Dietary Vitamin E Status Dictates Oxidative Stress Outcomes by Modulating Effects of Fish Oil Supplementation in Alzheimer Disease Model APP$_{swe}$/PS1$_{dE9}$ Mice

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Abstract

Quite a number of studies have examined the effects of fish oil supplementation on cognitive performance in different transgenic animal models of Alzheimer’s disease (AD). However, inconsistent and controversial outcomes have been derived from these experiments. In order to investigate whether the beneficial effect of fish oil supplementation on cognition was dietary VE status associated, fish oil dietary intervention was carried out in transgenic APP$_{swe}$/PS1$_{dE9}$ (APP/PS1) mice. Control mice (C57BL/6J mice) were fed a normal control diet. APP/PS1 mice were assigned to a normal control diet group and low VE diet + fish oil supplement, normal VE diet + fish oil supplement, and high VE diet + fish oil supplement groups, respectively. After 7 months of dietary intervention, we found that fish oil supplementation improved behavioral performance, alleviated brain beta-amyloid (Aβ) plaque burden, and attenuated the oxidative stress in APP/PS1 mice by increasing cortical GSH content and total antioxidant capacity, as well as by decreasing MDA level. Fish oil treatment increased cortical n-3 PUFA concentration and decreased n-6/n-3 PUFA ratio in APP/PS1 mice. Fatty acid transporters, Nrf2 and downstream targets involved in cortical and hippocampal antioxidant system were also modulated by fish oil-supplemented diet. Our data demonstrate that fish oil supplementation exerts an enhanced modulatory effect on the antioxidant system and fatty acid concentrations in APP/PS1 mice fed on lowly or averagely concentrated level of VE-containing diet than in mice fed with VE-rich diet. The current data do support previous findings that already dictate the beneficial effect of n-3 PUFAs on cognitive function. Moreover, the cognition promoting effects of n-3 PUFAs may be dietary VE status related.

Keywords N-3 polyunsaturated fatty acids · Docosahexaenoic acid (DHA) · Vitamin E · Cognition

Introduction

Despite decades of extensive basic and clinical research efforts, the cause of Alzheimer’s disease (AD) still remains an unsolved puzzle. Epidemiological studies have suggested that nutrition might be one of the most targeted modifiable factors for AD. Modification of dietary patterns and in vivo nutritional status also could potentially alter the risk of developing AD [1, 2]. Human- and animal-based dietary interventional studies indicate the potential cognition-protecting effect of dietary components, such as n-3 long chain polyunsaturated fatty acids (n-3 LC-PUFAs) and antioxidant vitamins [3, 4]. N-3 LC-PUFAs, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have neuronal protective functions and may potentially impact mental health outcomes [5,
Population-based prospective studies have consistently shown that diets rich in n-3 PUFAs were associated with decreased risk of developing AD [3]. Animal studies also demonstrate that alteration in brain DHA contents are positively correlated with the changes in cognitive and behavioral performance [7]. There are aligning lines of evidence demonstrating that dietary DHA supplementation decreases the amount of brain beta-amyloid (Ab) deposition in AD transgenic mice [8, 9]. However, lines of evidence from human studies lack generalization on the beneficial effect of n-3 LC-PUFA supplementation and its preventive impacts on cognitive decline or AD [10]. On one side, it may be comprehended that these unsatisfactory results may represent that the potential protective effect of a diet could not solely be attributed to the performance of an individual nutrient [11–13]. On the other hand, the complexity of dietary composition implies the potential interaction of different nutrients in vivo, which further infer that an optimal nutritional status may be required for a beneficial response to dietary DHA supplementation.

Brain enriched in PUFAs is highly susceptible to lipid peroxidation, particularly under the antioxidant-deficient environment. As a key lipophilic antioxidant in humans, vitamin E (α-tocopherol, α-TOH) plays an essential role in protecting cellular membrane-associated PUFAs from oxidative damage. Recently published studies report that vitamin E (VE) accumulates in specific PUFA-rich membrane domains to exert its functions of membrane stabilization and protection [14]. When feeding α-tocopherol transfer protein-null (α-TTP-null) mice with a VE-deficient diet, retinal structures of the α-TTP knockout species were found altered accompanying with decreased retina DHA concentration [15]. Decreased serum and cerebral-spinal fluid (CSF) VE concentration has also been observed in AD patients [16]. VE-deficient diet could also cause severe AD-like pathological changes as has been observed in the brains of AD animal models [17]. Choi et al. showed that in the adult zebrafish, low brain α-TOH levels were detected to be associated with the depletion of brain DHA-containing phospholipids [18]. α-TOH deficiency in embryos could be a result of a decrease in DHA concentration during zebrafish embryogenesis [19]. Interestingly, it has been noted that high dietary intake of PUFAs with concomitantly very low intake of VE might result in potential adverse effects of VE depletion, such as increased erythrocyte fragility and lipid peroxides [20]. Therefore, additional VE intake was recommended to compensate for the increase of dietary PUFAs in order to achieve the adequate balance of dietary PUFAs and VE [21]. Furthermore, some researchers pointed out that the optimal VE requirement should depend on the quality and quantity of PUFAs in the diet [22]. All these lines of evidence highlight that a rational balance was needed between VE and PUFAs to determine their role in maintaining optimal functions at the cellular level.

Given the complexity of AD etiology, increasing lines of evidence support the use of different neuroprotective nutrients as an adjuvant therapy for AD [23, 24]. This indicates the synergistic or combined effects of different nutrients in the preventive role of AD. Additionally, in previous studies, the influence of baseline nutritional status of individual nutrients on interventional outcomes has always been underestimated, understudied, and overlooked, which probably contribute to the inconsistently inconclusive outcomes of the different dietary interventional study trials. All these data indicate PUFAs associated function in regard to dietary VE intake, sufficing the hypothesis that dietary VE intake might modulate the response of AD animal models to fish oil supplementation and, consequently, affecting the efficacy of fish oil supplementation on cognitive performance. Therefore, in the present study, we carried out a dietary interventional study to elucidate the close connection between dietary VE and n-3 LC-PUFA intake in affecting cognitive performance in APP/PS1 double transgenic mice. The current study is intended to provide further proof and enrich the existing conclusions regarding the role of n-3 PUFAs in the prevention of AD.

Materials and Methods

Animals and Diet

Six male C57BL/6J (12 weeks) mice and 30 male APP<sub>swe/PS1</sub>dE9 transgenic mice (12 weeks) were from the experimental animal center of Capital Medical University. All diet was purchased from Open Source Animal Diets Co., Ltd. (Changzhou, China). The supplemented fish oil powder was provided by Royal DSM Company (DSM Nutritional Products, Ltd., product code: 50-1526-9, Heerlen, Holland). The content of fatty acids and vitamins in the fish oil powder is listed in Supporting Information 1. The control diet and fish oil-supplemented diet differ in the composition of the fat (5%) added in the diet (Table 1). To confirm the fatty acid and VE content, lipid profile and VE content were measured for all the diets prior to administration to the mice. The content of VE and fatty acids in the final used chow is listed in Supporting Information 2.

Treatment

C57BL/6J mice (control mice) were fed a normal control diet. APP<sub>swe/PS1</sub>dE9 transgenic mice were assigned to a normal control diet group (APP/PS1 group) and low VE diet + fish oil supplement (LVE+DHA), normal VE diet + fish oil supplement (NVE+DHA), and high VE diet + fish oil supplement (HVE+DHA) groups. The dietary intervention started at the age of 3 months and was maintained for 7 months. In order to balance the behavior across the groups, the behaviors of
**APPsw/PS1dE9** mice were measured using a Morris water maze before the dietary intervention. According to the baseline behavior, the APP/PS1 mice were assigned into control diet and fish oil-containing diet-treated groups. The experiments were performed in accordance with the guide for the care and use of laboratory animals (AEEI-2015-040).

### Behavioral Testing

At the end of the dietary intervention, the spatial learning and memory ability of animals was tested using a Morris water maze test as described previously [25, 26]. In brief, the mouse was placed in a pool (100 cm in diameter and 60 cm in height). A submerged platform was set inside the tank and the mice needed to learn to find the platform. Each mouse was given one trial per day for four consecutive days to find the hidden platform, and the full acquisition time course was recorded. The escape latency, the distance traveled to arrive at the hidden platform, and the target crossing of the mouse that crossed the quadrant in which the platform was recorded and the average swim speed were determined by using a computerized tracking system (Water Maze 2.6 Institute of Materia, Chinese Academy of Medical Sciences DMS-2, Beijing, China).

### Tissue Preparation

Following behavioral testing, all mice were euthanatized and sacrificed. Brains were removed and separated along the middle sagittal sulcus. The left side was used for the histological study. The right half of each brain was immediately placed in a cold saline solution and then the cortical and hippocampal regions were dissected. After that, the cortex and hippocampus were transferred into a separate prelabeled 1.5ml tube and stored at −80 °C for real-time PCR, western blotting protein analysis, and cortical fatty acid and VE content measurement. The liver tissues used for VE analysis were also removed and frozen at −80 °C.

### Histology

Beta-amyloid plaque levels in the hippocampus and cortex were measured by using a Congo red (Key Gen Bio Tech, Nanjing, China) staining method according to the description of Oksman et al. [9]. First, the tissues were mounted on slides, and after pretreatment, the slides were put in 4% paraformaldehyde overnight and thereafter rinsed twice in ddH2O. Then, the slides were incubated in NaCl-hydroxide-ethanol solution (saturated NaCl in 80% EtOH, 1% sodium hydroxide added) for 20 min. Slides were transferred in Congo red solution (0.2% Congo red in saturated NaCl in 80% EtOH, 1% sodium hydroxide added, filtered) for 20–30 min. After dehydration, slides were coverslipped and photographed (Panoramic MIDI, 3DHISTECH, Hungary, magnification ×200). The stained amyloid plaques in the cortex and hippocampus were quantified using image analysis software (Image-Pro Plus 6.0, Media Cybernetics, Inc., Rockville, MD, USA).

### Total Antioxidant Capacity, Malondialdehyde, and Glutathione Contents in the Cortex

Twenty milligrams of cortex tissues were homogenized on ice in PBS and centrifuged for 10 min at 12,000 rpm at 4 °C. The

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**Table 1 Composition of experimental diets (g/kg)**

<table>
<thead>
<tr>
<th>Ingredients (g/kg)</th>
<th>Control</th>
<th>APP/PS1</th>
<th>LVE+DHA</th>
<th>NVE+DHA</th>
<th>HVE+DHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>200</td>
<td>200</td>
<td>187.5</td>
<td>187.5</td>
<td>187.5</td>
</tr>
<tr>
<td>DL-methionine</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>179.25</td>
<td>179.25</td>
<td>175</td>
<td>175</td>
<td>175</td>
</tr>
<tr>
<td>Sucrose</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>Olive oil</td>
<td>48</td>
<td>48</td>
<td>0</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Fish oil powder</td>
<td>0</td>
<td>0</td>
<td>35.71</td>
<td>35.71</td>
<td>35.71</td>
</tr>
<tr>
<td>Lard</td>
<td>0</td>
<td>0</td>
<td>30.8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>0.04</td>
<td>0.04</td>
<td>0.026</td>
<td>0.04</td>
<td>0.2</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>0.0157</td>
<td>0.0157</td>
<td>0.0157</td>
<td>0.0157</td>
<td>0.0157</td>
</tr>
<tr>
<td><strong>g %</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Protein</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>67</td>
<td>67</td>
<td>67</td>
<td>67</td>
<td>67</td>
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<tr>
<td>Fat</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

**LVE+DHA**, low VE diet + fish oil supplement; **NVE+DHA**, normal VE diet + fish oil supplement; **HVE+DHA**, high VE diet + fish oil supplement.

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supernatant was collected for biomarker measurement. The total antioxidant capacity (T-AOC), malondialdehyde (MDA), total glutathione (T-GSH), and oxidized GSH (GSSG) were measured using commercial assay kits (Nanjing Jiancheng Institute, Nanjing, China) according to the manufacturer’s instruction. Two independent measurements were performed for each sample. Protein concentrations were determined by BCA protein assay kit (Pierce, Rockford, IL, USA) as described by the manufacturer.

**Real-Time PCR**

RNA was isolated using TRIzol reagent (Invitrogen, USA) as per the manufacturer’s instructions. The quality of RNA was assessed by using a microplate reader (Epoch, BioTek, USA). Three micrograms of RNA was reverse transcribed using a reverse transcription system kit (Thermo Fisher Scientific, USA) according to the manufacturer’s protocol. Real-time PCR was performed in a 20μl reaction system containing 10 μl SYBR Green Mix (SYBR Green, Kapa Biosystems, USA), 1 μl of cDNA, 8.2 μl ddH2O, and 0.4 μl 500 nM of each specific primer. The cycling parameters were as follows: 95 °C, 30 s; 95 °C, 30 s and 62 °C, 30 s; 65 and 95 °C. The primers used for PCR were as follows: nrf2, forward: 5′-TTACCCTGCTGCCATTAGTCAGTC-3′, reverse: 5′-GCTC TTCCATTTCCAGTCACTG3′; ho-1, forward: 5′-GAGC GAAACAAGCAGAACCC3′, reverse: 5′-ACCTCGTGAGACGCTTTAC3′; nqo1, forward: 5′-CCAGGGAGGAAGAGGATGCG3′, reverse: 5′-TCCTCGCTCCAGACGTCTCAG3′; gclc, forward: 5′-GGTAAAACCCA3′, reverse: 5′-GGTGTGGGCACA GGTAAAACCCA3′; gclm, forward: 5′-GGTAAAACCCA3′, reverse: 5′-GGTAAAACCCA3′; actin, forward: 5′-CCAT CGTACTGACTGACTGACT3′, reverse: 5′-CCAT CGTACTGACTGACTGACT3′.

**Fatty Acids and VE Content Measurement in the Cortex and Liver**

Fatty acid concentrations in the brain cortex were determined as the method described previously [27]. Briefly, total lipid was extracted from brain homogenates by methanol and chloroform (2:1, v/v). Samples were centrifuged at 3000 rpm for 10 min and the lower phase (chloroform and lipids) was removed. Chloroform was added to the upper phase, and the samples were centrifuged again at 3000 rpm for 10 min and the lower phase was combined with the first one. The chloroform fractions were dried and the fatty acids were derivatized to their methyl ester with methanol NaOH and boron trifluoride. The collected fatty acid methyl ester was dissolved in isoctane and analyzed by gas chromatograph (Shimadzu GC-2010, Japan) with a flame ionization detector. Separations were performed using a capillary column (SP2560, Supelco, Bellefonte, PA, USA) according to Connor’s description [28]. Fatty acid compositions of 5-6 brains per group are reported, and the fatty acid contents were expressed as g/100 g total fatty acids. VE (α- and γ-tocopherol) was quantitated in the cortex and liver using a published HPLC-electrochemical detection method [29].

**Statistical Analysis**

All data are expressed as mean ± SD and analyzed with SPSS software (SPSS Inc., Chicago, IL, USA). One-way ANOVA was conducted to analyze the differences in body weight, cortical antioxidant parameters, protein and mRNA expression, fatty acids, and VE content in brain and liver tissues. Statistical significance was set at P < 0.05.
Results

Body Weight

Mice were weighted every week and the change in body weight is summarized in Fig. 1. As shown in Fig. 1, the body weight did not differ between different treatment groups at baseline. At the end of the experiment, the NVE+DHA and HVE+DHA diet-treated APP/PS1 mice demonstrated significantly increased body weights as compared with the control diet-treated C57 and APP/PS1 mice and LVE+DHA diet-fed APP/PS1 mice \((P < 0.05)\).

Behavior

As indicated in Table 2, after 3 days of training, both C57 control mice and fish oil-treated APP/PS1 mice showed a significant decrease in escape latency (day 4 compared to day 1, \(P < 0.05\)), while the behavior training had no effect on the escape latency of APP/PS1 control mice (day 4 compared to day 1, \(P > 0.05\)). Compared with control diet-treated APP/PS1 mice, fish oil-containing diet-treated APP/PS1 mice have decreased escape latency, and the difference between the APP/PS1 control group and the LVE+DHA group reached statistical significance \((P < 0.05)\). Although the cognitive performance of APP/PS1 mice in NVE+DHA and HVE+DHA groups was improved as compared with the APP/PS1 mice in the control group (which was proved by the shorter escape latency on day 4), no statistical significance was observed \((P > 0.05)\).

Histology Results

As shown in Fig. 2, APP/PS1 control mice had a higher cortical and hippocampal amyloid plaque burden than the wild-type C57 control mice. The intake of fish oil-containing diet significantly reduced the cortical and hippocampal amyloid plaque burden in APP/PS1 mice.

Antioxidative Parameters in the Cortex

As shown in Fig. 3, APP/PS1 control mice exhibited lower cortical T-AOC level than C57 control mice \((P < 0.05)\). Fish oil supplementation increased cortical T-AOC in APP/PS1 mice as compared with the control diet-treated APP/PS1 mice \((P < 0.05)\). Compared to mice in the HVE+DHA group, the mice in the LVE+DHA and NVE+DHA groups have higher cortical T-AOC levels \((P < 0.05)\) (Fig. 3A).

As compared with C57 control mice, the APP/PS1 control mice have higher cortical MDA level \((P < 0.05)\). The mice in the LVE+DHA and NVE+DHA groups have lower cortical MDA content than APP/PS1 control mice \((P < 0.05)\). The mice in the HVE+DHA group have higher cortical MDA content compared to C57 control mice and LVE+DHA diet-treated mice \((P < 0.05)\) (Fig. 3B).

Compared with C57 control mice, APP/PS1 control mice have lower cortical T-GSH content \((P < 0.05)\). The fish oil supplementation increased cortical T-GSH content in APP/PS1 mice, especially in the NVE+DHA and HVE+DHA groups \((P < 0.05)\). Compared to C57 control mice, APP/PS1 control mice have lower cortical oxidized GSH (GSSG) level \((P < 0.05)\). Fish oil-containing diet significantly increased the
cortical GSSG content as compared with APP/PS1 control mice \((P<0.05)\), and the highest cortical GSSG status was observed in HVE+DHA diet-treated APP/PS1 mice \((P<0.05)\) (Fig. 3C, D).

### Cortical Fatty Acid Content

The relative amounts of different fatty acids in the lipid fraction of the cortical homogenates are listed in Table 3. A significant difference of cortical \(\alpha\)-linolenic acid (ALA), DHA, and total n-3PUFA amounts between different diet-treated mice was observed. The cortical DHA and total n-3 PUFA contents in LVE+DHA and NVE+DHA diet-treated mice were higher than those observed in C57, APP/PS1 control mice and HVE+DHA diet-fed mice \((P<0.05)\). Lower cortical ALA content was observed in the NVE+DHA and HVE+DHA groups \((P<0.05)\). Fish oil supplementation also changed the ratio of n-6 to n-3 PUFAs in the cortex demonstrated by a lower cortical ratio of n-6 to n-3 PUFAs in LVE+DHA and NVE+DHA diet-treated APP/PS1 mice \((P<0.05)\).

### VE Concentration in the Liver and Cortex

The changes of liver VE content were also observed. As shown in Table 4, compared with the control diet-treated C57 and APP/PS1 mice, the NVE+DHA and LVE+DHA diet-treated mice have slight decreased liver VE content. Higher total liver VE and \(\alpha\)-tocopherol status was observed in the HVE+DHA diet-treated APP/PS1 mice compared to NVE+DHA and LVE+DHA diet-treated mice \((P<0.05)\).

Compared with C57 control mice, APP/PS1 control mice have lower cortical total VE content \((P<0.05)\). Fish oil supplementation increased cortical VE status in APP/PS1 mice, and the NVE+DHA diet-treated mice have higher cortical total VE, \(\alpha\)-tocopherol, and \(\gamma\)-tocopherol concentrations \((P<0.05)\).

### CD36 and FABP5 Expression in the Cortex and Hippocampus

As shown in Fig. 4, APP/PS1 control mice demonstrated higher cortical and hippocampal CD36 mRNA expression as compared with C57 control mice \((P<0.05)\). Fish oil-containing diet downregulated CD36 mRNA expression in APP/PS1 mice, and the lowest cortical and hippocampal CD36 mRNA expression was observed in HVE+DHA diet-fed mice \((P<0.05)\). Compared to C57 control mice, APP/PS1 control mice showed higher cortical CD36 protein expression \((P<0.05)\). LVE+DHA and NVE+DHA diets further induced cortical CD36 protein expression \((P<0.05)\), while the HVE+DHA diet exhibited an inhibiting effect on cortical CD36 protein expression \((P<0.05)\). The hippocampal CD36 protein expression was upregulated by fish oil-containing diets, while the best upregulating effect was observed in the NVE+DHA group \((P<0.05)\).

APP/PS1 control mice have higher cortical and hippocampal FABP5 mRNA expression compared to C57 control mice \((P<0.05)\). LVE+DHA and NVE+DHA diets have no effect on cortical FABP5 mRNA expression; however, the HVE+DHA diet significantly induced FABP5 gene expression in the cortex \((P<0.05)\). LVE+DHA and NVE+DHA diets significantly downregulated the mRNA expression of FABP5 in the hippocampus \((P<0.05)\). The HVE+DHA diet has no effect on hippocampal FABP5 mRNA expression.

As compared with C57 control mice, the APP/PS1 control mice showed lower hippocampal FABP5 protein expression \((P<0.05)\). Fish oil supplementation significantly induced cortical and hippocampal FABP5 protein expressions, especially in the NVE+DHA and HVE+DHA groups \((P<0.05)\).

### \(\alpha\)-TTP and SRB1 Expression in the Cortex

Compared to C57 control mice, APP/PS1 control mice have lower cortical \(\alpha\)-TTP mRNA expression \((P<0.05)\). The LVE+DHA diet has no effect on the cortical \(\alpha\)-TTP mRNA expression.

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### Table 2 Performance of experimental animals in the water maze test

<table>
<thead>
<tr>
<th>Groups</th>
<th>Escape latency time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>Control</td>
<td>21.87 ± 3.54</td>
</tr>
<tr>
<td>APP/PS1</td>
<td>19.68 ± 5.32</td>
</tr>
<tr>
<td>LVE+DHA</td>
<td>45.23 ± 5.47</td>
</tr>
<tr>
<td>NVE+DHA</td>
<td>30.49 ± 3.40</td>
</tr>
<tr>
<td>HVE+DHA</td>
<td>27.45 ± 2.64</td>
</tr>
</tbody>
</table>

Data were expressed as mean ± SD, \(n=6\) for each group.

LVE+DHA, low VE diet + fish oil supplement; NVE+DHA, normal VE diet + fish oil supplement; HVE+DHA, high VE diet + fish oil supplement.

<sup>a</sup>: \(P<0.05\) vs control group; <sup>b</sup>: \(P<0.05\) vs APP/PS1 control group; <sup>c</sup>: \(P<0.05\) vs day 1.
Fig. 2 Deposit of amyloid plaque in the cortex (A) and hippocampus (B). Quantitation analysis of amyloid plaque in the cortex (C) and hippocampus (D) (n = 6 per group. Values are expressed as mean ± SE). a: control; b: APP/PS1; c: LVE+DHA; d: NVE+DHA; e: HVE+DHA. LVE+DHA, low VE diet + fish oil supplement; NVE+DHA, normal VE diet + fish oil supplement; HVE+DHA, high VE diet + fish oil supplement. *: P < 0.05 vs control group; #: P < 0.05 vs APP/PS1 group.
expression; however, the NVE+DHA and HVE+DHA diets slightly upregulated cortical α-TTP mRNA expression in APP/PS1 mice ($P < 0.05$). Compared to C57 control mice, the APP/PS1 mice have higher cortical SRB1 mRNA expression ($P < 0.05$). The LVE+DHA diet downregulated cortical SRB1 mRNA expression ($P < 0.05$), while the NVE+DHA and HVE+DHA diets have no effect on cortical SRB1 mRNA expression in APP/PS1 mice (Fig. 5A).

APP/PS1 control mice demonstrated lower cortical α-TTP protein expression as compared with C57 control mice ($P < 0.05$). Fish oil-containing diet downregulated the α-TTP protein expression, and the difference between the

Table 3  The content of fatty acids in the cortex

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>APP/PS1</th>
<th>LVE+DHA</th>
<th>NVE+DHA</th>
<th>HVE+DHA</th>
<th>$P$ value</th>
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<tbody>
<tr>
<td>SFA</td>
<td>64.59±0.06</td>
<td>64.97±1.47</td>
<td>58.6±1.52</td>
<td>59.35±2.05</td>
<td>61.33±2.31</td>
<td>0.066</td>
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<tr>
<td>MUFA</td>
<td>21.58±4.63</td>
<td>22.47±0.11</td>
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<td>25.41±3.46</td>
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<td>PUFA</td>
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<td>15.90±1.55</td>
<td>16.07±1.50</td>
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<tr>
<td>n-6 PUFA</td>
<td>7.31±0.38</td>
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<tr>
<td>LA</td>
<td>1.75±0.11</td>
<td>1.79±0.05</td>
<td>1.57±0.24</td>
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<td>1.28±0.22</td>
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</tr>
<tr>
<td>ARA</td>
<td>5.35±0.47</td>
<td>4.52±0.91</td>
<td>4.98±0.43</td>
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<td>6.45±0.31</td>
<td>5.98±0.69</td>
<td>8.94±1.26 $^{a}$</td>
<td>8.93±0.65 $^{a}$</td>
<td>7.20±0.62 $^{a,##}$</td>
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<td>0.88±0.07</td>
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<td>0.57±0.18 $^{a,##}$</td>
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<td>1.08±0.03</td>
<td>0.78±0.09 $^{a}$</td>
<td>0.79±0.04 $^{a}$</td>
<td>0.83±0.00 $^{a}$</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Data were expressed as mean±SE, $n=3$ for each group. One-way ANOVA analysis method was applied to analyze the difference between groups LVE+DHA, low VE diet + fish oil supplement; NVE+DHA, normal VE diet + fish oil supplement; HVE+DHA, high VE diet + fish oil supplement; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; LA, linoleic acid; ARA, arachidonic acid; ALA, alpha-linolenic acid; DHA, docosahexaenoic acid

*: compared with the C57 control group, $P < 0.05$; #: compared with APP/PS1 control group, $P < 0.05$; **: compared with the LVE+DHA group, $P < 0.05$; ##: compared with the NVE+DHA group, $P < 0.05$
APP/PS1 group and the NVE+DHA group reached statistical significance \( (P<0.05) \).

Cortical SRB1 protein was overexpressed in APP/PS1 control mice compared to C57 control mice \( (P<0.05) \). Fish oil-containing diet further increased cortical SRB1 protein expression \( (P<0.05) \), and the highest cortical SRB1 protein expression was observed in the NVE+DHA diet-treated APP/PS1 mice (Fig. 5B, C).

### Expression of Nrf2 and Downstream Targets in the Cortex

The gene encoding antioxidant protein expression in the cortex was measured in the present study. As shown in Fig. 6A, compared with C57 control mice, the APP/PS1 control mice have decreased mRNA expression of Nrf2, GCLC, GCLM, and NQO1 and increased mRNA expression of HO-1.

### Table 4 The content of VE in the liver and cortex

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>APP/PS1</th>
<th>LVE+DHA</th>
<th>NVE+DHA</th>
<th>HVE+DHA</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver VE (mg/g protein)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total VE</td>
<td>0.32 ± 0.02</td>
<td>0.39 ± 0.04</td>
<td>0.28 ± 0.00</td>
<td>0.26 ± 0.01</td>
<td>0.40 ± 0.03</td>
<td>0.028</td>
</tr>
<tr>
<td>( \alpha )-Tocopherol</td>
<td>0.16 ± 0.05</td>
<td>0.17 ± 0.02</td>
<td>0.14 ± 0.00</td>
<td>0.15 ± 0.01</td>
<td>0.29 ± 0.01</td>
<td>0.043</td>
</tr>
<tr>
<td>( \gamma )-Tocopherol</td>
<td>0.04 ± 0.02</td>
<td>0.04 ± 0.05</td>
<td>0.08 ± 0.00</td>
<td>0.07 ± 0.01</td>
<td>0.09 ± 0.01</td>
<td>0.065</td>
</tr>
<tr>
<td>Cortex VE (mg/mg protein)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total VE</td>
<td>45.30 ± 8.13</td>
<td>35.00 ± 5.78</td>
<td>41.40 ± 2.56</td>
<td>53.10 ± 8.99</td>
<td>42.20 ± 6.18</td>
<td>0.001</td>
</tr>
<tr>
<td>( \alpha )-Tocopherol</td>
<td>16.20 ± 6.17</td>
<td>12.60 ± 1.02</td>
<td>15.50 ± 2.32</td>
<td>18.10 ± 8.44</td>
<td>13.80 ± 5.71</td>
<td>0.654</td>
</tr>
<tr>
<td>( \gamma )-Tocopherol</td>
<td>29.10 ± 5.6</td>
<td>22.40 ± 4.89</td>
<td>25.90 ± 4.71</td>
<td>35.00 ± 10.10</td>
<td>28.40 ± 1.84</td>
<td>0.337</td>
</tr>
</tbody>
</table>

Data were expressed as mean ± SE, \( n=3 \) for each group. One-way ANOVA analysis method was applied to analyze the difference between groups. LVE+DHA, low VE diet + fish oil supplement; NVE+DHA, normal VE diet + fish oil supplement; HVE+DHA, high VE diet + fish oil supplement.

\( * \): compared with the C57 control group, \( P<0.05 \); \#: compared with the APP/PS1 control group, \( P<0.05 \); **: compared with the LVE+DHA group, \( P<0.05 \); ##: compared with the NVE+DHA group, \( P<0.05 \).
Fig. 5 α-TTP and SRB1 expression in the cortex. A Relative levels of mRNA expression in the cortex. B Protein levels of SRB1 and α-TTP in the cortex. C Densitometry analysis of SRB1 and α-TTP protein levels from B. Data are presented as mean ± SEM (n = 3). LVE+DHA, low VE diet + fish oil supplement; NVE+DHA, normal VE diet + fish oil supplement; HVE+DHA, high VE diet + fish oil supplement. #: significantly different from the corresponding values for the control group (P < 0.05); a: significantly different from the corresponding values for the APP/PS1 group (P < 0.05); b: significantly different from the corresponding values for the NVE+DHA group (P < 0.05)

Fig. 6 Nrf2 and downstream target expression in the cortex. A Relative levels of Nrf2 and downstream targets mRNA expression in the cortex. B Protein expression of Nrf2 and downstream targets mRNA expression in the cortex. C Densitometry analysis of protein levels from B. Data are presented as mean ± SEM (n = 3). LVE+DHA, low VE diet + fish oil supplement; NVE+DHA, normal VE diet + fish oil supplement; HVE+DHA, high VE diet + fish oil supplement. #: significantly different from the corresponding values for the control group (P < 0.05); #: significantly different from the corresponding values for the APP/PS1 group (P < 0.05); a: significantly different from the corresponding values for the LVE+DHA group (P < 0.05)
The LVE+DHA diet further decreased the mRNA expression of Nrf2, GCLC, and GCLM ($P<0.05$) but had no effect on the mRNA expression of HO-1 and NQO1. The NVE+DHA diet downregulated the mRNA expression of GCLM but increased NQO1 mRNA expression ($P<0.05$). The HVE+DHA diet increased the mRNA expression of Nrf2, GCLC, HO-1, and NQO1 ($P<0.05$).

As shown in Fig. 6B, the fish oil supplementation has no effect on the protein expression of Nrf2, GCLM, and NQO1 in the cortex ($P>0.05$). GCLC protein expression was upregulated by NVE+DHA and HVE+DHA diets ($P<0.05$). The cortical HO-1 protein expression was induced by the fish oil-containing diet ($P<0.05$), and the strongest inducing effect was observed in the LVE+DHA group ($P<0.05$).

Expression of Nrf2 Downstream Targets in the Hippocampus

As shown in Fig. 7, APP/PS1 control mice have lower hippocampal GCLC, GCLM mRNA expression, but higher NQO1 mRNA expression than C57 control mice ($P<0.05$). The fish oil-containing diet downregulated the mRNA expression of GCLC and GCLM ($P<0.05$), and significant downregulating effects were observed in the LVE+DHA diet-treated APP/PS1 mice ($P<0.05$). The LVE+DHA diet has no effect on the HO-1 mRNA expression, while the NVE+DHA and HVE+DHA diets significantly decreased HO-1 mRNA expression ($P<0.05$). LVE+DHA and NVE+DHA diets have no effect on the mRNA expression of NQO1; however, the HVE+DHA diet significantly decreased the NQO1 mRNA expression ($P<0.05$).

Compared with C57 control mice, APP/PS1 control mice have higher hippocampal GCLC and HO-1 protein expression, but lower GCLM protein expression ($P<0.05$). Fish oil-containing diets significantly induced the protein expression of GCLC, GCLM, and HO-1 ($P<0.05$). The increased NQO1 protein expression in the hippocampus was observed in the HVE+DHA-treated APP/PS1 mice ($P<0.05$).

Discussion

Our data demonstrated the dementia-preventing effect of fish oil supplementation in AD model mice (APP/PS1 mice). Also, the results of the present study indicated a potential correlation between dietary LC n-3 PUFAs and VE status in affecting cognitive performance and AD pathology in APP/PS1 mice.

In the current study, the APP/PS1 control mice exhibited a decline in memory retention as demonstrated by a lengthened latency in the probe trial. The delayed acquisition of the platform location in the water maze consistently indicated an...
impaired working memory and potential impairment in the hippocampus [30]. The fish oil-containing diet enhanced spatial learning and memory ability and reduced the AD-like pathology in APP/PS1 mice, as indicated by shortened latency in the probe trial and significant reduction of cortical and hippocampal Aβ plaque burden. These observations were consistent with earlier studies carried out in aged Tg2576 and 3×Tg-AD model mice [8, 31]. As a pathological hallmark of AD, Aβ is normally secreted by neurons. The abnormally high aggregation and accumulation of Aβ in the brain were reportedly involved in the development of AD via increased oxidative stress states [32, 33]. Similarly, the extensive cortical and hippocampal Aβ plaque deposition in the APP/PS1 control mouse demonstrated the typical pathological changes of AD in the present study. Decreased cortical T-AOC but increased MDA contents were also found in APP/PS1 control mice, which indicates the role of Aβ as an oxidant in the pathological process of AD. Fish oil supplementation (FOS) reduced cortical and hippocampal Aβ burden concomitantly with significantly increased cortical T-AOC and decreased MDA levels. These findings partly shed light on the involved mechanisms underlying the observed improvement of cognitive performance following fish oil supplementation in the APP/PS1 mice.

Our data also indicate that the effectiveness of FOS to improve experimental animals’ behaviors might be dietary VE status associated, demonstrated by the best cognitive performance observed in LVE+DHA diet-fed APP/PS1 mice. In agreement with previous reports, our results also indicate that the impacts of n-3 PUFAs on AD-like pathology potentially depend on the composition of the diet substrate [34]. VE is an essential antioxidant in maintaining normal neuron membrane structure and consequently promoting normal brain function [35]. Regarding AD preventive effects by VE, the data derived from experimental animals and human randomized controlled trials (RCTs) have been contradictory [36, 37]. With the exception of the dosage administered and the stage chosen for the interventional studies, the baseline in vivo VE nutritional statuses of the participants were also addressed as possibly contributing to the contradictory conclusions [38]. Additionally, differences between individual’s responses to antioxidants might contribute to the high variability in the results. It has been reported that VE does not biosimilarity function as an antioxidant in all AD patients [39]. Some researchers pointed out that specific AD patients, for whom VE does act as an antioxidant, are reacting favorably to treatment, and this subset can potentially gain cognitive benefit from dietary VE supplementation [40, 41]. Similarly, the APP/PS1 mice fed on low VE diet might be exposed to higher brain oxidative stress states owing to the Aβ-mediated overgeneration of reactive oxygen species (ROS). As a result, this inherent high tendency for oxidative stress possibly predisposed the APP/PS1 mice to easily benefit from the n-3 PUFA supplementation (the type of fatty acids containing abundance of double bonds and consequently endowing the mice with strong antioxidative capacity) to neutralize the oxidative stress in the brain.

The slight decrease in cortical T-AOC in the HVE+DHA group mice indicates that the FOS to APP/PS1 mice having high VE dietary intake seem to increase the oxidative stress, further supporting the evidence shown by the slight increase in cortical MDA level. The data presented here highlights the potential correlation between dietary VE and n-3 PUFA intake in affecting brain oxidative stress outcomes. Although VE has been considered as an antioxidant, α-tocopherol-mediated peroxidation seems to be the implication, and it is thought to act as a peroxidant to further cause oxidation and consequently resulting in damage to cells [42]. Therefore, we speculate that higher VE concomitant with higher dietary PUFA intake may have perhaps suppressed the beneficial effects of n-3 PUFAs on cognition and, hence, triggered peroxidation of the lipids in and around the cellular environment of the brain.

GSH plays an important role in the detoxification of ROS and maintains intracellular redox equilibrium [43, 44]. The decreased level of GSH in the brain is for one reason suggestive of the build up of the oxidative stress in AD [45]. In the brain of AD patients and AD animal models, depletion of GSH was observed indicating the protective role of GSH in this neurodegenerative disease [46]. Additionally, in both blood and brain of AD experimental animals, a decrease in GSH/GSSG ratio has also been reported [47]. In contrast to these findings, we detected a decreased content of both cortical GSSG and T-GSH, suggestive of inadequate GSH synthesis [48] or perhaps an accelerated breakdown (or over-consumption) of GSH in APP/PS1 control mice. FOS significantly increased the content of cortical T-GSH and restored GSH and GSSG ratio to optimal levels in the LVE+DHA and NVE+DHA diet-treated mice. The dramatic increase in cortical T-GSH and GSSG in the HVE+DHA group demonstrated that FOS to mice fed on high VE-containing diet increased the concentration of T-GSH and simultaneously promoted the transformation of reduced GSH to oxidized GSH (GSSG), implying the enhanced oxidative status following fish oil supplementation. Based on this observation, we speculate that the net alteration of T-GSH and/or GSSG will terminally determine the oxidative stress outcomes in the brain.

Scavenger receptors CD36 have been implicated in microglial and astrocyte binding to Aβ while mediating the uptake of fibrillar Aβ in vitro [49]. In the current study, we detected increased mRNA and protein expression of CD36 in the cortex and hippocampus in APP/PS1 control mice (Figs. 4A, B and 5A, B). These results demonstrate an upregulation of scavenger receptor expression following overgeneration of Aβ in the AD model animals. Also, FOS mitigated the deposition of Aβ plaque (Fig. 2), implying that the overexpression of CD36 mRNA in the cortex and hippocampus was downregulated. Moreover, we found that the fish...
oil-containing diet significantly increased brain total n-3 PUFAs and DHA concentrations with concomitantly decreased n-6/n-3 PUFA ratio in the APP/PS1 mice. These results are consistent with those in previous studies [50]. An adequate quantity of n-3 PUFAs and balance of n-3 to n-6 PUFA ratio in vivo were important determinants of normal brain function [51]. The increase of n-3 PUFAs in the brain might be mediated through the regulation of fatty acid transporter protein expression. CD36 is a fatty acid transporter expressed in the brain and is involved in the transportation of long chain fatty acids across endothelial cells of the blood-brain barrier (BBB) [52, 53]. Recent studies have indicated that CD36 might enhance intracellular metabolism of fatty acids by accelerating their uptake [54]. As an intracellular lipid-binding protein, FABP5 is also essential for uptake of exogenous polyunsaturated fatty acids in the brain (including DHA). Genetic deletion of FABP5 in mice reduced the central nervous system’s (CNS) access to DHA [55] and consequently resulting in cognitive decline [56]. A reduction in BBB transport of DHA as well as diminished brain DHA levels was also observed in FABP5−/− mice [57]. Besides, FABP5−/− mice have also been found to exhibit decreased hippocampal neurogenesis [58]. In this current study, the cortical and hippocampal protein expressions of CD36 and FABP5 were substantially induced following FOS in the APP/PS1 mice. This upregulation of CD36 and FABP5 might enhance the uptake and transport of n-3 PUFAs in the brain and consequently resulting in the decrease of n-6/n-3 PUFA ratio outcome. Compared to the HVE+DHA group, cortical CD36 and FABP5 protein expressions were observed to be the highest in the LVE+DHA and NVE+DHA groups, respectively (Fig. 4), and were also concomitantly consistent with the highest total n-3 PUFA content in the cortex (Table 4). These outcomes imply the depletive role of dietary VE status in modulating the impacts of fish oil on the brain’s fatty acid transporter. The regulating effects of VE on CD36 gene and protein expression have been reported by previous studies. VE-deficient rats have increased expression of CD36 mRNA, while α-tocopherol treatment downregulates the CD36 mRNA and protein expression in oxidized low density lipoprotein (oxLDL)-stimulated THP-1 (a human leukemic cell line) monocytes [59]. It was concluded that VE may modulate CD36 expression and ultimately modify the uptake of fatty acids. VE showed an inconsistent induction of fatty acid transporter protein expression in the cortex and hippocampus by FOS possibly indicating the complex mechanisms involved in the regulation of these molecular. Comprehensively, our data hints a synergistic role of VE and PUFA intake in modulating the content of n-3 PUFAs in the brain.

VE contents in the liver and cortex of experimental animals were also determined. The significant increase of liver total VE and α-tocopherol in the HVE+DHA group indicates that high VE diet enhances VE storage in the liver. The slight decrease of liver VE level in the LVE+DHA and NVE+DHA groups also indicated the possible utilization of VE by extra PUFA intake and, consequently, caused a reduction of stored VE in the liver. Nevertheless, this statement may also be opened to other interpretations. For instance, we did not measure the in vivo bioavailable VE levels in LVE+DHA and NVE+DHA diet-fed mice; therefore, it would be baseless to evidence large amounts of VE storage in the liver, implying that we cannot solely attribute this liver VE finding to uptakes by PUFAs alone.

Consistent results indicated that VE deficiency can lead to the destruction of neurons and increase the risk of neurodegenerative disorders [60]. Among all the forms of vitamin E, α-tocopherols and γ-tocopherols have been acknowledged to provide the greatest form of protection against cognitive decline and AD [61]. As per previous studies, we consistently detected a decrease of cortical total VE, α-tocopherol, and γ-tocopherol concentrations in APP/PS1 control mice indicating an increased oxidative stress and utilization of VE owing to the increased tendency of Aβ plaques [62]. α-TTP is a protein responsible for VE concentration in circulation [63] and for facilitating its release for distribution to peripheral tissues [64]. The decreased cortical α-TTP mRNA and protein expression in APP/PS1 control mice indicated that inhibition of α-TTP expression might contribute to the decreased VE status in this AD model. In the current study, a slight increase of cortical α-TTP mRNA expression was demonstrated after NVE+DHA and HVE+DHA diet treatments; however, the fish oil-containing diets have no effect on cortical α-TTP protein expression. It was reported that α-TTP knockout mice could also absorb VE from diet and maintain acceptable concentration of VE in tissues [65]. These findings support the theory that the brain is especially efficient at conserving VE [66, 67]. In the present study, the significant increase of cortical VE concentration was found in NVE+DHA diet-treated APP/PS1 mice; also, the cortical SRB1 expression was enhanced in the mice. These results indicate the involvement of SRB1 in regulating VE status in the brain. Recent studies indicate that SRB1 functions as an acceptor of α-tocopherol from plasma HDL to tissues [68, 69]. Abnormal disposition of α-tocopherol was observed in SRB1-deficient mice, accompanied by a 50% reduction of α-tocopherol concentration in the brain [70]. The predominant protein expression of SRB1 in the NVE+DHA group compared to the slight decrease of the protein expression in the HVE+DHA group further demonstrated the correlation between dietary VE and n-3 PUFAs in affecting their contents in the brain.

The Nrf2 was regarded as one of the most important mechanisms in cells for the protection against oxidative stress [71]. In the current study, we investigated the expression of Nrf2 and downstream targets in the brain of AD animal models. Our data indicates an extensive inhibition of cortical and hippocampal Nrf2-targeted antioxidant gene mRNA expression in APP/PS1
mice, except for the overexpression of cortical HO-1 mRNA and hippocampal NQO1 mRNA (Figs. 5A and 6A). The inhibition of the Nrf2-antioxidant system possibly explains the increased oxidative stress and neuronal-pathological damage in these AD animal models. FOS could significantly enhance these antioxidant gene expressions in a dietary VE status-dependent way. Interestingly, we did not observe any significant differences in these antioxidant expressions in the cortex at the posttranscriptional level; however, GCLC and HO-1 were found significantly induced in the hippocampus of the APP/PS1 control mice (Figs. 5B and 6B). These inconsistencies in protein expression in the cortex and hippocampus of the APP/PS1 control mice might indicate the potential degree of oxidative status or oxidative stress predisposition in different brain localizations rendering some areas of the brain to become more vulnerable to the impact(s). The protein expressions of GCLC and HO-1 were significantly enhanced in the APP/PS1 mice after fish oil-containing diet treatment. In the hippocampus, the induction of antioxidant protein expression seemed much more extensive than that in the cortex including GCLC, GCLM, HO-1, and NQO1. Data from clinical autopsies also demonstrated that the cellular damage occurring in AD is not uniformly widespread indicated by heavy damage in the hippocampus compared to the spared motor, somatosensory, and primary visual areas [72]. Additionally, regional expression of antioxidant capacity in the brain has also been reported in previous animal studies [73–75]. Ascorbate, GSH, and α-tocopherol concentrations were proven to be higher in the neuron-rich anterior regions (cerebral cortex and hippocampus) than in myelin-rich posterior regions (pons and medulla oblongata) in adult rodent brains [76, 77]. Additionally, different antioxidants have been documented to interact synergistically and help resynthesize those depleted when exposed to an oxidant [48, 78, 79]. In our study, the regional differences in antioxidative protein expression in response to FOS could be possibly attributed to the variability in localized antioxidant distribution and oxidative stress capacity and/or vulnerability. Besides, the dietary VE content-dependent induction of these antioxidant protein expressions further imply a potential correlation between VE and n-3 PUFAs in affecting antioxidative capacity and process. Limitation with this study exists. Vitamin E remains a fat-soluble micronutrient and is closely associated with lipid availability and metabolism. Therefore, it would be prudent to determine the states of lipid profiles of involved study subjects in future experiments to clarify the interactions of VE status, n-3 PUFA efficacy, and serum lipid interaction. Another shortcoming of the present study is the small number of experimental animal subjects, which potentially attenuates the substantial relevance of VE outcomes and n-3 PUFAs' efficacy on cognitive function. Thus, large-scale animal subjects or a human-based experiment is encouraged to uncover the accurate relationship(s) existing between VE and n-3 PUFAs and cognition.

Conclusion

In summary, our results indicate the cognitive-promoting effect of fish oil supplementation in diet potentially through the inhibition of AD pathological process, enhancement of anti-oxidant biomarkers, alteration of lipid profile, and regulation of Nrf2 antioxidant system in the brain. This present study demonstrated that dietary VE status might dictate oxidative stress outcomes by modulating the effects of n-3 fatty acids in the APP/PS1 AD model.

Authors’ Contributions Linhong Yuan designed the study; Shengqi Dong, Xiaochen Huang, and Jie Zhen carried out the lab work; Linhong Yuan and Nicholas Van Halm-Lutterodt contributed to the data interpretation and drafting of the manuscript; and Cui Zhou and Jiajia Wang performed the statistical analysis.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

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