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DHA diet reduces AD pathology in young APPswe/PS1ΔE9 transgenic mice: Possible Gender Effects

Sylvia E. Perez¹, Brian M. Berg¹, Kenneth A. Moore², Bin He¹, Scott E. Counts¹, Jason J. Fritz³, Yuan-Shih Hu⁴, Orly Lazarov⁴, James J. Lah³, and Elliott J. Mufson^{1,*}

¹Department of Neurological Sciences, Rush University Medical Center, Chicago, IL 60612

²Wyeth Nutrition, Collegeville, PA 19426

³Department of Neurology, Center of Neurodegenerative Disease, Emory University, Atlanta, GA 30322

⁴Department of Anatomy and Cell Biology, College of Medicine, University of Illinois at Chicago, Chicago, IL, 60612

Abstract

Epidemiological and clinical trial findings suggest that consumption of docosahexaenoic acid (DHA) lowers the risk of Alzheimer's disease (AD). We examined the effects of short-term (3 months) DHA enriched diet on plaque deposition and synaptic deficits in forebrain of young APPswe/PS1ΔE9 transgenic (tg) and non-transgenic (ntg) mice. Gas chromatography revealed a significant increase in DHA concomitant with a decrease of arachidonic acid in both brain and liver in mice fed with DHA. Female tg mice consumed relatively more food daily than ntg female mice, independent of diet. Plaque load was significantly reduced in the cortex, ventral hippocampus and striatum of female APPswe/PS1ΔE9 tg mice on DHA diet compared to female tg mice on control diet. LR11 levels were unchanged in mice on DHA. Moreover drebrin levels were significantly increased in the hippocampus of tg mice on the DHA diet. Finally, in vitro DHA treatment prevented amyloid toxicity in cell cultures. Our findings support the concept that increased DHA consumption may play an important role in preventing brain insults in AD.

Keywords

n-3 fatty acids; Alzheimer's disease; amyloid; transgenics; drebrin

Introduction

Alzheimer's disease (AD), a devastating neurodegenerative disorder, causes cognitive dysfunction in the elderly, and is characterized by brain β -amyloid (A β) plaques, neurofibrillary tangles and synaptic loss (Masliah et al., 1989; Braak and Braak, 1990; Arnold et al., 1991; Scheff et al., 2006). Currently, five FDA-approved drugs (tacrine/cognex, donepezil, rivastigmine, galantamine and memantine) are available for AD treatment (Klafki et al., 2006), but none prevent the progression of the disease. Research has focused on developing novel treatments to ameliorate the clinical and pathological sequelae of AD including natural products.

Address correspondence to: Elliott J. Mufson, Ph.D., Professor of Neurological Sciences, Alla V. and Solomon Jesmer Chair in Aging, Rush University Medical Center, 1735 W. Harrison Street, Suite 300, Chicago, IL 60612, 312-563-3558 tel., 312-563-3571 fax., emufson@rush.edu.

B.M. Berg's present address: Wyeth Nutrition, Collegeville, PA 19426

Epidemiological studies suggest that the consumption of fish/seafood rich in n (omega)-3 polyunsaturated fatty acids lowers the risk of developing AD (Kalmijn et al., 1997; Barberger-Gateau et al., 2002; Morris et al., 2003; Huang et al 2005). In fact, diet supplementation with the essential n-3 polyunsaturated fatty acid docosahexaenoic acid (DHA) improves cognition in people with mild AD or mild cognitive impairment (MCI) (Freund-Levi et al., 2006; Chiu et al., 2008), suggesting that brain DHA reduction (Prasad et al., 1998; Söderberg et al., 1991) play a role in the development of AD (Morris et al., 2003; Schaefer et al., 2006). However, despite mounting evidence for a role of DHA on the development of AD, the actions of DHA in brain remain unknown.

DHA dietary administration reduces A β and tau pathology (Lim et al., 2005; Oksman et al., 2006; Green et al., 2007), protects against dendritic pathology (Calon et al., 2004), cerebral blood volume alterations (Hooijmans et al., 2007) and enhances cognitive function (Calon et al., 2004) in animal models of AD. For instance, we demonstrated that DHA intake reduced deficits in striatal associated motor dysfunction in A β over-expressing APPswe/PS1 Δ E9 transgenic (tg) mice (Berg et al., 2005) suggesting widespread effects of DHA in the brain of these mutant mice. Although other studies showed that DHA dietary enrichment beginning at 6 months of age and onwards failed to reduce A β plaque deposition and improve cognition in APPswe/PS1 Δ E9 tg mice (Oksman et al., 2006; Hooijmans et al., 2007), the effects of the initiation of DHA dietary enrichment prior to plaque formation in these mice remains to be determined. Moreover, as most DHA animal supplementation studies have focused on its ability to reduce hippocampal A β deposition, there is little information regarding DHA's effect on other brain regions vulnerable in AD. To explore the effect of dietary DHA on AD-like pathology in the brains of APPswe/PS1 Δ E9 tg mice prior to extensive plaque deposition (Perez et al., 2005, 2007), 3 month old age-matched APPswe/PS1 Δ E9 tg and nontransgenic (ntg) mice were fed either a DHA supplemented or control diet for 3 months. At 6 months of age, brains were harvested and biochemically analyzed for the ability of DHA to alter A β plaque deposition and synaptic pathology in the cortex, hippocampus and striatum. We report that short-term DHA intake differentially reduced plaque pathology in the cortex, hippocampus and striatum in a gender-specific manner and prevented pre- and postsynaptic deficits in the brains of APPswe/PS1 Δ E9 tg mice.

Material and Methods

Animal groups and housing conditions

A total of thirty-six young (3 months) mice were used: 16 (8♂:8♀) heterozygous APPswe/PS1 Δ E9 tg and 20 (11♂:9♀) age-matched ntg mice. APPswe/PS1 Δ E9 mice coexpress human presenilin 1 (PS1) bearing the Δ E9 deletion mutation and a chimeric mouse-human amyloid precursor protein (APP) 695 with mutations (K595N, M596L) linked to Swedish FAD pedigrees (APPswe) under control of the mouse prion protein promoter (Borchelt et al., 1996a,b, 1997). Mice were obtained from an in-house breeding colony by crossing single APPswe line C3-3 and PS1 Δ E9 line S-9 tg mice and PS1 Δ E9 line S-9 tg with ntg littermate mice derived from breeding pairs provided by Dr. S. Sisodia (University of Chicago, IL). The background strains for APPswe are {C3H/HeJ $\times$ C57BL/6J F3} $\times$ C57BL/6J n1, and PS1 Δ E9 are C3H/HeJ $\times$ C57BL/6J F3. After genotyping, APPswe/PS1 Δ E9 tg and ntg mice were randomly fed one of two AIN-93G based diets (Harlan Teklad, Madison, WI): control diet (#TD06088) containing 0% DHA with milk fat and safflower oil as lipid source or DHA diet (#TD06087) containing 0.6% DHA (DHASCO, Martek Biosciences, Columbia, MD) with safflower oil as the main source of fat (Table I). Both diets contained all the adequate nutrients for healthy growth. Mice were fed control or DHA diet for 12 weeks before being sacrificed at 6 months of age. During treatment, food intake was measured 3 times a week and body weight recorded once a week. Animal care and

procedures were conducted according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Tissue processing

All mice were anesthetized with an injection of ketamine/xylazine (285mg/kg/9.5mg/kg) and perfused transcardially with ice-cold 0.9% sodium chloride. Brains were rapidly removed from the calvarium and hemisected using a stainless steel brain blocker. One hemisphere was sectioned into 1 mm coronal slabs on wet ice in the brain blocker. Tissue pieces were dissected from the cortex, hippocampus and striatum using fiduciary landmarks and frozen at -80°C until processed for biochemical assays. The other hemisphere was immersion-fixed in 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M phosphate buffer for 12 hours at 4°C , cryoprotected in 30% sucrose at 4°C until the brain sank, and then cut frozen on a sliding knife microtome in the coronal plane at a thickness of $40\text{ }\mu\text{m}$. The sections were stored at -20°C in a cryoprotectant solution (30% glycerol and 30% ethylene glycol in 0.1 M phosphate buffered saline) prior to use.

Immunofluorescence

Fixed tissue was processed as free-floating sections and single-labeled with a monoclonal antibody against A β (10D5, a gift from Elan Pharmaceuticals, San Francisco, CA). Sections were washed several times in Tris-buffered saline (TBS) and placed in TBS containing 0.25% Triton X-100, before incubation in the blocking solution of TBS with 0.25% Triton X-100 and 3% horse normal serum for 1 hour. Subsequently, the sections were incubated in mouse anti-human A β 10D5 antibodies for 24 hours in a medium containing TBS 0.25% Triton X-100 and 1% horse normal serum. Following 3 washes with 1% horse normal serum in TBS, sections were incubated with Cy3-conjugated donkey anti-mouse (1:300; Jackson ImmunoResearch Labs, West Grove, PA) for 1 hour. After several washes in TBS, sections were mounted on gelatin coated glass slides, dehydrated in graded alcohols, cleared in xylenes and coverslipped.

Amyloid plaque load analysis

For quantification of A β load, immunofluorescence images of the cortex, hippocampus and striatum of APP^{sw}/PS1 ΔE9 tg mice fed control or DHA diet were taken with a Zeiss confocal laser scanning microscope and volume of A β immunoreactive deposits were analyzed using Volocity 3.7 2006 (Improvision Inc, Waltham, MA). Z series of $10\text{ }\mu\text{m}$ depth were imaged from each section with $1\text{ }\mu\text{m}$ intervals between images. Images were converted in all planes into binary images after calibration of pixel size. Single components were considered noise and removed uniformly from all planes. Burden volume was automatically calculated as previously described (Lazarov et al., 2002).

Quantitative immunoblotting

Mice tissue samples from cortex, hippocampus and striatum were sonicated in ice-cold NP-40 buffer (150mM sodium chloride, 1% NP-40, 50mM Tris, pH 8.0) containing a protease inhibitor cocktail (Sigma, St. Louis, MO). Samples were prepared by centrifugation of whole cell lysates at 1000 rpm for 10 minutes at 4°C . Protein concentration of the resulting S1 supernatant was determined by the Bradford method (Bio-Rad, Hercules, CA) using bovine serum albumin as the protein standard. Sample proteins were denatured in Laemmli sample buffer (Bio-Rad, Hercules, CA) at 100°C for 5 minutes and kept on wet ice for 2 minutes. Fifty μg of total protein was used for immunoblotting of drebrin (DRB, 140 kDa), synaptotagmin (SYT, 65 kDa) and 10 μg protein was used for immunoblotting of synaptophysin (SYP, 38 kDa), and 50 μg for LR11 (250 kDa). β -actin immunoreactivity on the same blots served as an internal control for sample loading. Synaptic and LR11 proteins

were separated by gradient (8–16% Tris-HCl Gel) and 7.5 % sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, respectively, and transferred electrophoretically to polyvinylidene fluoride membranes (Immobilon P, Millipore, Bedford, MA). Membranes were blocked in TBS/0.05% Tween-20/5% non-fat milk for 30–60 minutes at room temperature. Membranes were incubated with primary antibodies of interest for 30–60 minutes at room temperature and then incubated overnight at 4°C (mouse anti-DRB, clone M2F6, 1:100, MBL International, Woburn, MA; chicken anti-SYT, 1:1000, Abcam, Cambridge, CA; mouse anti-SYP, clone SY38; 1:2000, Millipore, Bedford, MA; mouse anti-LR11, clone 48, 1:500, BD, San Jose, CA). After several rinses, the blots were incubated for 1 hour at room temperature with the following secondary antibodies depending on the primary antibody: horseradish peroxidase-conjugated (HRP) goat anti-mouse IgG (1:8,000; Pierce, Rockford, IL), HRP-rabbit anti-chicken IgG (1:4000; Millipore, Bedford, MA), IRDye-800 donkey anti-mouse for LR11 (1:10000; Rockland, Gilbertsville, PA) or IRDye-700 donkey anti-goat for β -actin (1:10000; Rockland, Gilbertsville, PA). Immunoreactive synaptic proteins were visualized by enhanced chemiluminescence (Thermo Scientific, Rockford, IL for synaptic proteins or Perkin-Elmer, Boston, MA for LR11) on a Kodak Image Station 440CF (Eastman Kodak, New Haven, Cincinnati, Ohio), which provides linear data over a dynamic range of four orders of magnitude. Bands were quantified using Kodak 1D image analysis software (Perkin-Elmer, Wellesley, MA). LR11 immunoblots were dried, scanned, and quantitated with an Odyssey Infrared Imaging System (Li-Cor Biosciences, Lincoln, NE). Immunoreactive signals of the synaptic and LR11 proteins were normalized to β -actin {(synaptic proteins: mouse anti- β -actin, clone AC-74, 1:4000, Sigma, St. Louis, MO or goat anti- β -actin for LR11, 1:1000, Santa Cruz Biotechnology, Santa Cruz, CA)} signals for quantitative analysis. Each sample was analyzed on three different western blots in independent experiments.

Fatty Acid Determination

Brain and liver fatty acids from APPswe/PS1 Δ E9 tg and ntg mice were extracted using Folch's method (Folch et al., 1957). Individual samples from brain and liver were homogenized (1 gram of tissue/10 mL of DI water) and then 1 mL homogenate was mixed with chloroform: methanol (2:1 v/v) for lipid extraction which was preserved in argon and stored at –80 °C. To convert the fatty acids and methyl esters, the extracted lipid was trans-methylated with toluene and boron trifluoride-methanol at 100 °C for 30 minutes according to Morrison and Smith (1959). Samples were collected with hexane, purged with nitrogen and stored at –20 °C. A 250 μ L sample of the hexane upper phase was transferred to a gas chromatography vial for fatty acid methyl ester profiling by gas chromatography.

Fatty acid methyl esters (FAME) profiles were analyzed on an Agilent 6890N gas chromatograph (GC) equipped with a flame ionization detector (FID). The different FAMEs were separated in a capillary column (RTTM-2560; 100 m $\times$ 0.25 mm and 0.2 μ m thickness; Restek, PA) containing helium as a carrier gas. GC inlet was run in split mode at 225 °C, pressure of 29.6 psi, split ratio of 80:1 with a split flow of helium at 80 ml/minute. The FID was run at 300 °C with a hydrogen flow of 30 ml/minute, air flow of 400 ml/min and makeup flow (nitrogen) of 25 ml/minute. The column was run in constant flow mode at 1.0 ml/min starting at 60 °C with no hold and rising to a final temperature of 249 °C with a 12 minute hold at the end for a total run time of 75 minutes. Fatty acids were identified from their retention times compared to the standards (Nu-Chek Prep: GLC-463, GLC-481-B, UC-59-M standards; Matreya 1181 standard). Standards and samples were run under the same conditions and relative amounts were determined using peak area calculations. Data were analyzed with Agilent Chemstation software and results were expressed as a percentage of the total normalized weight FAMEs.

Fatty acid content in the diets was determined using a modified Roese-Gottlieb extraction method (Folch et al., 1957) and total fat measured gravimetrically. Modification of the extraction method was achieved by acidifying the samples with acid ethanol before addition of anhydrous and petroleum ether. Approximately 500 mg of each sample was weighed and extracted with acid ethanol, anhydrous ether, and petroleum ether. The ether layer was dried under low heat (45 °C) and nitrogen to produce an oily residue (extracted residue), which was then weighed to quantify the total fat content. A methylation procedure was performed on the extracted residue with toluene, boron trifluoride/methanol, hexane, saturated sodium chloride, and anhydrous sodium sulfate. The hexane/toluene layer was transferred to a separate vial for storage and then quantified by GC as described above.

Human NT2 neuronal cell culture

Differentiated, post-mitotic hNT neuronal cultures were derived from the human teratocarcinoma cell line NTera 2/D1 (NT2; provided by Virginia M.-Y. Lee, University of Pennsylvania) based on a modification of previously published protocols (Andrews, 1984; Pleasure et al., 1992). NT2 cells were maintained in OptiMem (Invitrogen, Carlsbad, CA) with 5% fetal bovine serum (FBS). For differentiation, 2×10^6 cells were seeded into T75 flasks in 1:1 DMEM/F-12 media (Invitrogen, Carlsbad, CA)/10% FBS and treated twice a week with 10 μ M all-trans retinoic acid (RA; Sigma, St. Louis, MO) for four weeks. Following RA treatment, cells were trypsinized and transferred to new T75 flasks at a density of $4 \times 10^5/\text{cm}^2$. Cells were then treated with the mitotic inhibitors 1 μ M cytosine arabinoside (Sigma, St. Louis, MO) and 10 μ M fluorodeoxyuridine (Sigma) in 1:1 DMEM/F-12/10% FBS twice weekly for 2 weeks. This treatment resulted in a layer of small, phase-bright post-mitotic neuronal cells loosely attached atop a monolayer of large, flattened non-neuronal cells. Neuronal enrichment was achieved by gently removing the top neuronotypic layer with 0.005 % trypsin and replating at $1.25 \times 10^5/\text{cm}^2$ onto 2% Matrigel (BD Biosciences, San Jose, CA) and 10 μ M poly-D-lysine (Sigma). NT2 neurons cultured for an additional 2 weeks developed extensive neuritic networks (see Fig. 7), which have been shown functionally and morphologically to resemble dendrites and axons (Pleasure et al., 1992).

DHA preparation for cell culture experiments

DHA (Sigma, St. Louis, MO) was dissolved in ethanol as a 20 mg/ml stock solution. 4–16 μ M DHA was prepared by adding the appropriate stock volume to a microfuge tube, evaporating the ethanol under nitrogen, and adding 100 μ g/ml bovine serum albumin (Sigma) to achieve a 2:1 DHA:BSA molar ratio. The tube was then purged with nitrogen, sealed, and incubated at 37 °C for 2 hours in a shaking water bath.

A β neurotoxicity cell culture experiments

Differentiated NT2 neurons were preloaded with 4, 8, or 16 μ M DHA:BSA complex or BSA alone (control) in 1:1 DMEM/F-12 /10% FBS for 24 hours at 37 °C. Following several rinses, cultures were then challenged with 10 μ M A β _{1–42} in serum-free OptiMem. Neuronal viability was determined by propidium iodide or the Live/Dead cell viability assay (Invitrogen Carlsbad, CA) following 2 days of A β treatment.

Statistical analysis

Data were evaluated using the non-parametric Mann-Whitney rank-sum test or one-, two- or three-way Analysis Of Variance (ANOVA) followed with either Tukey's or Holm-Sidak test for multiple comparisons (SigmaStat 3.0; Aspire Software International, Leesburg, VA, USA). The level of statistical significance was set at 0.05 (two-sided).

Results

DHA diet does not affect body weight and feed intake in APPswe/PS1ΔE9 tg mice

Fatty acid contents in DHA and control diets were analyzed using gas chromatography. Briefly, the total percentage of saturated (SFA) and monounsaturated (MUFA) fatty acids were relatively similar in both DHA and control diets, whereas the total percentage of polyunsaturated fatty acids (PUFA) were higher in DHA diet than in control diet, due to a content of 0.62 % of n-3 DHA PUFA in the DHA versus its absence in the control diet (Table 1) (For a more complete fatty acid analysis see Table S1 in supplemental material).

To examine whether DHA diet affects the body weight of APPswe/PS1ΔE9 tg and ntg mice, food intake and body weight were recorded three times a week and once a week, respectively. Male and female APPswe/PS1ΔE9 tg and ntg mice were randomly assigned to control or DHA diets. A minimum of 4 female mice were assigned to each group (Table II). Body weight did not differ between the four groups analyzed at 3 months of age: ntg-control (con) (22.6 ± 4.1 g), ntg-DHA (22.6 ± 3.6 g), tg-con (21.4 ± 3.0 g) and tg-DHA (21.2 ± 3.2 g) (One-way ANOVA, $p > 0.05$). Females weighed significantly less than male mice independent of genotype (Two-way ANOVA, $p < 0.001$). Although there was no statistically significant difference in body weight between the different groups after three months of dietary treatment (Three-way ANOVA, $p > 0.05$), the body weight of female mice was significantly lower than male mice independent of the genotype and diet (Three-way ANOVA, $p < 0.001$) (Table II). However, after three months of feeding, feed intake (FI; g/day) adjusted for individual body weight (g) was significantly difference between genotypes (Fig. 1; Three-way ANOVA, $p < 0.001$), but not between diets. In fact the mean FI value for the ntg mice was significantly lower than for the APPswe/PS1ΔE9 tg mice (ntg = 0.130 ± 0.011 g, tg = 0.146 ± 0.01 g, mean $\pm$ standard deviation of the mean) (Fig. 1). Likewise FI in both genotypes was significantly higher in female mice compared to male mice (Fig. 1; Holm-Sidak test, $p < 0.05$). Moreover, FI in female tg mice was significantly higher than in female ntg mice (Fig. 1; Holm-Sidak test, $p < 0.001$). These data indicate that female APPswe/PS1ΔE9 tg mice consumed more food without an increase in body weight.

DHA diet increases brain and liver fatty acid levels in APPswe/PS1ΔE9 tg and ntg mice

To assess whether DHA diet affected brain as well as lipid metabolism reflected in the liver, fatty acid content was determined by gas chromatography in the brainstem harvested from APPswe/PS1ΔE9 tg and ntg mice maintained on the DHA and control diets. Brain levels of individual or total saturated fats (SFAs) were unchanged in tg and ntg mice fed either the DHA or control diet (Table III; Man Whitney rank sum test, $p > 0.05$). By contrast, five of the 13-monounsaturated fatty acids (MUFAs) showed statistical differences between some of the groups examined, whereas differences in the total amount of MUFAs did not reach statistical significance (Table III). Specifically, oleic (18:1n-9) MUFA, which was the most abundant brain MUFA, was increased in APPswe/PS1ΔE9 tg and ntg mice fed DHA compared to control diet (Man Whitney rank sum test, $p < 0.001$). In contrast, vaccenic acid levels (18:1) were significantly decreased (Man Whitney rank sum test, $p < 0.05$). Furthermore, the individual and total content of brain polyunsaturated fatty acids (PUFAs) revealed statistical differences for some of the examined groups (Table III). Although the total amount of PUFAs was significantly increased in ntg mice fed DHA diet, this increase did not reach statistical significance in APPswe/PS1ΔE9 tg mice (Mann Whitney rank sum test, $p > 0.05$). However, individual levels of the linoleic (18:2n-6), dihomo- γ -linolenic (20:3n-6), docosatetraenoic (22:4n-6) and docosahexaenoic (22:6n-3) (DHA) PUFAs were significantly increased in the brain of mice maintained on the DHA diet compared to the control diet, whereas arachidonic acid (ARA, 20:4n-6) levels were decreased in DHA groups (Fig. 2; Mann Whitney rank sum test, $p < 0.001$). The mean values of the n-3 PUFA

DHA were double those of the n-6 PUFA ARA in the brain of mice fed the DHA diet (Table III). No statistical differences in FA content were detected between genders in mice on control or DHA diet. In addition, the total liver MUFAs were significantly decreased whereas SFAs and PUFAs were significantly increased in APPswe/PS1ΔE9 tg mice on DHA diet compared tg mice on control diet (Mann Whitney rank sum test, $p < 0.05$). Changes in specific liver fatty acids are shown in S2 table as supplementary data. These findings suggest that DHA diet alters mainly brain PUFA levels, including DHA, independent of mouse genotype, indicating that the essential fatty acid DHA crosses the blood brain barrier to enter the brain.

DHA diet decreases cortical, hippocampal and striatal plaque load in female APPswe/PS1ΔE9 tg mice

To determine the effect of the DHA diet on Aβ plaque deposition, we quantified Aβ load throughout the cortex, hippocampus and striatum of APPswe/PS1ΔE9 tg mice maintained on control or DHA diets using Z series of images obtained with confocal microscopy. Cortical and striatal plaque deposition was significantly decreased (Two-way ANOVA, $p = 0.014$, $p = 0.009$, respectively) by 27–30% in tg mice given the DHA diet compared to control diet (Figs. 3 and 4A,C). Female APPswe/PS1ΔE9 tg mice on control diet displayed a significantly greater plaque load in cortical, ventral hippocampus and striatum than male tg mice fed the same control diet (Fig. 4; Multiple comparison tests, $p < 0.02$). Interestingly, Aβ plaque load analysis revealed that female tg mice fed DHA showed a significant reduction in plaque load in the cortex (Figs. 3 and 4A; Tukey-test multiple comparison test, $p = 0.007$), ventral hippocampus (Figs. 3 and 4B; Holm-Sidak multiple comparison test, $p = 0.023$) and the striatum (Figs 3 and 4C; Tukey-test multiple comparison test, $p = 0.03$) compared to female tg mice on the control diet (Fig. 4A–C). No such effect was seen in the male tg mice (Fig. 4A–C). These findings indicate DHA has a greater effect upon reducing plaque load in female than in male APPswe/PS1ΔE9 tg mice.

DHA diet does not affect LR11 protein levels in APPswe/PS1ΔE9 tg or ntg mice

LR11 (a.k.a SorLA), a member of the ApoE/low density lipoprotein receptor family, is modulated by fatty acids and cholesterol, involved in APP/Aβ production and is reduced in postmortem cortical tissue from subjects who died with a clinical diagnosis of mild cognitive impairment (MCI) (Sager et al., 2007) or AD (Sherzer et al., 2004; Offe et al., 2006; Dodson et al., 2006). DHA increased cortical LR11 levels in the cortex of aged wild-type and amyloid over expressing Tg2576 mice maintained on a DHA depleted background/diet (Ma et al., 2007). In the present study, quantitative western blotting revealed no significant differences in LR11 protein levels in the cortex, hippocampus or striatum of mutant and ntg mice fed either DHA or control diet (Fig. 5). Gender difference in LR11 levels were not detected in any of the structures examined across groups. These findings suggest the reduction of Aβ load in the cortex, hippocampus and striatum following dietary supplementation with DHA, are mediated by mechanisms independent of LR11 activity.

DHA diet up-regulates hippocampal drebrin and cortical synaptotagmin levels in APPswe/PS1ΔE9 tg mice

To measure the impact of the DHA upon presynaptic and postsynaptic markers, levels of drebrin, synaptophysin and synaptotagmin were measured by quantitative immunoblotting in the cortex, hippocampus and striatum of APPswe/PS1ΔE9 tg and ntg littermate mice on DHA and control diets. No differences in synaptotagmin levels were found in the cortex, striatum or hippocampus of tg and ntg mice fed either DHA or control diets. However, we found a significant interaction between genotype, diet and gender for cortical levels of synaptotagmin (Fig. 6A; Three-way ANOVA, $p = 0.039$). APPswe/PS1ΔE9 tg female mice fed DHA displayed a significant increase in cortical synaptotagmin levels compared to

double mutant female mice on the control diet (Fig. 6A; Holm-Sidak multiple comparison test, $p < 0.01$). This effect was not seen in male tg littermates. By contrast, the cortex of APPswe/PS1 Δ E9 tg mice showed lower levels of synaptophysin than ntg mice (Fig. 6B; Three-way ANOVA, $p < 0.05$), independent of the dietary regimen. Levels of the postsynaptic docking protein drebrin showed significant differences between genotypes in the cortex (Fig. 6C; Three-way ANOVA, $p < 0.01$) and hippocampus (Fig. 6D; Three-way ANOVA, $p < 0.05$) but not in the striatum. Specifically, cortical and hippocampal drebrin levels were significantly reduced in tg compared to ntg mice ($p < 0.05$). Notably, hippocampal drebrin levels were significantly increased in tg mice fed the DHA diet compared to those tg on the control diet (Holm-Sidak multiple comparison test, $p < 0.01$), whereas this dietary effect was not seen in ntg mice (Fig. 6D).

DHA protects against A β toxicity in NT2 neuronal cultures

We tested whether DHA was neuroprotective against A β by preloading differentiated NT2 neuronal cultures exposed to a range of DHA (4–16 μ M) prior to challenge with A β _{1–42}. After 2 days of A β _{1–42}:BSA treatment, cultures without DHA exhibited an apoptotic-like morphology (Fig. 7A–C) and a significant ~45 % increase in cell death measured by propidium iodide fluorescence (Fig. 7D). Notably, there was a significant increase in cell viability in A β _{1–42}-treated cultures preloaded with 4, 8 or 16 μ M DHA:BSA compared to A β _{1–42}-treated cultures without DHA (Fig. 7D; One-way ANOVA, $p < 0.05$). Equivalent results were obtained using a corroborative Live/Dead assay (data not shown).

Discussion

DHA may be a potential natural product treatment for AD (Kalmijn et al., 1997; Barberger-Gateau et al., 2002; Morris et al., 2003; Freund-Levi et al., 2006; Chiu et al., 2008). The present study is the first demonstration that young APPswe/PS1 Δ E9 tg mice maintained on a three month diet containing DHA display: 1) brain and liver changes in the ratio of n-6/n-3 PUFAs, 2) reduction of brain A β plaque load, and 3) an increase in levels of postsynaptic drebrin and presynaptic synaptotagmin proteins but preserved levels of LR11. Several of these biological effects were gender dependent. In addition, DHA protected against A β neurotoxicity in cell culture.

Our fatty acid analysis revealed that consumption of a diet containing 0.62% microalgal DHA translates to a 25% increase in DHA brain levels in APPswe/PS1 Δ E9 tg and ntg mice. Female APPswe/PS1 Δ E9 tg mice consumed more DHA diet per day relative to body weight than male tg littermates without an increase in body weight suggesting that female mutant mice are more active and require a greater daily caloric intake than male tg mice. Studies using the same (Oskman et al., 2006) and different APP/PS1 mice (Arendash et al., 2007) reported an increase in general activity in these double mutants maintained on a DHA or n-3 enriched diet. This increase in activity may be related to DHA induced antidepressant effects (Bourre, 2005; Venna et al., 2008). In fact, an apparent consequence of this increase in DHA consumption and brain DHA levels is an ~30% plaque load reduction in the cortex, striatum and ventral hippocampus seen in female APPswe/PS1 Δ E9 tg mice, which displayed more plaque pathology than male tg mice (present findings; Wang et al., 2003). This intriguing observation resulted from a sub-analysis, which reduced the number of subjects in each group from 9 to 4. Despite the reduced sample size, a significant gender effect was found suggesting that the observation is real. Although the mechanisms underlying this gender effect remain to be determined, several possibilities exist including that sex hormone levels affect AD plaque pathology in APPswe/PS1 Δ E9 (Wang et al., 2003) and in 3xTg-AD (Carroll et al., 2007) transgenic mice. Interestingly, n-3 PUFA concentration is positively associated with circulating concentration of estradiol and progesterone and negatively with circulating levels of testosterone (Childs et al., 2008). However, whether DHA interacts with

female sex hormones to modify AD pathology remains unknown. Consumption of a DHA enriched diet increased the relative cerebral blood volume indicative of larger brain circulation, probably due to vasodilatation in APPswe/PS1 Δ E9 tg mice (Hooijmans et al. 2007). This dietary effect on brain vasodilatation was associated with decreased vascular A β deposition (Hooijmans et al., 2007) supporting the suggestion that higher daily DHA consumption in female APPswe/PS1 Δ E9 tg mice reduces amyloid plaque load via an interaction between increased cerebral circulation and female hormones without changes in DHA/ARA brain levels even at a young age in these mice.

The current DHA-mediated plaque load reduction in the brain of young mutant mice support and expand studies in aged Tg2576 mice (Lim et al., 2005). However, these findings conflict with other studies using APPswe/PS1 Δ E9 tg mice including a report of no change in hippocampal plaque load but a reduction in insoluble A β in 10-month-old APPswe/PS1 Δ E9 male tg mice fed a DHA-enriched diet for 3–4 months (Oskman et al. (2006). By contrast, others did not find differences in soluble or insoluble hippocampal A β levels in APP/PS1 line 6.2 tg mice maintained on a high n-3 PUFAS diet starting at 2 and ending at 9 months of age (Arendash et al., 2007). DHA treatment of male APPswe/PS1 Δ E9 tg mice beginning at 6 months of age and ending at 18 months failed to show variation in hippocampal dentate gyrus plaque burden, whereas vascular A β was significantly decreased in the cingulate gyrus (Hooijmans et al., 2007). DHA supplementation reduced hippocampal soluble A β levels, which correlated with reduced intraneuronal A β staining in 3xTg-AD mice (Green et al., 2007). Conversely, high-fat diets increased insoluble A β _{1–42} and A β _{1–40} or plaque burden in other AD animal models (Ho et al., 2004; Hooijmans et al., 2007; Julien et al., 2008). *In vitro* investigations indicate that DHA ameliorates A β production (Oksman et al., 2006). Differences in brain plaque load and A β levels may be related to age, gender and/or type and duration of dietary treatment (Oskman et al. 2006; Arendash et al., 2007; Hooijmans et al., 2007) or genetic phenotype.

Although the mechanism(s) of action of DHA upon A β metabolism is unclear, studies in aged APPswe mice suggest an effect on APP trafficking or secretase activity (Lim et al., 2005), whereas the reduction of soluble A β in 3xTg-AD mice was attributed to a decrease in steady-state levels of presenilin 1 (Green et al., 2007). Whether DHA affects PS1 and/or APP processing in any age in APPswe/PS1 Δ E9 tg mice remains unknown. The sortilin receptor, LR11, which plays a role in APP trafficking and A β production (Andersen et al., 2005; Offe et al., 2006), is a risk factor for AD (Lee et al., 2007; Rogaeva et al., 2007), and is reduced in people with MCI (Sager et al., 2007) and sporadic AD (Scherzer et al., 2004; Dodson et al., 2006), we evaluated LR11 brain levels in mice fed DHA or control diet. In contrast to a previous report that DHA increased LR11 levels in aged Tg2576 mice (Ma et al., 2007), we found no change in LR11 protein expression in APPswe/PS1 Δ E9 tg or ntg mice. This discrepancy may be related to the age, differences in mouse strains, and/or other methodological differences. The ability of DHA supplementation to reduce amyloid pathology without increasing LR11 expression suggests that DHA may act through other mechanisms related to the present finding that increased brain DHA co-occurs with a decrease in ARA levels (Calon et al., 2004; Lim et al., 2005; Oskman et al., 2006; Green et al., 2007; Hooijmans et al., 2007). This decrease in ARA, a precursor for prostaglandin E2 (Tassoni et al., 2008; Miller, 2008) may decrease γ -secretase production (Qin et al., 2003) reducing fibrillar A β . Since DHA is incorporated into the lipid bilayer of cellular membranes playing a role in membrane fluidity (Wassall and Stillwell, 2008), increases in brain DHA may induce changes in the physical properties of the neuronal membrane affecting enzyme activity, receptor conformation and ion channel formation (see Yehuda et al., 1999). Both the amyloidogenic β and γ -secretases require cholesterol-lipid rich domains (i.e., lipid rafts) (Fassbender et al., 2001; Wahrle et al., 2002; Osenkowski et al., 2008), whereas the non-amyloidogenic pathway utilizes lipid non-raft domains with higher DHA-

containing phospholipids and lower cholesterol (Wolozin 2001, 2004; Wassall and Stillwell, 2008). Perhaps decreased DHA alters β and γ -secretase activity by changing the phospholipid composition and fluidity of cellular membranes and consequently modifying A β production. Moreover, increased brain DHA may also reduce inflammatory processes (see Rojo et al., 2008) as well as neuroprotective actions within the AD brain (Calon et al., 2004; Lim et al., 2005; Green et al., 2007).

We found that DHA levels of the postsynaptic protein drebrin were increased in the hippocampus of APPswe/PS1 Δ E9 tg mice compared to tg mice fed control diet. This contrasts to reports of increased cortical drebrin but not hippocampal levels in Tg2576 mice fed DHA (Calon et al., 2004). These discrepancies may be due to differences in the extent of AD pathology, genotype signature or a combination of genetic and environmental variables. Although the functional consequences of DHA-mediated increases in hippocampal drebrin remain unknown, DHA potentiates synaptic transmission, improves synaptic protein quantity, activates dendritic spine plasticity (Cansev et al., 2008), protects against loss of drebrin and its binding partner actin (Calon et al., 2004), and induces neurogenesis (Dagai et al., 2008; Venna et al., 2008). By contrast, cortical synaptotagmin levels were upregulated in APPswe/PS1 Δ E9 tg female mice fed DHA compared to female tg mice fed control diet. As synaptotagmin is a positive regulator of Ca²⁺-mediated exocytosis (Gardzinski et al., 2007) DHA may enhance presynaptic function. Finally, the gender effects of DHA on synaptotagmin and plaque burden, raises the intriguing possibility that DHA may be particularly beneficial for women, who are at a higher risk for AD than men (Andersen et al., 1999).

Although DHA diets appear to ameliorate AD pathology in APP over-expressing mice (present findings; Calon et al., 2004; Lim et al., 2005; Oksman et al., 2006; Green et al., 2007; Hooijmans et al., 2007), its ability to improve cognition remains controversial (Calon et al., 2004; Oksman et al., 2006; Arendash et al., 2007). A placebo-controlled clinical trial found that n-3 fatty acids supplementation versus placebo improve cognition in people with mild AD or MCI, but not moderate AD (Freund-Levi et al., 2006; Chiu et al., 2008). Conversely, a prospective interventional study failed to show benefits of higher intake in unsaturated fatty acids (low in EPA/DHA) on cognitive performance in healthy elderly humans (Witte et al., 2009). Since Witte et al. (2009) found that caloric restriction (CR) correlates with improved memory, studies combining CR and DHA should be undertaken in tg AD mice.

Our *in vitro* experiments revealed that high amounts of DHA increased cell viability in neuronal cell cultures challenged with A β ₁₋₄₂, suggesting that DHA may be neuroprotective against A β toxicity (Oksman et al., 2006; Sahlin et al., 2007). Whereas the mechanism for cellular neuroprotection is unclear, *in vivo* and *in vitro* studies suggest that DHA activates the pro-survival PI3K/Akt pathway while suppressing apoptotic caspase-3 activity (Calon et al., 2004; Akbar et al., 2005). The present finding suggests DHA may be a non-invasive natural product treatment for multiple AD brain insults.

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Figure 1.

Histogram showing the mean values of feed intake (FI) adjusted by body weight per day in APPswe/PS1ΔE9 tg and ntg mice. Statistical analysis revealed that FI was significantly higher in tg mice compared to ntg mice (Three-way ANOVA, & $p < 0.001$). Statistical evaluation by genders showed that FI in both female and male APPswe/PS1ΔE9 tg mice were significantly higher than their respective gender ntg mice (Holm-Sidak test, °, • $p < 0.001$). Female mice displayed significantly higher FI values than their male littermates in both genotypes (Holm-Sidak test, * $p < 0.05$, \$ $p < 0.001$). Data is represented as mean $\pm$ standard error of the mean. Abbreviations: tg, APPswe/PS1ΔE9 transgenic mice; ntg, non-transgenic mice.

Figure 2.

Histogram showing the mean values of n-3 DHA and the n-6 arachidonic (ARA) PUFAs in the brain of APPswe/PS1 Δ E9 tg and ntg mice on DHA and control diet. Statistical analysis revealed a significant increase in brain DHA concomitant with a decrease in ARA levels in either APPswe/PS1 Δ E9 tg or ntg mice on DHA diet in comparison with their littermate mice on control diet (Mann-Whitney rank sum test, * $\bullet$ p < 0.001). Data is represented as mean $\pm$ standard deviation of the mean. Abbreviations: ntg-con, non-transgenic mice on control diet; ntg-DHA, non-transgenic mice on DHA diet; tg-con, APPswe/PS1 Δ E9 transgenic mice on control diet; tg-DHA, APPswe/PS1 Δ E9 transgenic mice on DHA diet; FAMES, Fatty Acid Methyl Esters.

Figure 3.

Fluorescence microscopic images showing a reduction in plaque deposits in the cortex, ventral portion of the hippocampus and striatum in female APPswe/PS1 Δ E9 tg mice on DHA diet (**A, B, C**) than female APPswe/PS1 Δ E9 tg mice on control diet (**D, E, F**).

Abbreviations: con, control diet; DHA, DHA diet. Scale bars=200 μ m.

Figure 4.

Histograms showing the mean values of plaque load in cortex (A), ventral portion of the hippocampus (B) and striatum (C) in APPswe/PS1ΔE9 tg mice on DHA and control diet. Statistical evaluation revealed a significant decrease in plaque load in the cortex (A) and striatum (C) of APPswe/PS1ΔE9 tg mice on DHA diet compared to tg mice on control diet (Two-way ANOVA, cortex $p=0.014$ and striatum $p=0.009$). This plaque reduction was not statistically detected in the ventral portion of the hippocampus (B). Multiple comparison tests revealed significant differences in plaque load in the cortex (A), ventral portion of the hippocampus (B) and striatum (C) between female and male tg mice on control diet ($p<0.02$). Statistical analysis by gender also revealed a significant decrease in plaque load

in the cortex (**A**), ventral portion of the hippocampus (**B**) and striatum (**C**) in female tg mice given DHA diet compared to female tg mice on control diet (Holm-Sidak and Tukey tests, $*p<0.05$). Data is represented as mean $\pm$ standard error of the mean. Abbreviations: con, control diet; DHA, DHA diet.

Figure 5.

Representative immunoblot of LR11 protein from cortex, hippocampus and striatum of a APPswe/PS1ΔE9 tg and ntg mouse fed with DHA and control diets. Statistical analysis of the densitometry quantification of LR11 bands revealed no significant differences between the groups examined. Abbreviations, ntg-con, non-transgenic mice on control diet; ntg-DHA, non-transgenic mice on DHA diet; tg-con, APPswe/PS1ΔE9 transgenic mice on control diet; tg-DHA, APPswe/PS1ΔE9 transgenic mice on DHA diet; C, cortex; H, hippocampus; S, striatum.

Figure 6.

Histograms showing mean drebrin, synaptophysin and synaptotagmin levels in the cortex, hippocampus and in the striatum of APPswe/PS1ΔE9 tg and ntg mice on DHA and control diets. **A**, Holm-Sidak test revealed a significant increase in cortical synaptotagmin levels in female tg mice on DHA diet in comparison with female tg mice on control diet (* $p < 0.01$), but not in male tg mice. **B** and **C**, Statistical analysis revealed a significant decrease in synaptophysin and drebrin levels in the cortex of APPswe/PS1ΔE9 tg mice compared to ntg littermate mice respectively (Three-way ANOVA, synaptophysin * $p < 0.05$, drebrin * $p < 0.01$). **D**, Statistical analysis also revealed that the hippocampal drebrin levels in tg mice were significantly decreased compared to ntg mice (Holm-Sidak test, between genotypes * $p < 0.05$, between genotypes on control diet • $p < 0.01$). Notably, the Holm-Sidak statistical analysis revealed an increase of hippocampal drebrin levels in tg mice fed with DHA compared to tg mice on control diet (& $p < 0.01$). Abbreviations: ntg, non-transgenic mice; tg, APPswe/PS1ΔE9 transgenic mice; ntg-con, non-transgenic mice on control diet; ntg-DHA, non-transgenic mice on DHA diet; tg-con, APPswe/PS1ΔE9 transgenic mice on control diet; tg-DHA, APPswe/PS1ΔE9 transgenic mice on DHA diet.

Figure 7.

A–C, Images of NT2 neuronal cultures showing apoptotic profiles in the culture preloaded with 10 μ M $A\beta_{1-42}$:BSA (**B**), whereas NT2 neuronal cultures in the control (treated only with BSA) (**A**) or 16 μ M DHA:BSA with 10 μ M $A\beta_{1-42}$ (**C**) displayed a more healthy appearance with the presence of numerous neuritic processes. **D**, Statistical evaluation of NT2 neuronal cultures stained with propidium iodide revealed a significant increase in cell viability in NT2 neuronal cultures preloaded with 4, 8 or 16 μ M DHA:BSA and challenged with 10 μ M $A\beta_{1-42}$ compared to those treated only with 10 μ M $A\beta_{1-42}$:BSA (Holm-Sidak test, * $p < 0.05$, • $p < 0.01$).

Table I

Fatty acid content of the diets

		Control	DHA
	<i>Analyzed FAME content (% of total FAME)</i>		
SFAs			
	caprylic (8:0)	<0.1	<0.1
	nonanoic (9:0)	<0.1	<0.1
	capric (10:0)	0.7	0.5
	undecanoic (11:0)	<0.1	<0.1
	lauric (12:0)	0.8	1.5
	tridecanic (13:0)	<0.1	<0.1
	myristic (14:0)	2.6	3.4
	pentadenoic (15:0)	0.3	<0.1
	palmitic (16:0)	12	7.9
	magaric (17:0)	0.2	<0.1
	stearic (18:0)	4.9	2.1
	arachidic (20:0)	0.3	0.3
	behenic (22:0)	0.3	0.3
	lignoceric (24:0)	0.1	0.2
	<i>Total of SFAs</i>	22.2	16.1
MUFAs			
	undecenoic (11:1)	<0.1	<0.1
	dodecenoic (12:1)	<0.1	<0.1
	tridecenoic (13:1)	<0.1	<0.1
	myristoleic (14:1)	0.2	<0.1
	pentadecenoic (15:1)	<0.1	<0.1
	palmitoleic (16:1n-7)	0.5	0.5
	heptadecenoic (17:1)	<0.1	<0.1
	vaccenic (18:1)	1	0.6
	oleic (18:1n-9)	17.8	16.6
	nonadecenoic (19:1)	<0.1	<0.1
	gondonic (20:1n-9)	0.2	0.2
	erucic (22:1n-9)	<0.1	<0.1
	nervonic (24:1)	0.1	0.1
	<i>Total of MUFAs</i>	19.9	18
PUFAs			
	linoleic (18:2n-6)	55	54.8
	γ -linolenic (18:3n-6)	0.1	<0.1
	α -linolenic (18:3n-3)	0.3	0.2
	eicosadienoic (20:2n-6)	<0.1	<0.1
	eicosatrienoic (20:3n-3)	<0.1	<0.1
	dihomo- γ linolenic (20:3n-6)	0.1	<0.1

	Control	DHA
arachidonic (ARA, C20:4n-6)	<0.1	<0.1
eicosapentaenoic (20:5n-3)	<0.1	<0.1
docosadienoic (22:2n-6)	<0.1	<0.1
docosatrienoic (C22:3n-3)	<0.1	<0.1
docosatetraenoic (C22:4n-6)	<0.1	<0.1
docosapentaenoic (C22:5n-3)	<0.1	0.1
docosahexaenoic (DHA, C22:6n-3)	<0.1	9.73 [*]
<i>Total of PUFAs</i>	55.5	64.8
Total of n-6	55.1	54.8
Total of n-3	0.3	9.93
ratio n-6/n-3	183.7	5.5
Total fat	6.35	6.25

FAME = Fatty Acid Methyl Ester; SFA = saturated fatty acids

MUFA = monounsaturated fatty acids; PUFA= polyunsaturated fatty acids

^{*}
= 9.73% of 6.25% total fat = 0.61% DHA in diet

Table II

Body weight (bw) and feed intake (FI) adjusted by body weight in APPswe/PS1ΔE9 tg and ntg mice on control (con) or DHA diets

	ntg-con			ntg-DHA			tg-con			tg-DHA		
	M (n=6)	F (n=4)	Total	M (n=5)	F (n=5)	Total	M (n=4)	F (n=4)	Total	M (n=4)	F (n=4)	Total
3 mos bw (g)	24.93 ± 3.7*	19.22 ± 1.1	22.65 ± 4.1	24.7 ± 3.5	20.66 ± 2.8	22.68 ± 3.6	23.95 ± 1.9	19.02 ± 1.3	21.48 ± 3.0	23.75 ± 2.0	18.47 ± 1.4	21.11 ± 3.2
6 mos bw (g)	32.12 ± 6.0	23.38 ± 2.8	28.62 ± 6.5	30.98 ± 5.1	25.95 ± 2.6	28.46 ± 4.6	28.66 ± 2.5	22.50 ± 2.2	25.58 ± 3.9	31.15 ± 4.0	23.91 ± 1.6	27.53 ± 4.8
FI	0.122 ± 0.01	0.141 ± 0.0	0.129 ± 0.01	0.122 ± 0.0	0.139 ± 0.0	0.130 ± 0.01	0.134 ± 0.0	0.159 ± 0.01	0.147 ± 0.01	0.129 ± 0.0	0.163 ± 0.01	0.146 ± 0.02

* mean ± standard deviation of the mean; mos, months

M, male; F, female

Table III

Brain fatty acid content in APPsw/PS1ΔE9 tg and ntg mice on control (con) or DHA diet

	ntg-con (n=10)	ntg-DHA (n=10)	tg-con (n=8)	tg-DHA (n=7)	p value
SFAs					
caprylic (8:0)	0.029±0.09	0	0	0	ns
nonanoic (9:0)	0.038±0.1	0.102±0.2	0.222±0.3	0±0	ns
capric (10:0)	1.327±0.4	1.383±0.6	1.207±0.1	1.236±0.2	ns
undecanoic (11:0)	0.169±0.2	0.122±0.2	0.0428±0.1	0.0628±0.1	ns
lauric (12:0)	0.319±0.1	0.185±0.2	0.31±0.2	0.2±0.2	ns
tridecanic (13:0)	0	0	0	0	ns
myristic (14:0)	0.605±0.08	0.603±0.1	0.649±0.09	0.653±0.1	ns
pentadenoic (15:0)	0	0	0	0	ns
palmitic (16:0)	16.876±0.8	17.221±0.5	17.265±0.4	17.356±0.3	ns
magaric (17:0)	0.235±0.1	0.141±0.1	0.116±0.1	0.126±0.1	ns
stearic (18:0)	19.892±0.8	19.479±0.4	20.429±0.4	20.12±0.4	ns
arachidic (20:0)	0.718±0.07	0.671±0.05	0.674±0.08	0.659±0.05	ns
behenic (22:0)	0.552±0.2	0.546±0.1	0.556±0.1	0.54±0.07	ns
lignoceric (24:0)	0.601±0.4	0.515±0.5	0.818±0.1	0.756±0.09	ns
Total of SFAs	41.363±1.42	40.97±1.64	42.288±0.70	41.724±0.76	ns
MUFAs					
undecenoic (11:1)	0.175±0.22	0.0985±0.21 ^{\$}	0	0 ^{\$}	p=0.024
dodecenoic (12:1)	0	0	0	0	ns
tridecenoic (13:1)	0	0	0	0	ns
myristoleic (14:1)	0	0	0	0	ns
pentadecenoic (15:1)	0.074±0.15	0	0	0	ns
palmitoleic (16:1n-7)	0.338±0.2	0.405±0.2	0.261±0.2 [^]	0.439±0.2 [^]	p=0.029
heptadecenoic (17:1)	0.242±0.2 [*]	0.0421±0.09 [*]	0.046±0.1	0.0671±0.1	p=0.030
vaccenic (18:1)	3.862±0.157 [*]	3.615±0.11 [*]	3.866±0.095 [^]	3.615±0.065 [^]	p<0.001
oleic (18:1n-9)	17.212±0.4 [*]	18.254±0.5 [*]	16.909±0.3 [^]	18.267±0.3 [^]	p<0.001
nonadecenoic (19:1)	0	0	0	0	ns
gondonic (20:1n-9)	3.51±0.2	3.417±0.2	3.466±0.3	3.5±0.2	ns

	ntg-con (n=10)	ntg-DHA (n=10)	tg-con (n=8)	tg-DHA (n=7)	p value
PUFAs					
erucic (22:1n-9)	0.259±0.1	0.231±0.1	0.0526±0.1	0.237±0.1	ns
nervonic (24:1)	2.477±0.48	2.381±0.299	2.409±0.526	2.235±0.335	ns
Total of MUFAs	28.148±1.48	28.444±0.97	27.009±1.10	28.36±1.03	ns
linoleic (18:2n6)	0.433±0.2 *	0.682±0.06 *	0.525±0.07 ^	0.717±0.04 ^	p≤0.003
γ-linolenic (18:3n-6)	0	0	0	0	ns
α-linolenic (18:3n-3)	0	0	0	0	ns
eicosadienoic (20:2n-6)	0.038±0.1	0.0614±0.1	0	0.149±0.1	ns
eicosatrienoic (20:3n-3)	0	0	0	0	ns
dihomo-γ linolenic (20:3n-6)	0.208±0.1 *	0.632±0.05 *	0.121±0.1 ^	0.707±0.0 ^	p≤0.001
arachidonic (ARA, C20:4n-6)	6.711±0.3 *	5.145±0.3 *	6.87±0.4 ^	5.033±0.3 ^	p≤0.001
eicosapentaenoic (20:5n-3)	0	0	0	0	ns
docosadienoic (22:2n-6)	0	0	0	0	ns
docosatrienoic (C22:3n-3)	0	0	0	0	ns
docosatetraenoic (C22:4n-6)	2.667±0.1	1.629±0.1 \$	2.713±0.1	1.667±0.1 \$	p≤0.001
docosapentaenoic (C22:5n-3)	0	0	0	0	ns
docosahexaenoic (DHA, C22:6n-3)	9.371±0.4 *	12.004±0.4 *	9.703±0.4 ^	11.851±0.4 ^	p<0.001
Total of PUFAs	19.429±0.66 *	20.21±0.79 *	19.933±0.92	20.219±0.96	p<0.031
Total of n-6	10.057±0.55 *	8.149±0.50 *	10.23±0.55 ^	8.274±0.55 ^	p≤0.001
Total of n-3	9.371±0.4 *	12.004±0.4 *	9.703±0.4 ^	11.851±0.4 ^	p<0.001
ratio n-6/n-3	1.075±0.08 *	0.676±0.04 *	1.054±0.02 ^	0.693±0.03 ^	p≤0.001
ratio DHA/ARA	1.399±0.07 *	2.340±0.151 *	1.414±0.04 ^	2.360±0.12 ^	p≤0.001

mean percentage of total normalized weight Fatty Acid Methyl Esters ± SD

SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids
ntg-con, non-transgenic mice on control diet; ntg-DHA, non-transgenic mice on DHA diet
tg-con, APPswe/PS1ΔE9 transgenic mice on control diet; tg-DHA, APPswe/PS1ΔE9 transgenic mice on DHA diet

\$, significant differences between genotypes: ntg versus APPswe/PS1ΔE9 tg mice

^, *, significant differences between diets in APPswe/PS1ΔE9 tg and ntg mice respectively

ns, non significant

Table IV

Liver fatty acid content in APPswe/PS1ΔE9 tg and ntg fed with DHA or control (con) diet

	ntg-con (n=10)	ntg-DHA (n=10)	tg-con (n=8)	tg-DHA (n=8)	p value
SFAs					
caprylic (8:0)	0.0717±0.12	0.0855±0.13	0.0634±0.04 [^]	0.108±0.08 [^]	p=0.01
nonanoic (9:0)	0.0936±0.15	0.104±0.13	0.0838±0.11	0.132±0.14	ns
capric (10:0)	1.016±0.36	0.99±0.25	0.914±0.49	0.943±0.27	ns
undecanoic (11:0)	0.321±0.18	0.386±0.15	0.256±0.15	0.315±0.1	ns
lauric (12:0)	0.484±0.21	0.588±0.24	0.438±0.15	0.529±0.17	ns
tridecanic (13:0)	0	0.0354±0.10	0.0343±0.06	0.0251±0.05	ns
myristic (14:0)	0.529±0.08	0.455±0.07 ^{\$}	0.613±0.10	0.522±0.13 ^{\$}	p=0.035
pentadenoic (15:0)	0.108±0.06 [#]	0.0487±0.06	0.0704±0.05	0.0442±0.06 [#]	p=0.047
palmitic (16:0)	24.391±1.33 [*]	27.735±1.24 [*]	25.597±2.54 [^]	29.401±1.94 [^]	p≤0.001/p≤0.004
magaric (17:0)	0.175±0.08	0.181±0.04 ^{\$}	0.138±0.06	0.0934±0.07 ^{\$}	p=0.015
stearic (18:0)	11.707±3.52	12.495±3.12	10.337±2.13	11.37±2.64	ns
arachidic (20:0)	0.211±0.12	0.199±0.19	0.124±0.15	0.238±0.25	ns
behenic (22:0)	0.0326±0.05	0.023±0.04	0.0222±0.04	0.078±0.08	ns
lignoceric (24:0)	0	0	0	0	ns
Total of SFAs	39.181±3.79 [*]	43.352±2.94 [*]	38.686±3.01 [^]	43.834±2.14 [^]	p=0.013/p=0.005
MUFAs					
undecenoic (11:1)	0.693±0.28	0.73±0.32	0.463±0.21 [^]	0.746±0.22 [^]	p=0.015
dodecenoic (12:1)	0.0555±0.12	0.0537±0.06	0.0764±0.10	0.0482±0.09	ns
tridecenoic (13:1)	0	0	0	0	ns
myristoleic (14:1)	0	0	0	0	ns
pentadecenoic (15:1)	0.003±0.01	0	0	0	ns
palmitoleic (16:1n-7)	2.87±0.84	2.224±0.86 ^{\$}	3.835±1.30	3.006±0.93 ^{\$}	p=0.037
heptadecenoic (17:1)	0.11±0.09 [*]	0.0271±0.05 [*]	0.129±0.08	0.0206±0.05 [*]	p=0.030
vaccenic (18:1)	3.352±0.99 [*]	1.989±0.42 [*]	3.591±1.04 [^]	1.926±0.47 [^]	p=0.002/p=0.010
oleic (18:1n-9)	19.883±8.21 [*]	15.729±5.6 [*]	23.107±3.77 [^]	17±4.69 [^]	p=0.021/p=0.024

	ntg-con (n=10)	ntg-DHA (n=10)	tg-con (n=8)	tg-DHA (n=8)	p value
nonadecenoic (19:1)	0	0	0	0	ns
gondonic (20:1n-9)	0.585±0.26 [#]	0.425±0.20	0.592±0.26	0.383±0.27 [#]	p=0.033
erucic (22:1n-9)	0.0152±0.03	0.0249±0.05	0.0407±0.05	0.03±0.05	ns
nervonic (24:1)	0	0	0	0	ns
Total of MUFAs	27.596±9.86	21.203±6.91	31.873±5.90 [^]	23.179±6.05 [^]	p=0.028
PUFAs					
linoleic (18:2n6)	14.034±2.04	15.105±1.53	13.548±2.73	14.74±2.85	ns
γ-linolenic (18:3n-6)	0.382±0.18 [*]	0.162±0.08 [*]	0.328±0.2 [^]	0.194±0.08 [^]	p≤0.001/p≤0.010
α-linolenic (18:3n-3)	0	0	0	0	ns
eicosadienoic (20:2n-6)	0.278±0.07	0.282±0.06 ^{\$}	0.203±0.08	0.219±0.09 ^{\$}	p=0.011
eicosatrienoic (20:3n-3)	0	0	0	0	ns
dihomo-γ linolenic (20:3n-6)	1.015±0.23 [*]	1.445±0.20 [*]	1.022±0.16 [^]	1.262±0.18 [^]	p=0.01/p=0.021
arachidonic (ARA, C20:4n-6)	11.415±3.77 [*]	4.994±1.52 [*]	8.961±2.07 [^]	3.979±0.82 [^]	p=0.004/p≤0.001
eicosapentaenoic (20:5n-3)	0 [*]	1.39±0.51 [*]	0 [^]	1.171±0.51 [^]	p≤0.001
docosadienoic (22:2n-6)	0	0	0	0	ns
docosatrienoic (C22:3n-3)	0	0	0	0	ns
docosatetraenoic (C22:4n-6)	0.572±0.15 [*]	0.0358±0.05 [*]	0.5±0.14 [^]	0.0256±0.04 [^]	p≤0.001
docosapentaenoic (C22:5n-3)	0 [*]	0.501±0.12 [*]	0 [^]	0.463±0.08 [^]	p≤0.001
docosahexaenoic (DHA, C22:6n-3)	1.44±0.65 [*]	10.327±1.89 [*]	1.208±0.49 [^]	9.889±1.89 [^]	p≤0.001
Total of PUFAs	29.136±6.72 [*]	34.24±4.48 [*]	25.77±5.31 [^]	31.942±5.48 [^]	p<0.012/p<0.028
Total of n-6	27.697±6.11 [*]	22.023±2.68 [*]	24.562±4.85 [^]	20.419±3.54 [^]	p<0.017/p<0.05
Total of n-3	1.44±0.65 [*]	12.217±2.38 [*]	1.208±0.49 [^]	11.523±2.30 [^]	p≤0.001
ratio n-6/n-3	22.393±8.52 [*]	1.844±0.30 [*]	22.237±5.96 [^]	1.803±0.27 [^]	p≤0.001
ratio DHA/ARA	0.122±0.02 [*]	2.176±0.50 [*]	0.131±0.02 [^]	2.493±0.16 [^]	p≤0.001

mean percentage of total normalized weight Fatty Acid Methyl Esters ± SD

SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids

ntg-con, non-transgenic mice on control diet; ntg-DHA, non-transgenic mice on DHA diet

tg-con, APPswe/PS1ΔE9 transgenic mice on control diet; tg-DHA, APPswe/PS1ΔE9 transgenic mice on DHA diet

\$ significant differences between genotypes and
 # diets;
 ^ between diets in tg and
 * ntg mice
 ns, non significant