in the vessels. Once more, this pattern of staining did not differ overtly as a function of diet.

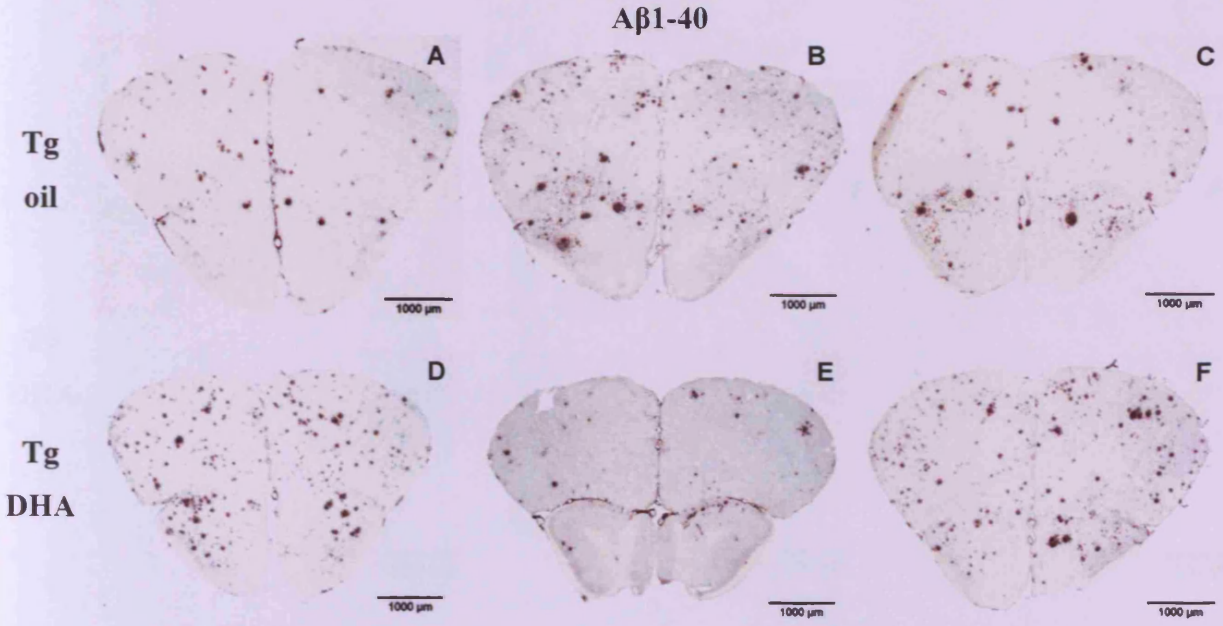


**Figure 6.2.** Immunohistochemical analysis of Aβ1-40 in frontal cortex from 21 month-old Tg2576 female mice on oil blend and DHA diets. 40 µm sections were immunostained for human Aβ1-40 (AB5074P antibody at 0.1% in TXTBS with 3% normal goat serum) using the ABC method (Vectastain Elite PK-6101 antibody at 0.5% in 0.1 M TBS with 1% normal goat serum) with DAB and nickel staining. Tg oil sections (A, B and C) and Tg DHA sections (D, E and F) show one representative section from each of the three Tg mice on the oil blend diet and from each of the three Tg mice on the DHA diet, respectively. Scale bar, 1000 µm.

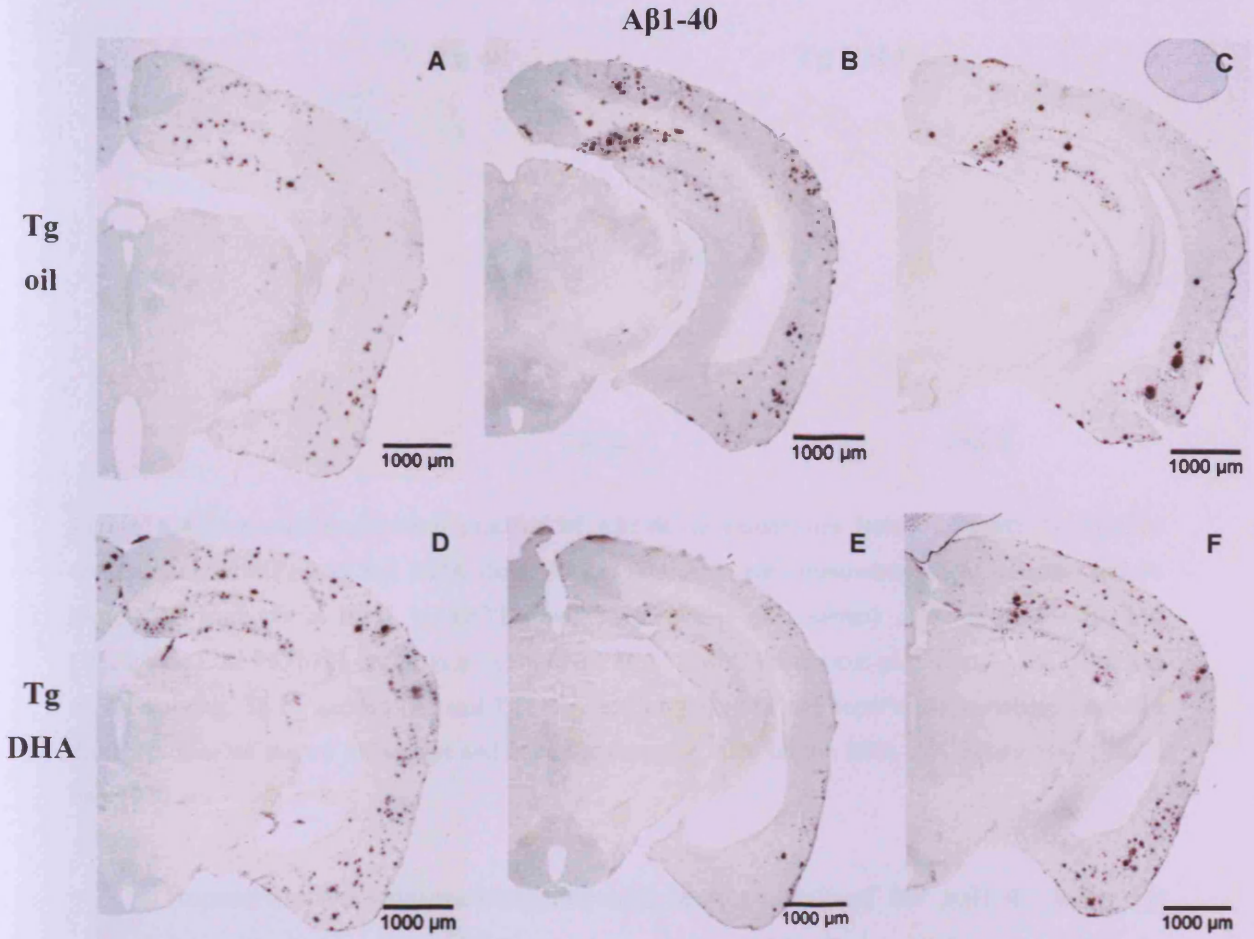


**Figure 6.3.** Immunohistochemical analysis of Aβ1-40 in cerebral cortex and hippocampus from 21 month-old Tg2576 female mice on oil blend and DHA diets. 40 µm sections were immunostained for human Aβ1-40 (AB5074P antibody at 0.1% in TXTBS with 3% normal goat serum) using the ABC method (Vectastain Elite PK-6101 antibody at 0.5% in 0.1 M TBS with 1% normal goat serum) with DAB and nickel staining. Tg oil sections (A, B and C) and Tg DHA sections (D, E and F) show one representative section from each of the three Tg mice on the oil blend diet and from each of the three Tg mice on the DHA diet, respectively. Scale bar, 1000 µm.

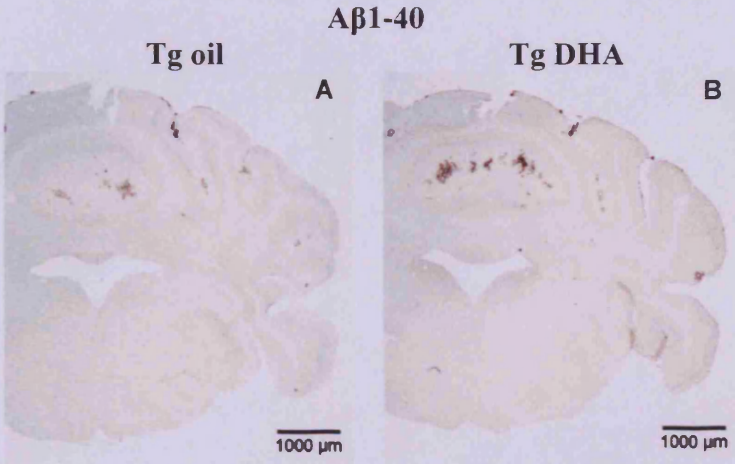


**Figure 6.4.** Immunohistochemical analysis of Aβ1-40 in cerebellum from 21 month-old Tg2576 female mice on oil blend and DHA diets. 40 µm sections were immunostained for human Aβ1-40 (AB5074P antibody at 0.1% in TXTBS with 3% normal goat serum) using the ABC method (Vectastain Elite PK-6101 antibody at 0.5% in 0.1 M TBS with 1% normal goat serum) with DAB and nickel staining. Tg oil section (A) and Tg DHA section (B) show one representative section from the three Tg mice on the oil blend diet and from the three Tg mice on the DHA diet, respectively. Scale bar, 1000 µm.

Representative immunohistochemical sections stained for Aβ1-42 from Tg mice on the oil blend and the DHA diets are presented in Figures 6.5, 6.6 and 6.7. In the frontal cortex (Figure 6.5), Aβ1-42 deposits appeared as small and diffuse spots. The deposits were denser in the anterior olfactory nucleus with the intra-bulbar anterior commissure relatively clear of deposits, but no obvious differences were observed between Tg mice fed the oil blend or the DHA diet. In the cerebral cortex and hippocampus (Figure 6.6), Aβ1-42 deposits were also present as diffuse plaques. In hippocampus, deposits appeared mostly concentrated along the hippocampus fissure, as observed for Aβ1-40 deposits. As with the Aβ1-40 immunohistochemical analysis, there was no obvious effect of the diets on the Aβ1-42 plaque load. In the cerebellum (Figure 6.7), very few or no deposits were detected.

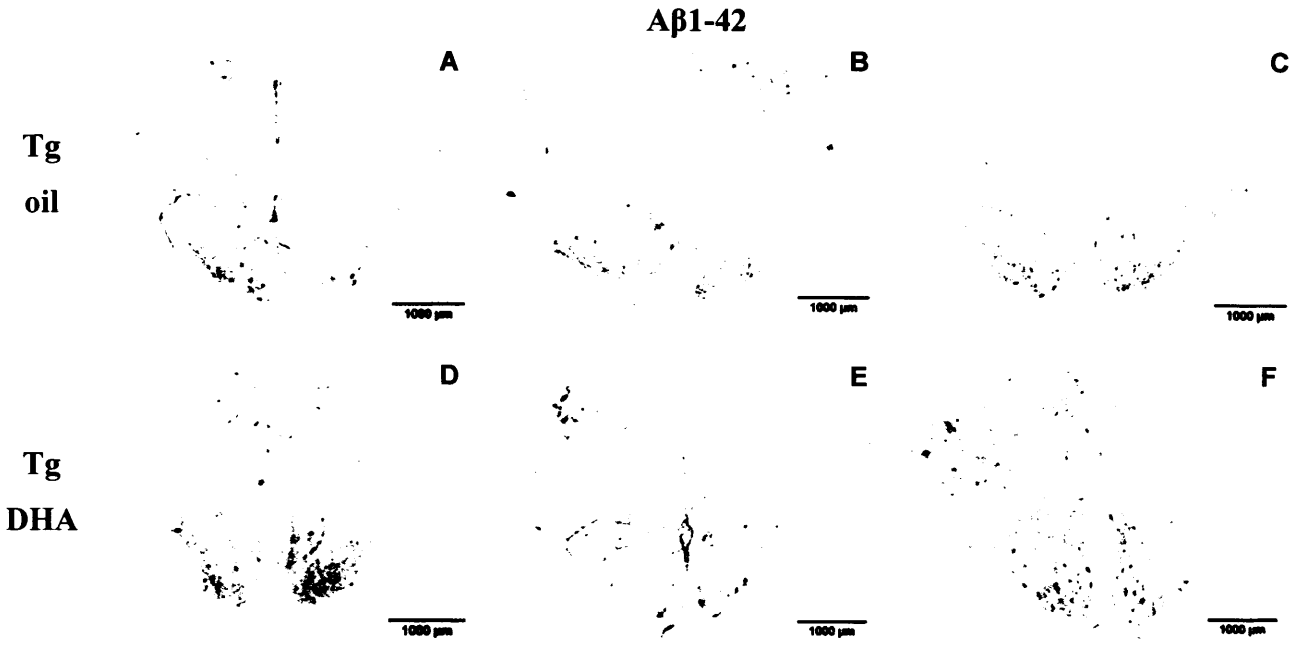


**Figure 6.5.** Immunohistochemical analysis of Aβ1-42 in frontal cortex from 21 month-old Tg2576 female mice on oil blend and DHA diets. 40 μm sections were immunostained for human Aβ1-42 (AB5078P antibody at 0.1% in TXTBS with 3% normal goat serum) using the ABC method (Vectastain Elite PK-6101 antibody at 0.5% in 0.1 M TBS with 1% normal goat serum) with DAB and nickel staining. Tg oil sections (A, B and C) and Tg DHA sections (D, E and F) show one representative section from each of the three Tg mice on the oil blend diet and from each of the three Tg mice on the DHA diet, respectively. Scale bar, 1000 μm.

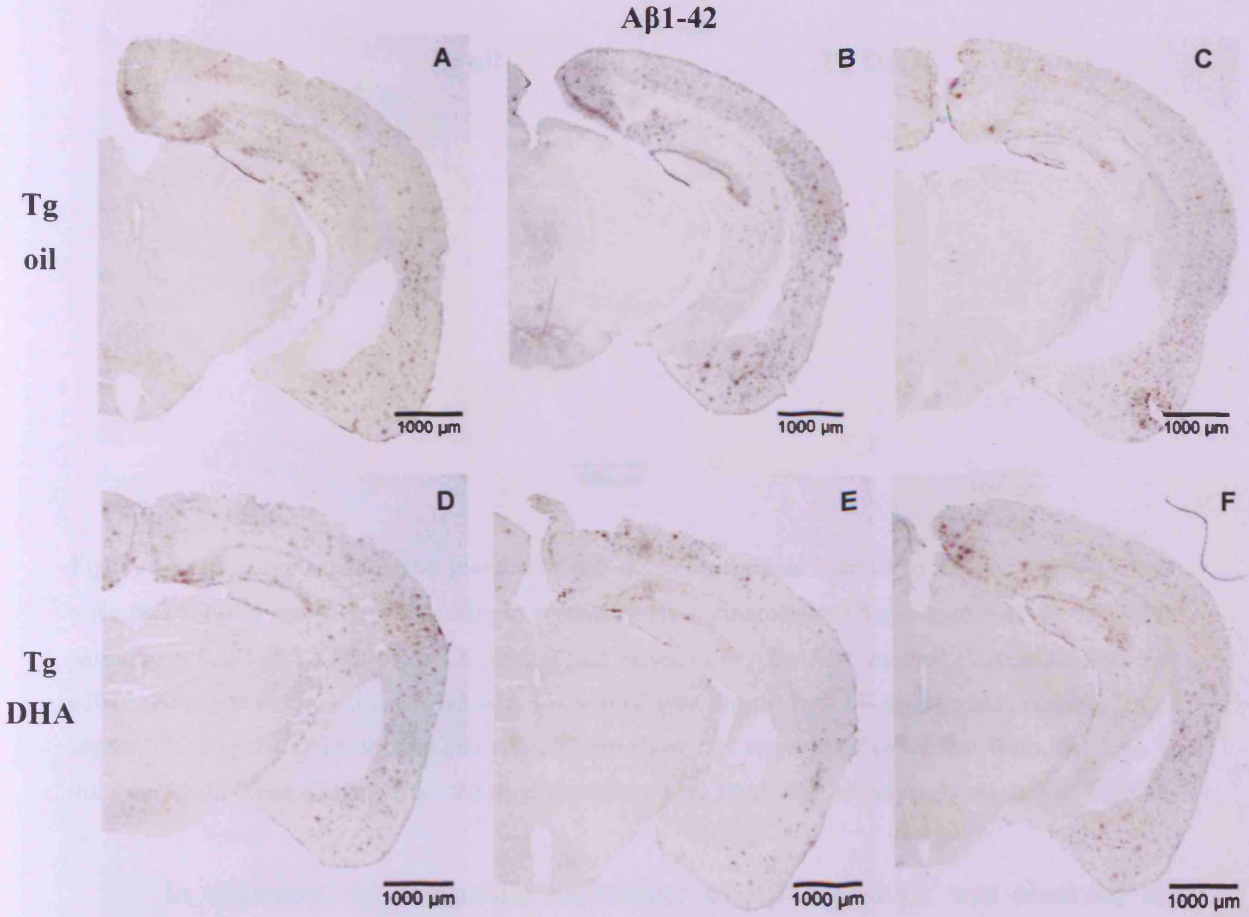


**Figure 6.6.** Immunohistochemical analysis of Aβ1-42 in cerebral cortex and hippocampus from 21 month-old Tg2576 female mice on oil blend and DHA diets. 40 µm sections were immunostained for human Aβ1-42 (AB5078P antibody at 0.1% in TXTBS with 3% normal goat serum) using the ABC method (Vectastain Elite PK-6101 antibody at 0.5% in 0.1 M TBS with 1% normal goat serum) with DAB and nickel staining. Tg oil sections (A, B and C) and Tg DHA sections (D, E and F) show one representative section from each of the three Tg mice on the oil blend diet and from each of the three Tg mice on the DHA diet, respectively. Scale bar, 1000 µm.

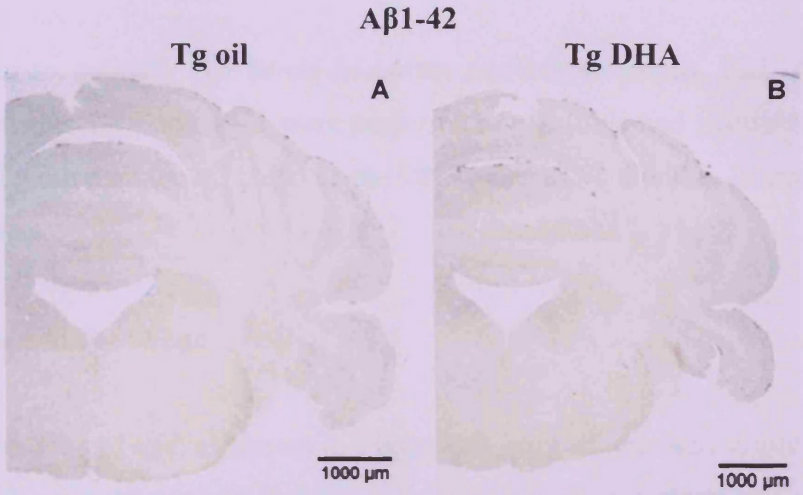


**Figure 6.7.** Immunohistochemical analysis of Aβ1-42 in cerebellum from 21 month-old Tg2576 female mice on oil blend and DHA diets. 40 µm sections were immunostained for human Aβ1-42 (AB5078P antibody at 0.1% in TXTBS with 3% normal goat serum) using the ABC method (Vectastain Elite PK-6101 antibody at 0.5% in 0.1 M TBS with 1% normal goat serum) with DAB and nickel staining. Tg oil section (A) and Tg DHA section (B) respectively show one representative section from the three Tg mice on the oil blend diet and from the three Tg mice on the DHA diet, respectively. Scale bar, 1000 µm.

In summary, no systematic differences in Aβ deposition was observed in frontal, hippocampal or cortical regions between aged Tg2576 females fed the oil blend or the DHA diet.

### 6.3.2 β-amyloid analysis by enzyme-linked immunosorbent assay

In order to quantify Aβ levels in cortex and hippocampus, ELISA for the human Aβ isoforms 1-40 and 1-42 were performed on soluble and insoluble protein extracts from Tg mice on the oil blend or the DHA diets at 12 months, 16 months and 21 months of age.

#### 6.3.2.1 Twelve months of age

At 12 months of age, as shown in Figure 6.8, both soluble and insoluble Aβ1-40 and Aβ1-42 were detected in Tg2576 mice on both the oil blend and the DHA diets with no significant effect of diet ($F(1,8) = 0.368$, $p = 0.561$) and no significant effect of gender ($F(1,8) = 0.114$, $p = 0.745$). However, the level of Aβ was significantly higher in cortex than hippocampus ($F(1,8) = 9.980$, $p = 0.013$), the level of insoluble Aβ was significantly higher than the level of soluble Aβ ($F(1,8) = 10.627$, $p = 0.012$) and the level of Aβ1-40 was significantly higher than the level of Aβ1-42 ($F(1,8) = 8.463$, $p = 0.020$). The statistical analysis also revealed a significant interaction of brain region by extract ($F(2,8) = 9.849$, $p = 0.014$) caused by significantly higher levels of insoluble Aβ in cortex than in hippocampus ($F(1,8) = 11.098$, $p = 0.010$) and significantly higher insoluble Aβ than soluble Aβ in cortex ($F(1,8) = 10.360$, $p = 0.012$). A significant interaction of brain region by Aβ isoform was found ($F(2,8) = 7.449$, $p = 0.026$) caused by higher levels of Aβ1-40 ($F(1,8) = 8.913$, $p = 0.017$) and Aβ1-42 ($F(1,8) = 13.560$, $p = 0.006$) in cortex than in hippocampus as well as higher levels of Aβ1-40 than Aβ1-42 in cortex ($F(1,8) = 9.555$, $p = 0.015$) and in hippocampus ($F(1,8) = 6.003$, $p = 0.040$). There was a significant interaction of Aβ isoform by extract ($F(1,8) = 10.030$, $p = 0.013$) caused by higher levels of soluble Aβ1-40 than soluble Aβ1-42 ($F(1,8) = 6.271$, $p = 0.037$) and of insoluble Aβ1-40 than insoluble Aβ1-42 ($F(1,8) = 9.140$, $p = 0.016$) as well as higher levels of insoluble Aβ1-40 ($F(1,8) = 10.380$, $p = 0.012$) and insoluble Aβ1-42 ($F(1,8) = 11.933$, $p = 0.009$) than their respective soluble isoforms. In addition, there was a three-ways significant interaction of brain region, diet and gender ($F(1,8) = 5.396$, $p = 0.049$ caused significantly higher levels of Aβ in cortex than hippocampus, in oil blend fed females ($F(1,8) = 6.595$, $p = 0.33$) but not males ($F < 0.001$) and in DHA fed males ($F(1,8) = 8.492$, $p = 0.019$) but not females ($F(1,8) = 0.719$, $p =$

0.421). The tests of simple main effect for this interaction also revealed no significant effect of diet (all $p > 0.2$) or gender (all $p > 0.2$). There was a four-ways significant interaction of brain region, Aβ isoform, diet and gender ($F(1,8) = 6.357$, $p = 0.036$) caused by some significant differences of Aβ levels in cortex and hippocampus ($p < 0.04$) and higher levels of Aβ1-40 than Aβ1-42 in the cortex of oil blend fed females ($F(1,8) = 6.652$, $p = 0.033$). The tests of simple main effect for this interaction also revealed no significant effect of diet (all $p > 0.2$) or gender (all $p > 0.2$). There was also a three-ways significant interaction of brain region, Aβ isoform and extract ($F(1,8) = 9.182$, $p = 0.016$). Tests of simple main effect for the interaction are detailed in Table 6.2.

Although, the effect of diet was not significant, the pair-wise comparison of each average level of Aβ obtained for Tg mice on DHA was numerically lower than the value obtained for Tg mice on oil blend diet, suggesting that DHA might reduce the level of Aβ in cortex and hippocampus. The failure to detect these changes at conventional levels of statistical significance might reflect the inherent variability in levels of Aβ in Tg2576 mice.

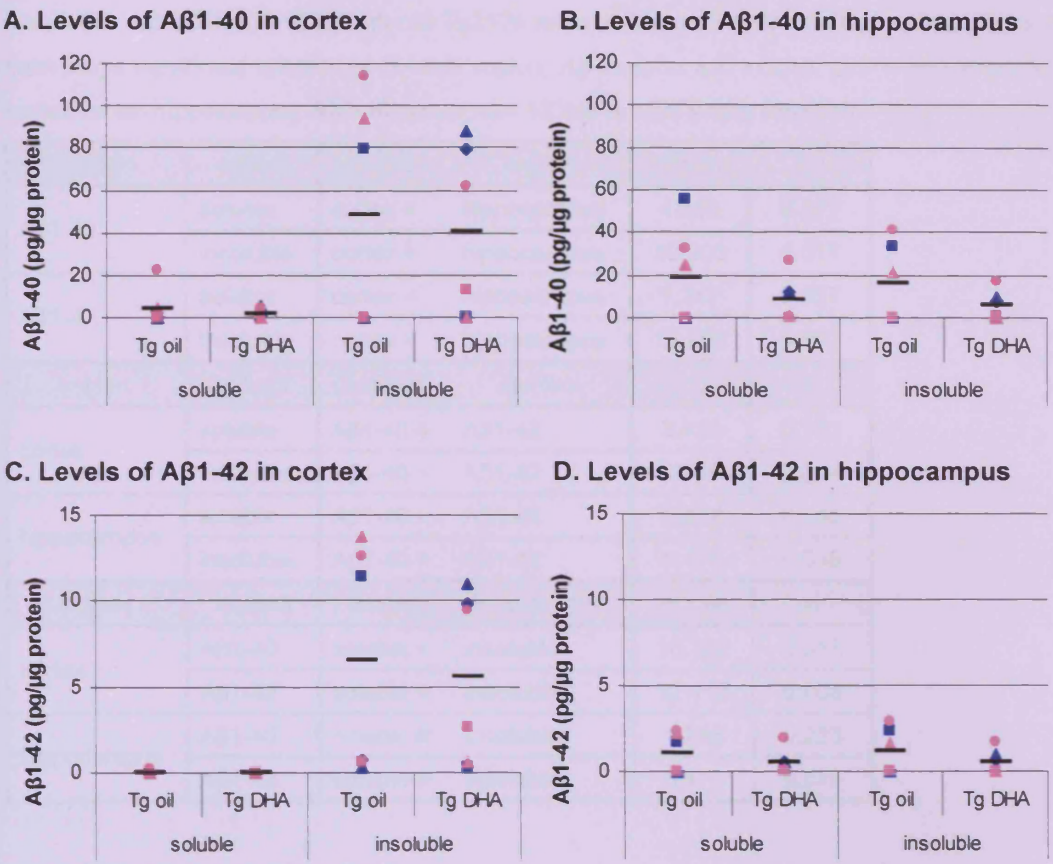


**Figure 6.8.** Levels of Aβ in cortex and hippocampus of 12 month-old male and female Tg2576 mice, analysed by ELISA (Biosource). Four species of Aβ were measured: soluble Aβ1-40 and Aβ1-42, and insoluble Aβ1-40 and Aβ1-42 in cortex and hippocampus of six Tg mice on oil blend diet (Tg oil) and six Tg mice on DHA diet (Tg DHA). The individual values are represented in blue (males) and pink (females), and the mean is represented by a black bar.

**Table 6.2.** Aβ ELISA in 12 month-old Tg2576 mice: subsequent tests of simple main effects of the three ways significant interaction of brain region, Aβ isoform and extract, pair wise comparisons of cortex versus hippocampus, Aβ1-40 versus Aβ1-42 and soluble versus insoluble.

| isoform | extract | region | region | F(1,8) | p |
|---|---|---|---|---|---|
| Aβ1-40 | soluble | cortex ≡ | hippocampus | 4.103 | 0.077 |
| | insoluble | cortex > | hippocampus | 10.803 | 0.011 |
| Aβ1-42 | soluble | cortex < | hippocampus | 7.247 | 0.027 |
| | insoluble | cortex > | hippocampus | 12.868 | 0.007 |
| region | extract | isoform | isoform | F(1,8) | p |
| cortex | soluble | Aβ1-40 ≡ | Aβ1-42 | 3.425 | 0.101 |
| | insoluble | Aβ1-40 > | Aβ1-42 | 9.789 | 0.014 |
| hippocampus | soluble | Aβ1-40 > | Aβ1-42 | 5.582 | 0.046 |
| | insoluble | Aβ1-40 > | Aβ1-42 | 6.176 | 0.038 |
| region | isoform | extract | extract | F(1,8) | p |
| cortex | Aβ1-40 | soluble < | insoluble | 10.122 | 0.013 |
| | Aβ1-42 | soluble < | insoluble | 12.112 | 0.008 |
| hippocampus | Aβ1-40 | soluble ≡ | insoluble | 1.745 | 0.223 |
| | Aβ1-42 | soluble ≡ | insoluble | 0.471 | 0.512 |

### 6.3.2.2 Sixteen months of age

Levels of Aβ measured in the cortex of 16 month-old Tg2576 males are presented in Figure 6.9. There was no significant effect of diet ($F(1,4) = 0.301$, $p = 0.613$). However, as previously observed, the average levels of Aβ obtained for Tg mice on the DHA diet were all lower than those obtained for Tg mice on the oil blend diet. The level of insoluble Aβ was significantly higher than the level of soluble Aβ ($F(1,4) = 11.386$, $p = 0.028$). The level of Aβ1-40 was significantly higher than the level of Aβ1-42 ($F(1,4) = 9.117$, $p = 0.039$). There was also a significant interaction of Aβ isoform by extract ($F(1,4) = 10.386$, $p = 0.032$) caused by a higher level of insoluble Aβ1-40 ($F(1,4) = 10.906$, $p = 0.030$) and insoluble Aβ1-42 ($F(1,4) = 22.242$, $p = 0.009$) than their respective soluble isoforms and higher levels of insoluble Aβ1-40 than insoluble Aβ1-42 ($F(1,4) = 9.696$, $p = 0.036$) but no significant difference between soluble isoforms ($F(1,4) = 2.292$, $p = 0.077$).

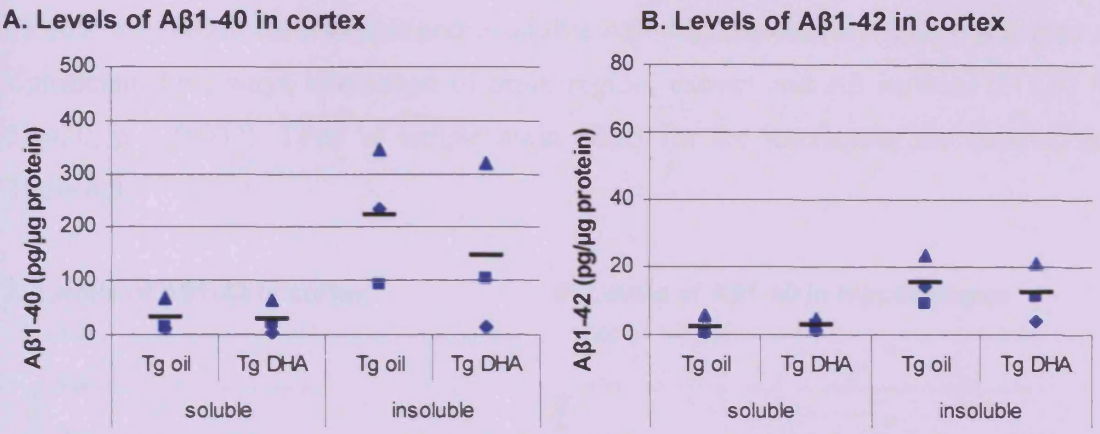


**Figure 6.9.** Levels of Aβ in cortex of 16 month-old male Tg2576 mice, analysed by ELISA (Biosource). Four species of Aβ were measured: soluble Aβ1-40 and Aβ1-42, and insoluble Aβ1-40 and Aβ1-42 in cortex of three Tg mice on oil blend diet (Tg oil) and three Tg mice on DHA diet (Tg DHA). The individual values are represented in blue and the mean is represented by a black bar.

### 6.3.2.3 Twenty one months of age

Levels of Aβ measured in the cortex and hippocampus of Tg2576 21 month-old females are presented in Figure 6.10. There was no significant effect of diet ($F(1,4) = 0.141$, $p = 0.726$). However, as previously observed at 12 and 16 months of age, the average levels of Aβ obtained for Tg mice on the DHA diet were all lower than those obtained for Tg mice on the oil blend diet. There was no significant difference of Aβ levels between cortex and hippocampus ($F(1,4) = 1.232$, $p = 0.329$) but the level of insoluble Aβ was significantly higher than the level of soluble Aβ ($F(1,4) = 14.962$, $p = 0.018$) and the level of Aβ1-40 was significantly higher than the level of Aβ1-42 ($F(1,4) = 13.045$, $p = 0.023$).

There was a significant interaction of brain region by extract ($F(1,4) = 54.323$, $p = 0.002$) caused by higher levels of insoluble Aβ than soluble in cortex ($F(1,4) = 24.081$, $p = 0.008$) and in hippocampus ($F(1,4) = 7.707$, $p = 0.050$) but no significantly different levels of soluble Aβ ($F(1,4) = 7.444$, $p = 0.053$) and insoluble Aβ ($F(1,4) = 0.128$, $p = 0.739$) between cortex and hippocampus. There was a significant interaction of extract by Aβ isoform ($F(1,4) = 14.392$, $p = 0.019$) caused by higher levels of insoluble Aβ1-40 ($F(1,4) = 14.710$, $p = 0.019$) and insoluble Aβ1-42 ($F(1,4) = 17.106$, $p = 0.014$) than their respective soluble isoforms and higher levels of soluble Aβ1-40 ($F(1,4) = 11.129$, $p = 0.029$) and insoluble Aβ1-40 ($F(1,4) =$

13.565, p = 0.021) than soluble and insoluble Aβ1-42, respectively. There was also a significant three ways interaction of brain region, extract and Aβ isoform (F(1,4) = 55.420, p = 0.002). Tests of simple main effect for the interaction are detailed in Table 6.3.

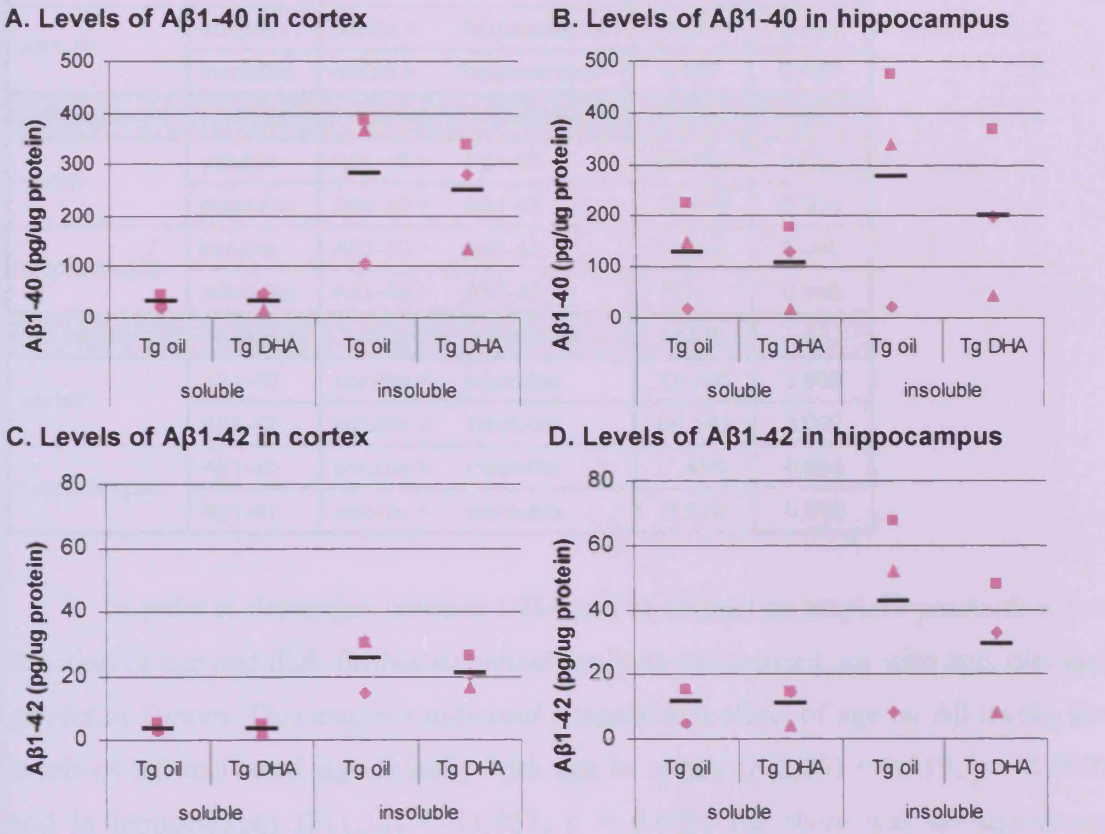


**Figure 6.10.** Levels of Aβ in cortex and hippocampus of 21 month-old female Tg2576 mice, analysed by ELISA (Biosource). Four species of Aβ were measured: soluble Aβ1-40 and Aβ1-42, and insoluble Aβ1-40 and Aβ1-42 in cortex and hippocampus of three Tg mice on oil blend diet (Tg oil) and three Tg mice on DHA diet (Tg DHA). The individual values are represented in pink and the mean is represented by a black bar.

**Table 6.3**. Aβ ELISA in 21 month-old Tg2576 mice: subsequent tests of simple main effects of the three ways significant interaction of brain region, Aβ isoform and extract, pair wise comparisons of cortex versus hippocampus, Aβ1-40 versus Aβ1-42 and soluble versus insoluble.

| isoform | extract | region | region | F(1,8) | p |
|---|---|---|---|---|---|
| Aβ1-40 | soluble | cortex ≡ | hippocampus | 6.972 | 0.058 |
| | insoluble | cortex ≡ | hippocampus | 0.718 | 0.445 |
| Aβ1-42 | soluble | cortex < | hippocampus | 13.608 | 0.021 |
| | insoluble | cortex ≡ | hippocampus | 2.847 | 0.167 |
| region | extract | isoform | isoform | F(1,8) | p |
| cortex | soluble | Aβ1-40 > | Aβ1-42 | 25.759 | 0.007 |
| | insoluble | Aβ1-40 > | Aβ1-42 | 22.479 | 0.009 |
| hippocampus | soluble | Aβ1-40 > | Aβ1-42 | 9.035 | 0.040 |
| | insoluble | Aβ1-40 > | Aβ1-42 | 8.221 | 0.046 |
| region | isoform | extract | extract | F(1,8) | p |
| cortex | Aβ1-40 | soluble < | insoluble | 22.706 | 0.009 |
| | Aβ1-42 | soluble < | insoluble | 55.446 | 0.002 |
| hippocampus | Aβ1-40 | soluble ≡ | insoluble | 7.456 | 0.052 |
| | Aβ1-42 | soluble < | insoluble | 8.970 | 0.040 |

In order to determine whether DHA had an impact on amyloid production as a function of age and diet, further statistical analysis was carried out with age, diet and gender as factors. This analysis indicated a significant effect of age on Aβ levels; the levels of Aβ increased significantly with age in cortex ($F(2,16) = 9.919$, $p = 0.002$) and in hippocampus ($F(1,12) = 11.837$, $p = 0.005$) but there was no significant interaction with the other factors ($F < 1$).

The level of insoluble Aβ was significantly higher than the level of soluble Aβ and the level of Aβ1-40 was significantly higher than the level of Aβ1-42 at all ages. However, 12 month-old mice presented significantly higher levels of Aβ in cortex than in hippocampus while the levels of Aβ between cortex and hippocampus were not significantly different at 21 months of age.

The DHA supplementation did not have a significant effect on Aβ levels - neither on the soluble nor on the insoluble Aβ levels, neither on Aβ1-40 nor on Aβ1-42 levels - at any age - 12, 16 or 21 months of age. However, the average Aβ level at all ages and for all forms of Aβ was always lower when the mice were fed with the DHA diet than when the mice were on oil blend diet, which may indicate a mild effect of DHA supplementation on the accumulation of Aβ in the brain of Tg2576 mice. Given the biological variation between mice, the number of animal used in the

experiments were probably insufficient for this to show significance.

## 6.4 Discussion

In agreement with previous studies, the immunohistochemical analysis showed that 21 month-old Tg2576 mice presented Aβ plaques containing Aβ1-40 or Aβ1-42 mainly in the frontal and cerebral cortex, and the hippocampus (Frautschy et al., 1998; Harigaya et al., 2006; Kawarabayashi et al., 2001). In the hippocampus, the plaques were particularly concentrated along the dentate gyrus and in the subiculum. Very few or no deposits were observed in the cerebellum of these animals but deposits formed by Aβ1-40 were observed in the form of angiopathy, as previously described (Harigaya et al., 2006; Kawarabayashi et al., 2001). As previously seen in the study of Kawarabayashi et al. (2001), Aβ plaques formed by Aβ1-40 had a different aspect from Aβ1-42 plaques; Aβ plaques formed by Aβ1-42 proteins appeared very diffuse while Aβ1-40 deposits were more scattered but formed larger clusters. For both Aβ1-40 and Aβ1-42, the Aβ load in cortex and hippocampus appeared very variable between subjects and no striking differences were observed between subjects on the DHA diet and subjects on the oil blend diet, although quantitative analysis was not performed on the immunohistochemical sections.

The analysis of Aβ by ELISA confirmed the presence of soluble as well as insoluble Aβ1-40 and Aβ1-42 in cortex and hippocampus of Tg2576 mice from the age of 12 months, and a significant progressive increase of the protein levels at 16 and 21 months, as expected and consistent with previous findings (Harigaya et al., 2006; Kawarabayashi et al., 2001). Over the three time points when Aβ levels were measured, the levels of insoluble Aβ1-40 and Aβ1-42 appeared mostly higher than levels of their soluble Aβ isoforms, as previously described (Kawarabayashi et al., 2001). Levels of Aβ1-40 were also mostly found higher than levels of Aβ1-42, as previously described (Kawarabayashi et al., 2001; Parachikova et al., 2008). Levels of soluble Aβ1-40 and Aβ1-42 first appeared higher in hippocampus than in cortex, and levels of insoluble Aβ1-40 and Aβ1-42 first appeared higher in cortex than in hippocampus. Then, at 21 months of age, only the level of soluble Aβ1-42 remained significantly higher in hippocampus. Although, the effect of diet was not significant at any time point, we observed that the average levels of Aβ obtained in the brain of Tg mice on DHA diet were all lower than those obtained for Tg mice on oil blend diet, at the three time points the Aβ levels were measured, suggesting that DHA

might reduce Aβ accumulation in cortex and hippocampus of Tg2576 mice.

Other studies have shown that DHA intake is associated with a reduction of Aβ levels in the brain of rodent AD models (Green et al., 2007; Hashimoto et al., 2005a; Hooijmans et al., 2009; Lim et al., 2005; Oksman et al., 2006). This contrasts with the results from the present study in which no statistically significant changes in amyloid deposition were observed. However, several factors may account for this discrepancy including differences in design and animal model. Hashimoto et al. (2005) used Wistar rats infused with Aβ1-40 as an AD model and DHA was injected instead of being present in the diet. Both of these procedures are very different from the natural processes and may affect the availability and forms of both Aβ and DHA in the brain and therefore have a different effect on the accumulation of Aβ in the brain. Oksman et al. (2006) and Hooijmans et al. (2009), used the APPswe/PS1dE9 mouse model that presents an accelerated amyloid pathology and enhanced generation of Aβ1-42, compared with Tg2576 mice (McGowan et al., 2006). In the study of Oksman et al. (2006), the benefit of dietary DHA supplementation was mainly seen on the accumulation of Aβ1-42 so it may be that higher levels of Aβ1-42 may enhance the appearance of the effect of DHA supplementation. Green at al. (2007) also used a mouse model presenting APPswe, the 3×TgAD transgenic model which develops both Aβ and tau pathology. The use of such a model may, in some ways, reflect better the pathology of AD but does not allow a focus on the Aβ pathology and the presence of tau may affect the action of DHA on the Aβ pathology.

As discussed previously in section 2.1.2.2, the choice of experimental diets is also critical in this kind of study. In the second experiment of Oksman et al. (2006), DHA supplementation (0.5% DHA, n-6/n-3 = 3) was associated with a significant reduction of Aβ1-40 and Aβ1-42 levels compared to Aβ levels obtained with the "typical Western diet" (0% DHA, 1% cholesterol, n-6/n-3 = 23), but not compared to the standard diet (0% DHA, n-6/n-3 = 8, equivalent of our oil blend diet). In the study of Green et al. (2007), DHA supplementation (1.27% DHA) was more effective alone than in combination with n-6 fatty acids such as arachidonic acid (1.27% DHA + 0.48% AA) or docosapentaenoic acid (1.25% DHA + 0.51% DPA). In addition, the effect of these diets was compared to a standard diet containing nearly twice as much 18:2n-6 as the DHA diet. Lim et al. (2005) also compared the effect of DHA supplementation (0.6% DHA, n-6/n-3 = 4) with DHA depletion (0% DHA, 6% safflower oil, n-6/n-3 = 85). Any benefit of DHA supplementation may be concealed

by the harmful effects of n-6 PUFA when these are administrated in combination. Moreover, by comparing the benefit of DHA supplementation with the harmful effect of n-3 PUFA depleted diets, or with n-6 PUFA or fat-enriched diets, the apparent effect of DHA supplementation on Aβ levels might be increased and may appear significant.

Two other studies carried out on the APPswe/PS1dE9 mouse model also indicated that DHA supplementation may not affect significantly the accumulation of Aβ in the brain (Arendash et al., 2007; Hooijmans et al., 2007). Although the Aβ load in the brain of APPswe/PS1dE9 mice was increased with a "typical Western diet" (0% DHA, n-6/n-3 = 23) compared to a standard diet (0% DHA, n-6/n-3 = 8), DHA supplementation (0.5% DHA, n-6/n-3 = 3) did not have a significant effect compared to either of the two other diets (Hooijmans et al., 2007). In the study of Arendash et al. (2007), there were no significant differences in hippocampal levels of either soluble or insoluble, Aβ1-40 or Aβ1-42 between high n-3 PUFA (4% fish oil, 6% safflower oil, n-6/n-3 = 3.8) and standard (standard diet, n-6/n-3 = 11.4) dietary groups of mice. However, in this study, the high n-3 PUFA diet also had a higher fat content (4% fish oil, 6% safflower oil) and n-6 PUFA content than the standard diet, which may negate any benefit of the high n-3 PUFA content. In terms of diet, we believe that our study is a better representation of the effect of DHA supplementation, as the two diets have the same fat content and the DHA diet contains about 1.8% DHA mainly compensated by saturated fatty acids and oleic acid in the control diet. Although, DHA supplementation might not have a significant effect on the accumulation of Aβ in brain, other mechanisms by which DHA has a beneficial effect on the pathology may be involved, including synthesis of anti-inflammatory metabolites or down regulation of inflammatory cytokine synthesis.

Although, the Aβ levels in our 12 month-old Tg2576 mice and older mice did not appear to be significantly decreased with DHA supplementation, this does not exclude the possibility that the levels of Aβ were not changed significantly at any time during the period the mice were fed with the experimental diets, from 4 months of age to the time they were sacrificed. In other mouse models of AD, Oksman et al. (2006) and Green et al. (2007) showed that levels of Aβ were reduced with DHA supplementation at an early stage of the development of the pathology but the treatment lost efficacy over time. This suggests that DHA may only delay the accumulation of Aβ in the brain with no apparent effect at a later stage of

development of the disease. This could explain why no significant change of Aβ load was seen in our cohort of aged mice. As shown in previous work, 12 month-old Tg2576 present an advanced stage of Aβ pathology (Harigaya et al., 2006; Kawarabayashi et al., 2001) so if DHA supplementation prevents or delays the Aβ pathology, the effect may only be seen at earlier stages. However, in the study of Hooijmans et al. (2009), DHA diet decreased Aβ load in the cingulate gyrus and vascular Aβ in 15 month-old mice but not in 8 month-old mice.

Regarding the variability of plaque load between individual Tg2576 mice, found in our experiments using both immunohistochemical analysis and ELISA, a number of studies have shown that Aβ load is very variable between Tg2576 subjects (Cacucci et al., 2008; Chapman et al., 1999; Hsiao et al., 1996) and it was suggested that the Tg2576 model may present a variable phenotype due to variations of the genetic background (Lassalle et al., 2008). The plaque burden was measured in hippocampus and neocortex of 9 Tg2576 mice (Cacucci et al., 2008) and around 0.01 to 0.17% of the hippocampal area and 0.01 to 0.38% of the neocortex area were occupied by plaques detected by Congo Red staining. The highest values were higher than the lowest by over 10-fold. In 15-17 month-old Tg2576 mice, levels of total brain Aβ1-40 and Aβ1-42 were also very variable with 2057 to 7209 pmol/g Aβ1-40 and 665 to 1396 pmol/g Aβ1-42 measured in 6 Tg mice (Chapman et al., 1999). These findings suggest that the number of animals used in each condition of our study may have been too low to detect a statistically significant effect of the DHA supplementation on Aβ levels. This shows the necessity of having a larger number of subjects of the same condition to reveal subtle differences in Aβ levels as a function of treatment. The effect of DHA might not have been detected in the statistical analysis because of the wide variation of Aβ levels observed between subjects on the same condition and the low number of subjects in each condition. Further work is therefore required to determine whether and to what extent DHA supplements modify the profile of Aβ production in Tg2576 mice. At one level, the lack of a robust effect of DHA on Aβ production is consistent with the relatively mild effects of DHA on learning and memory reported in Chapter 3. This point will be considered further in the general discussion.

# CHAPTER 7

# General discussion

Alzheimer's disease (AD) is the most frequent cause of dementia. Although symptomatic treatments are available to the patients, there is currently no cure for this fatal neurodegenerative disease. In addition, there is growing concern that once AD is clinically diagnosed, it is already too late to initiate effective treatment. Hence, strategies to reduce the risk or to prevent the onset of AD are particularly relevant and various nutritional approaches are gaining a lot of interest.

The n-3 polyunsaturated fatty acid known as docosahexaenoic acid (DHA, 22:6n-3) is a major fatty acid in the brain and its presence in the diet, mainly from fish products, is important for brain functions. Moreover, there is evidence that dietary DHA and the risk of AD are related. Although many studies have already been carried out in humans, in animal models and *in vitro*, the mechanisms by which DHA may interact with and potentially alleviate AD pathogenesis are not fully understood. However, there is some evidence that DHA has a beneficial effect on the β-amyloid pathology, one of the main neuropathological features of AD. Therefore, the aim of this thesis was to test the hypothesis that DHA may alleviate brain and behavioural changes in a transgenic mouse developing β-amyloid pathology.

A series of experiments were carried out on Tg2576 mice that overexpress a human APP mutant (APPswe). Transgenic mice and wild type littermates received either an oil blend control diet, corresponding to the average UK diet, or a DHA-enriched diet that contained approximately 1.8% of DHA, from the age of 4 months. Body weight and food consumption were monitored as general health information. In order to test the hypothesis that dietary DHA supplementation alleviates the cognitive impairment caused by the Aβ pathology, Tg and WT mice were tested on an open field foraging task and the T-maze forced choice alternation task. The blood plasma fatty acid composition and the brain phospholipid composition of both Tg and WT

mice were analysed in order to investigate the effect of the Aβ pathology, the dietary DHA supplementation and the duration of the feeding period on these parameters. Finally, the levels of Aβ in the brains of Tg2576 mice were analysed, using an immunohistochemical technique and an enzyme-linked immunosorbent assay to test the hypothesis that DHA supplementation would protect against the accumulation of the protein in Tg2576 mice.

This study showed that Tg2576 mice present significant spatial learning and memory impairments at the age of 8, 12 and 16 months. This is accompanied by an accumulation of Aβ1-40 and Aβ1-42, mainly in the frontal and cerebral cortex and the hippocampus, from 12 months of age, as previously described (Chapman et al., 1999; Frautschy et al., 1998; Harigaya et al., 2006; Hsiao et al., 1996; Kawarabayashi et al., 2001; Westerman et al., 2002). In addition, although food consumption was equivalent between Tg and WT mice, the body weight of Tg mice was significantly lower, suggesting that APP expression or Aβ pathology had a significant effect on mouse metabolism. The percentages of DHA-containing species of phosphatidylethanolamine (PE), PE 18:0p/22:6 (in hippocampus at 16 months of age), PE 18:1a/22:6 and PE 18:1p/22:6 or 18:2e/22:6 (in cerebellum at 16 months of age), were also significantly lower in the brain of Tg mice compared to WT mice. These findings were in agreement with the epidemiological studies suggesting that the Aβ pathology decreases levels of DHA in PE. In addition, the percentage of 20:4n-6 was significantly reduced in the plasma of 21 month-old Tg mice and in the cerebellum of 12 month-old Tg mice; and the proportion of total n-6 PUFA was also significantly reduced in the cortex of 12 month-old Tg mice, suggesting a reduction of desaturation and/or elongation enzymatic activity which may be caused by the development of Aβ pathology in Tg2576 mice. As these enzymes are also involved in DHA synthesis from 18:3n-3, this may also affect DHA levels in AD. However, the percentages of total n-6 PUFA and total PUFA were significantly higher in the cerebellum in Tg mice than in WT mice, at 12 months of age and, surprisingly, at 16 months, the n-3/n-6 ratio was significantly higher in Tg mice on the DHA diet than in WT mice on the same diet, for the three brain regions. Moreover, the percentage of brain PE 22:4n-6 was higher in Tg mice than WT mice on oil diet, possibly due to an increase of PE 16:0p/22:4. PE 16:0p/22:4 (in cortex at 12 months of age), PE 18:0a/20:4 (in cerebellum at 12 months of age) also appeared significantly higher in

Tg mice than WT suggesting that the Aβ pathology increases levels of n-6 PUFA in PE of Tg mice.

The DHA diet increased the proportion of DHA in plasma, cortex, hippocampus and cerebellum. Moreover, in the absence of dietary DHA, the proportion of DHA was essentially replaced by AA in plasma as well as in cortex, hippocampus and cerebellum. Since AA was not present in the diets, this suggested that while AA could be synthesised from 18:2n-6, dietary DHA is the best source of DHA. Moreover, the increased levels of plasma AA may lead to increased inflammation which could trigger the development of AD or exacerbate the disease. Although DHA was absorbed by the mice and we observed a significant increase in the brain, DHA supplementation only had a mild beneficial effect on spatial learning and memory impairments caused by the Aβ pathology. There was also evidence to suggest that DHA had a greater benefit as the mice aged and thus as Aβ pathology increased. This mild effect of DHA supplementation on cognitive performance was consistent with the lack of robust effect on the accumulation of Aβ in the brain of Tg mice. These findings suggest that DHA may alleviate spatial learning and memory impairments, perhaps by reducing the inflammatory and oxidative effects caused by Aβ, rather than reducing the accumulation of the protein *per se*. Moreover, phosphatidylethanolamine, one of the main phospholipids in cortex, hippocampus and cerebellum, was one of the main DHA-containing phospholipids and was the phospholipid the most effected by dietary DHA and the Aβ pathology. In PE, we observed an increase of DHA with the DHA diet, and a decrease of some DHA containing molecular species in Tg mice with a concomitant increase of AA. This suggests that, in the context of AD, PE may have a key role in the maintenance of brain DHA levels.

Although, the effect of diet on the levels of Aβ was not significant, we observed that (numerically) the average levels of Aβ obtained in the brain of Tg mice on the DHA diet was lower than for Tg mice on oil blend diet. As shown by Lim et al. (2005), this suggests that DHA might reduce Aβ accumulation in cortex and hippocampus of Tg2576 mice. Moreover, although, the Aβ levels in our 12 month-old Tg2576 mice and older mice did not appear to be significantly decreased with DHA supplementation, this does not exclude the possibility that the levels of Aβ were not

changed significantly at any time during the period the mice were fed with the experimental diets, from 4 months of age to the time they were sacrificed. In addition, both the Aβ1-40 and Aβ1-42 load in cortex and hippocampus appeared very variable between subjects and may be caused by variations of the genetic background (Hsiao et al., 1996; Lassalle et al., 2008). As shown in the study of Cacucci et al. (2008) using Tg2576 mice, although memory deficits correlated with Aβ load, the plaque burden was very variable between subjects (Cacucci et al., 2008). The relatively small number of subjects used in our Aβ analysis is one likely reason why we failed to observe a statistically significant reduction in Aβ with DHA supplementation. It would be of value to repeat some of the dietary manipulation and Aβ analysis with a larger number of subjects. In addition, assessing a broader range of mouse behaviours may provide a more sensitive test of the effect of DHA on cognition. Dietary manipulations may have more than one effect on mouse behaviour, therefore, as Wainwright (2001) suggested, a standardised battery of tests may be necessary for a complete assessment of the effects of dietary manipulations on behaviour (Wainwright, 2001).

A limitation of the present study might also be in the analysis of brain lipids. The analysis was carried out on whole brain tissue of different regions but without regard to the cell type or the cellular fraction. Astrocytes, microglial cells and neurons have very distinct functions and, therefore, may be differently affected by the Aβ pathology or the DHA supplementation. For instance, in the study of Wells et al. (1995), the decreased level of PE observed in AD patients was much more severe in the glial and neuronal cell body fractions than in the synaptosomal fraction suggesting that the study of specific plasma membrane fractions would reveal more information. Moreover, it has been shown that some cellular fractions, such as lipid rafts, have a particular role in the processing of APP and can vary in lipid composition (Hashimoto et al., 2005a). Therefore it may be of particular interest to carry out lipid studies in this context. Several studies have shown that dietary fish oil or DHA lowers cholesterol levels in plasma (Higuchi et al., 2008; Meyer et al., 2007; Riediger et al., 2008), reduces membrane-bound cholesterol that can cause neural membrane rigidity when present in excess (Yehuda et al., 2002) and is associated with a decreased accumulation of brain Aβ in mouse models (Li et al., 2003; Oksman

et al., 2006). Therefore, the beneficial effect of dietary DHA may also be due to a reduction of cholesterol rather then a direct effect of DHA *per se*.

Another questionable point in our study is the level of DHA in the DHA-enriched diet. As previously discussed in section 2.7, 1.8% DHA was fairly high and higher than in the diets used in other mouse studies. Although it seems logical to suppose that the more DHA in the diet the better, high levels of DHA might in fact be harmful. In this context, it is interesting to note that we observed a lower body weight in mice fed the DHA diet, relative to the oil-blend diet. Although this might suggest that DHA helped to maintain a healthy body weight, it might also indicate a toxic effect of the higher amount of DHA. Moreover, the brain is rich in unsaturated fatty acids which are prone to oxidative damage. Oxidative stress is known to play a role in AD, thus the benefit of supplementation with a highly peroxide prone fatty acid like DHA could aggravate the symptoms by increasing levels of peroxidised fatty acids. Lipid peroxidation of PUFA such as DHA and subsequent damages were reported *in vitro* (Alexander-North et al., 1994; Arita et al., 2003) and *in vivo* (Montine and Morrow, 2005). In addition, the level of oxidation of the PUFA in the diet was not monitored in our study. It remains possible, therefore that some of the DHA was already oxidised in the diet when fed to the mice. New studies with DHA dietary supplements in transgenic mice are currently underway in our laboratory and oxidative processes in the diet are being closely monitored.

Finally, the Tg2576 mouse model expresses a human APPswe constitutively. Therefore, the levels of Aβ may be so acute that a dietary intervention may not have the power to alleviate such pathology. Moreover, this mouse represents a model of early-onset Alzheimer's disease (EOAD) for which the onset of the disease is caused by genetic mutations affecting APP processing and, thus, the Aβ pathology is almost ineluctable. Therefore, a dietary intervention may have a relatively small impact of pathology processes in such models. In addition, EOAD occurs in a very small fraction of the AD population so if dietary DHA had a significant effect in this mouse model, it would only be possible to speculate on its benefit in this type of AD. Moreover, although low levels of DHA in blood or brain may be associated with an increased risk of cognitive decline and AD, it does not necessary means that the opposite is true. If a low level of DHA in the brain of AD patients is caused by increased DHA degradation by peroxidation or cell death, or a decreased transport

into the brain, increasing the intake may not have a significant effect and may not be sufficient to restore brain DHA level.

This study did show relatively mild beneficial effects of the DHA treatment on mouse cognition. If this trend is indicative of an action of DHA on the neural processes supporting memory, the question remains as to whether this effect is mediated by an effect on the accumulation of Aβ in the brain. For example, studies on the Tg2576 model have revealed beneficial effects of treatments on learning and memory that are without direct effects on Aβ levels in the brain. In a study on testing the effect of *Ginkgo biloba* extracts on memory and Aβ pathology, no reduction of the brain levels of soluble or insoluble of Aβ1-40 and Aβ1-42 was observed. Nevertheless, the authors reported a significant improvement of spatial memory in the treated Tg mice (Stackman et al., 2003). Short-term exercise in aged Tg2576 mice also alters neuroinflammation and can improve spatial learning without affecting the levels of insoluble Aβ1-40 and Aβ1-42 in the brain (Parachikova et al., 2008). Alpha-lipoic acid treatment has also been shown to improve spatial learning and memory retention in Tg2576 without affecting brain soluble and insoluble Aβ levels (Quinn et al., 2007). This suggests that spatial memory impairment can be alleviated without changes in Aβ production, deposition or clearance. Therefore, one might speculate that the beneficial effect of DHA on mouse performance might be due to the production of neuroprotectins and its anti-inflammatory and anti-oxidant properties rather than having a direct effect on Aβ processing. In the study of Hashimoto et al. (2002), DHA supplementation resulted in anti-oxidant effects in the hippocampus and the cortex of Aβ infused rats, suggesting that the preventive effect of DHA could be due to anti-oxidant properties of this fatty acid. In addition, in the study of Lukiw et al. (2005), DHA treatment in primary co-cultures of human neurons and glial cells was associated with neuroprotectin synthesis and anti-apoptotic and anti-inflammatory gene expression.

In conclusion, the series of experiments described in my thesis have shown that spatial learning and memory impairments in Tg2576 mice were only mildly modified by dietary DHA supplementation. DHA was absorbed and incorporated in the brain of DHA-fed mice, but it did not have a significant effect on the brain levels of Aβ. This suggests that DHA may alleviate cognitive impairments caused by the Aβ pathology through its anti-inflammatory and/or anti-oxidative properties. Moreover,

the DHA-rich brain phospholipid, phosphatidylethanolamine, may play a key role in this process. However, because of the lack of power of the different analyses, the outcomes of the study should be interpreted carefully. In spite of detailed lipid analyses, the use of two different tasks to assess learning and memory and two different immuno-detection methods for the analysis of changes in Aβ plaque formation, the different aspects of the study were carried out with small group sizes. Therefore, each study may lack power to detect relatively small effects of diet on learning and memory or Aβ levels, for example, compared to the variability between subjects of the same group.

In the behavioural studies, the observed power of the diet effect was always below 0.6 while a power of 0.8 or over is commonly considered acceptable. In addition, each behavioural analysis (looking at errors or percent of correct choice) included at least four cases of extreme outliers that also indicates a notable variability between subjects. These observations clearly indicate a lack of power of the analyses to detect any subtle affects of diet and the need for a larger number of mice in the studies to overcome the variability.

Partly due to the fact that brain samples from all the mice available for the study had to be divided between the different biochemical analyses, very small group sizes were used (typically n = 3 to 6 per condition, with males and females pooled together in some occasions). In addition to the small group sizes, gender was unequally distributed. At 12 months, analyses were carried out on both males and females and then, males only at 16 months and females only at 21 months. Therefore, any observed effect of age may be mixed with an effect of gender, as they were the only old mice available. Although the plasma fatty acid composition and the brain phospholipid composition appeared relatively consistent (with low SEM), this was not true for the analysis of Aβ plaque formation where levels of Aβ in hippocampus and cortex appeared very variable between Tg mice on the same diet. As indicated by the observed power of diet effect below 0.1 for each of the analysis at 12, 16 and 21 months, the group sizes where also too low to detect any significant but small effects of diet on the level of Aβ. Consequently, the analyses undertaken were partial in terms of gender and age, restricted as group sizes were very small and therefore lacked of power to detect subtle diet effects.

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# APPENDIX 1

Fatty acid composition of plasma from 12 month-old WT and Tg mice on the oil blend diet or the DHA diet from the age of 4 months. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 4) | WT oil (n = 4) | Tg DHA (n = 4) | WT DHA (n = 4) |
|---|---|---|---|---|
| 12:0 | 0.3 ± 0.2 | 0.1 ± tr. | 0.1 ± 0.1 | 0.2 ± 0.1 |
| 14:0 | 0.5 ± 0.1 | 0.4 ± tr. | 0.9 ± 0.2 | 0.8 ± 0.2 |
| 16:0 | 20.1 ± 0.8 | 19.4 ± 1.0 | 22.5 ± 0.6 | 20.9 ± 0.4 |
| 16:1n-7 | 1.8 ± 0.2 | 1.8 ± 0.2 | 2.4 ± 0.4 | 1.9 ± 0.2 |
| 18:0 | 9.7 ± 1.5 | 10.2 ± 0.6 | 8.5 ± 1.1 | 8.6 ± 1.1 |
| 18:1n-9 + 18:1n-7 | 22.4 ± 2.6 | 21.0 ± 1.4 | 20.9 ± 2.4 | 18.9 ± 0.9 |
| 18:2n-6 | 23.6 ± 1.0 | 25.1 ± 2.2 | 23.1 ± 2.3 | 25.1 ± 1.3 |
| 18:3n-6 | 0.2 ± tr. | 0.2 ± tr. | N.D. | 0.1 ± tr. |
| 18:3n-3 | 0.6 ± 0.1 | 0.7 ± 0.1 | 0.4 ± tr. | 0.5 ± 0.1 |
| 20:0 | 0.2 ± tr. | 0.2 ± 0.1 | 0.2 ± tr. | 0.2 ± tr. |
| 20:1 | 0.6 ± 0.2 | 0.6 ± 0.1 | 0.4 ± tr. | 0.5 ± tr. |
| 20:2 | 0.2 ± tr. | 0.1 ± tr. | 0.2 ± tr. | 0.3 ± tr. |
| 20:3n-6 | 1.0 ± 0.2 | 1.0 ± 0.1 | 0.8 ± 0.1 | 1.0 ± 0.2 |
| 20:4n-6 | 12.1 ± 2.5 | 13.2 ± 1.9 | 0.9 ± 0.1 | 1.4 ± 0.2 |
| 20:5n-3 | 0.3 ± 0.1 | 0.3 ± tr. | 3.0 ± 0.2 | 3.4 ± 0.5 |
| 22:0 | 0.2 ± 0.1 | 0.2 ± tr. | 0.3 ± 0.1 | 0.2 ± tr. |
| 22:1 | N.D. | 0.1 ± tr. | 0.1 ± tr. | N.D. |
| 22:2 | N.D. | N.D. | N.D. | N.D. |
| 22:4n-6 | 0.3 ± tr. | 0.2 ± tr. | N.D. | N.D. |
| 23:0 | 0.3 ± 0.1 | 0.2 ± 0.1 | N.D. | N.D. |
| 22:5n-3 | 0.3 ± tr. | 0.2 ± tr. | 0.5 ± 0.1 | 0.7 ± 0.1 |
| 22:6n-3 | 4.3 ± 0.7 | 3.9 ± 0.4 | 13.9 ± 1.2 | 14.7 ± 1.5 |
| 24:0 | 0.2 ± 0.1 | 0.2 ± tr. | 0.3 ± 0.1 | 0.2 ± tr. |
| 24:1 | 0.7 ± 0.1 | 0.5 ± 0.1 | 0.6 ± 0.1 | 0.6 ± tr. |
| Total SFA | 31.6 ± 1.1 | 31.0 ± 0.7 | 32.8 ± 1.4 | 31.1 ± 1.0 |
| Total MUFA | 25.4 ± 2.8 | 23.9 ± 1.5 | 24.3 ± 2.7 | 21.8 ± 1.0 |
| Total PUFA | 42.9 ± 2.3 | 45.1 ± 1.0 | 42.9 ± 1.3 | 47.0 ± 1.0 |
| Total n-3 FA | 5.5 ± 0.7 | 5.2 ± 0.2 | 17.8 ± 1.3 | 19.3 ± 1.6 |
| Total n-6 FA | 37.2 ± 1.7 | 39.8 ± 0.9 | 24.9 ± 2.5 | 27.5 ± 1.1 |
| n-3/n-6 ratio | 0.15 ± 0.01 | 0.13 ± 0.01 | 0.72 ± 0.14 | 0.70 ± 0.08 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 2

Fatty acid composition of plasma from 16 month-old WT and Tg mice on the oil blend diet or the DHA diet from the age of 4 months. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
|---|---|---|---|---|
| 12:0 | N.D. | N.D. | N.D. | N.D. |
| 14:0 | 0.2 ± tr. | 0.2 ± 0.1 | 0.1 ± tr. | 0.3 ± 0.1 |
| 16:0 | 21.3 ± 0.7 | 19.7 ± 1.0 | 15.1 ± 0.4 | 18.9 ± 1.6 |
| 16:1n-7 | 1.1 ± 0.1 | 1.2 ± 0.1 | 0.3 ± tr. | 0.6 ± 0.1 |
| 18:0 | 8.5 ± 0.5 | 10.1 ± 0.9 | 8.2 ± 0.2 | 6.7 ± 0.2 |
| 18:1n-9 | 21.4 ± 2.9 | 16.7 ± 2.8 | 11.2 ± 0.3 | 13.5 ± 0.8 |
| 18:1n-7 | 1.5 ± 0.1 | 1.7 ± 0.1 | 0.3 ± tr. | 0.4 ± 0.1 |
| 18:2n-6 | 30.7 ± 0.6 | 28.2 ± 1.5 | 29.7 ± 2.6 | 26.4 ± 1.2 |
| 18:3n-6 | 0.1 ± tr. | 0.1 ± tr. | N.D. | N.D. |
| 18:3n-3 | 0.7 ± 0.2 | 0.5 ± 0.2 | 0.4 ± tr. | 0.3 ± 0.1 |
| 20:0 | 0.2 ± tr. | 0.1 ± tr. | 0.2 ± tr. | 0.1 ± tr. |
| 20:1 | 0.1 ± 0.1 | 0.3 ± 0.1 | N.D. | 0.1 ± 0.1 |
| 20:1 | N.D. | 0.1 ± tr. | N.D. | N.D. |
| 20:1 | 0.4 ± 0.2 | 0.1 ± tr. | 0.2 ± tr. | 0.1 ± tr. |
| 20:2 | 0.2 ± tr. | 0.2 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:3n-6 | 1.0 ± 0.1 | 1.4 ± 0.3 | 0.3 ± tr. | 0.4 ± 0.1 |
| 20:4n-6 | 8.5 ± 1.6 | 13.0 ± 2.7 | 0.4 ± 0.1 | 0.5 ± 0.1 |
| 20:5n-3 | 0.3 ± tr. | 0.4 ± 0.1 | 3.5 ± 0.6 | 3.1 ± 0.2 |
| 22:0 | 0.5 ± 0.3 | 0.2 ± tr. | 0.8 ± 0.3 0.3 | 0.3 ± 0.1 |
| 22:1 | 0.1 ± tr. | N.D. | N.D. | N.D. |
| 22:2 | N.D. | N.D. | N.D. | N.D. |
| 22:4n-6 | 0.1 ± tr. | 0.1 ± tr. | N.D. | N.D. |
| 23:0 | N.D. | N.D. | N.D. | N.D. |
| 22:5n-3 | 0.2 ± tr. | 0.2 ± tr. | 0.4 ± 0.1 | 0.6 ± tr. |
| 22:6n-3 | 2.5 ± 0.4 | 5.1 ± 0.7 | 28.2 ± 2.2 | 27.2 ± 2.0 |
| 24:0 | 0.1 ± tr. | 0.1 ± tr. | 0.2 ± 0.1 | N.D. |
| 24:1 | 0.3 ± 0.1 | 0.3 ± tr. | 0.3 ± 0.1 | 0.3 ± tr. |
| Total SFA | 30.8 ± 0.9 | 30.3 ± 0.3 | 24.6 ± 0.3 | 26.3 ± 1.4 |
| Total MUFA | 24.9 ± 2.9 | 20.4 ± 3.0 | 12.4 ± 0.4 | 15.0 ± 1.0 |
| Total PUFA | 44.3 ± 2.0 | 49.3 ± 3.2 | 63.0 ± 0.2 | 58.7 ± 2.4 |
| Total n-3 FA | 3.7 ± 0.3 | 6.3 ± 0.7 | 32.5 ± 2.8 | 31.2 ± 1.9 |
| Total n-6 FA | 40.4 ± 2.0 | 42.9 ± 2.7 | 30.4 ± 2.6 | 27.4 ± 1.0 |
| n-3/n-6 ratio | 0.09 ± 0.01 | 0.15 ± 0.01 | 1.11 ± 0.20 | 1.14 ± 0.07 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 3

Fatty acid composition of plasma from 21 month-old WT and Tg mice on the oil blend diet or the DHA diet from the age of 4 months. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 3) | WT oil (n = 2) | Tg DHA (n = 3) | WT DHA (n = 3) |
|---|---|---|---|---|
| 12:0 | N.D. | N.D. | N.D. | N.D. |
| 14:0 | 0.2 ± tr. | 0.2 ± 0.1 | 0.4 ± 0.1 | 0.5 ± tr. |
| 16:0 | 18.6 ± 1.1 | 17.6 ± 2.0 | 21.4 ± 0.1 | 21.4 ± 0.7 |
| 16:1n-7 | 1.0 ± 0.2 | 1.2 ± tr. | 1.2 ± 0.1 | 1.1 ± 0.1 |
| 18:0 | 12.7 ± 0.2 | 14.7 ± 1.8 | 9.1 ± 0.3 | 10.3 ± 0.3 |
| 18:1n-9 | 21.9 ± 0.9 | 18.9 ± 0.6 | 15.1 ± 0.2 | 16.4 ± 0.6 |
| 18:1n-7 | 1.7 ± 0.4 | 1.8 ± 0.2 | 0.7 ± tr. | 1.0 ± 0.2 |
| 18:2n-6 | 25.4 ± 2.01 | 21.1 ± 3.3 | 27.2 ± 0.8 | 24.7 ± 0.7 |
| 18:3n-6 | 0.1 ± tr. | 0.1 ± tr. | N.D. | N.D. |
| 18:3n-3 | 0.4 ± tr. | 0.3 ± tr. | 0.6 ± tr. | 0.6 ± tr. |
| 20:0 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:1 | 0.2 ± 0.1 | N.D. | N.D. | N.D. |
| 20:1 | 0.2 ± 0.1 | 0.2 ± tr. | 0.2 ± 0.1 | 0.2 ± 0.1 |
| 20:1 | 0.1 ± tr. | 0.1 ± tr. | 0.2 ± 0.1 | 0.1 ± 0.1 |
| 20:2 | 0.2 ± tr. | 0.2 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:3n-6 | 1.0 ± 0.1 | 1.1 ± 0.4 | 0.4 ± tr. | 0.5 ± 0.1 |
| 20:4n-6 | 11.8 ± 1.3 | 17.2 ± 3.2 | 0.6 ± tr. | 1.3 ± 0.2 |
| 20:5n-3 | 0.2 ± tr. | 0.2 ± tr. | 3.3 ± 0.4 | 3.1 ± 0.4 |
| 22:0 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 22:1 | N.D. | N.D. | N.D. | N.D. |
| 22:2 | N.D. | N.D. | N.D. | N.D. |
| 22:4n-6 | 0.1 ± tr. | 0.2 ± tr. | N.D. | N.D. |
| 23:0 | 0.1 ± tr. | N.D. | N.D. | N.D. |
| 22:5n-3 | 0.1 ± tr. | 0.1 ± tr. | 0.5 ± tr. | 0.6 ± 0.1 |
| 22:6n-3 | 3.6 ± 0.6 | 4.1 ± 0.4 | 18.4 ± 0.2 | 17.4 ± 0.7 |
| 24:0 | N.D. | N.D. | N.D. | N.D. |
| 24:1 | 0.3 ± tr. | 0.3 ± 0.1 | 0.3 ± 0.1 | 0.3 ± tr. |
| Total SFA | 31.7 ± 1.0 | 32.8 ± 0.3 | 31.2 ± 0.2 | 32.4 ± 1.0 |
| Total MUFA | 25.4 ± 1.5 | 22.5 ± 0.6 | 17.6 ± 0.3 | 19.2 ± 0.9 |
| Total PUFA | 43.0 ± 0.5 | 44.7 ± 0.2 | 51.2 ± 0.4 | 48.4 ± 0.2 |
| Total n-3 FA | 4.4 ± 0.5 | 4.8 ± 0.3 | 22.9 ± 0.7 | 21.7 ± 1.1 |
| Total n-6 FA | 38.4 ± 0.7 | 39.7 ± 0.5 | 28.3 ± 0.7 | 26.5 ± 0.9 |
| n-3/n-6 ratio | 0.11 ± 0.02 | 0.12 ± 0.01 | 0.81 ± 0.04 | 0.82 ± 0.07 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 4

Fatty acid composition of cortex from 12 month-old WT and Tg mice on the oil blend diet or the DHA diet. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 6) | WT oil (n = 6) | Tg DHA (n = 6) | WT DHA (n = 6) |
|---|---|---|---|---|
| 12:0 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.3 ± 0.2 | 0.1 ± tr. |
| X1 | 0.8 ± 0.1 | 0.9 ± 0.1 | 1.0 ± 0.2 | 0.8 ± 0.1 |
| 16:0 | 19.8 ± 0.3 | 19.7 ± 0.4 | 19.8 ± 0.3 | 20.3 ± 0.4 |
| 16:1n-7 | 0.7 ± tr. | 0.6 ± tr. | 0.7 ± tr. | 0.7 ± tr. |
| X2 | 0.6 ± 0.1 | 0.7 ± 0.2 | 0.6 ± 0.1 | 0.5 ± 0.1 |
| X3 | 0.3 ± 0.1 | 0.3 ± 0.1 | 0.3 ± 0.1 | 0.2 ± tr. |
| 18:0 | 21.8 ± 0.1 | 21.7 ± 0.1 | 21.1 ± 0.1 | 21.7 ± 0.4 |
| 18:1n-9 | 13.7 ± 0.2 | 13.7 ± 0.2 | 14.5 ± 0.1 | 14.9 ± 0.2 |
| 18:1n-7 | 3.9 ± 0.1 | 3.9 ± 0.1 | 3.8 ± 0.1 | 3.8 ± 0.1 |
| 18:2n-6 | 0.6 ± tr. | 0.6 ± tr. | 0.8 ± tr. | 0.7 ± tr. |
| 18:3n-6 | N.D. | N.D. | N.D. | N.D. |
| 18:3n-3 | N.D. | 0.1 ± tr. | N.D. | N.D. |
| 20:0 | 0.2 ± tr. | 0.2 ± tr. | 0.2 ± tr. | 0.2 ± tr. |
| 20:1 | 1.0 ± 0.1 | 1.0 ± 0.1 | 0.9 ± tr. | 1.0 ± tr. |
| 20:1 | 0.3 ± tr. | 0.3 ± tr. | 0.3 ± tr. | 0.3 ± tr. |
| 20:1 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:2 | 0.1 ± tr. | 0.1 ± tr. | 0.2 ± tr. | 0.2 ± tr. |
| 20:3n-6 | 0.4% ± tr. | 0.4 ± tr. | 1.1 ± tr. | 1.0 ± tr. |
| 20:4n-6 | 10.9 ± 0.1 | 10.8 ± 0.2 | 7.0 ± 0.1 | 6.7 ± 0.1 |
| 20:3n-3 | N.D. | 0.1 ± tr. | N.D. | N.D. |
| 20:5n-3 | N.D. | N.D. | 0.3 ± tr. | 0.3 ± tr. |
| 22:0 | 0.2 ± tr. | 0.2 ± tr. | 0.2 ± tr. | 0.2 ± tr. |
| 22:1 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 22:2 | N.D. | N.D. | 0.1 ± tr. | 0.1 ± tr. |
| 22:4n-6 | 2.9 ± 0.1 | 2.8 ± 0.1 | 1.2 ± tr. | 1.1 ± 0.1 |
| 23:0 | 0.4 ± 0.1 | 0.2 ± 0.1 | 0.2 ± tr. | 0.2 ± tr. |
| 22:5n-3 | 0.1 ± tr. | 0.1 ± tr. | 0.4 ± tr. | 0.3 ± tr. |
| 22:6n-3 | 19.8 ± 0.4 | 19.7 ± 0.3 | 23.7 ± 0.5 | 23.0 ± 0.9 |
| 24:0 | 0.3 ± tr. | 0.2 ± 0.1 | 0.3 ± tr. | 0.3 ± tr. |
| 24:1 | 1.1 ± 0.1 | 1.3 ± 0.1 | 1.1 ± 0.1 | 1.2 ± 0.1 |
| Total SAT | 42.7 ± 0.3 | 42.5 ± 0.3 | 42.0 ± 0.4 | 42.9 ± 0.8 |
| Total MUFA | 20.8 ± 0.3 | 21.1 ± 0.3 | 21.4 ± 0.3 | 22.0 ± 0.2 |
| Total PUFA | 34.8 ± 0.4 | 34.7 ± 0.4 | 34.7 ± 0.5 | 33.5 ± 1.0 |
| Total n-3 FA | 19.9 ± 0.4 | 19.9 ± 0.3 | 24.5 ± 0.5 | 23.8 ± 1.0 |
| Total n-6 FA | 14.7 ± 0.1 | 14.6 ± 0.3 | 10.0 ± 0.2 | 9.5 ±.0.2 |
| n-3/n-6 ratio | 1.35 ± 0.02 | 1.37 ± 0.04 | 2.45 ± 0.06 | 2.52 ± 0.1 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 5

Fatty acid composition of hippocampus from 12 month-old WT and Tg mice on the oil blend diet or the DHA diet. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 6) | WT oil (n = 6) | Tg DHA (n = 6) | WT DHA (n = 6) |
|---|---|---|---|---|
| 12:0 | N.D. | N.D. | N.D. | N.D. |
| X1 | 1.8 ± 0.2 | 1.2 ± 0.1 | 1.0 ± 0.3 | 1.3 ± 0.1 |
| 16:0 | 16.5 ± 0.2 | 16.2 ± 0.2 | 16.6 ± 0.3 | 16.6 ± 0.3 |
| 16:1n-7 | 0.5 ± tr. | 0.5 ± tr. | 0.6 ± tr. | 0.6 ± tr. |
| X2 | 1.6 ± 0.1 | 1.4 ± 0.2 | 0.8 ± 0.3 | 1.3 ± 0.2 |
| X3 | 0.9 ± tr. | 0.8 ± 0.1 | 0.6 ± 0.1 | 0.8 ± 0.1 |
| 18:0 | 21.7 ± 0.1 | 22.1 ± 0.3 | 21.9 ± 0.2 | 21.5 ± 0.1 |
| 18:1n-9 | 14.1 ± 0.3 | 14.1 ± 0.3 | 15.4 ± 0.2 | 15.4 ± 0.3 |
| 18:1n-7 | 3.6 ± tr. | 3.7 ± 0.1 | 3.5 ± 0.1 | 3.5 ± 0.1 |
| 18:2n-6 | 0.6 ± tr. | 0.6 ± tr. | 0.8 ± tr. | 0.7 ± tr. |
| 18:3n-6 | 0.1 ± tr. | N.D. | N.D. | N.D. |
| 18:3n-3 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:0 | 0.3 ± tr. | 0.3 ± tr. | 0.3 ± tr. | 0.3 ± tr. |
| 20:1 | 1.2 ± 0.1 | 1.2 ± 0.1 | 1.1 ± 0.1 | 1.1 ± 0.1 |
| 20:1 | 0.3 ± tr. | 0.3 ± tr. | 0.3 ± tr. | 0.3 ± tr. |
| 20:1 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:2 | 0.1 ± tr. | 0.1 ± tr. | 0.2 ± tr. | 0.2 ± tr. |
| 20:3n-6 | 0.4 ± tr. | 0.4 ± tr. | 1.0 ± tr. | 0.9 ± tr. |
| 20:4n-6 | 12.3 ± 0.2 | 12.3 ± 0.2 | 8.3 ± 0.1 | 7.9 ± 0.2 |
| 20:3n-3 | 0.1 ± tr. | 0.1 ± tr. | N.D. | 0.1 ± tr. |
| 20:5n-3 | N.D. | N.D. | 0.4 ± tr. | 0.4 ± tr. |
| 22:0 | 0.3 ± tr. | 0.3 ± tr. | 0.3 ± tr. | 0.3 ± tr. |
| 22:1 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 22:2 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 22:4n-6 | 3.2 ± 0.1 | 3.1 ± 0.1 | 1.5 ± 0.1 | 1.4 ± 0.1 |
| 23:0 | 0.2 ± tr. | 0.2 ± tr. | 0.2 ± tr. | 0.2 ± tr. |
| 22:5n-3 | 0.1 ± tr. | 0.1 ± tr. | 0.5 ± tr. | 0.4 ± tr. |
| 22:6n-3 | 17.3 ± 0.2 | 18.4 ± 0.4 | 22.1 ± 0.6 | 22.0 ± 0.4 |
| 24:0 | 0.4 ± 0.1 | 0.5 ± 0.1 | 0.4 ± tr. | 0.4 ± 0.1 |
| 24:1 | 1.9 ± 0.2 | 1.9 ± 0.1 | 1.9 ± 0.2 | 2.0 ± 0.1 |
| Total SAT | 39.4 ± 0.7 | 39.5 ± 0.9 | 39.7 ± 0.8 | 39.3 ± 0.8 |
| Total MUFA | 21.8 ± 1.4 | 22.0 ± 1.4 | 22.9 ± 1.1 | 23.1 ± 1.2 |
| Total PUFA | 34.4 ± 0.8 | 35.2 ± 1.3 | 35.0 ± 1.5 | 34.2 ± 0.8 |
| Total n-3 FA | 17.6 ± 0.4 | 18.7 ± 0.9 | 23.1 ± 1.5 | 23.0 ± 0.9 |
| Total n-6 FA | 16.6 ± 0.5 | 16.3 ± 0.6 | 11.6 ± 0.5 | 10.9 ± 0.5 |
| n-3/n-6 ratio | 1.06 ± 0.03 | 1.14 ± 0.05 | 2.00 ± 0.16 | 2.11 ± 0.15 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 6

Fatty acid composition of cerebellum from 12 month-old WT and Tg mice on the oil blend diet or the DHA diet. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 6) | WT oil (n = 6) | Tg DHA (n = 6) | WT DHA (n = 6) |
|---|---|---|---|---|
| 12:0 | N.D. | N.D. | N.D. | N.D. |
| X1 | 0.9 ± 0.2 | 1.0 ± 0.2 | 0.9 ± 0.2 | 1.0 ± 0.2 |
| 16:0 | 15.4 ± 0.4 | 15.5 ± 0.2 | 16.4 ± 0.2 | 16.1 ± 0.5 |
| 16:1n-7 | 0.5 ± 0.1 | 0.4 ± tr. | 0.6 ± tr. | 0.6 ± tr. |
| X2 | 0.9 ± 0.2 | 1.3 ± 0.1 | 1.0 ± 0.2 | 1.0 ± 0.1 |
| X3 | 0.6 ± 0.1 | 0.7 ± 0.1 | 0.6 ± 0.1 | 0.7 ± 0.1 |
| 18:0 | 19.4 ± 0.2 | 19.5 ± 0.1 | 18.6 ± 0.1 | 18.6 ± 0.1 |
| 18:1n-9 | 18.2 ± 0.1 | 18.4 ± 0.3 | 19.8 ± 0.3 | 20.0 ± 0.3 |
| 18:1n-7 | 4.3 ± 0.1 | 4.5 ± 0.1 | 4.2 ± tr. | 4.3 ± tr. |
| 18:2n-6 | 0.7 ± tr. | 0.6 ± tr. | 0.9 ± 0.1 | 0.8 ± tr. |
| 18:3n-6 | N.D. | N.D. | N.D. | N.D. |
| 18:3n-3 | 0.1 ± 0.1 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:0 | 0.5 ± tr. | 0.5 ± tr. | 0.4 ± tr. | 0.5 ± tr. |
| 20:1 | 3.6 ± 0.2 | 3.6 ± 0.1 | 3.2 ± 0.1 | 3.5 ± 0.1 |
| 20:1 | 0.6 ± tr. | 0.6 ± tr. | 0.5 ± tr. | 0.6 ± tr. |
| 20:1 | 0.4 ± 0.2 | 0.2 ± 0.1 | 0.1 ± tr. | 0.2 ± 0.1 |
| 20:2 | 0.2 ± tr. | 0.2 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:3n-6 | 0.4 ± tr. | 0.4 ± tr. | 0.8 ± tr. | 0.8 ± tr. |
| 20:4n-6 | 7.2 ± 0.1 | 6.9 ± 0.1 | 3.5 ± 0.1 | 3.3 ± 0.1 |
| 20:3n-3 | 0.2 ± 0.1 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:5n-3 | N.D. | N.D. | 0.4 ± tr. | 0.3 ± tr/ |
| 22:0 | 0.9 ± 0.2 | 0.8 ± 0.1 | 0.8 ± 0.2 | 0.9 ± 0.1 |
| 22:1 | 0.3 ± tr. | 0.4 ± tr. | 0.4 ± 0.1 | 0.3 ± tr. |
| 22:2 | N.D. | N.D. | N.D. | N.D. |
| 22:4n-6 | 1.9 ± 0.1 | 1.8 ± tr. | 0.6 ± 0.1 | 0.5 ± tr. |
| 23:0 | 0.9 ± 0.2 | 0.7 ± tr. | 0.5 ± tr. | 0.6 ± 0.1 |
| 22:5n-3 | 0.1 ± tr. | 0.1 ± tr. | 0.3 ± tr. | 0.3 ± tr. |
| 22:6n-3 | 16.2 ± 0.5 | 15.8 ± 0.3 | 19.8 ± 0.5 | 18.7 ± 0.7 |
| 24:0 | 1.3 ± 0.1 | 1.2 ± 0.1 | 1.2 ± tr. | 1.4 ± 0.1 |
| 24:1 | 4.3 ± 0.2 | 4.5 ± 0.1 | 4.1 ± 0.2 | 4.5 ± 0.2 |
| Total SAT | 38.3 ± 0.2 | 38.3 ± 0.1 | 37.9 ± 0.2 | 38.2 ± 0.4 |
| Total MUFA | 32.2 ± 0.4 | 32.6 ± 0.4 | 32.8 ± 0.4 | 34.0 ± 0.5 |
| Total PUFA | 27.0 ± 0.6 | 26.1 ± 0.4 | 29.1 ± 0.7 | 25.1 ± 0.7 |
| Total n-3 FA | 16.7 ± 0.5 | 16.1 ± 0.3 | 20.7 ± 0.5 | 19.6 ± 0.7 |
| Total n-6 FA | 10.1 ± 0.1 | 9.8 ± 0.1 | 5.8 ± 0.3 | 5.4 ± 0.2 |
| n-3/n-6 ratio | 1.65 ± 0.02 | 1.65 ± 0.03 | 3.57 ± 0.15 | 3.68 ± 0.19 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 7

Fatty acid composition of cortex from 16 month-old WT and Tg mice on the oil blend diet or the DHA diet. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
|---|---|---|---|---|
| 12:0 | N.D. | N.D. | N.D. | N.D. |
| X1 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 16:0 | 19.2 ± 0.7 | 20.0 ± 1.0 | 20.4 ± 0.6 | 19.2 ± 0.1 |
| 16:1n-7 | 0.3 ± tr. | 0.3 ± tr. | 0.4 ± tr. | 0.3 ± tr. |
| X2 | 0.2 ± tr. | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.2 ± tr. |
| X3 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 18:0 | 23.9 ± 0.2 | 23.7 ± 0.3 | 23.6 ± 0.1 | 23.5 ± 0.2 |
| 18:1n-9 | 14.7 ± 0.2 | 14.4 ± 0.1 | 15.6 ± 0.3 | 16.0 ± 0.3 |
| 18:1n-7 | 3.4 ± tr.. | 3.3 ± 0.1 | 3.0 ± 0.1 | 3.1 ± 0.1 |
| 18:2n-6 | 0.6 ± tr. | 0.4 ± tr. | 0.6 ± tr. | 0.5 ± tr. |
| 18:3n-6 | N.D. | N.D. | N.D. | N.D. |
| 18:3n-3 | N.D. | N.D. | N.D. | N.D. |
| 20:0 | 0.2 ± tr. | 0.1 ± tr. | 0.2 ± tr. | 0.2 ± tr. |
| 20:1 | 0.7 ± tr. | 0.6 ± 0.1 | 0.7 ± tr. | 0.7 ± tr. |
| 20:1 | 0.2 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.2 ± tr. |
| 20:1 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | N.D. |
| 20:2 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:3n-6 | 0.3 ± tr. | 0.3 ± tr. | 0.9 ± tr. | 0.8 ± tr. |
| 20:4n-6 | 11.2 ± 0.1 | 11.0 ± 0.4 | 5.6 ± 0.2 | 6.6 ± 0.2 |
| 20:3n-3 | N.D. | N.D. | N.D. | N.D. |
| 20:5n-3 | N.D. | N.D. | 0.4 ± tr. | 0.3 ± tr. |
| 22:0 | 0.2 ± tr. | 0.2 ± tr. | 0.2 ± tr. | 0.2 ± tr. |
| 22:1 | 0.1 ± tr. | N.D. | N.D. | N.D. |
| 22:2 | N.D. | N.D. | N.D. | N.D. |
| 22:4n-6 | 2.7 ± tr. | 2.5 ± tr. | 0.8 ± tr. | 1.0 ± tr. |
| 23:0 | 0.2 ± tr. | 0.2 ± tr. | N.D. | N.D. |
| 22:5n-3 | 0.1 ± tr. | 0.1 ± tr. | 0.4 ± tr. | 0.3 ± tr. |
| 22:6n-3 | 20.3 ± 0.7 | 21.0 ± 0.8 | 25.5 ± 0.1 | 25.3 ± 0.4 |
| 24:0 | 0.2 ± tr. | 0.1 ± 0.1 | 0.2 ± tr. | 0.2 ± tr. |
| 24:1 | 0.9 ± tr. | 0.9 ± 0.1 | 0.9 ± 0.1 | 1.0 ± tr. |
| Total SAT | 43.9 ± 0.5 | 44.3 ± 0.7 | 44.6 ± 0.6 | 43.2 ± 0.2 |
| Total MUFA | 20.3 ± 0.3 | 19.8 ± 0.3 | 20.8 ± 0.5 | 21.3 ± 0.3 |
| Total PUFA | 35.4 ± 0.7 | 35.5 ± 1.2 | 34.3 ± 0.3 | 35.0 ± 0.2 |
| Total n-3 FA | 20.5 ± 0.7 | 21.1 ± 0.8 | 26.2 ± tr. | 26.0 ± 0.4 |
| Total n-6 FA | 14.8 ± 0.1 | 14.3 ± 0.4 | 7.9 ± 0.3 | 8.9 ± 0.2 |
| n-3/n-6 ratio | 1.38 ± 0.04 | 1.48 ± 0.04 | 3.32 ± 0.13 | 2.93 ± 0.11 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 8

Fatty acid composition of hippocampus from 16 month-old WT and Tg mice on the oil blend diet or the DHA diet. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
|---|---|---|---|---|
| 12:0 | N.D. | N.D. | 0.1 ± tr. | 0.1 ± 0.1 |
| X1 | 0.4 ± tr. | 0.4 ± tr. | 0.4 ± 0.1 | 0.6 ± 0.1 |
| 16:0 | 16.9 ± 0.2 | 18.0 ± 0.9 | 16.7 ± 0.6 | 17.8 ± 0.3 |
| 16:1n-7 | 0.3 ± tr. | 0.2 ± 0.1 | 0.3 ± tr. | 0.2 ± 0.1 |
| X2 | 0.9 ± 0.1 | 0.6 ± 0.1 | 0.9 ± 0.2 | 0.8 ± 0.3 |
| X3 | 0.5 ± 0.1 | 0.3 ± tr. | 0.4 ± 0.1 | 0.4 ± 0.1 |
| 18:0 | 25.4 ± 0.1 | 25.0 ± 0.1 | 24.9 ± 0.1 | 25.0 ± 0.2 |
| 18:1n-9 | 16.0 ± 0.1 | 16.2 ± 0.3 | 17.2 ± 0.3 | 17.3 ± 0.3 |
| 18:1n-7 | 3.1 ± 0.1 | 3.2 ± 0.1 | 2.7 ± 0.1 | 2.8 ± 0.1 |
| 18:2n-6 | 0.6 ± tr. | 0.5 ± tr. | 0.7 ± tr. | 0.7 ± tr. |
| 18:3n-6 | N.D. | N.D. | N.D. | N.D. |
| 18:3n-3 | 0.1 ± tr. | N.D. | 0.1 ± tr. | N.D. |
| 20:0 | 0.2 ± tr. | 0.2 ± tr. | 0.2 ± tr. | 0.1 ± 0.1 |
| 20:1 | 0.9 ± tr. | 0.6 ± 0.3 | 0.7 ± tr. | 0.5 ± 0.2 |
| 20:1 | 0.2 ± tr. | 0.1 ± 0.1 | 0.1 ± tr. | 0.1 ± tr. |
| 20:1 | 0.1 ± 0.1 | 0.3 ± 0.2 | 0.2 ± tr. | 0.1 ± 0.1 |
| 20:2 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.3 ± 0.2 |
| 20:3n-6 | 0.3 ± tr. | 0.3 ± tr. | 0.7 ± tr. | 0.8 ± tr. |
| 20:4n-6 | 12.4 ± tr. | 12.0 ± 0.6 | 7.2 ± 0.1 | 7.5 ± 0.1 |
| 20:3n-3 | N.D. | N.D. | N.D. | N.D. |
| 20:5n-3 | N.D. | N.D. | 0.4 ± tr. | 0.4 ± tr. |
| 22:0 | 0.2 ± 0.1 | 0.2 ± tr. | 0.2 ± tr. | 0.1 ± 0.1 |
| 22:1 | 0.1 ± 0.1 | 0.1 ± tr. | N.D. | N.D. |
| 22:2 | N.D. | N.D. | N.D. | N.D. |
| 22:4n-6 | 3.1 ± 0.1 | 2.8 ± 0.1 | 1.0 ± tr. | 1.1 ± tr. |
| 23:0 | 0.2 ± 0.1 | 0.1 ± 0.1 | N.D. | N.D. |
| 22:5n-3 | N.D. | N.D. | 0.4 ± tr. | 0.2 ± 0.1 |
| 22:6n-3 | 16.8 ± 0.4 | 16.9 ± 0.6 | 22.8 ± 0.1 | 22.0 ± 0.5 |
| 24:0 | 0.2 ± 0.1 | 0.2 ± tr. | 0.2 ± 0.1 | N.D. |
| 24:1 | 1.3 ± 0.1 | 1.4 ± 0.1 | 1.3 ± 0.1 | 1.1 ± 0.1 |
| Total SAT | 43.0 ± 0.3 | 43.9 ± 0.9 | 42.3 ± 0.6 | 43.1 ± 0.5 |
| Total MUFA | 21.9 ± 0.3 | 22.1 ± 0.6 | 22.5 ± 0.5 | 22.0 ± 0.3 |
| Total PUFA | 33.3 ± 0.4 | 32.7 ± 1.3 | 33.5 ± 0.1 | 33.0 ± 0.3 |
| Total n-3 FA | 16.8 ± 0.4 | 17.0 ± 0.6 | 23.7 ± tr. | 22.6 ± 0.6 |
| Total n-6 FA | 16.4 ± 0.1 | 15.6 ± 0.6 | 9.7 ± 0.1 | 10.0 ± 0.2 |
| n-3/n-6 ratio | 1.02 ± 0.02 | 1.09 ± 0.02 | 2.44 ± 0.02 | 2.26 ± 0.08 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 9

Fatty acid composition of cerebellum from 16 month-old WT and Tg mice on the oil blend diet or the DHA diet. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
|---|---|---|---|---|
| 12:0 | 0.1 ± tr. | 0.1 ± tr. | N.D. | N.D. |
| X1 | 0.5 ± 0.1 | 0.5 ± 0.1 | 0.2 ± 0.1 | 0.4 ± tr. |
| 16:0 | 16.0 ± 0.5 | 17.4 ± 0.9 | 18.1 ± 0.8 | 18.6 ± 0.2 |
| 16:1n-7 | 0.2 ± 0.1 | 0.2 ± tr. | 0.4 ± tr. | 0.4 ± tr. |
| X2 | 0.7 ± tr. | 0.8 ± 0.1 | 0.3 ± 0.1 | 0.7 ± 0.1 |
| X3 | 0.5 ± tr. | 0.5 ± 0.1 | 0.2 ± 0.1 | 0.5 ± 0.1 |
| 18:0 | 22.2 ± 0.1 | 22.5 ± 0.2 | 21.4 ± 0.2 | 21.0 ± 0.2 |
| 18:1n-9 | 22.2 ± 0.7 | 20.8 ± 0.3 | 22.3 ± 0.5 | 23.0 ± 0.9 |
| 18:1n-7 | 4.6 ± tr. | 4.5 ± 0.1 | 4.2 ± 0.2 | 4.4 ± 0.2 |
| 18:2n-6 | 0.7 ± tr. | 0.6 ± tr. | 0.7 ± tr. | 0.6 ± tr. |
| 18:3n-6 | N.D. | N.D. | N.D. | 0.1 ± tr. |
| 18:3n-3 | N.D. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:0 | 0.5 ± 0.1 | 0.4 ± tr. | 0.4 ± tr. | 0.3 ± tr. |
| 20:1 | 3.7 ± 0.3 | 3.0 ± 0.1 | 2.7 ± 0.1 | 2.6 ± 0.2 |
| 20:1 | 0.4 ± 0.1 | 0.4 ± tr. | 0.3 ± tr. | 0.3 ± tr. |
| 20:1 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.1 ± tr. | 0.1 ± tr. |
| 20:2 | 0.1 ± 0.1 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:3n-6 | 0.3 ± tr. | 0.3 ± tr. | 0.5 ± tr. | 0.5 ± tr. |
| 20:4n-6 | 6.4 ± 0.3 | 6.5 ± 0.3 | 2.4 ± 0.2 | 2.8 ± 0.1 |
| 20:3n-3 | N.D. | N.D. | N.D. | N.D. |
| 20:5n-3 | N.D. | N.D. | 0.4 ± tr. | 0.3 ± tr. |
| 22:0 | 0.3 ± 0.2 | 0.4 ± tr. | 0.4 ± tr. | 0.4 ± tr. |
| 22:1 | 0.3 ± 0.1 | 0.1 ± 0.1 | 0.1 ± tr. | 0.2 ± tr. |
| 22:2 | N.D. | 0.1 ± tr. | N.D. | 0.1 ± tr. |
| 22:4n-6 | 1.8 ± 0.2 | 1.6 ± 0.1 | 0.6 ± tr. | 0.7 ± 0.1 |
| 23:0 | N.D. | N.D. | N.D. | N.D. |
| 22:5n-3 | N.D. | N.D. | 0.3 ± tr. | 0.2 ± tr. |
| 22:6n-3 | 13.9 ± 0.9 | 15.5 ± 0.5 | 19.9 ± 0.8 | 18.0 ± 1.1 |
| 24:0 | 0.8 ± 0.1 | 0.6 ± 0.1 | 0.8 ± tr. | 0.7 ± 0.1 |
| 24:1 | 3.6 ± 0.3 | 3.0 ± 0.1 | 3.0 ± 0.2 | 3.0 ± 0.3 |
| Total SAT | 39.9 ± 0.5 | 41.4 ± 0.7 | 41.1 ± 0.7 | 41.0 ± tr. |
| Total MUFA | 35.0 ± 1.5 | 32.1 ± 0.2 | 33.1 ± 0.8 | 33.9 ± 1.5 |
| Total PUFA | 23.4 ± 1.1 | 24.8 ± 0.9 | 25.0 ± 1.0 | 23.4 ± 1.2 |
| Total n-3 FA | 13.9 ± 0.9 | 15.6 ± 0.6 | 20.7 ± 0.8 | 18.6 ± 1.2 |
| Total n-6 FA | 9.3± 0.1 | 9.0 ± 0.4 | 4.2 ± 0.3 | 4.7 ± 0.1 |
| n-3/n-6 ratio | 1.50 ± 0.08 | 1.73 ± 0.04 | 4.93 ± 0.16 | 3.95 ± 0.22 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 10

Fatty acid composition of phosphatidylethanolamine (PE) from cortex of 12 month-old WT and Tg mice on the oil blend diet or the DHA diet. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 4) | WT oil (n = 4) | Tg DHA (n = 4) | WT DHA (n = 4) |
|---|---|---|---|---|
| 12:0 | N.D. | N.D. | N.D. | N.D. |
| X1 | 2.1 ± 0.4 | 2.1 ± 0.3 | 1.5 ± 0.5 | 2.0 ± 0.3 |
| 16:0 | 4.6 ± 0.2 | 5.4 ± 0.6 | 5.5 ± 0.8 | 5.1 ± 0.3 |
| 16:1n-7 | 0.3 ± 0.1 | 0.4 ± 0.1 | 0.4 ± 0.2 | 0.3 ± 0.1 |
| X2 | 5.4 ± 0.5 | 5.0 ± 1.2 | 4.6 ± 1.0 | 5.5 ± 0.5 |
| X3 | 2.2 ± 0.2 | 2.1 ± 0.5 | 2.0 ± 0.5 | 2.4 ± 0.1 |
| 18:0 | 22.1 ± 1.0 | 21.7 ± 0.5 | 21.9 ± 0.7 | 21.6 ± 0.5 |
| 18:1n-9 | 8.6 ± 0.2 | 8.7 ± 0.2 | 9.6 ± 0.2 | 10.1 ± 0.3 |
| 18:1n-7 | 1.8 ± 0.1 | 1.8 ± tr. | 1.6 ± tr. | 1.7 ± 0.1 |
| 18:2n-6 | 0.4 ± tr. | 0.3 ± tr. | 0.5 ± 0.1 | 0.4 ± tr. |
| 18:3n-6 | N.D. | N.D. | N.D. | N.D. |
| 18:3n-3 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:0 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:1 | 1.5 ± 0.1 | 1.9 ± 0.1 | 1.5 ± 0.1 | 1.7 ± 0.1 |
| 20:1 | 0.2 ± 0.1 | 0.3 ± tr. | 0.2 ± 0.1 | 0.2 ± 0.1 |
| 20:1 | 0.2 ± tr. | 0.2 ± tr. | 0.2 ± tr. | 0.2 ± tr. |
| 20:2 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:3n-6 | 0.4 ± tr. | 0.3 ± tr. | 1.1 ± 0.1 | 0.9 ± tr. |
| 20:4n-6 | 13.3 ± 0.4 | 13.0 ± 0.4 | 7.7 ± 0.2 | 7.4 ± 0.2 |
| 20:3n-3 | 0.1 ± 0.1 | N.D. | 0.1 ± tr. | N.D. |
| 20:5n-3 | N.D. | N.D. | 0.5 ± tr. | 0.5 ± tr. |
| 22:0 | N.D. | N.D. | N.D. | N.D. |
| 22:1 | N.D. | 0.1 ± tr. | N.D. | N.D. |
| 22:2 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 22:4n-6 | 5.6 ± 0.2 | 5.6 ± 0.3 | 2.0 ± tr. | 1.9 ± 0.1 |
| 23:0 | 0.6 ± 0.1 | 0.4 ± 0.1 | N.D. | N.D. |
| 22:5n-3 | 0.2 ± tr. | 0.2 ± tr. | 0.8 ± tr. | 0.8 ± tr. |
| 22:6n-3 | 30.1 ± 0.8 | 30.1 ± 0.6 | 37.9 ± 0.7 | 37.1 ± 0.6 |
| 24:0 | N.D. | N.D. | N.D. | N.D. |
| 24:1 | N.D. | N.D. | N.D. | N.D. |
| Total SAT | 27.5 ± 1.0 | 27.7 ± 0.7 | 27.5 ± 1.3 | 26.8 ± 0.2 |
| Total MUFA | 12.6 ± 0.3 | 13.4 ± 0.4 | 13.6 ± 0.1 | 14.1 ± 0.5 |
| Total PUFA | 50.3 ± 0.6 | 49.8 ± 1.0 | 50.8 ± 0.7 | 49.2 ± 0.3 |
| Total n-3 FA | 30.5 ± 0.8 | 30.4 ± 0.6 | 39.4 ± 0.8 | 38.4 ± 0.6 |
| Total n-6 FA | 19.6 ± 0.4 | 19.3 ± 0.7 | 11.3 ± 0.2 | 10.6 ± 0.3 |
| n-3/n-6 ratio | 1.56 ± 0.07 | 1.58 ± 0.06 | 3.50 ± 0.11 | 3.64 ± 0.16 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 11

Fatty acid composition of phosphatidylethanolamine (PE) from cortex of 16 month-old WT and Tg mice on the oil blend diet or the DHA diet. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
|---|---|---|---|---|
| 12:0 | N.D. | N.D. | N.D. | N.D. |
| X1 | 2.7 ± 0.4 | 3.1 ± 0.4 | 3.3 ± 0.2 | 3.7 ± 0.3 |
| 16:0 | 7.1 ± 0.5 | 7.1 ± 0.5 | 7.0 ± 0.5 | 7.6 ± 0.7 |
| 16:1n-7 | 0.3 ± tr. | 0.3 ± 0.1 | 0.3 ± tr. | 0.4 ± 0.1 |
| X2 | 4.4 ± 0.7 | 5.3 ± 0.2 | 5.9 ± 0.3 | 5.7 ± tr. |
| X3 | 2.2 ± 0.2 | 2.0 ± 0.1 | 2.2 ± 0.3 | 2.0 ± 0.2 |
| 18:0 | 27.8 ± 0.3 | 26.9 ± 0.3 | 25.7 ± 0.4 | 26.3 ± 0.2 |
| 18:1n-9 | 9.6 ± 0.3 | 9.2 ± 0.3 | 10.6 ± 0.4 | 11.0 ± 0.2 |
| 18:1n-7 | 2.2 ± 0.2 | 2.0 ± 0.2 | 1.7 ± 0.4 | 1.9 ± 0.2 |
| 18:2n-6 | 0.2 ± tr. | 0.2 ± tr. | 0.3 ± tr. | 0.3 ± tr. |
| 18:3n-6 | N.D. | N.D. | N.D. | N.D. |
| 18:3n-3 | N.D. | N.D. | 0.1 ± tr. | N.D. |
| 20:0 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:1 | 0.8 ± 0.4 | 0.8 ± 0.4 | 0.7 ± 0.4 | 0.8 ± 0.4 |
| 20:1 | 0.1 ± tr. | 0.1 ± tr. | N.D. | 0.1 ± tr. |
| 20:1 | 0.4 ± 0.4 | 0.5 ± 0.4 | 0.4 ± 0.3 | 0.4 ± 0.3 |
| 20:2 | 0.1 ± tr. | 0.1 ± tr. | 0.2 ± tr. | 0.2 ± tr. |
| 20:3n-6 | 0.2 ± tr. | 0.2 ± tr. | 0.7 ± tr. | 0.6 ± tr. |
| 20:4n-6 | 12.1 ± 0.1 | 11.9 ± 0.5 | 5.1 ± 0.3 | 6.1 ± 0.2 |
| 20:3n-3 | N.D. | N.D. | 0.1 ± tr. | N.D. |
| 20:5n-3 | N.D. | N.D. | 0.4 ± tr. | 0.3 ± tr. |
| 22:0 | N.D. | N.D. | N.D. | N.D. |
| 22:1 | N.D. | N.D. | N.D. | N.D. |
| 22:2 | N.D. | N.D. | 0.1 ± tr. | N.D. |
| 22:4n-6 | 4.1 ± 0.1 | 3.8 ± 0.1 | 0.9 ± 0.1 | 1.2 ± 0.1 |
| 23:0 | 0.3 ± tr. | 0.2 ± tr. | N.D. | N.D. |
| 22:5n-3 | 0.1 ± tr. | 0.1 ± tr. | 0.3 ± 0.1 | 0.5 ± tr. |
| 22:6n-3 | 25.0 ± 1.2 | 26.1 ± 1.0 | 34.1 ± 1.4 | 30.9 ± 0.9 |
| 24:0 | N.D. | N.D. | N.D. | N.D. |
| 24:1 | N.D. | N.D. | N.D. | N.D. |
| Total SAT | 35.2 ± 0.6 | 34.3 ± 0.2 | 32.8 ± 0.9 | 34.0 ± 0.9 |
| Total MUFA | 13.4 ± 0.6 | 12.8 ± 0.7 | 13.6 ± 0.8 | 14.6 ± tr. |
| Total PUFA | 42.1 ± 1.2 | 42.5 ± 1.4 | 42.1 ± 1.6 | 40.1 ± 1.0 |
| Total n-3 FA | 25.2 ± 1.2 | 26.3 ± 1.0 | 34.9 ± 1.4 | 31.7 ± 0.9 |
| Total n-6 FA | 16.7 ± 0.2 | 16.2 ± 0.5 | 7.0 ± 0.4 | 8.1 ± 0.3 |
| n-3/n-6 ratio | 1.51 ± 0.08 | 1.62 ± 0.05 | 5.03 ± 0.27 | 3.90 ± 0.14 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 12

Fatty acid composition of phosphatidylethanolamine (PE) from hippocampus of 12 month-old WT and Tg mice on the oil blend diet or the DHA diet. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 4) | WT oil (n = 4) | Tg DHA (n = 4) | WT DHA (n = 4) |
|---|---|---|---|---|
| 12:0 | N.D. | N.D. | N.D. | N.D. |
| X1 | 1.7 ± 0.2 | 1.4 ± 0.6 | 0.8 ± 0.5 | 1.3 ± 0.4 |
| 16:0 | 6.4 ± 0.5 | 7.1 ± 0.3 | 6.5 ± 0.7 | 6.6 ± 0.8 |
| 16:1n-7 | 0.8 ± 0.3 | 0.6 ± 0.1 | 0.8 ± 0.2 | 0.9 ± 0.5 |
| X2 | 2.0 ± 0.2 | 2.8 ± 1.3 | 2.5 ± 0.6 | 1.7 ± 0.2 |
| X3 | 1.1 ± 0.2 | 1.8 ± 0.4 | 1.2 ± 0.5 | 0.7 ± 0.1 |
| 18:0 | 23.6 ± 0.5 | 22.8 ± 0.6 | 23.1 ± 0.9 | 22.3 ± 0.3 |
| 18:1n-9 | 11.3 ± 0.8 | 11.7 ± 1.5 | 13.7 ± 0.9 | 13.2 ± 1.0 |
| 18:1n-7 | 1.7 ± 0.2 | 2.1 ± 0.1 | 1.9 ± 0.1 | 1.9 ± 0.1 |
| 18:2n-6 | 0.3 ± tr. | 0.2 ± tr. | 0.4 ± tr. | 0.3 ± tr. |
| 18:3n-6 | N.D. | N.D. | N.D. | N.D. |
| 18:3n-3 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:0 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:1 | 1.9 ± 0.5 | 2.4 ± 0.3 | 1.9 ± 0.5 | 2.0 ± 0.4 |
| 20:1 | N.D. | N.D. | N.D. | 0.1 ± 0.1 |
| 20:1 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:2 | 0.1 ± tr. | 0.1 ± tr. | 0.5 ± 0.1 | 0.3 ± 0.1 |
| 20:3n-6 | 0.3 ± tr. | 0.2 ± tr. | 0.8 ± tr. | 0.6 ± 0.1 |
| 20:4n-6 | 15.9 ± 0.3 | 14.5 ± 0.4 | 9.5 ± 0.1 | 10.8 ± 1.2 |
| 20:3n-3 | N.D. | N.D. | N.D. | 0.1 ± 0.1 |
| 20:5n-3 | N.D. | N.D. | 0.4 ± 0.1 | 0.5 ± 0.2 |
| 22:0 | N.D. | N.D. | N.D. | N.D. |
| 22:1 | 0.1 ± tr. | N.D. | N.D. | N.D. |
| 22:2 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 22:4n-6 | 6.7 ± 0.2 | 6.0 ± 0.5 | 2.5 ± 0.1 | 3.5 ± 1.1 |
| 23:0 | N.D. | 0.1 ± 0.1 | N.D. | N.D. |
| 22:5n-3 | 0.1 ± tr. | 0.1 ± tr. | 0.6 ± tr. | 0.5 ± 0.2 |
| 22:6n-3 | 25.6 ± 0.5 | 25.7 ± 0.7 | 32.6 ± 1.4 | 32.3 ± 1.9 |
| 24:0 | N.D. | N.D. | N.D. | N.D. |
| 24:1 | N.D. | N.D. | N.D. | N.D. |
| Total SAT | 30.2 ± 0.9 | 30.1 ± 0.6 | 29.7 ± 1.5 | 29.0 ± 0.6 |
| Total MUFA | 15.8 ± 1.2 | 16.9 ± 1.8 | 18.5 ± 1.2 | 18.2 ± 1.0 |
| Total PUFA | 49.2 ± 0.6 | 47.0 ± 1.3 | 47.4 ± 1.4 | 49.0 ± 0.5 |
| Total n-3 FA | 25.8 ± 0.5 | 25.9 ± 0.7 | 33.7 ± 1.4 | 33.4 ± 2.2 |
| Total n-6 FA | 23.2 ± 0.4 | 21.0 ± 0.9 | 13.1 ± 0.1 | 15.2 ± 2.3 |
| n-3/n-6 ratio | 1.12 ± 0.03 | 1.24 ± 0.05 | 2.57 ± 0.12 | 2.37 ± 0.39 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 13

Fatty acid composition of phosphatidylethanolamine (PE) from hippocampus of 16 month-old WT and Tg mice on the oil blend diet or the DHA diet. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
|---|---|---|---|---|
| 12:0 | N.D. | N.D. | N.D. | N.D. |
| X1 | 3.2 ± 0.1 | 3.0 ± 0.3 | 2.4 ± 0.4 | 2.1 ± 0.6 |
| 16:0 | 6.2 ± 0.3 | 6.7 ± 0.2 | 6.9 ± 0.2 | 7.0 ± 0.3 |
| 16:1n-7 | 0.3 ± 0.1 | 0.3 ± tr. | 0.4 ± tr. | 0.4 ± tr. |
| X2 | 5.3 ± 0.3 | 5.2 ± 0.4 | 4.5 ± 0.4 | 4.6 ± 0.8 |
| X3 | 3.0 ± 0.2 | 3.2 ± 0.3 | 2.7 ± 0.2 | 2.7 ± 0.1 |
| 18:0 | 26.6 ± 1.8 | 24.4 ± 0.4 | 25.8 ± 0.7 | 25.6 ± 0.8 |
| 18:1n-9 | 10.3 ± 0.1 | 10.5 ± 0.4 | 12.5 ± 0.4 | 12.2 ± 0.6 |
| 18:1n-7 | 1.5 ± tr. | 1.8 ± 0.2 | 1.4 ± 0.1 | 1.4 ± tr. |
| 18:2n-6 | 0.3 ± tr. | 0.2 ± tr. | 0.4 ± tr. | 0.3 ± tr. |
| 18:3n-6 | N.D. | N.D. | N.D. | N.D. |
| 18:3n-3 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:0 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:1 | 1.5 ± tr. | 1.4 ± 0.3 | 1.3 ± 0.3 | 1.0 ± 0.1 |
| 20:1 | 0.1 ± tr. | 0.1 ± 0.1 | 0.1 ± tr. | N.D. |
| 20:1 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:2 | 0.1 ± tr. | 0.1 ± tr. | 0.3 ± tr. | 0.3 ± tr. |
| 20:3n-6 | 0.3 ± tr. | 0.3 ± tr. | 0.6 ± tr. | 0.5 ± tr. |
| 20:4n-6 | 13.8 ± 0.4 | 13.8 ± 0.5 | 6.7 ± 0.4 | 7.9 ± 0.3 |
| 20:3n-3 | N.D. | N.D. | N.D. | N.D. |
| 20:5n-3 | N.D. | N.D. | 0.5 ± tr. | 0.4 ± tr. |
| 22:0 | N.D. | N.D. | N.D. | N.D. |
| 22:1 | N.D. | N.D. | N.D. | N.D. |
| 22:2 | 0.1 ± tr. | N.D. | N.D. | N.D. |
| 22:4n-6 | 4.7 ± 0.2 | 4.4 ± tr. | 1.1 ± 0.1 | 1.4 ± 0.1 |
| 23:0 | 0.3 ± 0.2 | 0.2 ± tr. | N.D. | N.D. |
| 22:5n-3 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.6 ± tr. | 0.6 ± tr. |
| 22:6n-3 | 22.2 ± 1.0 | 24.1 ± 0.5 | 31.6 ± 0.6 | 31.2 ± 0.7 |
| 24:0 | N.D. | N.D. | N.D. | N.D. |
| 24:1 | N.D. | N.D. | N.D. | N.D. |
| Total SAT | 33.1 ± 1.5 | 31.4 ± 0.3 | 32.8 ± 0.4 | 32.7 ± 0.6 |
| Total MUFA | 13.9 ± 0.1 | 14.2 ± 0.7 | 15.8 ± 0.7 | 15.2 ± 0.6 |
| Total PUFA | 41.6 ± 1.5 | 43.0 ± 0.7 | 41.8 ± 0.9 | 42.7 ± 1.1 |
| Total n-3 FA | 22.4 ± 1.0 | 24.3 ± 0.6 | 32.8 ± 0.5 | 32.3 ± 0.8 |
| Total n-6 FA | 19.0 ± 0.6 | 18.6 ± 0.4 | 8.7 ± 0.5 | 10.1 ± 0.4 |
| n-3/n-6 ratio | 1.17 ± 0.04 | 1.30 ± 0.04 | 3.78 ± 0.21 | 3.19 ± 0.12 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 14

Fatty acid composition of phosphatidylethnolamine (PE) from cerebellum of 12 month-old WT and Tg mice on the oil blend diet or the DHA diet. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 4) | WT oil (n = 4) | Tg DHA (n = 4) | WT DHA (n = 4) |
|---|---|---|---|---|
| 12:0 | N.D. | N.D. | N.D. | N.D. |
| X1 | 2.8 ± 1.0 | 3.7 ± 0.4 | 2.5 ± 0.7 | 3.1 ± 0.5 |
| 16:0 | 5.1 ± 0.4 | 5.2 ± 0.2 | 5.4 ± 0.5 | 5.9 ± 0.5 |
| 16:1n-7 | 0.3 ± 0.1 | 0.3 ± 0.1 | 0.4 ± 0.1 | 0.6 ± 0.2 |
| X2 | 7.1 ± 1.1 | 8.3 ± 0.6 | 6.6 ± 0.9 | 5.9 ± 1.2 |
| X3 | 4.5 ± 0.2 | 5.4 ± 0.2 | 4.6 ± 0.2 | 3.7 ± 0.9 |
| 18:0 | 19.2 ± 0.7 | 17.8 ± 1.0 | 18.8 ± 1.3 | 17.7 ± 0.7 |
| 18:1n-9 | 17.3 ± 0.4 | 17.8 ± 0.5 | 19.3 ± 0.4 | 19.9 ± 0.9 |
| 18:1n-7 | 2.0 ± 0.1 | 2.6 ± 0.5 | 2.0 ± 0.1 | 2.3 ± tr. |
| 18:2n-6 | 0.4 ± 0.1 | 0.4 ± tr. | 0.5 ± 0.1 | 0.5 ± tr. |
| 18:3n-6 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 18:3n-3 | 0.1 ± tr. | 0.1 ± 0.1 | 0.1 ± tr. | 0.2 ± tr. |
| 20:0 | 0.4 ± tr. | 0.4 ± 0.1 | 0.2 ± 0.1 | 0.3 ± 0.1 |
| 20:1 | 5.5 ± 0.4 | 6.0 ± tr. | 4.4 ± 0.1 | 5.6 ± 0.2 |
| 20:1 | 0.4 ± tr. | 0.3 ± 0.1 | 0.4 ± 0.1 | 0.5 ± 0.1 |
| 20:1 | 0.2 ± tr. | 0.2 ± tr. | 0.2 ± 0.1 | 0.1 ± tr. |
| 20:2 | 0.1 ± tr. | 0.2 ± 0.1 | 0.1 ± 0.1 | 0.1 ± tr. |
| 20:3n-6 | 0.3 ± tr. | 0.3 ± tr. | 0.5 ± tr. | 0.6 ± tr. |
| 20:4n-6 | 8.4 ± 0.6 | 8.2 ± 0.1 | 3.3 ± 0.3 | 3.2 ± 0.2 |
| 20:3n-3 | 0.1 ± tr. | N.D. | 0.1 ± 0.1 | 0.1 ± 0.1 |
| 20:5n-3 | 0.1 ± tr. | 0.1 ± tr. | 0.3 ± 0.1 | 0.4 ± tr. |
| 22:0 | N.D. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 22:1 | 0.2 ± tr. | 0.1 ± tr. | N.D. | 0.1 ± tr. |
| 22:2 | N.D. | N.D. | N.D. | 0.1 ± 0.1 |
| 22:4n-6 | 2.6 ± 0.2 | 2.6 ± 0.1 | 0.7 ± 0.1 | 0.7 ± 0.1 |
| 23:0 | 0.1 ± tr. | 0.1 ± 0.1 | N.D. | N.D. |
| 22:5n-3 | 0.1 ± tr. | 0.1 ± tr. | 0.4 ± tr. | 0.5 ± tr. |
| 22:6n-3 | 22.5 ± 1.8 | 19.4 ± 1.1 | 28.5 ± 1.6 | 27.7 ± 0.3 |
| 24:0 | N.D. | N.D. | N.D. | N.D. |
| 24:1 | N.D. | N.D. | N.D. | N.D. |
| Total SAT | 24.9 ± 0.6 | 23.6 ± 1.0 | 24.6 ± 0.8 | 24.0 ± 1.2 |
| Total MUFA | 25.9 ± 0.8 | 27.4 ± 0.4 | 26.9 ± 0.3 | 29.0 ± 1.2 |
| Total PUFA | 34.8 ± 2.4 | 31.6 ± 1.4 | 34.8 ± 1.7 | 34.2 ± 0.7 |
| Total n-3 FA | 22.9 ± 1.7 | 19.7 ± 1.2 | 29.5 ± 1.6 | 28.9 ± 0.4 |
| Total n-6 FA | 11.8 ± 0.8 | 11.6 ± 0.3 | 5.2 ± 0.4 | 5.1 ± 0.2 |
| n-3/n-6 ratio | 1.95 ± 0.10 | 1.69 ± 0.09 | 5.77 ± 0.53 | 5.71 ± 0.21 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 15

Fatty acid composition of phosphatidylethanolamine (PE) from cerebellum of 16 month-old WT and Tg mice on the oil blend diet or the DHA diet. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
|---|---|---|---|---|
| 12:0 | N.D. | N.D. | N.D. | N.D. |
| X1 | 4.0 ± 0.2 | 2.8 ± 0.4 | 3.3 ± 0.1 | 3.6 ± 0.6 |
| 16:0 | 5.2 ± 0.1 | 5.4 ± 0.3 | 5.3 ± 0.2 | 5.7 ± 0.4 |
| 16:1n-7 | 0.4 ± 0.1 | 0.4 ± 0.1 | 0.3 ± tr. | 0.3 ± tr. |
| X2 | 7.4 ± 0.3 | 6.6 ± 0.5 | 7.4 ± 0.3 | 7.1 ± tr. |
| X3 | 5.1 ± 0.4 | 4.6 ± 0.1 | 4.9 ± 0.5 | 4.7 ± 0.1 |
| 18:0 | 18.5 ± 0.1 | 19.2 ± 0.5 | 17.5 ± 0.6 | 18.2 ± 0.2 |
| 18:1n-9 | 20.1 ± 1.2 | 18.8 ± 0.9 | 21.7 ± 1.1 | 21.4 ± 0.5 |
| 18:1n-7 | 2.5 ± 0.1 | 2.8 ± 0.4 | 2.4 ± 0.2 | 2.7 ± 0.5 |
| 18:2n-6 | 0.4 ± tr. | 0.3 ± tr. | 0.4 ± tr. | 0.3 ± tr. |
| 18:3n-6 | N.D. | N.D. | N.D. | N.D. |
| 18:3n-3 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:0 | 0.2 ± tr. | 0.2 ± 0.1 | 0.2 ± tr. | 0.1 ± tr. |
| 20:1 | 6.0 ± 0.4 | 5.0 ± 0.2 | 5.3 ± 0.3 | 4.8 ± 0.4 |
| 20:1 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.1 ± 0.1 | N.D. |
| 20:1 | 0.2 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:2 | 0.2 ± tr. | 0.1 ± tr. | N.D. | N.D. |
| 20:3n-6 | 0.3 ± tr. | 0.3 ± tr. | 0.3 ± tr. | 0.4 ± tr. |
| 20:4n-6 | 7.5 ± 0.4 | 8.0 ± 0.3 | 2.1 ± 0.2 | 2.7 ± 0.1 |
| 20:3n-3 | N.D. | N.D. | N.D. | N.D. |
| 20:5n-3 | N.D. | N.D. | 0.4 ± tr. | 0.3 ± tr. |
| 22:0 | N.D. | N.D. | N.D. | N.D. |
| 22:1 | N.D. | 0.1 ± tr. | 0.1 ± tr. | N.D. |
| 22:2 | N.D. | N.D. | N.D. | N.D. |
| 22:4n-6 | 2.6 ± tr. | 2.2 ± tr. | 0.4 ± tr. | 0.5 ± tr. |
| 23:0 | N.D. | N.D. | N.D. | N.D. |
| 22:5n-3 | N.D. | 0.1 ± tr. | 0.4 ± tr. | 0.4 ± tr. |
| 22:6n-3 | 19.1 ± 1.3 | 22.9 ± 0.8 | 27.2 ± 1.1 | 26.5 ± 1.7 |
| 24:0 | N.D. | N.D. | N.D. | N.D. |
| 24:1 | N.D. | N.D. | N.D. | N.D. |
| Total SAT | 24.0 ± 0.1 | 24.7 ± 0.7 | 23.0 ± 0.5 | 24.0 ± 0.5 |
| Total MUFA | 29.4 ± 1.5 | 27.4 ± 0.7 | 30.0 ± 1.5 | 29.4 ± 0.8 |
| Total PUFA | 30.2 ± 1.7 | 34.0 ± 0.6 | 31.3 ± 1.3 | 31.2 ± 1.7 |
| Total n-3 FA | 19.2 ± 1.3 | 23.1 ± 0.8 | 28.0 ± 1.1 | 27.3 ± 1.6 |
| Total n-6 FA | 10.8 ± 0.5 | 10.8 ± 0.3 | 3.3 ± 0.2 | 3.9 ± 0.2 |
| n-3/n-6 ratio | 1.78 ± 0.08 | 2.14 ± 0.14 | 8.63 ± 0.34 | 7.04 ± 0.54 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 16

Fatty acid composition of phosphatidylcholine (PC) from cortex of 12 month-old WT and Tg mice on the oil blend diet or the DHA diet. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 4) | WT oil (n = 4) | Tg DHA (n = 4) | WT DHA (n = 4) |
|---|---|---|---|---|
| 12:0 | N.D. | N.D. | N.D. | N.D. |
| X1 | N.D. | N.D. | 0.1 ± tr. | N.D. |
| 16:0 | 40.3 ± 1.6 | 39.1 ± 2.3 | 39.9 ± 2.8 | 40.2 ± 1.8 |
| 16:1n-7 | 0.6 ± 0.1 | 0.6 ± 0.2 | 0.7 ± 0.2 | 0.7 ± 0.2 |
| X2 | N.D. | N.D. | N.D. | N.D. |
| X3 | N.D. | N.D. | N.D. | N.D. |
| 18:0 | 13.7 ± 0.6 | 13.6 ± 0.8 | 13.6 ± 0.9 | 13.9 ± 0.5 |
| 18:1n-9 | 22.2 ± 0.3 | 22.5 ± 0.7 | 23.6 ± 1.1 | 23.9 ± 0.6 |
| 18:1n-7 | 6.4 ± 0.2 | 6.6 ± 0.2 | 5.9 ± 0.2 | 5.8 ± 0.2 |
| 18:2n-6 | 0.7 ± 0.1 | 0.7 ± tr. | 0.9 ± tr. | 0.8 ± tr. |
| 18:3n-6 | N.D. | N.D. | N.D. | N.D. |
| 18:3n-3 | 0.1 ± tr. | N.D. | N.D. | N.D. |
| 20:0 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:1 | 1.0 ± tr. | 1.0 ± 0.1 | 0.6 ± 0.2 | 1.0 ± 0.1 |
| 20:1 | 0.5 ± tr. | 0.6 ± tr. | 0.5 ± 0.1 | 0.6 ± 0.1 |
| 20:1 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:2 | 0.2 ± tr. | 0.2 ± tr. | 0.3 ± tr. | 0.3 ± tr. |
| 20:3n-6 | 0.4 ± tr. | 0.4 ± tr. | 1.1 ± 0.1 | 0.9 ± 0.1 |
| 20:4n-6 | 7.2 ± 0.4 | 7.5 ± 0.5 | 4.4 ± 0.2 | 3.9 ± 0.2 |
| 20:3n-3 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.2 ± 0.1 |
| 20:5n-3 | N.D. | N.D. | 0.2 ± tr. | 0.2 ± tr. |
| 22:0 | 0.1 ± tr. | 0.1 ± tr. | N.D. | 0.1 ± tr. |
| 22:1 | 0.1 ± tr. | 0.1 ± tr. | N.D. | 0.1 ± tr. |
| 22:2 | N.D. | N.D. | N.D. | N.D. |
| 22:4n-6 | 0.7 ± tr. | 0.7 ± tr. | 0.2 ± tr. | 0.2 ± tr. |
| 23:0 | 0.2 ± tr. | 0.1 ± tr. | N.D. | N.D. |
| 22:5n-3 | N.D. | N.D. | 0.1 ± tr. | 0.1 ± tr. |
| 22:6n-3 | 5.3 ± 0.6 | 5.7 ± 0.5 | 7.5 ± 0.9 | 6.8 ± 0.5 |
| 24:0 | N.D. | N.D. | N.D. | N.D. |
| 24:1 | N.D. | 0.1 ± 0.1 | N.D. | N.D. |
| Total SAT | 54.4 ± 1.4 | 53.1 ± 1.7 | 53.7 ± 2.1 | 54.3 ± 1.4 |
| Total MUFA | 30.8 ± 0.4 | 31.5 ± 0.9 | 31.5 ± 1.1 | 32.2 ± 0.8 |
| Total PUFA | 14.7 ± 1.1 | 15.4 ± 1.0 | 14.7 ± 1.2 | 13.4 ± 0.7 |
| Total n-3 FA | 5.5 ± 0.6 | 5.8 ± 0.5 | 7.9 ± 0.9 | 7.4 ± 0.5 |
| Total n-6 FA | 9.0 ± 0.5 | 9.3 ± 0.5 | 6.6 ± 0.3 | 5.8 ± 0.3 |
| n-3/n-6 ratio | 0.61 ± 0.03 | 0.63 ± 0.03 | 1.19 ± 0.09 | 1.27 ± 0.06 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 17

Fatty acid composition of phosphatidylcholine (PC) from cortex of 16 month-old WT and Tg mice on the oil blend diet or the DHA diet. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
|---|---|---|---|---|
| 12:0 | N.D. | N.D. | N.D. | N.D. |
| X1 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 16:0 | 50.7 ± 1.1 | 52.5 ± 0.7 | 52.0 ± 1.2 | 52.2 ± 1.4 |
| 16:1n-7 | 0.5 ± 0.1 | 0.4 ± tr. | 0.5 ± 0.1 | 0.5 ± tr. |
| X2 | N.D. | N.D. | N.D. | N.D. |
| X3 | N.D. | N.D. | N.D. | N.D. |
| 18:0 | 13.2 ± 0.4 | 12.6 ± 0.2 | 13.1 ± 0.3 | 12.2 ± 0.3 |
| 18:1n-9 | 20.4 ± 0.5 | 19.7 ± 0.5 | 21.2 0.6 | 21.6 ± 0.9 |
| 18:1n-7 | 5.7 ± 0.1 | 5.6 ± 0.3 | 4.9 ± 0.4 | 5.2 ± 0.4 |
| 18:2n-6 | 0.5 ± tr. | 0.5 ± 0.1 | 0.5 ± tr. | 0.5 ± tr. |
| 18:3n-6 | N.D. | 0.1 ± 0.1 | N.D. | N.D. |
| 18:3n-3 | N.D. | N.D. | N.D. | N.D. |
| 20:0 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:1 | 0.3 ± 0.2 | 0.3 ± 0.1 | 0.3 ± 0.1 | 0.3 ± 0.1 |
| 20:1 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.1 ± 0.1 |
| 20:1 | 0.2 ± 0.1 | 0.1 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 |
| 20:2 | 0.1 ± tr. | 0.1 ± tr. | 0.2 ± 0.1 | 0.1 ± tr. |
| 20:3n-6 | 0.2 ± tr. | 0.2 ± tr. | 0.5 ± tr. | 0.4 ± tr. |
| 20:4n-6 | 4.9 ± 0.3 | 4.6 ± 0.3 | 1.9 ± 0.1 | 2.4 ± 0.3 |
| 20:3n-3 | N.D. | N.D. | N.D. | N.D. |
| 20:5n-3 | N.D. | N.D. | 0.1 ± tr. | 0.1 ± tr. |
| 22:0 | N.D. | N.D. | N.D. | N.D. |
| 22:1 | N.D. | N.D. | N.D. | N.D. |
| 22:2 | N.D. | N.D. | N.D. | N.D. |
| 22:4n-6 | 0.3 ± tr. | 0.3 ± tr. | N.D. | 0.1 ± tr. |
| 23:0 | N.D. | N.D. | N.D. | N.D. |
| 22:5n-3 | N.D. | N.D. | N.D. | N.D. |
| 22:6n-3 | 2.7 ± 0.2 | 2.7 ± 0.1 | 4.4 ± 0.4 | 4.0 ± 0.4 |
| 24:0 | N.D. | N.D. | N.D. | N.D. |
| 24:1 | N.D. | N.D. | N.D. | N.D. |
| Total SAT | 64.0 ± 1.1 | 65.1 ± 0.9 | 65.1 ± 1.3 | 64.5 ± 1.6 |
| Total MUFA | 27.1 ± 0.6 | 26.3 ± 0.7 | 27.2 ± 0.7 | 27.8 ± 1.2 |
| Total PUFA | 8.8 ± 0.5 | 8.4 ± 0.2 | 7.6 ± 0.6 | 7.6 ± 0.7 |
| Total n-3 FA | 2.7 ± 0.2 | 2.7 ± 0.1 | 4.5 ± 0.4 | 4.1 ± 0.4 |
| Total n-6 FA | 5.9 ± 0.3 | 5.6 ± 0.1 | 2.9 ± 0.2 | 3.4 ± 0.3 |
| n-3/n-6 ratio | 0.46 ± 0.02 | 0.49 ± 0.01 | 1.54 ± 0.09 | 1.23 ± 0.04 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 18

Fatty acid composition of phosphatidylcholine (PC) from hippocampus of 12 month-old WT and Tg mice on the oil blend diet or the DHA diet. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 4) | WT oil (n = 4) | Tg DHA (n = 4) | WT DHA (n = 4) |
|---|---|---|---|---|
| 12:0 | N.D. | N.D. | N.D. | N.D. |
| X1 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 16:0 | 44.0 ± 0.8 | 46.0 ± 1.2 | 43.7 ± 1.0 | 44.6 ± 1.2 |
| 16:1n-7 | 0.5 ± 0.4 | 1.1 ± 0.4 | 1.3 ± 0.7 | 0.8 ± 0.5 |
| X2 | N.D. | N.D. | N.D. | N.D. |
| X3 | N.D. | N.D. | N.D. | N.D. |
| 18:0 | 13.5 ± 0.4 | 13.1 ± 0.3 | 13.3 ± 0.1 | 14.0 ± 0.4 |
| 18:1n-9 | 20.1 ± 0.3 | 19.3 ± 0.1 | 21.9 ± 0.2 | 21.8 ± 0.7 |
| 18:1n-7 | 9.1 ± 0.3 | 8.5 ± 0.7 | 8.5 ± 0.3 | 8.3 ± 1.4 |
| 18:2n-6 | 0.7 ± tr. | 0.6 ± tr. | 0.9 ± 0.1 | 0.7 ± 0.1 |
| 18:3n-6 | N.D. | 0.2 ± 0.2 | N.D. | N.D. |
| 18:3n-3 | N.D. | 0.1 ± tr. | N.D. | N.D. |
| 20:0 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:1 | 0.6 ± tr. | 0.5 ± tr. | 0.6 ± tr. | 0.5 ± 0.1 |
| 20:1 | 0.5 ± tr. | 0.4 ± 0.1 | 0.6 ± tr. | 0.3 ± 0.1 |
| 20:1 | 0.1 ± tr. | N.D. | 0.1 ± tr. | N.D. |
| 20:2 | 0.1 ± 0.1 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:3n-6 | 0.2 ± tr. | 0.2 ± tr. | 0.5 ± tr. | 0.4 ± tr. |
| 20:4n-6 | 7.3 ± 0.3 | 6.7 ± 0.2 | 4.2 ± 0.2 | 3.9 ± 0.3 |
| 20:3n-3 | N.D. | N.D. | 0.1 ± 0.1 | N.D. |
| 20:5n-3 | N.D. | N.D. | 0.1 ± 0.1 | 0.3 ± 0.2 |
| 22:0 | N.D. | N.D. | N.D. | N.D. |
| 22:1 | N.D. | N.D. | N.D. | N.D. |
| 22:2 | N.D. | N.D. | N.D. | N.D. |
| 22:4n-6 | 0.4 ± 0.1 | 0.3 ± 0.1 | 0.1 ± tr. | 0.1 ± tr. |
| 23:0 | N.D. | N.D. | N.D. | N.D. |
| 22:5n-3 | N.D. | N.D. | N.D. | 0.2 ± 0.2 |
| 22:6n-3 | 2.7 ± 0.1 | 2.8 ± 0.3 | 3.9 ± 0.1 | 3.7 ± 0.2 |
| 24:0 | N.D. | N.D. | N.D. | N.D. |
| 24:1 | N.D. | N.D. | N.D. | N.D. |
| Total SAT | 57.6 ± 0.6 | 59.2 ± 1.4 | 57.1 ± 1.0 | 58.7 ± 1.6 |
| Total MUFA | 30.9 ± 0.2 | 29.8 ± 0.7 | 33.0 ± 0.8 | 31.7 ± 1.1 |
| Total PUFA | 11.4 ± 0.4 | 10.9 ± 0.7 | 9.9 ± 0.3 | 9.6 ± 0.5 |
| Total n-3 FA | 2.8 ± 0.1 | 2.8 ± 0.2 | 4.1 ± 0.1 | 4.3 ± 0.5 |
| Total n-6 FA | 8.5 ± 0.3 | 7.9 ± 0.4 | 5.6 ± 0.3 | 5.2 ± 0.3 |
| n-3/n-6 ratio | 0.32 ± tr. | 0.35 ± 0.01 | 0.75 ± 0.04 | 0.84 ± 0.14 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 19

Fatty acid composition of phosphatidylcholine (PC) from hippocampus of 16 month-old WT and Tg mice on the oil blend diet or the DHA diet. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
|---|---|---|---|---|
| 12:0 | N.D. | N.D. | N.D. | N.D. |
| X1 | N.D. | N.D. | 0.1 ± 0.1 | 0.1 ± tr. |
| 16:0 | 50.8 ± 0.6 | 51.9 ± 0.6 | 51.8 ± 1.4 | 52.3 ± 0.7 |
| 16:1n-7 | 0.7 ± 0.1 | 0.6 ± 0.1 | 0.6 ± tr. | 0.6 ± 0.1 |
| X2 | N.D. | N.D. | N.D. | N.D. |
| X3 | N.D. | N.D. | N.D. | N.D. |
| 18:0 | 14.4 ± 0.5 | 13.9 ± 0.4 | 14.0 ± 0.4 | 13.4 ± 0.3 |
| 18:1n-9 | 19.8 ± 0.1 | 19.9 ± 0.2 | 22.1 ± 0.5 | 21.6 ± 0.4 |
| 18:1n-7 | 5.7 ± 0.5 | 5.4 ± 0.5 | 4.5 ± 0.5 | 5.1 ± 0.4 |
| 18:2n-6 | 0.4 ± tr. | 0.4 ± tr. | 0.5 ± tr. | 0.5 ± tr. |
| 18:3n-6 | N.D. | N.D. | N.D. | 0.2 ± 0.2 |
| 18:3n-3 | N.D. | N.D. | N.D. | 0.1 ± tr. |
| 20:0 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:1 | 0.4 ± tr. | 0.4 ± tr. | 0.4 ± tr. | 0.3 ± tr. |
| 20:1 | 0.2 ± tr. | 0.2 ± tr. | 0.2 ± tr. | 0.2 ± tr. |
| 20:1 | N.D. | N.D. | N.D. | N.D. |
| 20:2 | N.D. | N.D. | N.D. | N.D. |
| 20:3n-6 | 0.2 ± tr. | 0.2 ± tr. | 0.3 ± tr. | 0.3 ± tr. |
| 20:4n-6 | 5.4 ± 0.3 | 5.1 ± 0.3 | 2.5 ± 0.3 | 2.8 ± 0.1 |
| 20:3n-3 | N.D. | N.D. | N.D. | N.D. |
| 20:5n-3 | N.D. | N.D. | 0.1 ± tr. | 0.1 ± tr. |
| 22:0 | N.D. | N.D. | N.D. | N.D. |
| 22:1 | N.D. | N.D. | N.D. | N.D. |
| 22:2 | N.D. | N.D. | N.D. | N.D. |
| 22:4n-6 | 0.2 ± tr. | 0.2 ± tr. | N.D. | N.D. |
| 23:0 | N.D. | N.D. | N.D. | N.D. |
| 22:5n-3 | N.D. | N.D. | N.D. | N.D. |
| 22:6n-3 | 1.7 ± 0.1 | 1.8 ± 0.1 | 2.8 ± 0.2 | 2.5 ± 0.1 |
| 24:0 | N.D. | N.D. | N.D. | N.D. |
| 24:1 | N.D. | N.D. | N.D. | N.D. |
| Total SAT | 65.3 ± 1.0 | 65.9 ± 0.9 | 65.9 ± 1.2 | 65.7 ± 0.8 |
| Total MUFA | 26.7 ± 0.6 | 26.4 ± 0.7 | 27.7 ± 0.9 | 27.8 ± 0.8 |
| Total PUFA | 7.9 ± 0.5 | 7.7 ± 0.4 | 6.3 ± 0.5 | 6.4 ± 0.5 |
| Total n-3 FA | 1.7 ± 0.1 | 1.8 ± 0.1 | 3.0 ± 0.2 | 2.6 ± 0.2 |
| Total n-6 FA | 6.2 ± 0.3 | 5.8 ± 0.3 | 3.3 ± 0.3 | 3.7 ± 0.3 |
| n-3/n-6 ratio | 0.28 ± 0.01 | 0.31 ± 0.02 | 0.90 ± 0.03 | 0.70 ± 0.03 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 20

Fatty acid composition of phosphatidylcholine (PC) from cerebellum of 12 month-old WT and Tg mice on the oil blend diet or the DHA diet. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 4) | WT oil (n = 4) | Tg DHA (n = 4) | WT DHA (n = 4) |
|---|---|---|---|---|
| 12:0 | N.D. | N.D. | N.D. | N.D. |
| X1 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 |
| 16:0 | 44.4 ± 0.9 | 44.0 ± 1.8 | 46.2 ± 2.0 | 43.5 ± 0.2 |
| 16:1n-7 | 0.4 ± 0.1 | 0.4 ± 0.1 | 0.4 ± 0.1 | 0.5 ± 0.1 |
| X2 | 0.1 ± tr. | 0.1 ± tr. | N.D. | 0.1 ± tr. |
| X3 | N.D. | N.D. | N.D. | N.D. |
| 18:0 | 16.4 ± 0.3 | 16.8 ± 0.2 | 15.8 ± 0.7 | 15.4 ± 0.3 |
| 18:1n-9 | 19.8 ± 0.4 | 19.6 ± 0.5 | 19.3 ± 0.9 | 20.5 ± 0.4 |
| 18:1n-7 | 5.9 ± 0.2 | 6.5 ± 0.8 | 5.1 ± 0.4 | 5.7 ± 0.2 |
| 18:2n-6 | 0.6 ± 0.1 | 0.6 ± tr. | 0.7 ± 0.1 | 0.7 ± 0.1 |
| 18:3n-6 | 0.1 ± tr. | N.D. | 0.1 ± 0.1 | 0.1 ± tr. |
| 18:3n-3 | N.D. | 0.1 ± 0.1 | 0.2 ± tr. | 0.1 ± tr. |
| 20:0 | 0.3 ± tr. | 0.2 ± 0.1 | 0.3 ± 0.1 | 0.3 ± tr. |
| 20:1 | 1.6 ± 0.1 | 1.7 ± 0.2 | 1.4 ± 0.1 | 1.7 ± 0.1 |
| 20:1 | 0.4 ± tr. | 0.5 ± tr. | 0.4 ± 0.1 | 0.5 ± 0.1 |
| 20:1 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:2 | 0.1 ± tr. | 0.2 ± tr. | 0.2 ± tr. | 0.1 ± 0.1 |
| 20:3n-6 | 0.2 ± tr. | 0.2 ± tr. | 0.4 ± tr. | 0.4 ± tr. |
| 20:4n-6 | 2.6 ± 0.2 | 2.5 ± 0.2 | 0.8 ± 0.1 | 0.8 ± tr. |
| 20:3n-3 | 0.1 ± tr. | 0.1 ± tr. | N.D. | 0.1 ± tr. |
| 20:5n-3 | 0.1 ± tr. | N.D. | 0.1 ± 0.1 | 0.1 ± tr. |
| 22:0 | 0.1 ± tr. | N.D. | 0.1 ± tr. | 0.1 ± tr. |
| 22:1 | 0.1 ± tr. | 0.1 ± tr. | N.D. | 0.1 ± tr. |
| 22:2 | N.D. | N.D. | N.D. | N.D. |
| 22:4n-6 | 0.3 ± tr. | 0.2 ± 0.1 | N.D. | 0.1 ± tr. |
| 23:0 | N.D. | N.D. | N.D. | N.D. |
| 22:5n-3 | N.D. | N.D. | N.D. | 0.1 ± tr. |
| 22:6n-3 | 6.1 ± 0.4 | 5.8 ± 0.7 | 8.1 ± 0.8 | 8.5 ± 0.3 |
| 24:0 | N.D. | N.D. | N.D. | N.D. |
| 24:1 | N.D. | N.D. | N.D. | N.D. |
| Total SAT | 61.2 ± 0.7 | 61.0 ± 1.9 | 62.4 ± 2.6 | 59.4 ± 0.5 |
| Total MUFA | 28.3 ± 0.5 | 28.9 ± 1.2 | 26.8 ± 1.5 | 29.2 ± 0.7 |
| Total PUFA | 10.2 ± 0.5 | 9.8 ± 0.9 | 10.6 ± 1.0 | 11.1 ± 0.3 |
| Total n-3 FA | 6.3 ± 0.3 | 6.1 ± 0.6 | 8.4 ± 0.8 | 8.9 ± 0.4 |
| Total n-6 FA | 3.7 ± 0.3 | 3.5 ± 0.3 | 2.0 ± 0.3 | 2.1 ± 0.1 |
| n-3/n-6 ratio | 1.70 ± 0.11 | 1.71 ± 0.06 | 4.25 ± 0.46 | 4.22 ± 0.27 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 21

Fatty acid composition of phosphatidylcholine (PC) from cerebellum of 16 month-old WT and Tg mice on the oil blend diet or the DHA diet. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
|---|---|---|---|---|
| 12:0 | N.D. | N.D. | N.D. | N.D. |
| X1 | 0.1 ± tr. | N.D. | N.D. | N.D. |
| 16:0 | 43.2 ± 0.3 | 44.2 ± 1.2 | 45.2 ± 0.3 | 45.1 ± 0.8 |
| 16:1n-7 | 0.7 ± 0.1 | 0.6 ± 0.2 | 0.4 ± tr. | 0.5 ± tr. |
| X2 | N.D. | N.D. | N.D. | N.D. |
| X3 | N.D. | N.D. | N.D. | N.D. |
| 18:0 | 17.5 ± 0.3 | 16.8 ± 0.4 | 15.2 ± 0.2 | 15.6 ± 0.1 |
| 18:1n-9 | 21.6 ± 0.5 | 20.2 ± 0.2 | 22.3 ± 0.5 | 21.8 ± 0.6 |
| 18:1n-7 | 7.2 ± 0.2 | 7.7 ± 0.4 | 5.9 ± 0.5 | 6.7 ± 0.4 |
| 18:2n-6 | 0.6 ± tr. | 0.5 ± tr. | 0.6 ± tr. | 0.6 ± tr. |
| 18:3n-6 | N.D. | N.D. | N.D. | N.D. |
| 18:3n-3 | 0.1 ± tr. | 0.1 ± tr. | N.D. | 0.1 ± tr. |
| 20:0 | 0.2 ± tr. | 0.2 ± tr. | 0.2 ± tr. | 0.2 ± tr. |
| 20:1 | 1.5 ± tr. | 1.4 ± 0.1 | 1.2 ± tr. | 1.2 ± 0.1 |
| 20:1 | 0.1 ± 0.1 | 0.4 ± tr. | 0.4 ± tr. | 0.4 ± tr. |
| 20:1 | 0.3 ± 0.1 | N.D. | N.D. | N.D. |
| 20:2 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:3n-6 | 0.1 ± tr. | 0.1 ± 0.1 | 0.2 ± tr. | 0.2 ± tr. |
| 20:4n-6 | 1.8 ± 0.2 | 1.9 ± 0.3 | 0.3 ± tr. | 0.5 ± tr. |
| 20:3n-3 | N.D. | N.D. | N.D. | N.D. |
| 20:5n-3 | N.D. | N.D. | 0.1 ± tr. | 0.1 ± tr. |
| 22:0 | N.D. | N.D. | N.D. | N.D. |
| 22:1 | N.D. | N.D. | N.D. | 0.1 ± tr. |
| 22:2 | N.D. | N.D. | N.D. | N.D. |
| 22:4n-6 | 0.1 ± 0.1 | 0.1 ± 0.1 | N.D. | N.D. |
| 23:0 | N.D. | N.D. | N.D. | N.D. |
| 22:5n-3 | N.D. | N.D. | N.D. | N.D. |
| 22:6n-3 | 4.6 ± 0.5 | 5.5 ± 0.9 | 7.7 ± 0.4 | 6.8 ± 0.4 |
| 24:0 | N.D. | N.D. | N.D. | N.D. |
| 24:1 | N.D. | N.D. | N.D. | N.D. |
| Total SAT | 61.0 ± 0.2 | 61.3 ± 1.6 | 60.7 ± 0.5 | 60.9 ± 0.7 |
| Total MUFA | 31.5 ± 0.8 | 30.3 ± 0.4 | 30.2 ± 1.0 | 30.7 ± 0.4 |
| Total PUFA | 7.5 ± 0.8 | 8.4 ± 1.2 | 9.1 ± 0.5 | 8.4 ± 0.4 |
| Total n-3 FA | 4.7 ± 0.5 | 5.6 ± 0.9 | 7.9 ± 0.4 | 7.0 ± 0.4 |
| Total n-6 FA | 2.7 ± 0.3 | 2.7 ± 0.3 | 1.1 ± 0.1 | 1.3 ± tr. |
| n-3/n-6 ratio | 1.77 ± 0.05 | 2.07 ± 0.21 | 6.91 ± 0.14 | 5.43 ± 0.39 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 22

Fatty acid composition of phosphatidylserine (PS) from cortex of 12 month-old WT and Tg mice on the oil blend diet or the DHA diet. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 4) | WT oil (n = 4) | Tg DHA (n = 4) | WT DHA (n = 4) |
|---|---|---|---|---|
| 12:0 | N.D. | N.D. | N.D. | N.D. |
| X1 | 0.1 ± tr. | 0.2 ± 0.1 | N.D. | 0.1 ± tr. |
| 16:0 | 1.3 ± 0.1 | 1.3 ± tr. | 0.9 ± 0.1 | 0.9 ± 0.2 |
| 16:1n-7 | 0.5 ± tr. | 0.4 ± 0.1 | 0.5 ± tr. | 0.5 ± tr. |
| X2 | N.D. | N.D. | N.D. | N.D. |
| X3 | N.D. | N.D. | N.D. | N.D. |
| 18:0 | 44.6 ± 2.0 | 42.8 ± 1.9 | 44.3 ± 1.4 | 45.2 ± 0.3 |
| 18:1n-9 | 12.6 ± 1.0 | 12.2 ± 0.7 | 12.5 ± 0.5 | 12.5 ± 0.5 |
| 18:1n-7 | 0.6 ± 0.2 | 0.6 ± 0.2 | 0.6 ± 0.2 | 0.8 ± tr. |
| 18:2n-6 | 0.2 ± tr. | 0.2 ± tr. | 0.3 ± tr. | 0.2 ± tr. |
| 18:3n-6 | N.D. | N.D. | N.D. | N.D. |
| 18:3n-3 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:0 | 0.3 ± tr. | 0.3 ± tr. | 0.3 ± tr. | 0.3 ± tr. |
| 20:1 | 0.7 ± 0.1 | 0.6 ± 0.1 | 0.5 ± tr. | 0.6 ± tr. |
| 20:1 | 0.4 ± tr. | 0.3 ± tr. | 0.3 ± 0.1 | 0.3 ± 0.1 |
| 20:1 | 0.2 ± tr. | 0.2 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:2 | N.D. | N.D. | N.D. | N.D. |
| 20:3n-6 | 0.3 ± tr. | 0.3 ± 0.1 | 0.6 ± tr. | 0.5 ± tr. |
| 20:4n-6 | 2.1 ± 0.1 | 2.1 ± 0.3 | 1.3 ± 0.1 | 1.3 ± 0.1 |
| 20:3n-3 | 0.3 ± tr. | 0.3 ± tr. | 0.2 ± 0.1 | 0.3 ± tr. |
| 20:5n-3 | N.D. | N.D. | 0.1 ± tr. | 0.1 ± tr. |
| 22:0 | 0.3 ± 0.1 | 0.4 ± 0.1 | 0.4 ± tr. | 0.4 ± tr. |
| 22:1 | 0.3 ± 0.1 | 0.4 ± 0.1 | 0.3 ± 0.1 | 0.4 ± 0.1 |
| 22:2 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 22:4n-6 | 2.8 ± 0.1 | 2.2 ± 0.4 | 1.0 ± tr. | 0.9 ± tr. |
| 23:0 | 0.9 ± 0.1 | 0.7 ± 0.2 | 0.2 ± tr. | 0.2 ± tr. |
| 22:5n-3 | 0.1 ± tr. | 0.2 ± 0.1 | 0.4 tr. | 0.4 ± tr. |
| 22:6n-3 | 30.8 ± 2.9 | 33.8 ± 3.1 | 34.7 ± 1.7 | 33.4 ± 0.8 |
| 24:0 | 0.1 ± tr. | 0.1 ± tr. | 0.2 ± tr. | 0.2 ± tr. |
| 24:1 | 0.2 ± 0.1 | 0.1 ± tr. | 0.1 ± tr. | 0.2 ± tr. |
| Total SAT | 47.6 ± 2.1 | 45.6 ± 2.2 | 46.2 ± 1.4 | 47.3 ± 0.3 |
| Total MUFA | 15.4 ± 1.1 | 14.7 ± 0.8 | 15.0 ± 0.7 | 15.3 ± 0.7 |
| Total PUFA | 36.9 ± 3.0 | 39.4 ± 2.8 | 38.7 ± 1.7 | 37.3 ± 0.7 |
| Total n-3 FA | 31.3 ± 2.9 | 34.4 ± 3.2 | 35.5 ± 1.8 | 34.2 ± 0.8 |
| Total n-6 FA | 5.4 ± 0.1 | 4.9 ± 0.6 | 3.1 ± 0.1 | 2.9 ± 0.1 |
| n-3/n-6 ratio | 5.76 ± 0.40 | 7.76 ± 1.99 | 11.38 ± 0.89 | 11.91 ± 0.67 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 23

Fatty acid composition of phosphatidylserine (PS) from cortex of 16 month-old WT and Tg mice on the oil blend diet or the DHA diet. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
|---|---|---|---|---|
| 12:0 | N.D. | N.D. | N.D. | N.D. |
| X1 | 0.1 ± tr. | 0.2 ± tr. | 0.1 ± tr. | 0.1 ± 0.1 |
| 16:0 | 1.6 ± tr. | 1.9 ± 0.1 | 1.9 ± 0.1 | 1.6 ± tr. |
| 16:1n-7 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.2 ± 0.1 |
| X2 | N.D. | N.D. | N.D. | N.D. |
| X3 | N.D. | N.D. | N.D. | N.D. |
| 18:0 | 54.1 ± 1.1 | 52.9 ± 1.5 | 53.4 ± 2.0 | 52.6 ± 1.3 |
| 18:1n-9 | 12.0 ± 0.5 | 12.5 ± 0.3 | 12.3 ± 0.6 | 13.6 ± 0.4 |
| 18:1n-7 | 0.2 ± 0.2 | 0.5 ± 0.2 | 0.2 ± 0.2 | 0.4 ± 0.2 |
| 18:2n-6 | 0.2 ± tr. | 0.1 ± tr. | 0.2 ± tr. | 0.2 ± tr. |
| 18:3n-6 | N.D. | N.D. | N.D. | N.D. |
| 18:3n-3 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:0 | 0.2 ± tr. | 0.2 ± tr. | 0.2 ± tr. | 0.2 ± tr. |
| 20:1 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.1 ± 0.1 | 0.1 ± 0.1 |
| 20:1 | N.D. | N.D. | N.D. | N.D. |
| 20:1 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.1 ± 0.1 |
| 20:2 | N.D. | N.D. | N.D. | N.D. |
| 20:3n-6 | 0.2 ± tr. | 0.2 ± tr. | 0.3 ± tr. | 0.3 ± tr. |
| 20:4n-6 | 1.4 ± 0.1 | 1.5 ± 0.1 | 0.6 ± tr. | 0.8 ± tr. |
| 20:3n-3 | N.D. | N.D. | N.D. | N.D. |
| 20:5n-3 | N.D. | N.D. | N.D. | N.D. |
| 22:0 | 0.2 ± tr. | 0.2 ± tr. | 0.1 ± 0.1 | 0.2 ± tr. |
| 22:1 | 0.1 ± tr. | 0.1 ± tr. | N.D. | 0.1 ± tr. |
| 22:2 | N.D. | N.D. | N.D. | N.D. |
| 22:4n-6 | 1.9 ± tr. | 1.8 ± 0.1 | 0.4 ± tr. | 0.5 ± 0.1 |
| 23:0 | 0.4 ± tr. | 0.3 ± 0.1 | N.D. | N.D. |
| 22:5n-3 | N.D. | N.D. | 0.1 ± 0.1 | 0.2 ± tr. |
| 22:6n-3 | 26.9 ± 1.5 | 27.3 ± 1.1 | 29.7 ± 2.0 | 28.8 ± 1.1 |
| 24:0 | N.D. | N.D. | N.D. | N.D. |
| 24:1 | N.D. | N.D. | N.D. | N.D. |
| Total SAT | 56.5 ± 1.1 | 55.4 ± 1.3 | 55.7 ± 1.9 | 54.6 ± 1.3 |
| Total MUFA | 12.8 ± 0.7 | 13.4 ± 0.6 | 12.8 ± 0.5 | 14.5 ± 0.3 |
| Total PUFA | 30.6 ± 1.5 | 31.1 ± 1.1 | 31.4 ± 2.1 | 30.8 ± 1.1 |
| Total n-3 FA | 27.0 ± 1.5 | 27.4 ± 1.1 | 29.9 ± 2.1 | 29.0 ± 1.0 |
| Total n-6 FA | 3.7 ± 0.1 | 3.6 ± 0.1 | 1.5 ± tr. | 1.8 ± 0.1 |
| n-3/n-6 ratio | 7.39 ± 0.50 | 7.67 ± 0.60 | 20.07 ± 1.45 | 16.52 ± 0.37 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 24

Fatty acid composition of phosphatidylserine (PS) from hippocampus of 12 month-old WT and Tg mice on the oil blend diet or the DHA diet. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 4) | WT oil (n = 4) | Tg DHA (n = 4) | WT DHA (n = 4) |
|---|---|---|---|---|
| 12:0 | 0.1 ± tr. | N.D. | N.D. | 0.1 ± tr. |
| X1 | 0.1 ± tr. | N.D. | 0.1 ± 0.1 | 0.1 ± 0.1 |
| 16:0 | 2.0 ± 0.4 | 2.0 ± tr. | 2.0 ± 0.3 | 1.8 ± 0.2 |
| 16:1n-7 | 0.4 ± 0.1 | 0.3 ± 0.1 | 0.5 ± 0.1 | 0.3 ± 0.1 |
| X2 | N.D. | N.D. | N.D. | N.D. |
| X3 | N.D. | 0.1 ± 0.1 | N.D. | N.D. |
| 18:0 | 43.8 ± 1.3 | 45.2 ± 1.9 | 42.9 ± 0.9 | 43.2 ± 1.0 |
| 18:1n-9 | 20.2 ± 1.6 | 19.4 ± 1.7 | 20.6 ± 1.2 | 20.3 ± 2.0 |
| 18:1n-7 | N.D. | N.D. | N.D. | N.D. |
| 18:2n-6 | 0.3 ± tr. | 0.2 ± tr. | 0.5 ± 0.1 | 0.4 ± tr. |
| 18:3n-6 | N.D. | N.D. | N.D. | N.D. |
| 18:3n-3 | 0.2 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:0 | 0.3 ± tr. | 0.3 ± tr. | 0.3 ± tr. | 0.3 ± 0.1 |
| 20:1 | 0.7 ± 0.1 | 0.6 ± 0.1 | 0.6 ± 0.1 | 0.6 ± 0.1 |
| 20:1 | 0.5 ± tr. | 0.3 ± 0.1 | 0.5 ± tr. | 0.4 ± tr. |
| 20:1 | 0.1 ± tr. | 0.2 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:2 | N.D. | N.D. | N.D. | N.D. |
| 20:3n-6 | 0.2 ± tr. | 0.2 ± tr. | 0.5 ± tr. | 0.5 ± 0.1 |
| 20:4n-6 | 2.8 ± 0.2 | 2.7 ± 0.3 | 1.8 ± 0.2 | 1.7 ± 0.1 |
| 20:3n-3 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 |
| 20:5n-3 | N.D. | N.D. | 0.1 ± tr. | 0.1 ± tr. |
| 22:0 | 0.4 ± tr. | 0.3 ± 0.1 | 0.4 ± 0.1 | 0.4 ± 0.1 |
| 22:1 | 0.2 ± 0.1 | 0.2 ± tr. | 0.4 ± 0.1 | 0.2 ± 0.1 |
| 22:2 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 22:4n-6 | 2.0 ± 0.4 | 2.0 ± 0.2 | 0.8 ± 0.1 | 0.9 ± 0.3 |
| 23:0 | 0.4 ± 0.4 | 0.2 ± 0.2 | N.D. | N.D. |
| 22:5n-3 | N.D. | N.D. | 0.2 ± 0.1 | 0.1 ± 0.1 |
| 22:6n-3 | 25.2 ± 1.8 | 25.3 ± 0.4 | 27.4 ± 1.7 | 28.0 ± 1.1 |
| 24:0 | N.D. | N.D. | N.D. | 0.1 ± 0.1 |
| 24:1 | N.D. | N.D. | 0.1 ± 0.1 | 0.1 ± 0.1 |
| Total SAT | 46.9 ± 1.2 | 48.0 ± 2.0 | 45.6 ± 1.0 | 45.7 ± 1.0 |
| Total MUFA | 22.1 ± 1.5 | 21.1 ± 2.0 | 22.7 ± 1.2 | 22.1 ± 1.7 |
| Total PUFA | 30.9 ± 2.3 | 30.8 ± 0.6 | 31.6 ± 2.0 | 32.0 ± 1.4 |
| Total n-3 FA | 25.4 ± 1.9 | 25.5 ± 0.4 | 27.9 ± 1.8 | 28.5 ± 1.2 |
| Total n-6 FA | 5.3 ± 0.5 | 5.2 ± 0.3 | 3.6 ± 0.2 | 3.4 ± 0.3 |
| n-3/n-6 ratio | 4.81 ± 0.14 | 4.98 ± 0.26 | 7.86 ± 0.51 | 8.59 ± 0.52 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 25

Fatty acid composition of phosphatidylserine (PS) from hippocampus of 16 month-old WT and Tg mice on the oil blend diet or the DHA diet. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
|---|---|---|---|---|
| 12:0 | N.D. | N.D. | N.D. | N.D. |
| X1 | 0.3 ± 0.2 | 0.2 ± 0.1 | 0.3 ± 0.2 | 0.3 ± 0.1 |
| 16:0 | 2.2 ± 0.4 | 2.3 ± 0.3 | 2.8 ± 0.3 | 2.3 ± 0.5 |
| 16:1n-7 | 0.5 ± 0.1 | 0.3 ± tr. | 0.5 ± 0.1 | 0.5 ± 0.1 |
| X2 | N.D. | N.D. | N.D. | N.D. |
| X3 | N.D. | N.D. | N.D. | N.D. |
| 18:0 | 54.6 ± 0.8 | 54.2 ± 0.2 | 55.0 ± 0.3 | 53.2 ± 1.0 |
| 18:1n-9 | 16.5 ± 0.3 | 17.4 ± 0.5 | 15.9 ± 0.9 | 16.8 ± 1.0 |
| 18:1n-7 | 0.5 ± 0.3 | N.D. | 0.2 ± 0.2 | 0.2 ± 0.2 |
| 18:2n-6 | 0.2 ± tr. | 0.2 ± tr. | 0.3 ± tr. | 0.3 ± 0.1 |
| 18:3n-6 | N.D. | N.D. | N.D. | N.D. |
| 18:3n-3 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.3 ± tr. |
| 20:0 | 0.2 ± 0.1 | 0.3 ± tr. | 0.2 ± tr. | 0.3 ± tr. |
| 20:1 | 0.4 ± tr. | 0.3 ± tr. | 0.3 ± tr. | 0.3 ± tr. |
| 20:1 | N.D. | N.D. | N.D. | N.D. |
| 20:1 | N.D. | 0.1 ± 0.1 | N.D. | N.D. |
| 20:2 | N.D. | N.D. | N.D. | N.D. |
| 20:3n-6 | 0.2 ± tr. | 0.2 ± tr. | 0.3 ± tr. | 0.3 ± tr. |
| 20:4n-6 | 1.7 ± 0.1 | 1.7 ± tr. | 0.8 ± 0.1 | 1.0 ± 0.2 |
| 20:3n-3 | N.D. | N.D. | N.D. | N.D. |
| 20:5n-3 | N.D. | N.D. | N.D. | N.D. |
| 22:0 | N.D. | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.1 ± 0.1 |
| 22:1 | 0.2 ± 0.1 | 0.2 ± tr. | 0.1 ± 0.1 | N.D. |
| 22:2 | N.D. | N.D. | N.D. | N.D. |
| 22:4n-6 | 2.0 ± 0.1 | 1.7 ± 0.1 | 0.5 ± tr. | 0.5 ± 0.1 |
| 23:0 | 0.3 ± 0.1 | N.D. | N.D. | N.D. |
| 22:5n-3 | N.D. | N.D. | N.D. | 0.1 ± 0.1 |
| 22:6n-3 | 20.1 ± 0.2 | 20.8 ± 0.5 | 22.4 ± 1.0 | 23.4 ± 0.7 |
| 24:0 | N.D. | N.D. | N.D. | N.D. |
| 24:1 | N.D. | N.D. | N.D. | N.D. |
| Total SAT | 57.2 ± 0.6 | 56.8 ± 0.5 | 58.2 ± 0.4 | 56.0 ± 0.7 |
| Total MUFA | 18.1 ± 0.5 | 18.3 ± 0.5 | 17.1 ± 1.0 | 17.9 ± 1.0 |
| Total PUFA | 24.4 ± 0.2 | 24.7 ± 0.6 | 24.5 ± 1.1 | 25.8 ± 1.1 |
| Total n-3 FA | 20.3 ± 0.1 | 20.9 ± 0.5 | 22.6 ± 1.0 | 23.7 ± 0.8 |
| Total n-6 FA | 4.1 ± 0.2 | 3.7 ± 0.1 | 1.9 ± 0.1 | 2.1 ± 0.4 |
| n-3/n-6 ratio | 4.96 ± 0.20 | 5.59 ± 0.09 | 11.99 ± 0.09 | 11.73 ± 1.54 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 26

Fatty acid composition of phosphatidylserine (PS) from cerebellum of 12 month-old WT and Tg mice on the oil blend diet or the DHA diet. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 4) | WT oil (n = 4) | Tg DHA (n = 4) | WT DHA (n = 4) |
|---|---|---|---|---|
| 12:0 | N.D. | N.D. | N.D. | N.D. |
| X1 | 0.3 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 |
| 16:0 | 2.1 ± 0.3 | 2.0 ± 0.3 | 1.9 ± 0.3 | 2.3 ± 0.3 |
| 16:1n-7 | 0.3 ± 0.2 | 0.3 ± 0.1 | 0.4 ± 0.1 | 0.6 ± 0.2 |
| X2 | 0.1 ± tr. | 0.1 ± 0.1 | 0.1 ± 0.1 | N.D. |
| X3 | N.D. | N.D. | N.D. | 0.1 ± tr. |
| 18:0 | 45.3 ± 2.3 | 44.2 ± 1.5 | 41.2 ± 1.4 | 41.9 ± 1.4 |
| 18:1n-9 | 24.9 ± 0.7 | 26.1 ± 1.3 | 25.8 ± 1.1 | 26.3 ± 1.2 |
| 18:1n-7 | 0.5 ± 0.3 | 0.6 ± 0.3 | 0.5 ± 0.3 | 0.8 ± 0.3 |
| 18:2n-6 | 0.4 ± 0.1 | 0.4 ± 0.1 | 0.4 ± tr. | 0.5 ± 0.1 |
| 18:3n-6 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.3 ± 0.1 | 0.2 ± tr. |
| 18:3n-3 | 0.2 ± 0.1 | 0.6 ± 0.4 | 0.1 ± 0.1 | 0.2 ± tr. |
| 20:0 | 0.8 ± 0.1 | 0.7 ± 0.2 | 0.9 ± 0.3 | 0.7 ± 0.1 |
| 20:1 | 1.9 ± 0.3 | 2.2 ± 0.2 | 1.6 ± 0.2 | 2.3 ± 0.1 |
| 20:1 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.2 ± 0.1 | 0.4 ± 0.1 |
| 20:1 | 0.2 ± tr. | 0.1 ± 0.1 | 0.2 ± 0.1 | 0.1 ± tr. |
| 20:2 | 0.2 ± 0.1 | 0.5 ± 0.4 | 0.4 ± 0.2 | 0.2 ± tr. |
| 20:3n-6 | 0.3 ± 0.1 | 0.3 ± 0.1 | 0.4 ± tr. | 0.6 ± 0.1 |
| 20:4n-6 | 2.0 ± 0.2 | 1.9 ± 0.2 | 1.2 ± 0.2 | 1.0 ± 0.1 |
| 20:3n-3 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.2 ± 0.1 | 0.3 ± 0.1 |
| 20:5n-3 | 0.2 ± tr. | 0.3 ± 0.1 | 0.3 ± 0.1 | 0.2 ± 0.1 |
| 22:0 | 0.5 ± 0.1 | 0.5 ± 0.1 | 0.5 ± 0.2 | 0.7 ± 0.2 |
| 22:1 | 0.4 ± 0.1 | 0.5 ± 0.3 | 0.6 ± 0.1 | 0.5 ± tr. |
| 22:2 | N.D. | N.D. | N.D. | 0.3 ± 0.2 |
| 22:4n-6 | 1.6 ± 0.1 | 1.5 ± 0.1 | 0.6 ± tr. | 0.5 ± 0.1 |
| 23:0 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.1 ± 0.1 | N.D. |
| 22:5n-3 | N.D. | N.D. | 0.2 ± 0.1 | 0.3 ± 0.1 |
| 22:6n-3 | 17.3 ± 1.3 | 16.5 ± 0.9 | 21.6 ± 0.9 | 18.8 ± 0.7 |
| 24:0 | 0.1 ± 0.1 | N.D. | N.D. | N.D. |
| 24:1 | 0.1 ± tr. | N.D. | 0.1 ± 0.1 | N.D. |
| Total SAT | 49.0 ± 2.1 | 47.6 ± 1.1 | 44.5 ± 1.4 | 45.6 ± 1.1 |
| Total MUFA | 28.4 ± 1.3 | 29.9 ± 1.1 | 29.3 ± 1.6 | 31.0 ± 1.7 |
| Total PUFA | 22.3 ± 1.6 | 22.2 ± 1.5 | 25.7 ± 0.9 | 23.0 ± 1.0 |
| Total n-3 FA | 17.8 ± 1.3 | 17.5 ± 1.2 | 22.5 ± 0.9 | 19.7 ± 0.8 |
| Total n-6 FA | 4.3 ± 0.4 | 4.1 ± 0.4 | 2.9 ± 0.1 | 2.9 ± 0.3 |
| n-3/n-6 ratio | 4.17 ± 0.25 | 4.38 ± 0.59 | 7.86 ± 0.20 | 7.01 ± 0.41 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 27

Fatty acid composition of phosphatidylserine (PS) from cerebellum of 16 month-old WT and Tg mice on the oil blend diet or the DHA diet. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
|---|---|---|---|---|
| 12:0 | N.D. | N.D. | N.D. | N.D. |
| X1 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.2 ± 0.2 | N.D. |
| 16:0 | 2.9 ± 0.5 | 2.7 ± 0.7 | 2.6 ± 0.2 | 2.4 ± 0.1 |
| 16:1n-7 | 0.9 ± 0.5 | 0.5 ± 0.2 | 0.4 ± tr. | 0.4 ± tr. |
| X2 | N.D. | N.D. | N.D. | N.D. |
| X3 | N.D. | N.D. | N.D. | N.D. |
| 18:0 | 45.4 ± 1.3 | 44.3 ± 1.1 | 42.8 ± 0.5 | 42.8 ± 1.2 |
| 18:1n-9 | 32.3 ± 0.7 | 31.2 ± 0.5 | 31.8 ± 1.2 | 32.5 ± 0.9 |
| 18:1n-7 | 0.4 ± 0.4 | 0.7 ± 0.3 | 0.7 ± 0.3 | 0.4 ± 0.4 |
| 18:2n-6 | 0.3 ± 0.1 | 0.3 ± 0.1 | 0.3 ± tr. | 0.3 ± tr. |
| 18:3n-6 | N.D. | N.D. | N.D. | N.D. |
| 18:3n-3 | 0.2 ± 0.1 | 0.3 ± 0.1 | 0.2 ± tr. | 0.2 ± tr. |
| 20:0 | 0.5 ± 0.1 | 0.5 ± tr. | 0.4 ± 0.1 | 0.4 ± tr. |
| 20:1 | 2.3 ± 0.3 | 1.8 ± 0.1 | 1.5 ± 0.3 | 1.3 ± 0.1 |
| 20:1 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.1 ± 0.1 |
| 20:1 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.1 ± tr. | 0.1 ± tr. |
| 20:2 | N.D. | N.D. | N.D. | N.D. |
| 20:3n-6 | 0.1 ± 0.1 | 0.2 ± tr. | 0.2 ± tr. | 0.3 ± tr. |
| 20:4n-6 | 1.3 ± 0.2 | 1.5 ± 0.1 | 0.6 ± 0.1 | 0.8 ± 0.1 |
| 20:3n-3 | N.D. | N.D. | N.D. | 0.1 ± 0.1 |
| 20:5n-3 | N.D. | N.D. | 0.1 ± tr. | N.D. |
| 22:0 | 0.5 ± tr. | 0.6 ± tr. | 0.5 ± 0.1 | 0.6 ± tr. |
| 22:1 | 0.4 ± tr. | 0.3 ± tr. | 0.3 ± tr. | 0.4 ± tr. |
| 22:2 | N.D. | N.D. | N.D. | N.D. |
| 22:4n-6 | 0.9 ± 0.2 | 1.1 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 |
| 23:0 | N.D. | N.D. | N.D. | N.D. |
| 22:5n-3 | N.D. | N.D. | 0.2 ± tr. | 0.2 ± tr. |
| 22:6n-3 | 11.1 ± 2.1 | 13.8 ± 1.9 | 16.5 ± 1.3 | 15.9 ± 0.7 |
| 24:0 | N.D. | N.D. | 0.1 ± 0.1 | 0.2 ± 0.1 |
| 24:1 | N.D. | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.2 ± tr. |
| Total SAT | 49.4 ± 1.9 | 48.0 ± 1.8 | 46.4 ± 0.7 | 46.5 ± 1.0 |
| Total MUFA | 36.4 ± 0.6 | 34.8 ± 0.2 | 35.1 ± 1.3 | 35.5 ± 1.2 |
| Total PUFA | 14.1 ± 2.4 | 17.1 ± 1.9 | 18.3 ± 1.5 | 17.9 ± 0.6 |
| Total n-3 FA | 11.4 ± 2.1 | 14.1 ± 1.8 | 16.9 ± 1.3 | 16.3 ± 0.7 |
| Total n-6 FA | 2.7 ± 0.3 | 3.0 ± 0.2 | 1.4 ± 0.2 | 1.6 ± 0.2 |
| n-3/n-6 ratio | 4.12 ± 0.31 | 4.61 ± 0.48 | 12.73 ± 1.31 | 10.76 ± 1.66 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 28

Fatty acid composition of phosphatidylinositol (PI) from cortex of 12 month-old WT and Tg mice on the oil blend diet or the DHA diet. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 4) | WT oil (n = 4) | Tg DHA (n = 4) | WT DHA (n = 4) |
|---|---|---|---|---|
| 12:0 | N.D. | N.D. | N.D. | N.D. |
| X1 | 0.2 ± 0.1 | N.D. | 0.4 ± 0.3 | 0.2 ± 0.1 |
| 16:0 | 6.0 ± 1.1 | 5.4 ± 0.9 | 6.3 ± 0.7 | 7.5 ± 0.7 |
| 16:1n-7 | 1.1 ± 0.1 | 1.0 ± 0.1 | 0.9 ± 0.3 | 1.2 ± 0.1 |
| X2 | N.D. | N.D. | N.D. | N.D. |
| X3 | N.D. | N.D. | N.D. | N.D. |
| 18:0 | 35.3 ± 1.8 | 34.4 ± 1.8 | 34.5 ± 1.5 | 37.0 ± 0.7 |
| 18:1n-9 | 5.8 ± 0.2 | 5.4 ± 0.3 | 6.1 ± 0.2 | 6.0 ± 0.2 |
| 18:1n-7 | 2.5 ± tr. | 2.4 ± 0.1 | 2.6 ± 0.1 | 2.0 ± 0.7 |
| 18:2n-6 | 0.7 ± tr. | 0.6 ± tr. | 0.8 ± 0.1 | 0.8 ± tr. |
| 18:3n-6 | N.D. | N.D. | 0.2 ± 0.2 | N.D. |
| 18:3n-3 | 0.1 ± tr. | 0.1 ± tr. | 0.2 ± tr. | 0.2 ± 0.1 |
| 20:0 | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. | 0.1 ± tr. |
| 20:1 | 0.7 ± 0.1 | 0.6 ± 0.1 | 0.6 ± tr. | 0.6 ± 0.1 |
| 20:1 | 0.8 ± 0.1 | 0.7 ± 0.1 | 0.8 ± 0.1 | 0.8 ± 0.1 |
| 20:1 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.2 ± 0.1 | 0.1 ± 0.1 |
| 20:2 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.6 ± 0.1 | 0.7 ± 0.2 |
| 20:3n-6 | 0.3 ± tr. | 0.3 ± 0.1 | 1.1 ± 0.1 | 0.9 ± 0.1 |
| 20:4n-6 | 38.7 ± 2.3 | 41.6 ± 2.8 | 33.5 ± 2.5 | 32.2 ± 0.9 |
| 20:3n-3 | 1.9 ± 0.4 | 1.3 ± 0.3 | 2.1 ± 0.6 | 0.7 ± 0.2 |
| 20:5n-3 | N.D. | 0.1 ± tr. | 2.0 ± 0.4 | 1.8 ± 0.1 |
| 22:0 | N.D. | 0.7 ± 0.4 | 0.3 ± 0.1 | 0.1 ± 0.1 |
| 22:1 | 1.9 ± 0.6 | 0.6 ± 0.4 | 0.6 ± 0.4 | 0.2 ± 0.1 |
| 22:2 | 0.1 ± tr. | 0.2 ± tr. | 0.1 ± 0.1 | 0.1 ± tr. |
| 22:4n-6 | 0.6 ± 0.4 | 1.0 ± 0.4 | 1.5 ± 0.8 | 2.2 ± 1.1 |
| 23:0 | 0.2 ± 0.2 | 0.1 ± 0.1 | 0.1 ± 0.1 | N.D. |
| 22:5n-3 | N.D. | N.D. | 0.1 ± tr. | N.D. |
| 22:6n-3 | 2.6 ± 0.5 | 3.0 ± 0.4 | 3.4 ± 0.5 | 3.5 ± 0.2 |
| 24:0 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.6 ± 0.5 | 0.8 ± 0.7 |
| 24:1 | N.D. | N.D. | 0.3 ± 0.3 | 0.4 ± 0.4 |
| Total SAT | 41.7 ± 2.5 | 40.8 ± 2.6 | 42.0 ± 1.9 | 45.5 ± 0.9 |
| Total MUFA | 12.8 ± 0.9 | 10.8 ± 0.9 | 12.0 ± 1.0 | 11.2 ± 0.7 |
| Total PUFA | 45.2 ± 2.9 | 48.3 ± 3.1 | 45.5 ± 2.4 | 43.1 ± 1.0 |
| Total n-3 FA | 4.6 ± 0.7 | 4.5 ± 0.6 | 7.8 ± 1.1 | 6.1 ± 0.4 |
| Total n-6 FA | 40.3 ± 2.4 | 43.5 ± 2.5 | 37.0 ± 1.9 | 36.2 ± 0.6 |
| n-3/n-6 ratio | 0.11 ± 0.01 | 0.10 ± 0.01 | 0.21 ± 0.03 | 0.17 ± 0.01 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 29

Fatty acid composition of phosphatidylinositol (PI) from cortex of 16 month-old WT and Tg mice on the oil blend diet or the DHA diet. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
|---|---|---|---|---|
| 12:0 | N.D. | N.D. | N.D. | N.D. |
| X1 | 1.0 ± 0.6 | 0.6 ± 0.2 | 0.4 ± 0.2 | 0.5 ± tr. |
| 16:0 | 10.1 ± 1.0 | 8.5 ± 0.6 | 9.1 ± 0.5 | 9.6 ± 0.3 |
| 16:1n-7 | 0.8 ± 0.4 | 0.4 ± 0.2 | 0.7 ± 0.5 | 0.3 ± 0.3 |
| X2 | N.D. | N.D. | N.D. | N.D. |
| X3 | N.D. | N.D. | N.D. | N.D. |
| 18:0 | 45.4 ± 1.2 | 45.8 ± 1.6 | 46.7 ± 1.6 | 45.0 ± 1.8 |
| 18:1n-9 | 5.9 ± 0.4 | 5.7 ± tr. | 7.1 ± 0.2 | 6.5 ± 0.1 |
| 18:1n-7 | 1.6 ± 0.1 | 1.6 ± tr. | 1.6 ± 0.1 | 1.7 ± 0.2 |
| 18:2n-6 | 0.4 ± 0.1 | 0.3 ± tr. | 0.6 ± 0.1 | 0.4 ± tr. |
| 18:3n-6 | N.D. | N.D. | N.D. | N.D. |
| 18:3n-3 | 0.2 ± 0.1 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.2 ± 0.1 |
| 20:0 | 0.1 ± 0.1 | N.D. | 0.1 ± 0.1 | 0.1 ± 0.1 |
| 20:1 | N.D. | N.D. | N.D. | 0.1 ± 0.1 |
| 20:1 | N.D. | N.D. | N.D. | N.D. |
| 20:1 | 0.2 ± tr. | 0.1 ± 0.1 | 0.2 ± 0.2 | 0.1 ± 0.1 |
| 20:2 | N.D. | N.D. | 0.3 ± tr. | 0.3 ± 0.1 |
| 20:3n-6 | N.D. | N.D. | 0.8 ± tr. | 0.7 ± tr. |
| 20:4n-6 | 33.0 ± 1.9 | 35.2 ± 0.8 | 28.1 ± 2.4 | 30.9 ± 1.6 |
| 20:3n-3 | N.D. | N.D. | N.D. | N.D. |
| 20:5n-3 | N.D. | N.D. | 1.7 ± 0.2 | 1.2 ± 0.1 |
| 22:0 | N.D. | N.D. | N.D. | N.D. |
| 22:1 | N.D. | N.D. | N.D. | N.D. |
| 22:2 | N.D. | N.D. | N.D. | N.D. |
| 22:4n-6 | N.D. | N.D. | N.D. | N.D. |
| 23:0 | N.D. | N.D. | N.D. | N.D. |
| 22:5n-3 | N.D. | N.D. | N.D. | N.D. |
| 22:6n-3 | 1.3 ± 0.1 | 1.7 ± 0.1 | 2.6 ± 0.1 | 2.5 ± tr. |
| 24:0 | N.D. | N.D. | N.D. | N.D. |
| 24:1 | N.D. | N.D. | N.D. | N.D. |
| Total SAT | 55.6 ± 1.8 | 54.3 ± 1.0 | 55.9 ± 2.1 | 54.6 ± 1.7 |
| Total MUFA | 8.5 ± 0.6 | 7.7 ± 0.3 | 9.5 ± 0.6 | 8.6 ± 0.3 |
| Total PUFA | 34.9 ± 2.0 | 37.4 ± 0.7 | 34.2 ± 2.7 | 36.2 ± 1.6 |
| Total n-3 FA | 1.5 ± 0.1 | 1.9 ± 0.1 | 4.4 ± 0.2 | 3.9 ± tr. |
| Total n-6 FA | 33.4 ± 2.0 | 35.5 ± 0.8 | 29.5 ± 2.5 | 32.0 ± 1.6 |
| n-3/n-6 ratio | 0.04 ± tr. | 0.05 ± tr. | 0.15 ± 0.01 | 0.12 ± 0.01 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 30

Fatty acid composition of phosphatidylinositol (PI) from hippocampus of 12 month-old WT and Tg mice on the oil blend diet or the DHA diet. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 4) | WT oil (n = 4) | Tg DHA (n = 4) | WT DHA (n = 4) |
|---|---|---|---|---|
| 12:0 | 0.2 ± 0.2 | 0.1 ± 0.1 | 0.2 ± 0.2 | 0.1 ± 0.1 |
| X1 | 0.6 ± 0.3 | 0.3 ± 0.1 | 0.3 ± 0.2 | 0.5 ± 0.4 |
| 16:0 | 7.9 ± 1.2 | 9.1 ± 1.0 | 7.0 ± 1.7 | 6.7 ± 1.5 |
| 16:1n-7 | 0.9 ± 0.1 | 0.6 ± 0.1 | 0.9 ± 0.2 | 1.0 ± 0.3 |
| X2 | N.D. | N.D. | N.D. | N.D. |
| X3 | N.D. | N.D. | N.D. | N.D. |
| 18:0 | 40.0 ± 1.2 | 40.7 ± 2.7 | 41.0 ± 1.1 | 40.6 ± 1.3 |
| 18:1n-9 | 9.7 ± 0.6 | 8.6 ± 0.7 | 10.4 ± 0.9 | 9.3 ± 1.2 |
| 18:1n-7 | 3.8 ± 0.7 | 3.5 ± 0.1 | 3.5 ± 0.8 | 3.0 ± 1.2 |
| 18:2n-6 | 0.7 ± tr. | 0.7 ± 0.1 | 0.9 ± 0.1 | 0.8 ± 0.1 |
| 18:3n-6 | 0.5 ± 0.2 | 0.4 ± 0.1 | 0.3 ± 0.2 | 0.2 ± 0.1 |
| 18:3n-3 | 0.1 ± 0.1 | 0.1 ± 0.1 | N.D. | N.D. |
| 20:0 | 0.1 ± tr. | 0.1 ± 0.1 | N.D. | 0.1 ± 0.1 |
| 20:1 | 0.2 ± 0.1 | 0.3 ± 0.1 | 0.4± 0.2 | 0.4 ± 0.3 |
| 20:1 | 1.0 ± 0.1 | 0.7 ± 0.3 | 0.7± 0.2 | 0.8 ± 0.3 |
| 20:1 | N.D. | 0.1 ± 0.1 | 0.3± 0.3 | 0.1 ± 0.1 |
| 20:2 | 0.2 ± 0.1 | 0.1 ± 0.1 | 0.2± 0.1 | 0.6 ± 0.1 |
| 20:3n-6 | N.D. | N.D. | 0.3 ± 0.1 | 0.4 ± 0.2 |
| 20:4n-6 | 32.4 ± 1.7 | 33.1 ± 1.8 | 29.2 ± 1.3 | 30.5 ± 1.2 |
| 20:3n-3 | N.D. | N.D. | 0.5 ± 0.5 | 0.6 ± 0.6 |
| 20:5n-3 | N.D. | N.D. | 1.4 ± 0.2 | 1.5 ± 0.2 |
| 22:0 | 0.1 ± 0.1 | 0.3 ± 0.1 | N.D. | 0.2 ± 0.2 |
| 22:1 | 0.2 ± 0.2 | N.D. | 0.4 ± 0.4 | 0.3 ± 0.3 |
| 22:2 | N.D. | N.D. | N.D. | N.D. |
| 22:4n-6 | 0.5 ± 0.4 | N.D. | 0.3 ± 0.3 | 0.3 ± 0.3 |
| 23:0 | N.D. | N.D. | N.D. | N.D. |
| 22:5n-3 | N.D. | N.D. | N.D. | N.D. |
| 22:6n-3 | 1.0 ± 0.3 | 1.2 ± 0.2 | 1.5 ± 0.5 | 2.2 ± 0.4 |
| 24:0 | N.D. | N.D. | N.D. | N.D. |
| 24:1 | N.D. | N.D. | N.D. | N.D. |
| Total SAT | 48.2 ± 1.2 | 50.1 ± 3.7 | 48.3 ± 1.3 | 47.7 ± 0.7 |
| Total MUFA | 15.9 ± 1.1 | 13.8 ± 1.6 | 16.7 ± 1.2 | 14.8 ± 1.6 |
| Total PUFA | 35.3 ± 2.2 | 35.7 ± 2.2 | 34.7 ± 2.3 | 37.0 ± 2.1 |
| Total n-3 FA | 1.0 ± 0.3 | 1.4 ± 0.1 | 3.4 ± 0.9 | 4.3 ± 0.9 |
| Total n-6 FA | 34.0 ± 1.9 | 34.3 ± 2.1 | 31.1 ± 1.4 | 32.2 ± 1.3 |
| n-3/n-6 ratio | 0.03 ± 0.01 | 0.04 ± tr. | 0.11 ± 0.02 | 0.13 ± 0.03 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 31

Fatty acid composition of phosphatidylinositol (PI) from hippocampus of 16 month-old WT and Tg mice on the oil blend diet or the DHA diet. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
|---|---|---|---|---|
| 12:0 | N.D. | 0.1 ± 0.1 | N.D. | N.D. |
| X1 | 0.6 ± 0.3 | 1.6 ± 0.4 | 0.5 ± 0.3 | 0.7 ± 0.3 |
| 16:0 | 8.9 ± 1.1 | 9.7 ± 1.4 | 10.7 ± 0.9 | 10.4 ± 1.2 |
| 16:1n-7 | 1.8 ± 0.1 | 2.5 ± 0.2 | 1.5 ± 0.5 | 2.9 ± 0.6 |
| X2 | N.D. | N.D. | N.D. | N.D. |
| X3 | N.D. | N.D. | N.D. | N.D. |
| 18:0 | 51.0 ± 2.4 | 48.3 ± 1.8 | 51.0 ± 1.8 | 48.1 ± 2.6 |
| 18:1n-9 | 6.4 ± 0.9 | 6.6 ± 1.2 | 9.1 ± 0.5 | 8.6 ± 1.1 |
| 18:1n-7 | 0.5 ± 0.5 | 0.9 ± 0.5 | 1.4 ± tr. | 1.3 ± 0.1 |
| 18:2n-6 | 0.9 ± 0.1 | 0.8 ± 0.1 | 1.0 ± 0.2 | 1.0 ± 0.1 |
| 18:3n-6 | N.D. | N.D. | N.D. | N.D. |
| 18:3n-3 | 0.7 ± 0.4 | 0.4 ± 0.2 | 0.6 ± 0.3 | 0.7 ± 0.1 |
| 20:0 | 0.6 ± 0.5 | 0.1 ± 0.1 | 0.1 ± 0.1 | N.D. |
| 20:1 | N.D. | N.D. | N.D. | N.D. |
| 20:1 | N.D. | N.D. | N.D. | N.D. |
| 20:1 | N.D. | N.D. | 0.2 ± 0.1 | 0.2 ± 0.2 |
| 20:2 | 0.1 ± 0.1 | N.D. | N.D. | 0.1 ± 0.1 |
| 20:3n-6 | N.D. | N.D. | 0.1 ± 0.1 | 0.3 ± 0.1 |
| 20:4n-6 | 28.2 ± 1.1 | 28.7 ± 2.0 | 22.1 ± 1.3 | 24.2 ± 1.0 |
| 20:3n-3 | N.D. | N.D. | N.D. | N.D. |
| 20:5n-3 | N.D. | N.D. | 1.3 ± 0.1 | 1.0 ± 0.1 |
| 22:0 | N.D. | N.D. | 0.1 ± 0.1 | 0.3 ± 0.1 |
| 22:1 | N.D. | N.D. | N.D. | N.D. |
| 22:2 | N.D. | N.D. | N.D. | N.D. |
| 22:4n-6 | N.D. | N.D. | N.D. | N.D. |
| 23:0 | N.D. | N.D. | N.D. | N.D. |
| 22:5n-3 | N.D. | N.D. | N.D. | N.D. |
| 22:6n-3 | 0.1 ± 0.1 | 0.2 ± 0.2 | 0.4 ± 0.4 | 0.3 ± 0.3 |
| 24:0 | N.D. | N.D. | N.D. | N.D. |
| 24:1 | N.D. | N.D. | N.D. | N.D. |
| Total SAT | 60.5 ± 2.0 | 58.2 ± 0.4 | 61.9 ± 1.1 | 58.7 ± 1.3 |
| Total MUFA | 8.7 ± 1.5 | 10.1 ± 1.8 | 12.1 ± 0.9 | 12.9 ± 1.4 |
| Total PUFA | 30.1 ± 0.7 | 30.1 ± 1.7 | 25.4 ± 0.7 | 27.6 ± 1.3 |
| Total n-3 FA | 0.8 ± 0.4 | 0.6 ± 0.3 | 2.2 ± 0.7 | 2.1 ± 0.4 |
| Total n-6 FA | 29.1 ± 1.0 | 29.5 ± 2.0 | 23.2 ± 1.3 | 25.5 ± 1.0 |
| n-3/n-6 ratio | 0.03 ± 0.02 | 0.02 ± 0.01 | 0.10 ± 0.04 | 0.08 ± 0.01 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 32

Fatty acid composition of phosphatidylinositol (PI) from cerebellum of 12 month-old WT and Tg mice on the oil blend diet or the DHA diet. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 4) | WT oil (n = 4) | Tg DHA (n = 4) | WT DHA (n = 4) |
|---|---|---|---|---|
| 12:0 | N.D. | N.D. | N.D. | 0.1 ± tr. |
| X1 | 0.3 ± 0.3 | 0.1 ± 0.1 | N.D. | 0.4 ± 0.4 |
| 16:0 | 7.3 ± 0.9 | 6.1 ± 0.6 | 6.5 ± 1.0 | 8.7 ± 1.3 |
| 16:1n-7 | 0.8 ± 0.2 | 0.6 ± 0.3 | 0.6 ± 0.2 | 1.4 ± 0.4 |
| X2 | N.D. | N.D. | N.D. | N.D. |
| X3 | 0.5 ± 0.3 | 0.3 ± 0.3 | N.D. | 0.6 ± 0.2 |
| 18:0 | 40.6 ± 2.5 | 36.2 ± 0.6 | 34.4 ± 0.3 | 33.2 ± 1.2 |
| 18:1n-9 | 7.4 ± 0.5 | 6.3 ± 0.7 | 8.4 ± 1.0 | 10.0 ± 0.5 |
| 18:1n-7 | 1.9 ± 0.2 | 1.5 ± 0.6 | 1.8 ± 0.3 | 2.4 ± 0.2 |
| 18:2n-6 | 0.8 ± 0.3 | 1.5 ± 0.3 | 1.0 ± 0.3 | 1.2 ± 0.4 |
| 18:3n-6 | 0.2 ± 0.2 | 0.3 ± 0.2 | 0.4 ± 0.2 | 0.6± 0.1 |
| 18:3n-3 | 0.7 ± 0.2 | 0.9 ± 0.3 | 1.8 ± 1.1 | 0.7 ± 0.2 |
| 20:0 | 0.7 ± 0.3 | 2.4 ± 0.5 | 1.6 ± 0.9 | 2.0 ± 0.8 |
| 20:1 | 1.0 ± 0.4 | 1.0 ± 0.6 | 0.8 ± 0.3 | 1.2 ± 0.3 |
| 20:1 | N.D. | 1.0 ± 0.7 | 0.6 ± 0.4 | 1.5 ± 0.5 |
| 20:1 | 0.3 ± 0.1 | 1.1 ± 0.3 | 0.6 ± 0.3 | 0.3 ± 0.1 |
| 20:2 | 0.7 ± 0.3 | 2.2 ± 0.8 | 1.6 ± 0.7 | 0.6 ± 0.2 |
| 20:3n-6 | 0.3 ± 0.2 | 0.3 ± 0.3 | 1.5 ± 0.2 | 1.9 ± 0.4 |
| 20:4n-6 | 32.3 ± 2.7 | 31.4 ± 1.7 | 26.8 ± 1.2 | 23.1 ± 0.9 |
| 20:3n-3 | 0.2 ± 0.1 | 0.1 ± 0.1 | 0.5 ± 0.2 | 0.8 ± 0.3 |
| 20:5n-3 | 0.5 ± 0.3 | 2.1 ± 0.7 | 2.4 ± 0.5 | 2.3 ± 0.3 |
| 22:0 | 0.1 ± 0.1 | 0.6 ± 0.4 | 1.2 ± 0.6 | 0.3 ± 0.3 |
| 22:1 | 0.1 ± 0.1 | 0.4 ± 0.4 | 0.2 ± 0.1 | N.D. |
| 22:2 | N.D. | N.D. | N.D. | 0.3 ± 0.3 |
| 22:4n-6 | N.D. | N.D. | N.D. | N.D. |
| 23:0 | N.D. | N.D. | N.D. | N.D. |
| 22:5n-3 | N.D. | N.D. | N.D. | N.D. |
| 22:6n-3 | 3.4 ± 0.4 | 3.6 ± 0.7 | 7.2 ± 0.4 | 6.7 ± 0.2 |
| 24:0 | N.D. | N.D. | N.D. | N.D. |
| 24:1 | N.D. | N.D. | N.D. | N.D. |
| Total SAT | 48.7 ± 3.2 | 45.3 ± 1.3 | 43.7 ± 0.7 | 44.2 ± 1.5 |
| Total MUFA | 11.3 ± 1.2 | 11.9 ± 1.5 | 13.1 ± 1.9 | 16.7 ± 0.9 |
| Total PUFA | 39.2 ± 3.5 | 42.4 ± 1.7 | 43.2 ± 2.3 | 38.2 ± 1.9 |
| Total n-3 FA | 4.7 ± 0.7 | 6.7 ± 0.4 | 10.1 ± 0.9 | 10.4 ± 0.9 |
| Total n-6 FA | 33.7 ± 2.8 | 33.5 ± 1.5 | 29.7 ± 0.6 | 26.8 ± 1.1 |
| n-3/n-6 ratio | 0.14 ± 0.01 | 0.20 ± 0.01 | 0.34 ± 0.03 | 0.39 ± 0.02 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 33

Fatty acid composition of phosphatidylinositol (PI) from cerebellum of 16 month-old WT and Tg mice on the oil blend diet or the DHA diet. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
|---|---|---|---|---|
| 12:0 | N.D. | N.D. | N.D. | N.D. |
| X1 | 0.5 ± 0.5 | 0.1 ± 0.1 | 0.2 ± 0.2 | 0.2 ± 0.2 |
| 16:0 | 11.3 ± 1.4 | 10.6 ± 2.0 | 11.1 ± 0.5 | 10.1 ± 0.2 |
| 16:1n-7 | 2.2 ± 0.7 | 2.3 ± 1.4 | 1.0 ± tr. | 0.8 ± 0.1 |
| X2 | N.D. | N.D. | N.D. | N.D. |
| X3 | N.D. | N.D. | N.D. | N.D. |
| 18:0 | 44.2 ± 1.1 | 43.6 ± 0.1 | 41.6 ± 0.8 | 43.0 ± 1.6 |
| 18:1n-9 | 10.1 ± 1.6 | 9.8 ± 1.5 | 12.1 ± 0.9 | 11.7 ± 0.9 |
| 18:1n-7 | 1.8 ± 0.2 | 1.8 ± 0.2 | 2.3 ± 0.3 | 2.3 ± 0.2 |
| 18:2n-6 | 1.1 ± 0.4 | 1.2 ± 0.5 | 0.9 ± 0.1 | 0.6 ± 0.3 |
| 18:3n-6 | N.D. | N.D. | N.D. | N.D. |
| 18:3n-3 | 0.9 ± 0.3 | 0.8 ± 0.4 | 0.5 ± 0.1 | 0.5 ± tr. |
| 20:0 | 0.2 ± 0.2 | 0.1 ± 0.1 | 0.2 ± 0.1 | 0.1 ± 0.1 |
| 20:1 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.5 ± 0.1 | 0.6 ± 0.1 |
| 20:1 | N.D. | N.D. | 0.1 ± 0.1 | N.D. |
| 20:1 | N.D. | N.D. | N.D. | N.D. |
| 20:2 | N.D. | N.D. | N.D. | N.D. |
| 20:3n-6 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.9 ± tr. | 1.0 ± tr. |
| 20:4n-6 | 25.0 ± 3.6 | 27.3 ± 4.4 | 19.6 ± 1.8 | 21.5 ± 1.2 |
| 20:3n-3 | N.D. | N.D. | N.D. | N.D. |
| 20:5n-3 | N.D. | N.D. | 2.1 ± 0.1 | 1.8 ± 0.1 |
| 22:0 | N.D. | N.D. | N.D. | N.D. |
| 22:1 | N.D. | N.D. | N.D. | N.D. |
| 22:2 | N.D. | N.D. | N.D. | N.D. |
| 22:4n-6 | N.D. | N.D. | N.D. | N.D. |
| 23:0 | N.D. | N.D. | N.D. | N.D. |
| 22:5n-3 | N.D. | N.D. | N.D. | N.D. |
| 22:6n-3 | 2.4 ± 0.4 | 2.2 ± 1.1 | 6.9 ± 0.2 | 5.8 ± 0.4 |
| 24:0 | N.D. | N.D. | N.D. | N.D. |
| 24:1 | N.D. | N.D. | N.D. | N.D. |
| Total SAT | 55.8 ± 2.0 | 54.3 ± 2.1 | 52.8 ± 1.3 | 53.3 ± 1.5 |
| Total MUFA | 14.2 ± 1.6 | 14.0 ± 2.6 | 16.0 ± 0.9 | 15.4 ± 0.8 |
| Total PUFA | 29.4 ± 3.3 | 31.5 ± 4.6 | 30.9 ± 1.5 | 31.1 ± 1.6 |
| Total n-3 FA | 3.2 ± 0.1 | 3.0 ± 0.7 | 9.5 ± 0.2 | 8.0 ± 0.4 |
| Total n-6 FA | 26.2 ± 3.3 | 28.6 ± 3.9 | 21.5 ± 1.7 | 23.1 ± 1.2 |
| n-3/n-6 ratio | 0.13 ± 0.02 | 0.10 ± 0.01 | 0.45 ± 0.04 | 0.35 ± tr. |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 34

Fatty acid composition of sphingomyeline (Sph) from cortex of 12 month-old WT and Tg mice on the oil blend diet or the DHA diet. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 4) | WT oil (n = 4) | Tg DHA (n = 4) | WT DHA (n = 4) |
|---|---|---|---|---|
| 12:0 | 0.2 ± 0.2 | N.D. | 0.1 ± 0.1 | N.D. |
| X1 | 0.9 ± 0.4 | 0.6 ± 0.4 | 0.3 ± 0.3 | 0.2 ± 0.2 |
| 16:0 | 4.5 ± 0.7 | 4.6 ± 0.9 | 4.0 ± 0.7 | 3.9 ± 0.7 |
| 16:1n-7 | 2.8 ± 0.5 | 2.2 ± 0.3 | 2.3 ± 0.4 | 2.5 ± 0.5 |
| X2 | N.D. | N.D. | N.D. | 0.1 ± 0.1 |
| X3 | N.D. | N.D. | N.D. | N.D. |
| 18:0 | 68.5 ± 3.7 | 70.5 ± 1.9 | 60.7 ± 7.7 | 69.6 ± 4.1 |
| 18:1n-9 | 3.8 ± 0.4 | 3.7 ± 0.7 | 3.8 ± 0.6 | 4.1 ± 1.0 |
| 18:1n-7 | N.D. | N.D. | N.D. | N.D. |
| 18:2n-6 | 1.1 ± 0.2 | 0.9 ± 0.2 | 1.1 ± 0.3 | 1.1 ± 0.4 |
| 18:3n-6 | 0.6 ± 0.6 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.5 ± 0.2 |
| 18:3n-3 | 0.5 ± tr. | 0.3 ± 0.1 | 0.5 ± 0.1 | 0.3 ± 0.1 |
| 20:0 | 2.0 ± 0.2 | 2.0 ± 0.1 | 2.0 ± 0.3 | 2.3 ± 0.1 |
| 20:1 | 0.3 ± 0.2 | 0.1 ± 0.1 | 1.6 ± 1.3 | 0.1 ± 0.1 |
| 20:1 | 1.3 ± 0.6 | 1.1 ± 0.3 | 1.5 ± 0.6 | 1.1 ± 0.2 |
| 20:1 | N.D. | N.D. | N.D. | 0.1 ± 0.1 |
| 20:2 | 0.3 ± 0.3 | N.D. | N.D. | N.D. |
| 20:3n-6 | N.D. | N.D. | N.D. | N.D. |
| 20:4n-6 | 0.2 ± 0.2 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.1 ± 0.1 |
| 20:3n-3 | 1.4 ± 0.9 | 1.8 ± 0.5 | 3.6 ± 1.9 | 1.7 ± 0.6 |
| 20:5n-3 | 0.1 ± 0.1 | N.D. | 0.2 ± 0.2 | 0.1 ± 0.1 |
| 22:0 | 2.1 ± 0.4 | 2.3 ± 0.1 | 3.8 ± 1.0 | 2.4 ± 0.4 |
| 22:1 | 0.5 ± 0.3 | 1.2 ± 0.9 | 2.3 ± 1.2 | 1.2 ± 0.7 |
| 22:2 | N.D. | 0.2 ± 0.1 | 0.1 ± 0.1 | 0.1 ± 0.1 |
| 22:4n-6 | N.D. | N.D. | N.D. | 0.6 ± 0.6 |
| 23:0 | 0.9 ± 0.2 | 1.0 ± 0.1 | 2.4 ± 0.8 | 1.3 ± 0.3 |
| 22:5n-3 | N.D. | N.D. | N.D. | N.D. |
| 22:6n-3 | 0.1 ± 0.1 | 0.2 ± 0.2 | 0.2 ± 0.2 | N.D. |
| 24:0 | 2.4 ± 0.4 | 1.4 ± 0.3 | 3.6 ± 0.9 | 1.6 ± 0.7 |
| 24:1 | 5.4 ± 1.4 | 5.5 ± 1.4 | 6.0 ± 1.3 | 5.0 ± 1.2 |
| Total SAT | 80.6 ± 3.2 | 81.9 ± 2.3 | 76.6 ± 5.2 | 81.1 ± 3.9 |
| Total MUFA | 14.1 ± 2.5 | 13.8 ± 2.1 | 17.5 ± 3.8 | 14.1 ± 3.2 |
| Total PUFA | 4.4 ± 1.0 | 3.6 ± 0.7 | 5.7 ± 1.8 | 4.5 ± 1.2 |
| Total n-3 FA | 2.2 ± 1.0 | 2.3 ± 0.5 | 4.4 ± 1.8 | 2.0 ± 0.6 |
| Total n-6 FA | 1.97 ± 0.7 | 1.1 ± 0.2 | 1.2± 0.3 | 2.3 ± 0.7 |
| n-3/n-6 ratio | 1.56 ± 0.53 | 2.12 ± 0.60 | 4.01 ± 1.81 | 0.93 ± 0.33 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 35

Fatty acid composition of sphingomyelin (Sph) from cortex of 16 month-old WT and Tg mice on the oil blend diet or the DHA diet. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
|---|---|---|---|---|
| 12:0 | N.D. | N.D. | N.D. | N.D. |
| X1 | 0.1 ± 0.1 | 0.5 ± 0.3 | 0.3 ± 0.2 | 0.2 ± 0.2 |
| 16:0 | 6.9 ± 0.8 | 7.5 ± 1.0 | 6.8 ± 0.9 | 6.7 ± 1.0 |
| 16:1n-7 | 0.5 ± 0.5 | 0.6 ± 0.6 | 0.8 ± 0.8 | 0.8 ± 0.4 |
| X2 | N.D. | N.D. | N.D. | N.D. |
| X3 | N.D. | N.D. | N.D. | N.D. |
| 18:0 | 85.6 ± 1.0 | 84.2 ± 1.9 | 84.2 ± 2.2 | 85.0 ± 1.2 |
| 18:1n-9 | 3.7 ± 0.5 | 3.9 ± 0.6 | 4.1 ± 0.7 | 3.9 ± 0.5 |
| 18:1n-7 | N.D. | N.D. | N.D. | N.D. |
| 18:2n-6 | 0.3 ± 0.1 | 0.4 ± tr. | 0.5 ± tr. | 0.4 ± 0.1 |
| 18:3n-6 | N.D. | N.D. | N.D. | N.D. |
| 18:3n-3 | 0.2 ± 0.2 | N.D. | N.D. | N.D. |
| 20:0 | 1.5 ± tr. | 1.6 ± 0.1 | 1.6 ± tr. | 1.6 ± 0.1 |
| 20:1 | N.D. | N.D. | N.D. | N.D. |
| 20:1 | N.D. | N.D. | N.D. | N.D. |
| 20:1 | 0.1 ± 0.1 | N.D. | 0.1 ± 0.1 | 0.1 ± 0.1 |
| 20:2 | 0.8 ± 0.1 | 0.8 ± 0.1 | 0.8 ± 0.1 | 0.9 ± 0.1 |
| 20:3n-6 | N.D. | N.D. | N.D. | N.D. |
| 20:4n-6 | N.D. | N.D. | N.D. | N.D. |
| 20:3n-3 | N.D. | N.D. | N.D. | N.D. |
| 20:5n-3 | N.D. | N.D. | N.D. | N.D. |
| 22:0 | 0.3 ± 0.3 | 0.6 ± 0.3 | 0.7 ± 0.4 | 0.5 ± 0.3 |
| 22:1 | N.D. | N.D. | N.D. | N.D. |
| 22:2 | N.D. | N.D. | N.D. | N.D. |
| 22:4n-6 | N.D. | N.D. | N.D. | N.D. |
| 23:0 | N.D. | N.D. | N.D. | N.D. |
| 22:5n-3 | N.D. | N.D. | N.D. | N.D. |
| 22:6n-3 | N.D. | N.D. | N.D. | N.D. |
| 24:0 | N.D. | N.D. | N.D. | N.D. |
| 24:1 | N.D. | N.D. | 0.2 ± 0.2 | N.D. |
| Total SAT | 94.3 ± 0.4 | 93.9 ± 1.2 | 93.3 ± 1.7 | 93.8 ± 0.5 |
| Total MUFA | 4.3 ± 0.7 | 4.5 ± 1.0 | 5.1 ± 1.4 | 4.8 ± 0.4 |
| Total PUFA | 1.3 ± 0.3 | 1.2 ± tr. | 1.3 ± 0.1 | 1.3 ± 0.1 |
| Total n-3 FA | 0.2 ± 0.2 | N.D. | N.D. | N.D. |
| Total n-6 FA | 0.3 ± 0.1 | 0.4 ± tr. | 0.5 ± tr. | 0.4 ± 0.1 |
| n-3/n-6 ratio | 0.71 ± 0.71 | 0.00 ± tr. | 0.00 ± tr. | 0.00 ± tr. |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 36

Fatty acid composition of sphingomyelin (Sph) from hippocampus of 12 month-old WT and Tg mice on the oil blend diet or the DHA diet. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 4) | WT oil (n = 4) | Tg DHA (n = 4) | WT DHA (n = 4) |
|---|---|---|---|---|
| 12:0 | 0.1 ± 0.1 | 0.1 ± tr. | 0.2 ± 0.2 | 0.4 ± 0.3 |
| X1 | 0.8 ± 0.6 | 1.1 ± 0.6 | 0.6 ± 0.4 | N.D. |
| 16:0 | 7.6 ±1.3 | 7.3 ± 0.6 | 8.7 ± 2.2 | 4.4 ± 0.9 |
| 16:1n-7 | 1.6 ± 0.2 | 1.6 ± 0.1 | 1.9 ± 0.1 | 1.7 ± 0.3 |
| X2 | N.D. | N.D. | N.D. | N.D. |
| X3 | N.D. | N.D. | N.D. | N.D. |
| 18:0 | 71.5 ± 1.0 | 75.4 ± 1.8 | 70.8 ± 2.6 | 74.9 ± 0.7 |
| 18:1n-9 | 9.9 ± 0.3 | 6.8 ± 2.2 | 9.3 ± 0.7 | 8.1 ± 1.8 |
| 18:1n-7 | N.D. | N.D. | N.D. | N.D. |
| 18:2n-6 | 0.7 ± tr. | 0.6 ± tr. | 1.0 ± 0.2 | 0.5 ± 0.2 |
| 18:3n-6 | 0.1 ± 0.1 | N.D. | 0.2 ± 0.1 | N.D. |
| 18:3n-3 | 0.1 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.1 ± 0.1 |
| 20:0 | 1.3 ± 0.1 | 1.3 ± 0.1 | 1.2 ± 0.1 | 1.6 ± 0.3 |
| 20:1 | 0.3 ± 0.3 | 0.5 ± 0.3 | 0.6 ± 0.4 | 0.2 ± 0.2 |
| 20:1 | 1.1 ± 0.2 | 0.4 ± 0.2 | 0.7 ± 0.3 | 0.7 ± 0.1 |
| 20:1 | N.D. | N.D. | N.D. | N.D. |
| 20:2 | N.D. | N.D. | N.D. | N.D. |
| 20:3n-6 | N.D. | N.D. | N.D. | N.D. |
| 20:4n-6 | N.D. | N.D. | N.D. | N.D. |
| 20:3n-3 | 0.8 ± 0.5 | 0.8 ± 0.1 | 0.8 ± 0.2 | 1.3 ± 0.3 |
| 20:5n-3 | 0.2 ± 0.2 | N.D. | 0.1 ± 0.1 | N.D. |
| 22:0 | 1.3 ± 0.2 | 1.0 ± 0.4 | 0.8 ± 0.4 | 1.4 ± 0.5 |
| 22:1 | N.D. | N.D. | N.D. | N.D. |
| 22:2 | N.D. | N.D. | N.D. | 0.1 ± 0.1 |
| 22:4n-6 | 0.4 ± 0.3 | 0.4 ± 0.3 | 0.5 ± 0.4 | 1.4 ± 1.0 |
| 23:0 | N.D. | N.D. | N.D. | N.D. |
| 22:5n-3 | N.D. | N.D. | N.D. | N.D. |
| 22:6n-3 | N.D. | N.D. | N.D. | N.D. |
| 24:0 | 0.4 ± 0.1 | 0.2 ± 02. | 0.3 ± 0.1 | 0.7 ± 0.5 |
| 24:1 | 1.8 ± 0.7 | 2.2 ± 0.8 | 2.0 ± 0.8 | 2.5 ± 1.1 |
| Total SAT | 82.2 ± 0.9 | 85.3 ± 1.9 | 82.1 ± 0.9 | 83.4 ± 0.9 |
| Total MUFA | 14.7 ± 1.0 | 11.6 ± 2.0 | 14.5 ± 0.6 | 13.3 ± 1.6 |
| Total PUFA | 2.3 ± 0.6 | 2.0 ± 0.2 | 2.7 ± 0.5 | 3.4 ± 1.4 |
| Total n-3 FA | 1.1 ± 0.4 | 1.0 ± 0.2 | 1.1 ± 0.3 | 1.4 ± 0.4 |
| Total n-6 FA | 1.2 ± 0.5 | 1.1 ± 0.3 | 1.6 ± 0.2 | 1.9 ± 1.1 |
| n-3/n-6 ratio | 1.15 ± 0.59 | 1.20 ± 0.40 | 0.68 ± 0.14 | 0.66 ± 0.17 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 37

Fatty acid composition of sphingomyelin (Sph) from hippocampus of 16 month-old WT and Tg mice on the oil blend diet or the DHA diet. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
|---|---|---|---|---|
| 12:0 | N.D. | N.D. | 0.1 ± 0.1 | N.D. |
| X1 | 0.8 ± 0.4 | 0.7 ± 0.5 | 1.2 ± 1.1 | 0.4 ± 0.2 |
| 16:0 | 10.7 ± 1.2 | 11.3 ± 2.6 | 10.5 ± 2.6 | 9.1 ± 0.8 |
| 16:1n-7 | 4.0 ± 1.1 | 2.6 ± 0.5 | 3.3 ± 0.7 | 3.0 ± 1.2 |
| X2 | N.D. | N.D. | N.D. | N.D. |
| X3 | N.D. | N.D. | N.D. | N.D. |
| 18:0 | 75.3 ± 1.4 | 74.9 ± 5.0 | 74.7 ± 5.1 | 78.7 ± 1.9 |
| 18:1n-9 | 5.6 ± 0.6 | 5.8 ± 0.8 | 6.3 ± 0.7 | 6.4 ± 0.2 |
| 18:1n-7 | N.D. | 0.1 ± 0.1 | 0.1 ± 0.1 | N.D. |
| 18:2n-6 | 0.8 ± 0.1 | 0.6 ± 0.1 | 0.6 ± 0.1 | 0.7 ± tr. |
| 18:3n-6 | N.D. | N.D. | N.D. | N.D. |
| 18:3n-3 | 0.6 ± 0.3 | 0.6 ± 0.3 | 0.5 ± 0.2 | 0.4 ± 0.2 |
| 20:0 | 1.1 ± 0.1 | 1.1 ± 0.1 | 1.1 ± tr. | 0.7 ± 0.4 |
| 20:1 | N.D. | N.D. | N.D. | N.D. |
| 20:1 | N.D. | N.D. | N.D. | N.D. |
| 20:1 | N.D. | 0.1 ± 0.1 | 0.1 ± 0.1 | N.D. |
| 20:2 | 0.4 ± 0.4 | 0.6 ± 0.5 | 0.1 ± 0.1 | N.D. |
| 20:3n-6 | 0.4 ± 0.2 | 0.8 ± 0.2 | 0.8 ± 0.1 | 0.5 ± 0.2 |
| 20:4n-6 | N.D. | N.D. | N.D. | N.D. |
| 20:3n-3 | N.D. | 0.1 ± 0.1 | N.D. | N.D. |
| 20:5n-3 | N.D. | N.D. | N.D. | N.D. |
| 22:0 | 0.1 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 | N.D. |
| 22:1 | 0.3 ± 0.2 | 0.4 ± 0.2 | 0.4 ± 0.2 | 0.2 ± 0.2 |
| 22:2 | N.D. | N.D. | N.D. | N.D. |
| 22:4n-6 | N.D. | N.D. | N.D. | N.D. |
| 23:0 | N.D. | N.D. | N.D. | N.D. |
| 22:5n-3 | N.D. | N.D. | N.D. | N.D. |
| 22:6n-3 | N.D. | N.D. | N.D. | N.D. |
| 24:0 | N.D. | N.D. | N.D. | N.D. |
| 24:1 | N.D. | N.D. | N.D. | N.D. |
| Total SAT | 87.1 ± 1.8 | 87.6 ± 2.5 | 86.6 ± 2.6 | 88.5 ± 1.6 |
| Total MUFA | 10.0 ± 1.5 | 9.0 ± 1.5 | 10.2 ± 1.6 | 9.5 ± 1.0 |
| Total PUFA | 2.1 ± 0.3 | 2.7 ± 0.8 | 2.0 ± 0.2 | 1.6 ± 0.5 |
| Total n-3 FA | 0.6 ± 0.3 | 0.7 ± 0.4 | 0.5 ± 0.2 | 0.4 ± 0.2 |
| Total n-6 FA | 1.2 ± 0.3 | 1.4 ± 0.1 | 1.4 ± 0.1 | 1.2 ± 0.3 |
| n-3/n-6 ratio | 0.35 ± 0.19 | 0.53 ± 0.30 | 0.35 ± 0.18 | 0.31 ± 0.15 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 38

Fatty acid composition of sphingomyelin (Sph) from cerebellum of 12 month-old WT and Tg mice on the oil blend diet or the DHA diet. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 4) | WT oil (n = 4) | Tg DHA (n = 4) | WT DHA (n = 4) |
|---|---|---|---|---|
| 12:0 | N.D. | N.D. | 0.1 ± 0.1 | 0.4 ± 0.4 |
| X1 | 1.2 ± 0.5 | 1.4 ± 1.0 | 1.1 ± 0.7 | 1.1 ± 1.1 |
| 16:0 | 10.3 ± 1.0 | 9.7 ± 1.5 | 9.7 ± 2.1 | 8.5 ± 1.3 |
| 16:1n-7 | 1.5 ± 0.4 | 1.9 ± 0.2 | 1.7 ± 0.6 | 1.9 ± 0.3 |
| X2 | N.D. | N.D. | 0.3 ± 0.3 | 0.1 ± 0.1 |
| X3 | 0.1 ± 0.1 | 0.2 ± 0.2 | 0.2 ± 0.2 | 0.4 ± 0.3 |
| 18:0 | 55.5 ± 4.4 | 54.4 ± 3.9 | 49.8 ± 5.9 | 58.8 ± 2.9 |
| 18:1n-9 | 5.2 ± 1.4 | 4.0 ± 0.1 | 3.2 ± 1.4 | 6.3 ± 0.8 |
| 18:1n-7 | 1.0 ± 0.7 | 1.3 ± 1.1 | 2.0 ± 1.0 | 0.6 ± 0.6 |
| 18:2n-6 | 1.6 ± 0.3 | 2.2 ± 0.6 | 1.3 ± 0.5 | 1.8 ± 0.4 |
| 18:3n-6 | 1.0 ± 0.6 | 0.9 ± 0.6 | 0.8 ± 0.4 | 1.1 ± 0.4 |
| 18:3n-3 | 2.7 ± 0.8 | 2.3 ± 0.3 | 2.8 ± 0.4 | 1.1 ± 0.4 |
| 20:0 | 3.3 ± 0.7 | 5.3 ± 1.5 | 4.0 ± 2.0 | 3.8 ± 0.5 |
| 20:1 | 1.4 ± 0.7 | 1.5 ± 0.8 | 1.0 ± 0.6 | 2.2 ± 0.8 |
| 20:1 | N.D. | 0.1 ± 0.1 | 0.7 ± 0.5 | 0.8 ± 0.6 |
| 20:1 | 1.4 ± 0.6 | 1.5 ± 0.5 | 0.5 ± 0.3 | 0.3 ± 0.2 |
| 20:2 | 4.2 ± 0.4 | 5.8 ± 1.3 | 2.2 ± 0.9 | 1.7 ± 0.8 |
| 20:3n-6 | 1.2 ± 0.5 | 1.6 ± 0.5 | 1.3 ± 0.5 | 1.0 ± 0.4 |
| 20:4n-6 | 0.3 ± 0.2 | 0.2 ± 0.2 | 3.1 ± 2.5 | 1.3 ± 0.6 |
| 20:3n-3 | 1.8 ± 0.6 | 2.2 ± 0.9 | 1.5 ± 0.6 | 0.8 ± 0.3 |
| 20:5n-3 | 1.4 ± 0.3 | 1.4 ± 0.5 | 1.1 ± 0.1 | 0.8 ± 0.5 |
| 22:0 | 0.5 ± 0.3 | 0.7 ± 0.2 | 2.8 ± 1.3 | 1.5 ± 0.8 |
| 22:1 | 1.6 ± 0.9 | 0.6 ± 0.4 | 1.3 ± 0.9 | 0.4 ± 0.4 |
| 22:2 | N.D. | N.D. | 0.7 ± 0.4 | 0.7 ± 0.6 |
| 22:4n-6 | N.D. | 0.3 ± 0.2 | 1.0 ± 1.0 | 0.4 ± 0.3 |
| 23:0 | N.D. | N.D. | N.D. | N.D. |
| 22:5n-3 | N.D. | N.D. | N.D. | N.D. |
| 22:6n-3 | N.D. | N.D. | 0.4 ± 0.4 | N.D. |
| 24:0 | N.D. | 0.1 ± 0.1 | N.D. | 0.8 ± 0.8 |
| 24:1 | 2.8 ± 1.7 | 0.8 ± 0.8 | 5.7 ± 3.6 | 1.8 ± 1.8 |
| Total SAT | 69.6 ± 3.1 | 70.2 ± 3.1 | 66.4 ± 4.3 | 73.7 ± 2.6 |
| Total MUFA | 15.0 ± 2.6 | 11.5 ± 0.7 | 16.0 ± 1.9 | 14.1 ± 1.4 |
| Total PUFA | 14.1 ± 1.5 | 16.8 ± 3.5 | 15.9 ± 3.6 | 10.6 ± 2.7 |
| Total n-3 FA | 5.8 ± 0.6 | 5.9 ± 1.6 | 5.7 ± 0.5 | 2.6 ± 1.1 |
| Total n-6 FA | 4.1 ± 1.3 | 5.1 ± 1.5 | 7.3 ± 3.0 | 5.6 ± 1.1 |
| n-3/n-6 ratio | 2.54 ± 1.35 | 1.46 ± 0.47 | 1.45 ± 0.76 | 0.41 ± 0.17 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).

# APPENDIX 39

Fatty acid composition of sphingomyelin (Sph) from cerebellum of 16 month-old WT and Tg mice on the oil blend diet or the DHA diet. Results are represented as mean percentage of total fatty acids ± SEM.

| Fatty acid | Tg oil (n = 3) | WT oil (n = 3) | Tg DHA (n = 3) | WT DHA (n = 3) |
|---|---|---|---|---|
| 12:0 | N.D. | N.D. | 0.1 ± 0.1 | N.D. |
| X1 | 0.5 ± 0.5 | 0.6 ± 0.6 | 0.4 ± 0.4 | 0.4 ± 0.4 |
| 16:0 | 17.1 ± 1.9 | 14.7 ± 1.6 | 14.3 ± 1.4 | 12.4 ± 0.9 |
| 16:1n-7 | 3.6 ± 0.8 | 3.3 ± 1.0 | 2.4 ± 0.5 | 3.0 ± 0.8 |
| X2 | N.D. | N.D. | N.D. | N.D. |
| X3 | N.D. | N.D. | N.D. | N.D. |
| 18:0 | 59.9 ± 3.1 | 64.7 ± 3.1 | 64.8 ± 1.0 | 69.1 ± 2.5 |
| 18:1n-9 | 11.1 ± 2.5 | 8.6 ± 2.6 | 6.9 ± 0.9 | 6.5 ± 0.2 |
| 18:1n-7 | N.D. | N.D. | N.D. | N.D. |
| 18:2n-6 | 1.2 ± 0.4 | 0.9 ± 0.2 | 0.9 ± 0.2 | 0.7 ± tr. |
| 18:3n-6 | N.D. | N.D. | N.D. | N.D. |
| 18:3n-3 | 1.3 ± 0.3 | 1.2 ± 0.2 | 1.0 ± tr. | 0.7 ± 0.1 |
| 20:0 | 1.4 ± tr. | 1.3 ± 0.1 | 1.5 ± 0.1 | 1.4 ± tr. |
| 20:1 | N.D. | N.D. | N.D. | N.D. |
| 20:1 | N.D. | N.D. | N.D. | N.D. |
| 20:1 | 0.3 ± 0.2 | 0.1 ± 0.1 | 0.2 ± tr. | N.D. |
| 20:2 | N.D. | 0.3 ± 0.3 | 0.6 ± 0.6 | 0.2 ± 0.2 |
| 20:3n-6 | N.D. | 0.4 ± 0.4 | 0.3 ± 0.3 | 0.4 ± 0.4 |
| 20:4n-6 | 0.6 ± 0.6 | N.D. | N.D. | 0.6 ± 0.6 |
| 20:3n-3 | N.D. | N.D. | N.D. | 0.1 ± 0.1 |
| 20:5n-3 | 0.4 ± 0.4 | 0.1 ± 0.1 | 0.2 ± 0.2 | 0.2 ± 0.2 |
| 22:0 | 0.9 ± 0.5 | 0.8 ± 0.4 | 1.4 ± 0.5 | 1.5 ± 0.1 |
| 22:1 | N.D. | N.D. | 0.5 ± 0.5 | N.D. |
| 22:2 | N.D. | N.D. | N.D. | N.D. |
| 22:4n-6 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.3 ± 0.1 | 0.1 ± 0.1 |
| 23:0 | N.D. | N.D. | N.D. | N.D. |
| 22:5n-3 | N.D. | N.D. | N.D. | N.D. |
| 22:6n-3 | N.D. | N.D. | N.D. | N.D. |
| 24:0 | 0.3 ± 0.3 | 0.5 ± 0.2 | 1.1 ± 0.4 | 0.5 ± 0.3 |
| 24:1 | 1.3 ± 0.7 | 2.3 ± 1.2 | 3.4 ± 0.4 | 2.2 ± 0.3 |
| Total SAT | 79.6 ± 2.1 | 82.1 ± 2.1 | 83.2 ± 0.8 | 84.8 ± 1.8 |
| Total MUFA | 16.3 ± 2.4 | 14.3 ± 2.3 | 13.3 ± 1.1 | 11.7 ± 1.1 |
| Total PUFA | 3.5 ± 0.8 | 3.1 ± 0.1 | 3.2 ± 0.5 | 3.1 ± 0.9 |
| Total n-3 FA | 1.6 ± 0.2 | 1.3 ± 0.2 | 1.2 ± 0.2 | 0.9 ± 0.1 |
| Total n-6 FA | 1.9 ± 0.6 | 1.5 ± 0.3 | 1.4 ± 0.3 | 1.9 ± 0.7 |
| n-3/n-6 ratio | 0.98 ± 0.19 | 0.90 ± 0.16 | 0.87 ± 0.03 | 0.62 ± 0.19 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; N.D., not detected; tr., trace (less than 0.05).