High-fat diet aggravates amyloid-beta and tau pathologies in the 3xTg-AD mouse model

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Abstract

To investigate potential dietary risk factors of Alzheimer’s disease (AD), triple transgenic (3xTg-AD) mice were exposed from 4 to 13 months of age to diets with a low n-3:n-6 polyunsaturated fatty acid (PUFA) ratio incorporated in either low-fat (5% w/w) or high-fat (35% w/w) formulas and compared with a control diet. The n-3:n-6 PUFA ratio was decreased independently of the dietary treatments in the frontal cortex of 3xTg-AD mice compared to non-transgenic littermates. Consumption of a high-fat diet with a low n-3:n-6 PUFA ratio increased amyloid-β (Aβ) 40 and 42 concentrations in detergent-insoluble extracts of parieto-temporal cortex homogenates from 3xTg-AD mice. Low n-3:n-6 PUFA intake ratio increased insoluble tau regardless of total fat consumption, whereas high-fat diet incorporating a low n-3:n-6 PUFA ratio also increased soluble tau compared to controls. Moreover, the high-fat diet decreased cortical levels of the postsynaptic marker drebrin, while leaving presynaptic proteins synaptophysin, SNAP-25 and syntaxin 3 unchanged. Overall, these results suggest that high-fat consumption combined with low n-3 PUFA intake promote AD-like neuropathology.

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Keywords: Polyunsaturated fatty acid; Dietary fat; Amyloid-beta; tau; Drebrin; PAK; Cofilin; LR11; Docosahexaenoic acid; GFAP; Synaptophysin

1. Introduction

From a therapeutic point of view, environmental factors are easier to modify than genetic factors. Dietary fats have been shown to influence the risk of developing cardiovascular diseases, peripheral metabolic diseases and, more recently, Alzheimer’s disease (AD) (Mattson, 2004; Pasinetti et al., 2007). Epidemiological studies have first established an association between a high intake of saturated fats and an increased risk of developing cognitive impairment and AD (Luchsinger et al., 2002; Morris et al., 2003a; Parrott and Greenwood, 2007). The most useful animal models of AD include transgenic mice modeling amyloid-β (Aβ\textsubscript{40} and Aβ\textsubscript{42}) accumulation caused by the overexpression of a mutant amyloid precursor protein (APP) gene (ex: Tg2576 mouse line with APP\textsubscript{K670N,M671L} or the combination of mutant APP with mutant PS1 (McGowan et al., 2006). Studies in the Tg2576 mouse model of AD have evidenced the deleterious

Abbreviations: 3xTg-AD, triple transgenic mouse model of Alzheimer’s disease; ApoE, apolipoprotein E; APP, amyloid precursor protein; ARA, arachidonic acid; CTF, C-terminal fragment; DHA, docosahexaenoic acid; Diet A, low-fat control diet; Diet B, low-fat with low n-3:n-6 PUFA ratio diet; Diet C, high-fat with low n-3:n-6 PUFA ratio diet (high-fat diet); GFAP, glial fibrillary acidic protein; DPA, docosapentaenoic acid; DTA, docosatetraenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; LNA, linolenic acid; LR11, sortilin-related receptor SorLA/LR11; MUFA, monounsaturated fatty acids; NonTg, non-transgenic; n.s., non significant; O.D., relative optical density; PAK, p21-activated kinase; PSD-95, postsynaptic density-95; PUFA, polyunsaturated fatty acids; SNAP-25, synaptosome-associated protein-25; SFA, saturated fatty acid.

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effect of high caloric intake (based on saturated fat) on cognitive performance and Aβ burden in the brain (Ho et al., 2004; Li et al., 2003) or in the small intestinal enterocytes (Galloway et al., 2008). Conversely, dietary calorie restriction is associated with improved cognitive performance and reduced Aβ pathologies in old monkeys (Qin et al., 2006). High cholesterol consumption also leads to increased Aβ burden in the APPK670N,M671L/PS1M146V transgenic animal model of AD (Refolo et al., 2000).

Later on, epidemiological analyses based on semiquantitative food frequency questionnaire showed that individuals reporting a reduced consumption of n-3 polyunsaturated fatty acid (PUFA) had an increased risk of developing AD (Morris et al., 2003a; Morris et al., 2003b). This is also supported by a prospective follow-up analysis of blood n-3 PUFA content (Schaefer et al., 2006). A recent randomized clinical trial has shown a beneficial effect of n-3 PUFA on cognitive functions limited to patient with very mild AD (Freund-Levi et al., 2006). In animal models, Aβ deposition, synaptic marker defects and cognitive impairment were all shown to be reduced after exposure to a diet enriched in docosahexaenoic acid (DHA), a n-3 PUFA commonly found in fatty fish (Calon et al., 2004, 2005; Lim et al., 2005). Detrimental effects of high-fat diet and the protective effect of DHA on Aβ pathology are supported by a more recent analysis in the APPK670N,M671L/PS1E9 mouse model of AD (Oksman et al., 2006). Taken together, these data suggest that dietary lipids regulate AD neuropathology, at least in terms of Aβ content.

AD is also characterized by the presence of neurofibrillary tangles in the brain, formed by insoluble hyperphosphorylated tau protein displaying a paired helical filament (PHF) structure (Binder et al., 2005; Lee et al., 2001; Tremblay et al., 2007). Interestingly, the conversion of normal tau into its PHF form usually correlates better with cognitive symptoms of AD than measures of Aβ pathology (Arriagada et al., 1992; Bennett et al., 2004; Giannakopoulos et al., 2003; Nagy et al., 1995; Näslund et al., 2000; Tremblay et al., 2007). For instance, very high post mortem concentrations of Aβ can be found in the brain of patients with no detectable cognitive deficit (Dickson et al., 1992; Forman et al., 2007; Tremblay et al., 2007). This has led to the generation of the 3xTg-AD mouse model of AD that develops both Aβ and tau pathologies consequent to the expression of three mutated genes, amyloid-beta precursor protein (APPswe), presenilin-1 (PS1M146V), and tau (P301L) (Oddo et al., 2003a; Oddo et al., 2003b). A first group of investigators found that DHA treatment was associated with reduced accumulation of phosphorylated and total tau in the soluble fractions of protein extracted from the whole brain of 3xTg-AD mice (Green et al., 2007). In a second study, 3xTg-AD mice performed better in the Morris water maze paradigm and accumulated less tau in their brain following calorie restriction (Halagappa et al., 2007).

Taken together, these data suggest that underconsumption of n-3 PUFA coupled to ingestion of high level of calories from fat, which are commonplace in our modern society, might increase the incidence of AD by accelerating its pathogenic process (Calon and Cole, 2007; Calon, 2006; Pasinetti et al., 2007). Because the effects of dietary fats in AD remain very hard to demonstrate in clinical trials due to ethical and financial constraints (Calon, 2006), animal models of AD provide an opportunity to monitor precisely the intake of food in relation with subsequent alterations in brain markers of AD pathology. Since n-3 PUFA and total fats were the subject of separate studies, which were rather focused on Aβ pathology, we exposed the 3xTg-AD animal model of AD to n-3 PUFA deprivation alone (Diet B) or combined with a high-fat westernized diet (Diet C) to determine the quantitative impact of these precisely formulated diets on markers of Aβ, tau and synaptic pathologies.

2. Materials and methods

2.1. Material

Unless otherwise noted, reagents were obtained from Sigma–Aldrich (St. Louis, MO). Antibodies used: anti-actin at 1:5000 dilution (Chemicon International, Temecula, CA), anti-Amyloid β/A4 Protein Precursor (770–771) (C31) (1:50; Bachem, Torrance, CA) for C-terminal fragment of APP (CTF-α and CTF-β), and anti-Aβ clone 6E10 (1:200; Chemicon International) for APP measures, anti-amyloid oligomers A11 (1:1000; Millipore, Temecula, CA), anti-beta-amyloid (1:10,000; Sigma–Aldrich), anti-LR11/SorLA/gp250 (1:1000; BD Biosciences, San Jose, CA), anti-PK1/2/3 (1:1000; Cell Signaling Technology, Beverly, MA), anti-Drebrin monoclonal clones MX823 (1:500; Progen, Heidelberg, Germany) and M2F6 (1:1000; MBL, Woburn, MA), anti-glial fibrillary acidic protein (GFAP) (1:10,000; Sigma–Aldrich), anti-LS1/SorLa/gp250 (1:1000; BD Biosciences, San Jose, CA), anti-PAK1/2/3 (1:1000; Cell Signaling Technology, Beverly, MA), anti-lipopolysaccharide-associated protein (1:5000; Upstate Biotechnology, Lake Placid, NY), anti-synaptosome-associated protein (1:5000; SNAP-25) (Covance), anti-Synaptophysin clone SVP-38 (1:10,000; Chemicon International), anti-Syntaxin 3 (1:5000; Novus Biologicals, Littleton, CO), anti-total tau clone tau-13 (1:5000; Covance, Berkeley, CA), and anti-phosphorylated tau clones PHF1 (1:500; Novus Biologicals), CP13 (1:1000; gift from Dr Peter Davies, Albert Einstein College of Medicine), and AT270 (1:500; Pierce). Immunodetection was revealed with Horseradish Peroxidase-conjugated AffiniPure Donkey Anti-Rabbit or Goat Anti-Mouse (1:60,000 each; Jackson ImmunoResearch, West Grove, PA) secondary antibodies and detected by ECL (GE Healthcare, Baie d’Urfé, QC, Canada).

2.2. Animals and diets

Triple-transgenic mice (3xTg-AD) harboring three mutant genes, amyloid-beta precursor protein (APPswe), presenilin-1 (PS1M146V), and tau (P301L), were from a colony maintained in our animal facilities and generated from

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founding mice obtained from Dr Frank LaFerla (Oddo et al., 2003b). The non-transgenic (NonTg) mice used here are littermates from the original PS1-knockin mice and are on the same background as the 3xTg-AD mice (C57BL6/129SvJ). 3xTg-AD mice and non-transgenic age-matched controls were assigned randomly into three groups according to their dietary treatment at the age of 4 months. Mice were fed until the age of 13.2 ± 0.2 months (mean ± S.E.M.) with either: Diet A, a 5% fat (w/w) control diet similar to laboratory chow found in most institutions; Diet B, a 5% fat (w/w) diet with a very low amount of linolenic acid and, thereby, a low n-3:n-6 PUFA ratio; and Diet C, a high-fat westernized diet with 35% fat (w/w) and a similarly low n-3:n-6 PUFA ratio. Diet formulas and composition in fatty acids, as confirmed by gas chromatography, are detailed in Table 1. The formula of each purified diet (produced in collaboration with Dr Matthew Ricci from Research Diets Inc.) has been precisely determined to avoid any batch to batch variations. The diets are exactly the same in terms of fibers, vitamins, minerals, and antioxidants and do not contain phytoestrogens.

Table 1
Comparison of dietary treatments.

<table>
<thead>
<tr>
<th></th>
<th>Diet A (control)</th>
<th>Diet B (low fat and low n-3:n-6 ratio)</th>
<th>Diet C (high fat and low n-3:n-6 ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% (w/w) fat</td>
<td>% (cal/cal)</td>
<td>% (w/w)</td>
<td>% (cal/cal)</td>
</tr>
<tr>
<td>Protein</td>
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<td>21</td>
<td>27</td>
</tr>
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<td>Carbohydrate</td>
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<td>68</td>
<td>25</td>
</tr>
<tr>
<td>Fat</td>
<td>5</td>
<td>12</td>
<td>35</td>
</tr>
<tr>
<td>Total (kcal/g)</td>
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<td>100 kcal</td>
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<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g/kg</th>
<th>g/kg</th>
<th>g/kg</th>
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<td>Casein</td>
<td>200</td>
<td>800</td>
<td>800</td>
</tr>
<tr>
<td>L-cysteine</td>
<td>3</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Corn starch</td>
<td>150</td>
<td>600</td>
<td>600</td>
</tr>
<tr>
<td>Sucrose</td>
<td>500</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>Cellulose, BW200</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>10</td>
<td>90</td>
<td>225</td>
</tr>
<tr>
<td>Lard</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Canola oil</td>
<td>40</td>
<td>360</td>
<td>135</td>
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<td>Cholesterol, USP</td>
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<tr>
<td>Ethoxyquin</td>
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<td>Minerals (S19101)</td>
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<td>35</td>
</tr>
<tr>
<td>Vitamins (V15908)</td>
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<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Choline bitartrate</td>
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<td>0</td>
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<tr>
<td>Total</td>
<td>1000.6</td>
<td>3902</td>
<td>740.5</td>
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</table>

Fatty acid (FA) content (as determined by gas chromatography in the pelleted diet)

<table>
<thead>
<tr>
<th>FA</th>
<th>g/kg</th>
<th>g/kg</th>
<th>g/kg</th>
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<tbody>
<tr>
<td>C18:2n-6 LA</td>
<td>13.44</td>
<td>36.04</td>
<td>140.72</td>
</tr>
<tr>
<td>C20:4 n-6 ARA</td>
<td>0</td>
<td>0</td>
<td>0.39</td>
</tr>
<tr>
<td>C22:4 n-6 DTA</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C22:5 n-6 DPA</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total n-6 PUFA</td>
<td>13.44</td>
<td>36.25</td>
<td>142.56</td>
</tr>
<tr>
<td>C18:3 n-3 LNA</td>
<td>3.37</td>
<td>0.47</td>
<td>1.98</td>
</tr>
<tr>
<td>C20:5 n-3 EPA</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C22:6 n-3 DHA</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total n-3 PUFA</td>
<td>3.37</td>
<td>0.47</td>
<td>1.98</td>
</tr>
<tr>
<td>n-3:n-6 ratio</td>
<td>0.25</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Total fatty acids</td>
<td>44</td>
<td>47</td>
<td>247</td>
</tr>
</tbody>
</table>

Abbreviations: ARA, arachidonic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; DTA, docosatetraenoic acid; EPA, eicosapentaenoic acid; FA, fatty acids; LA, linoleic acid; LNA, linolenic acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acid; USP, United States Pharmacopeia.
Animals were sacrificed under deep anesthesia with ketamine/xylazine and perfused via transcardiac infusion with phosphate saline buffer (PBS; 1X: 1 mM KH$_2$PO$_4$, 10 mM Na$_2$HPO$_4$, 137 mM NaCl, 2.7 mM KCl, pH 7.4) containing a cocktail of protease inhibitors (SIGMAFAST™ Protease Inhibitor Tablets, Sigma–Aldrich, St. Louis, MO) along with phosphatase inhibitors (50 mM sodium fluoride and 1 mM sodium pyrophosphate). Frozen extracts of the frontal cortex and the parieto-temporal cortex were dissected and kept at −80°C.

2.3. Lipid extraction and gas chromatography

The procedures were similar to previous publications (Julien et al., 2006; Lepage and Roy, 1986). Approximately 20 mg of frozen frontal cortex tissue was homogenized with BHT-Methanol (Sigma, St. Louis, MO, USA) and with 22:3 n-3 methyl ester as an internal standard (NuChek Prep company, Elysian, MN, USA) at a concentration of 500 μg/g of tissue. Two volumes of chloroform (J.T. Baker, Phillipsburg, NJ, USA) and NaH$_2$PO$_4$ buffer solution were added to the resulting homogenate. After centrifugation at 3500 rpm for 7 min, the lower layer was collected (Folch et al., 1957). This procedure was repeated twice and the two extracts were pooled and brought to dryness with a stream of nitrogen. Lipid extracts were transmethylated with BF$_3$–MeOH (Alltech, State college, PA, USA) at 100 °C for 60 min. After cooling down, water and hexane (J.T. Baker, Phillipsburg, NJ, USA) were added. A 3-min centrifugation allowed separation of the phases and the upper layer was collected. These last steps were performed twice to pool the hexane extracts. Hexane was dried down to about 100 μL, transferred to a gas chromatography autosampler vial and capped under nitrogen. Fatty acid methyl esters were quantified on a model 6890 series gas chromatograph (Agilent Technologies, Palo Alto, CA) with a FAST-GC method. Each μL of each sample was injected at a 25:1 split ratio. The identification of the fatty acid methyl ester peak was performed for each sample by comparison to the peak retention times of a 28-component methyl standard (462, Nu-Chek Prep, Elysian, MN, USA) (Julien et al., 2006).

2.4. Biochemical assessment of tau and Aβ pathologies

After adding 8 volumes of Tris-buffered saline (TBS) containing Complete™ protease inhibitors cocktail (Roche, Indianapolis, IN), 10 μg/ml of pepstatin A, 0.1 mM EDTA and phosphatase inhibitors (1 mM each of sodium vanadate and sodium pyrophosphate, 50 mM sodium fluoride), frozen samples were sonicated briefly (3 × 10 s) and centrifuged at 100,000 × g for 20 min at 4 °C to produce a lysis buffer-soluble fraction (detergent-soluble fraction). The pellets (detergent-insoluble fractions) were homogenized in 175 μL of 90% formic acid followed by a short sonication (3 × 10 s). The resultant suspension was centrifuged (15,000 × g; 4 °C; 20 min) and 20 μL of the supernatant was neutralized with 1:13 dilution of Tris-base 2 M (pH 10) to be used for ELISA (see below). The rest of the supernatant was dried out by SpeedVac (Thermo Savant, Waltham, MA), solubilized in Laemmli’s buffer and processed for Western immunoblotting.

Insoluble Aβ40 and Aβ42 were measured by the β Amyloid [1–40] and [1–42] ELISA kits (Biosource, Camarillo, CA). Soluble Aβ40 and Aβ42 were measured using Human β Amyloid (1–42) ELISA kit WAKO, High Sensitive (WAKO, Osaka, Japan). The two ELISAs were done according to the manufacturer recommendations and the plates were read at 450 nm using a Synergy™ HT multi-detection microplate reader (Biotek, Winooski, VT).

2.5. Western Immunoblotting

For Western immunoblotting, protein concentration was determined using bicinchoninic acid assays (Pierce, Rockford, IL). Equal amounts of protein per sample (15 μg of total protein per lane) were added to Laemmli’s loading buffer, heated to 95 °C for 5 min before loading, and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Proteins were electroblotted onto PVDF membranes (Immobilon, Millipore, Massachusetts) before blocking in 5% nonfat dry milk and 1% bovine serum albumin (BSA) in PBS -Tween 20 for 1 h. Membranes were immunoblotted with appropriate primary and secondary antibodies followed by chemiluminescence reagents (ECL, Amersham/Pharmacia biotech, Piscataway, NJ or Supersignal, Pierce, Rockford, IL). Band intensities were quantified using a KODAK Image Station 4000 MM Digital Imaging System (Molecular Imaging Software version 4.0.5f7, KODAK, New Haven, CT).

2.6. Oxidized proteins

Levels of oxidized proteins were evaluated with an OxyBlot™ protein oxidation detection kit (S7150) from Chemicon International, as described (Calon et al., 2004; Lim et al., 2001).

2.7. Soluble amyloid oligomers

A dot blot assay with the A11 antibody was performed to detect soluble amyloid oligomers in TBS fractions of brain cortex (Kayed et al., 2003). Briefly, 1 μL aliquots (3 μg) in duplicates were applied onto a low fluorescence PVDF membrane (GE Healthcare) previously activated with methanol and equilibrated in TBS. The membrane was blocked (2 h at room temperature) with SeaBlock buffer (Pierce biotechnology, Rockford, IL) and incubated with anti-oligomer
antibody (A11, 1:1000) diluted in SeaBlock overnight at 4 °C. Washes were performed in TBS containing 0.01% Tween-20. The membrane was incubated with anti-rabbit IgG conjugated with Qdot®705 (Invitrogen, Burlington, ON, Canada) in SeaBlock (1:1200) for 1 h at room temperature. After washing, the blot was exposed (ex 465 em 700) with a KODAK Image Station 4000 MM Digital Imaging System. Fluorescent dots were quantified using Molecular Imaging Software version 4.0.5f7 (Kodak) and data were normalized to actin.

2.8. Data and statistical analyses

Statistical analysis were performed either with an ANOVA (equal variance) followed by Tukey-Kramer or Newman–Keuls post-hoc tests or a Welch’s ANOVA (unequal variance) followed by a Dunnett’s post-hoc test. In addition, logarithmic transformations were done to reduce variance to provide more normally distributed measures, when needed. Coefficients of correlation and significance of the degree of linear relationship between parameters were determined with a simple regression model and the threshold for statistical significance was set $P<0.05$. Logarithmic transformation was used to establish homogeneity of variance and improve normality, when necessary. JMP Statistical Analysis Software (version 5.0.1) or Prism (Macintosh version 4.0c) were used for all analyses.

3. Results

3.1. A high-fat westernized diet increased animal weights

Decreasing the n-3:n-6 PUFA dietary ratio alone (Diet B) had no effect on body weight (Table 2). However, feeding the animals with a high-fat westernized diet (Diet C) increased the weight of non-transgenic (NonTg) mice by 69% (Welsh-ANOVA: $P<0.0001$, F(groups)2,19 = 17.61) compared to Diet A ($P=0.0001$; Dunnett’s test) and by 62% compared to Diet B ($P=0.0009$; Dunnett’s test). Interestingly, 3xTg-AD mice were heavier than NonTg on the same diet, in the case of Diet A (+31%, $P=0.0166$) and Diet B (+26%, $P=0.0098$), but not Diet C. This shows that 3xTg-AD mice fed low-fat diets spontaneously gained more weight than their non-transgenic age-matched controls. Two-way ANOVA confirmed that the diets ($P<0.0001$, $F_{2,59} = 12.4$) and the transgenes ($P=0.0286$, $F_{1.59} = 5.3$) had significant separate effects on animal weight, with no interaction between the two variables ($P=0.3577$, $F_{2.59} = 1.0$).

3.2. Transgene expression and dietary treatments altered brain fatty acid profiles

The detailed effects of dietary treatment and transgene expression on brain fatty acid profiles are given in Table 2 along with the results of statistical analyses. As expected, the reduction of the dietary n-3:n-6 PUFA ratio (Diet B) was translated into a 33% and 26% increase of n-6 PUFA in the brain of NonTg (ANOVA, $F$ (groups)2,25 = 141.6; $P<0.0001$) and 3xTg-AD (ANOVA, $F$ (groups)2,18 = 59.0; $P<0.0001$), respectively (Table 2). Diet B also led to a 16% and 19% decrease of n-3 PUFA in the brain of NonTg (ANOVA, $F$ (groups)2,25 = 47.7; $P<0.0001$) and 3xTg-AD mice (ANOVA, $F$ (groups)2,18 = 34.8; $P<0.0001$), respectively (Table 2). In comparison to Diet A, Diet B led to a 19% decrease of DHA (ANOVA, $F$ (groups)2,25 = 46.1; $P<0.0001$) and a 37% decrease of the n-3:n-6 PUFA ratio (ANOVA, $F$ (groups)2,25 = 225.9; $P<0.0001$) in the brain of NonTg (Table 2). Compared to Diet A, Diet C also caused a reduction in DHA of 8% (ANOVA, $F$ (groups)2,25 = 46.1; $P<0.01$) accompanied by a 23% reduction of the n-3:n-6 PUFA ratio (ANOVA, $F$ (groups)2,25 = 225.9; $P<0.0001$) in the brain of NonTg (Table 2).

The 3xTg-AD animals differed from NonTg in terms of basic fatty acid profile and their response to diets. Fig. 1 illustrates the direct comparison between 3xTg-AD mice and NonTg age-matched controls on the same diet. Concentrations of DHA in the cortex were similar between 3xTg-AD and NonTg fed Diet A (Fig. 1). Importantly however, the low dietary intake of n-3 PUFA relative to n-6 PUFA led to decreased brain DHA in a more marked manner in 3xTg-AD than in NonTg, indicating that the effect of the n-3 PUFA dietary depletion on DHA levels were more profound in 3xTg-AD animals (Fig. 1A). This was confirmed by two-way ANOVA showing a significant interaction between the two variables (diets and transgenes), underscoring a greater vulnerability of the transgenic animals to n-3 PUFA depletion (Table 2). Accordingly, the n-3:n-6 PUFA ratio was significantly decreased in 3xTg-AD mice compared to NonTg in all diet groups, although the effect gained in statistical significance in the Diet C group. To isolate the transgenes as a variable, two-way ANOVA were performed and revealed that transgene expression in 3xTg-AD was associated with increased levels of docosatetraenoic acid (DTA) and total n-6 PUFA and a decrease in DHA, total n-3 PUFA and n-3:n-6 PUFA ratio (Table 2).

3.3. The high-fat diet increased cortical load of $A_{beta}$40 and $A_{beta}$42

Measurement of $A_{beta}$ burden in formic acid extracts from the detergent-insoluble protein fraction revealed no significant effect of n-3 PUFA deprivation (Diet B) whereas Diet C strikingly increased $A_{beta}$40 (+871% versus controls; ANOVA (log), $F$ (groups)2,19 = 7.52, $P=0.0039$; Newman–Keuls post-hoc test) and $A_{beta}$42 (+912% versus controls; ANOVA (log), $F$ (groups)2,19 = 8.79, $P=0.0020$; Newman–Keuls post-hoc test) in the parietotemporal cortex of 3xTg-AD mice (Fig. 2). Importantly, the cortical detergent-insoluble $A_{beta}$240 ratio was higher in the group of 3xTg-AD mice fed with Diet C compared to Diet B (+154%; ANOVA, $F$ (groups)2,19 = 3.66; $P=0.044$; Newman–Keuls post-hoc test) (Fig. 2). Dietary
Table 2
Animal weight and brain fatty acid profile of 3×Tg-AD mice fed with a low n-3:n-6 PUFA ratio incorporated into a low-fat or a high-fat formulation.

<table>
<thead>
<tr>
<th></th>
<th>Non-transgenic mice</th>
<th>Statistical test</th>
<th>Two-way ANOVA (P-values)</th>
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<tbody>
<tr>
<td></td>
<td>Control (Diet A)</td>
<td>Low n-3:n-6 ratio</td>
<td>Low n-3:n-6 ratio</td>
</tr>
<tr>
<td></td>
<td>5% (w/w) fat</td>
<td>(Diet B)</td>
<td>35% (w/w) fat (Diet C)</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>31.4 ± 1.3</td>
<td>32.8 ± 1.7</td>
<td>53.1 ± 19*§§</td>
</tr>
<tr>
<td>Brain fatty acid (% nC18:0)</td>
<td>13(4.9)</td>
<td>9(4.5)</td>
<td>6(2.4)</td>
</tr>
<tr>
<td>C16:0</td>
<td>23.3 ± 0.3</td>
<td>224.8 ± 0.3*</td>
<td>23.2 ± 0.3</td>
</tr>
<tr>
<td>C18:0</td>
<td>20.6 ± 0.2</td>
<td>21.4 ± 0.2**</td>
<td>20.4 ± 0.1</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.08 ± 0.03</td>
<td>0.26 ± 0.1</td>
<td>0.08 ± 0.03</td>
</tr>
<tr>
<td>C22:0</td>
<td>0.12 ± 0.04</td>
<td>0.2 ± 0.1</td>
<td>0.21 ± 0.07</td>
</tr>
<tr>
<td>Total SFA</td>
<td>44.2 ± 0.3</td>
<td>4.3 ± 0.5</td>
<td>44.2 ± 0.2</td>
</tr>
<tr>
<td>C18:1n-9</td>
<td>15.4 ± 0.38</td>
<td>13.7 ± 0.3**</td>
<td>14.5 ± 0.2*</td>
</tr>
<tr>
<td>Total MUFA</td>
<td>21.7 ± 0.6</td>
<td>200.3 ± 0.4</td>
<td>20.3 ± 0.3</td>
</tr>
<tr>
<td>C18:2n-6 LA</td>
<td>1.2 ± 0.1</td>
<td>1.3 ± 0.2</td>
<td>2.01 ± 0.09*</td>
</tr>
<tr>
<td>C20:4 n-6 ARA</td>
<td>9.9 ± 0.2</td>
<td>11.4 ± 0.2**</td>
<td>10.6 ± 0.1*</td>
</tr>
<tr>
<td>C22:4n-6 DPA</td>
<td>2.78 ± 0.07</td>
<td>365.0 ± 0.09****</td>
<td>3.41 ± 0.07****</td>
</tr>
<tr>
<td>C22:5n-6 HPA</td>
<td>0.02 ± 0.06</td>
<td>0.26 ± 0.2****</td>
<td>0.50 ± 0.02****</td>
</tr>
<tr>
<td>Total n-6 PUFA</td>
<td>14.6 ± 0.2</td>
<td>194.0 ± 0.2****</td>
<td>17.6 ± 0.2****</td>
</tr>
<tr>
<td>C18:3n-3 LNA</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>C20:5n-3 EPA</td>
<td>0.03 ± 0.03</td>
<td>0.11 ± 0.04</td>
<td>0.05 ± 0.03</td>
</tr>
<tr>
<td>C22:6n-3 DHA</td>
<td>17.2 ± 0.3</td>
<td>14.0 ± 0.2****</td>
<td>15.7 ± 0.2****</td>
</tr>
<tr>
<td>Total n-3 PUFA</td>
<td>19.5 ± 0.3</td>
<td>163.3 ± 0.1****</td>
<td>18.0 ± 0.2****</td>
</tr>
<tr>
<td>Total PUFA</td>
<td>34.1 ± 0.4</td>
<td>357.0 ± 0.1*</td>
<td>35.5 ± 0.2****</td>
</tr>
</tbody>
</table>

Abbreviations: *, females; 3×Tg-AD, triple transgenic mouse model of Alzheimer’s disease; ARA, arachidonic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; DTA, docosatetraenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; LNA, linolenic acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids. Values are expressed as means ± S.E.M.

* P<0.05, ** P<0.01 and *** P<0.001 versus control diet (same genotype).
† P<0.05, †† P<0.01, ††† P<0.001 and †††† P<0.0001 versus non-transgenic mice (same diet).
§ P<0.05, §§ P<0.01 versus non-transgenic mice (same diet)
treatments did not alter levels of $\mathrm{A}\beta_{40}$ and $\mathrm{A}\beta_{42}$ in the fraction containing soluble proteins, although Diet C caused a trend toward an increase of both $\mathrm{A}\beta$ species (Fig. 2). Overall, interindividual variations were important in all animal groups, as is often the case in samples from human brain as well (Tremblay et al., 2007).

To investigate whether increased $\mathrm{A}\beta_{40}$ and $\mathrm{A}\beta_{42}$ were a consequence of increased production of APP or its processing by $\alpha$- and $\beta$-secretase, we measured both full length APP and $\alpha$- and $\beta$-APP carboxy terminal fragment (CTF) products on Western immunoblots. Fig. 3 shows that the dietary treatments did not significantly modulate the protein expression of APP nor its CTF. Moreover, dietary treatments exerted no significant effect on soluble $\mathrm{A}\beta$ oligomers as measured in a dot blot assay with the oligomer-specific antibody A11, which does not bind to fibrils or monomers (Kayed et al., 2003) (Fig. 3).

3.4. Low dietary n-3:n-6 PUFA ratio and high-fat diet increased tau levels in 3xTg-AD mice

Diet B-induced n-3 PUFA dietary depletion led to a statistically significant rise (+97% versus controls; ANOVA (log), $F(\text{groups})_{2,18} = 3.79$; $P = 0.0424$; Newman–Keuls post-hoc test) in the total amount of tau protein found in the formic acid extract from the cortex of 3xTg-AD mice but not in the TBS-soluble protein fraction (Fig. 4). However, animals fed with Diet C diet had higher levels of total tau protein in both the soluble (+153% versus controls; ANOVA (log), $F(\text{groups})_{2,15} = 4.70$; $P = 0.0120$; Newman–Keuls post-hoc test) and insoluble fractions (+69% versus controls; ANOVA (log), $F(\text{groups})_{2,18} = 3.79$; $P = 0.0424$; Newman–Keuls post-hoc test) compared to 3xTg-AD mice fed Diet A. Diet C also increased total soluble tau compared to Diet B (+164% versus controls; ANOVA (log), $F(\text{groups})_{2,15} = 4.70$; $P = 0.0173$; Newman–Keuls post-hoc test). On the other hand, phospho-

rulyated tau remained unchanged between groups (Fig. 4). The present data indicate that the impact of high-fat-based caloric intake on tau pathology was less evident than on $\mathrm{A}\beta$ accumulation.

3.5. Low dietary n-3:n-6 PUFA ratio combined with a high-fat westernized diet decreased postsynaptic marker drebrin

High-fat intake combined with a low n-3:n-6 PUFA ratio (Diet C) induced a decrease in drebrin compared to Diet A in 3xTg-AD mice (~30.2% versus Diet A; ANOVA, $F(\text{groups})_{3,41} = 3.0$; $P < 0.01$; Newman–Keuls post-hoc test) in homogenates from the parieto-temporal cortex (Fig. 5). This reduction was observed in the detergent-soluble fraction (membrane) from cortex homogenates, but not in the TBS-soluble fraction (not shown), in accordance with previous data (Calon et al., 2004). No significant effects were detected on the expression of syntaxin 3, PSD-95, synaptophysin and SNAP-25 (Fig. 5). Two-way ANOVA revealed an effect of dietary treatments on levels of drebrin ($P < 0.01$), and an effect of transgenics on levels of PSD-95 (decrease; $P < 0.05$) and syntaxin 3 (increase; $P < 0.05$).

3.6. The high-fat diet increased glial fibrillary acidic protein (GFAP) in both NonTg and 3xTg-AD mice

To determine whether the effects of dietary treatments were related to glial activation, we measured the levels of GFAP in parieto-temporal cortex of NonTg and 3xTg-AD mice (Table 3). Although one-way ANOVA did not detect any significant alteration between individual groups, two-way ANOVA indicated that the high intake of calories from fats significantly increased actin-normalized GFAP ($P = 0.0095$). This effect of the high-fat diet was thus present in both NonTg and 3xTg-AD mice.
Fig. 2. High-fat diet potentiates the accumulation of Aβ in the brain cortex of 3xTg-AD mice. Increased Aβ load was found in the formic acid–soluble protein fraction from homogenates generated from the parieto-temporal cortex of 3xTg-AD mice fed low-fat and low n-3:n-6 PUFA ratio diet (Diet B) or high-fat and low n-3:n-6 PUFA ratio diet (Diet C), compared with control diet (Diet A). Aβ concentrations were determined using ELISA. Values are expressed as means ± S.E.M. (n = 5–10) and statistical analyses were performed using ANOVA followed by a Newman–Keuls post-hoc test. Abbreviations: Aβ, amyloid-β peptide; Ctrl, control diet.

3.7. Altered LR11, coflin and p21-activated kinase (PAK) concentrations in 3xTg-AD mice

To identify alterations of AD-related proteins that could be associated with the accumulation of Aβ/tau after high-fat diet intake, we measured by immunoblotting the concentrations of LR11, ApoE, coflin and p21-activated kinase (PAK) in the parieto-temporal cortex of NonTg and 3xTg-AD mice (Table 3). Two-way ANOVA of the data showed that transgene expression downregulated actin-normalized levels of LR11 (P < 0.0001), coflin (P < 0.0001) and PAK (P < 0.0001) (Table 3). Moreover, two-way ANOVA indicated that dietary treatments significantly altered coflin (P < 0.0001), but revealed an interaction between transgenes and diets on coflin levels (P = 0.0251), showing that dietary effects on coflin depended on the transgenic status (Table 3). No significant effect of transgenes or dietary treatments on ApoE or total oxidized proteins was detected (Table 3). Except for the specific case of GFAP described above, diets had little effects on markers shown in Table 3, which thus cannot be readily correlated with the rise in Aβ and tau levels induced by the high-fat diet.

4. Discussion

Consumption of high-fat meals containing a low n-3:n-6 PUFA ratio is prevalent in our modern society and has been reproduced here in an animal model of AD. The present study shows that a high-fat westernized treatment amplified various
aspects of AD neuropathology, supporting the contention that dietary factors may alter the progression of AD in humans.

4.1. Effects of transgene expression and dietary treatments on brain fatty acids

Our results first indicated that the brain of 13-month-old 3xTg-AD mice is spontaneously enriched in n-6 PUFA compared to n-3 PUFA. Such a decrease in n-3:n-6 PUFA ratio is detected in APP

K670N,M671L mice only when crossed with animals expressing mutant human PS1

M146L (Calon et al., 2005; Yao et al., in press), suggesting that the addition of PS1 and/or tau transgenes played a role in the modification of the cortical PUFA profile. Since n-3 PUFA are enriched in synapses (Breckenridge et al., 1973; Jones et al., 1997), the decrease in n-3 PUFA may contribute to synapse-related deficits. Evidence that n-3 PUFA are decreased in AD brain have also been reported (Lukiw et al., 2005; Prasad et al., 1998; Söderberg et al., 1991) although this remains a matter of controversy (Calon and Cole, 2007; Plourde et al., 2007), as some studies found no changes (Corrigan et al., 1998; Skinner et al., 1993) or even increased DHA (Pamplona et al., 2005). Nevertheless, the present observations suggest an important effect of the transgene expression on the accumulation of PUFA in the brain.

The present study design allowed the investigation of the effect of low n-3:n-6 ratio in dietary PUFA when carbohydrates:fat calories percentages were fixed at 68:12 (Diet B) or 20:60 (Diet C). Despite similar n-3:n-6 ratios, the absolute amount of n-3 PUFA in formulas was under 0.05% (w/w) in Diet B and close to 0.2% in Diet C (Table 1). This means that mice fed Diet B were exposed to less dietary n-3 PUFA than those fed Diet C, as testified by brain fatty acid measurements of PUFA (Table 2). DHA depletion is normally characterized by a compensatory increase of n-6 DTA and n-6 DPA (Pawlosky and Salem, 2001). Accordingly, n-6 DTA

Fig. 3. Dietary fats did not alter the production and processing of APP. (A) An immunoblot image is shown where each lane represents an individual 3xTg-AD mouse fed with either Diet A, B or C. Dietary treatments did not alter the levels of (B) full length APP; (C) ~9-kD C-terminal fragment (CTF) produced by α-secretase; (D) ~11-kD CTF produced by β-secretase; and (E) soluble amyloid oligomers. Measurements were made in the detergent-soluble fractions using antibodies recognizing the C-terminal segment of APP (C31) and in the TBS-soluble fractions for Aβ oligomers (anti-amyloid oligomer; A11). Values are expressed as means ± S.E.M. (n = 5–10) and statistical analyses were performed using ANOVA. Abbreviations: APP, amyloid precursor protein; CTF, C-terminal fragment; C and Ctrl, control; A and Diet A, low-fat control diet; B and Diet B, low-fat with low n-3:n-6 PUFA ratio diet (low-fat diet); C and Diet C, high-fat with low n-3:n-6 PUFA ratio diet (High-fat diet); O.D., optical density.
A decreased synthesis or a reduced incorporation of DHA in brain phospholipids (Rapoport et al., 2007).

4.2. Effects of dietary treatments on Aβ pathology

One of the most important results of our study is that high caloric intake from fat increased Aβ40 and Aβ42 burden in the cortex of 3xTg-AD mice. This response to the high-fat diet was more striking in some animals than others, showing significant interindividual variability. The observed changes were not correlated with alterations in total APP, C-terminal fragments (α and β cleavage products) of APP or soluble oligomers. These observations are consistent with previous observations in Tg2576 mice fed with a high-fat diet similar to present Diet C (Ho et al., 2004) and suggest that high-fat treatment does not promote the accumulation of Aβ through a change in the production and metabolism of APP. Given the mounting hypothesis that obesity and metabolic disorders are
linked to Aβ accumulation in AD (Erol, 2008; Luchsinger and Mayeux, 2007), it is tempting to speculate that these increases in weight and brain Aβ following the high-fat diet share common mechanisms.

Diet C had a more pronounced effect on Aβ_{42} than Aβ_{40} concentrations, an observation relevant to AD because the Aβ_{42/40} ratio is also increased in AD brain (Ingelsson et al., 2004; Julien et al., 2008). It was recently shown that all mutations in presenilins that are linked to early-onset familial Alzheimer’s disease lead to increased Aβ_{42/40} ratio in post mortem brain samples and within cultured cells transfected with the mutant genes (Citron et al., 1997; Kumar-Singh et al., 2006; Scheuner et al., 1996). Hence, this property of Diet C to increase Aβ_{42/40} ratio in the cortex of 3xTg-AD mice is likely to be relevant to AD pathology. Our results also demonstrate that an 8-month exposure to a low n-3:n-6 dietary PUFA ratio had no effect on both Aβ_{40} and Aβ_{42}, when given as a part of a low-fat diet. In line with our data, a previous n-3 PUFA deprivation study showed that old Tg2576 mice exposed to a safflower-based diet had increased soluble Aβ_{40} with no change in concentrations of soluble Aβ_{42}, insoluble Aβ_{40} and insoluble Aβ_{42} (Lim et al., 2005). Similarly, Green et al.

Fig. 5. Selective downregulation of the postsynaptic membrane protein drebrin in 3xTg-AD mice fed a high-fat westernized diet. Effects of low-fat and low n-3:n-6 PUFA ratio diet (Diet B) or high-fat and low n-3:n-6 PUFA ratio diet (Diet C) on the levels of drebrin, syntaxin 3, PSD-95, synaptophysin and SNAP-25 in detergent-soluble (membrane) fractions from the parieto-temporal cortex of 3xTg-AD mice. Animals were fed dietary treatments from 4 to 13 months. Values are expressed as means ± S.E.M. normalized to actin (n = 5–10). *P < 0.05 versus control diet (Diet A), same genotype; ANOVA followed by a Newman–Keuls post-hoc test. Abbreviations: 3xTg-AD, triple transgenic mouse model of Alzheimer’s disease; A, Diet A; B, Diet B; C, Diet C; NonTg, non-transgenic; O.D., optical density, PUFA, polyunsaturated fatty acids; PSD-95, postsynaptic density-95; SNAP-25, synaptosome-associated protein-25.
Table 3

Levels of GFAP, LR11, ApoE, Cofilin, PAK and oxidized proteins from parieto-temporal cortex of 3xTg-AD fed with low-fat or high-fat diets, incorporating either low n-3:n-6 PUFA ratio.

<table>
<thead>
<tr>
<th>Proteins (relative O.D.)</th>
<th>(Diet A)</th>
<th>(Diet B)</th>
<th>(Diet C)</th>
<th>(Diet A)</th>
<th>(Diet B)</th>
<th>(Diet C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (⇢;⇡)</td>
<td>10–13 (3–4;7–9)</td>
<td>9 (4;5)</td>
<td>6 (2;4)</td>
<td>7 (4;3)</td>
<td>10 (8;2)</td>
<td>4–5 (3;1–2)</td>
</tr>
<tr>
<td>GFAP</td>
<td>0.27 ± 0.02</td>
<td>0.27 ± 0.04</td>
<td>0.34 ± 0.03</td>
<td>0.25 ± 0.01</td>
<td>0.5 ± 0.2</td>
<td>0.0095 n.s. n.s.</td>
</tr>
<tr>
<td>LR11</td>
<td>0.70 ± 0.03</td>
<td>0.78 ± 0.07</td>
<td>0.64 ± 0.04</td>
<td>0.47 ± 0.04</td>
<td>0.50 ± 0.06</td>
<td>0.45 ± 0.07 n.s. &lt;0.0001 n.s.</td>
</tr>
<tr>
<td>ApoE</td>
<td>1.8 ± 0.2</td>
<td>1.30 ± 0.09</td>
<td>1.30 ± 0.04</td>
<td>1.5 ± 0.4</td>
<td>1.6 ± 0.4 n.s. n.s. n.s.</td>
<td></td>
</tr>
<tr>
<td>Cofilin</td>
<td>1.15 ± 0.08</td>
<td>0.90 ± 0.06</td>
<td>1.48 ± 0.04</td>
<td>0.7 ± 0.1</td>
<td>0.79 ± 0.06</td>
<td>1.0 ± 0.05 §§ §§ §§</td>
</tr>
<tr>
<td>PAK</td>
<td>0.85 ± 0.05</td>
<td>0.83 ± 0.06</td>
<td>0.83 ± 0.06</td>
<td>0.7 ± 0.1</td>
<td>0.79 ± 0.06</td>
<td>1.0 ± 0.05 §§ §§ §§</td>
</tr>
<tr>
<td>Oxidized proteins</td>
<td>2.0 ± 0.1</td>
<td>2.2 ± 0.2</td>
<td>2.0 ± 0.1</td>
<td>2.0 ± 0.1</td>
<td>2.0 ± 0.1</td>
<td>2.0 ± 0.1</td>
</tr>
</tbody>
</table>

Author’s personal copy

Abbreviations:
- ⇢ females; 3xTg-AD, triple transgenic mouse model of Alzheimer’s disease; ApoE, apolipoprotein E; GFAP, glial fibrillary acidic protein; LR11, sortilin-related receptor SOCS-LR11.
- O.D., optical density; PAK, p21-activated kinase. Values are expressed as means ± S.E.M. normalized to actin.
- § P < 0.05 versus low fat low n-3:n-6 PUFA diet (same genotype).
- §§ P < 0.001 versus control diet (same genotype).
- §§§ P < 0.0001 versus non-transgenic mice (same diet).

Also observed only a weak effect of long-term treatment with DHA limited to soluble Aβ40 (Green et al., 2007). Overall, this suggests that very high amounts of fat intake has a greater impact on Aβ accumulation than the n-3:n-6 dietary PUFA ratio alone.

4.3. Effects of dietary treatments on tau pathology

The only previous report on the impact of dietary fats on tau pathology showed that DHA supplementation in 3XTg-AD mice reduced the levels of total soluble tau (Green et al., 2007). However, this report did not examine the effect of lowering the n-3:n-6 PUFA ratio nor increasing fat intake. Our measures in homogenates from 3XTg-AD mice showed that a low n-3:n-6 dietary PUFA ratio was sufficient to raise the levels of insoluble tau whereas high calorie intake from fat was necessary to increase total tau in TBS-soluble fractions as well. It is likely that tau deposited in the formic acid extracts better represents the pathogenic form of this microtubule protein. Indeed, massive conversion of tau protein into its insoluble form is a major feature of AD pathogenesis and it correlates well with expression of cognitive deficit (Arriagada et al., 1992; Ballatore et al., 2007; Julien et al., 2008; Tremblay et al., 2007). For example, while levels of soluble tau remains unaltered in cortex samples from advanced AD patients, insoluble tau is increased by over 2000% (Julien et al., 2008). Thus, the present data, which demonstrate for the first time that n-3 PUFA deprivation can upregulate insoluble tau, might provide an important mechanism by which dietary fat modulates a pathogenic process tightly related to the clinical expression of the disease.

4.4. Effects of dietary treatments on synaptic pathology

Drebrin is a dendritic spine protein, which plays an important role in synaptic function (Hayashi and Shirao, 1999; Sekino et al., 2007). Proteic and mRNA levels of drebrin have been shown to be massively decreased in AD (Calon et al., 2004; Counts et al., 2006; Harigaya et al., 1996; Hatanpää et al., 1999; Julien et al., 2008; Shim and Lubec, 2002), an observation probably explained by deactivation of cell survival pathways and activation of specific caspases (Calon et al., 2005; Klaiman et al., 2008). Thus, drebrin loss qualifies as another important marker of AD neuropathology. Along with its effect on Aβ and tau accumulation, Diet C induced a concomitant decrease in drebrin in detergent-soluble fractions, which contained membrane proteins. Albeit in a less striking manner, this observation is in agreement with experiments in aged Tg2576 mice where n-3 PUFA depletion induced a massive translocation of drebrin out of the membrane (Calon et al., 2004, 2005). In addition, the change in synaptic proteins was selective to membrane drebrin, as reported (Calon et al., 2004, 2005). Therefore, increased consumption of fat led to a selective reduction in drebrin, driving 3XTg-AD mice neuropathology closer to the human disease.
4.5. Effect of dietary treatments and transgenes on other AD-relevant brain markers

Alterations of LR11 (Offe et al., 2006; Scherzer et al., 2004), cofilin (Zhao et al., 2006), PAK (Zhao et al., 2006) and GFAP (Panter et al., 1985) have been reported in AD brain and mechanisms through which these proteins can play a role in AD are supported by compelling evidence. LR11, a member of the ApoE/low-density lipoprotein receptor family, was identified as a probable genetic risk factor for late-onset sporadic AD (Rogaeva et al., 2007). LR11 was shown to downregulate Aβ production (Offe et al., 2006) and to be increased by DHA treatment in aged Tg2576 mice (Ma et al., 2007). Here, we measured a decrease of LR11 in the cortex of 3xTg-AD mice, an observation not reported in other transgenic mouse models of AD (PS1/APP and Tg2576) (Dodson et al., 2006; Ma et al., 2007). Our results thus raise the hypothesis that LR11 reduction in 3xTg-AD is related to the accumulation of neurofibrillary tangles. On the other hand, LR11 levels were not influenced by our experimental dietary treatments. Based on studies on AD brain and with transgenic models, it has been proposed that drebrin loss in AD is closely linked to aberrant PAK activity and cofilin-induced disruption of the actin cytoskeletal network (Heredia et al., 2006; Nguyen et al., 2008; Zhao et al., 2006). Cortical PAK and cofilin were both found to be decreased in 3xTg-AD mice whereas the effects of dietary treatments on cofilin levels were difficult to interpret. Therefore, these transgene-induced changes in LR11, cofilin or PAK are interesting to further characterize the 3xTg-AD model, but cannot be directly associated with the effect of dietary treatments on the markers of AD neuropathology Aβ, tau and drebrin.

GFAP is a commonly used marker of astrocyte activation occurring in AD and in transgenic animal models (Calon et al., 2005; Frautschy et al., 1998; Jacobsen et al., 2006; Lim et al., 2000; Wirths et al., in press; Wyss-Coray, 2006). Increased GFAP concentrations were detected in the cortex of mice fed with a high-fat diet, suggesting the presence of glial-neuron injury and the presence of gliosis following high-fat intake independently of the transgene status. This observation suggests that excessive consumption of calories from fat may increase reactive astrocytes in the brain, an event previously shown to be associated with Aβ pathology (Wyss-Coray, 2006). Indeed, genetic or pharmacological induction of microgliosis assessed with GFAP immunodetection has been shown to increase Aβ burden (Kitazawa et al., 2005; Tan et al., 2002). However, reduction of the accumulation of Aβ has also been reported (Wyss-Coray et al., 2001) suggesting that the link between neuroinflammation and the regulation of Aβ is complex (Wyss-Coray, 2006). These inflammatory changes are likely to appear independently from the accumulation of Aβ and tau as they were found in NonTg animals as well. Whether the rise in GFAP played a role in the aggravation of Aβ and tau pathologies or in drebrin loss remains to be determined.

5. Conclusion

Overall, the present data indicate that the accumulation of neuropathological markers of AD in 3xTg-AD mice depends on the dietary intake of PUFA and, more importantly, total intake of calories from fat. Given the fact that diets combining low n-3 PUFA and high-fat content are frequent, dietary interventions aiming at optimizing fat consumption might be relevant for prevention of AD, at least in people with a genetic predisposition.

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Disclosure statement: The authors declare that they have no actual or potential conflict of interest. The use of animals was approved by the Laval university animal ethics committee in accordance with the standards of the Canadian Council on Animal Care.

References


