Effects of Dietary Supplementation with EPA-enriched Phosphatidylcholine and Phosphatidylethanolamine on Glycerophospholipid Profile in Cerebral Cortex of SAMP8 Mice fed with High-fat Diet

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Abstract: The destruction of lipid homeostasis is associated with nervous system diseases such as Alzheimer’s disease (AD). It has been reported that dietary EPA-enriched phosphatidylcholine (EPA-PC) and phosphatidylethanolamine (EPA-PE) could improve brain function. However, it was unclear that whether EPA-PC and EPA-PE intervention could change the lipid composition of cerebral cortex in AD mice. All the senescence-accelerated mouse-prone 8 (SAMP8) mice were fed with a high-fat diet for 8 weeks. After another 8 weeks of intervention with EPA-PC and EPA-PE (1%, w/w), the cerebral cortex lipid levels were determined by lipidomics. Results demonstrated that dietary supplementation with EPA-PE and EPA-PC for 8 weeks significantly increased the amount of choline plasmalogen (pPC) and Lyso-phosphatidylethanolamine (LPE) in the cerebral cortex of SAMP8 mice fed with high fat diet. Meanwhile, administration with EPA-PE and EPA-PC could significantly decrease the level of docosapentaenoic acid (DPA)-containing phosphatidylserine (PS) as well as increase the levels of arachidonic acid (AA)-containing phosphatidylethanolamine and PS in cerebral cortex. EPA-PE and EPA-PC could restore the lipid homeostasis of dementia mice to a certain degree, which might provide a potential novel therapy strategy and direction of dietary intervention in patients with cognitive impairment.

Key words: Alzheimer’s disease, lipid profile, EPA, phosphatidylcholine, phosphatidylethanolamine

Abbreviations: AA, Arachidonic acid; Ββ, Amyloid beta; AD, Alzheimer’s disease; APP, amyloid precursor protein; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; EPA-EE, EPA-enriched ethyl esters; EPA-PC, EPA-enriched phosphatidylcholine; EPA-PE, EPA-enriched phosphatidylethanolamine; EPA-PL, EPA-enriched phospholipids; EPA-TG, EPA-enriched triacylglycerol; ePC, Alkyl ether analog of phosphatidylcholine; ePE, Alkyl ether analog of phosphatidylethanolamine; GPC, glycerophosphocholine; HPLC-ELSD, high-performance liquid chromatography with evaporative light scattering detector; LPE, Lyso-phosphatidylethanolamine; LPC, Lyso-phosphatidylcholine; PC, Phosphatidylcholine; PE, Phosphatidylethanolamine; PL, phospholipid; PLA₂, pancreatic phospholipase A₂; pPC, choline plasmalogen; pPE, Ethanolamine plasmalogen; PS, Phosphatidylinerine; SAMP8 mice, senescence-accelerated mouse-prone 8; TG, triacylglycerol

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Accepted October 22, 2020 (received for review July 31, 2020)
Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online
http://www.jstage.jst.go.jp/browse/jos/ http://mc.manuscriptcentral.com/jjocs
1 Introduction

Alzheimer’s disease (AD), a neurodegenerative disease, has the main manifestations including progressive memory loss, cognitive dysfunction, inattention, emotional disorders, personality changes and other characteristics\(^5\). As AD patients cannot take care of themselves, especially in the late stage of disease, long-term care of Alzheimer’s patients will be a serious social burden in the aging society. It has been reported that dietary intervention can affect the occurrence and development of AD\(^9\). Saturated fatty acid intake may increase the AD risk, while intake of unsaturated fatty acids may play an important role in alleviating AD\(^8\). Our study was to investigate the effects of dietary EPA-PC and EPA-PE on the AD brain lipid profiles, and the lipid composition in cerebral cortex of high-fat diet-induced SAMP8 mice with cognitive deficiency was evaluated by lipidomic analysis.

2 Materials and Method

2.1 EPA-PC and EPA-PE preparation

EPA-enriched phospholipids were isolated from sea cucumber Cucumaria frondosa (Nanshan Aquatic Market, Qingdao, China). Total lipids was extracted from the comminuted sea cucumber by chloroform-methanol (2:1) for 24 hours according to the previous method\(^14\). Then the extraction was mixed with NaCl solution (0.15M) and kept for 24 h to obtain the chloroform phase. EPA-PC was purified from total lipids by silica-gel column chromatography using chloroform, chloroform-methanol (9:1), acetone, chloroform-methanol (2:1) and methanol sequentially as eluents. EPA-PC was obtained after removal of the methanol eluent under vacuum. EPA-PE was enzymatically synthesized by catalyzing transphosphatidylation of EPA-PC using phospholipase D in ethanolamine-containing buffer system. The purity of EPA-PC and EPA-PE was above 90% analyzed by high-performance liquid chromatography with evaporative light scattering detector (HPLC-ELSD). The fatty acid composition of EPA-PC and EPA-PE was determined by an Agilent 6890 gas chromatography with a flame-ionization detector and the relative content of each fatty acid was shown in Table 1.

2.2 Animals and diet

Male SAMP8 mice purchased from NanjingQingzilang Technology Co., Ltd were housed at SPF environment under the temperature of 20 ± 2°C and the humidity of 60% with a 12/12 h light/dark cycle (light starting at 8 a.m.). SAMP8 mice were fed with high fat diet for 8 weeks, and then were randomly divided into three groups including high fat group (HF), EPA-PC group and EPA-PE group (n = 8/group). The diet of the three groups was prepared according to the AIN-93M rodent feed formula. Mice in EPA-PC group and EPA-PE group were fed with high fat diet containing 1% (w/w) EPA-PC and 1% (w/w) EPA-PE, respectively. The mice were continuously treated with corresponding diets for 8 weeks. The study protocols were approved by the ethical committee of experimental animal care at Ocean University of China (Qingdao, China, approval no. SPXY2019031). The ingredients and fatty acid composition of experimental diets are shown in Tables 2 and 3, respectively.
### Table 1  Main fatty acid composition of EPA-PC and EPA-PE.

| fatty acid composition (%) | EPA-PC (Mean ± SD) | EPA-PE (Mean ± SD) |
|---------------------------|---------------------|---------------------|
| Palmitic acid (C16:0)     | 29.28 ± 0.29        | 28.84 ± 0.21        |
| Palmitoleic acid (C16:1)  | 0.18 ± 0.01         | 0.17 ± 0.03         |
| Stearic acid (C18:0)      | 14.36 ± 0.20        | 14.20 ± 0.17        |
| Oleic acid (C18:1)        | 1.64 ± 0.06         | 1.30 ± 0.08         |
| Linoleic acid (C18:2)     | 0.37 ± 0.03         | 0.40 ± 0.01         |
| EPA (C20:5)               | 38.39 ± 0.04        | 38.49 ± 0.31        |
| DHA (C22:6)               | 7.35 ± 0.19         | 7.60 ± 0.34         |
| Other fatty acids         | 8.43 ± 0.43         | 9.00 ± 0.31         |

### Table 2  Composition of experimental diets (g/kg diet).

| Ingredients g/kg | HF | EPA-PC | EPA-PE |
|-----------------|----|--------|--------|
| Casein          | 140| 140    | 140    |
| Corn starch     | 430.7| 430.7 | 430.7 |
| Sucrose         | 100| 100    | 100    |
| Corn oil        | 46 | 46     | 46     |
| Lard            | 184| 174    | 174    |
| Mineral mix a   | 35 | 35     | 35     |
| Vitamin mix b   | 10 | 10     | 10     |
| Cellulose       | 50 | 50     | 50     |
| L-Cystine       | 2.5| 2.5    | 2.5    |
| Choline bitartrate | 1.8 | 1.8 | 1.8 |
| t-butylhydroquinone | 0.008 | 0.008 | 0.008 |
| EPA-PC          | -  | 10     | -      |
| EPA-PE          | -  | -      | 10     |

*AIN-93M mineral mix.  
**AIN-93M vitamin mix.

Note: "-", not detected.

### Table 3  Fatty acid composition of experimental diets.

| fatty acid composition (%) | HF (Mean ± SD) | EPA-PC (Mean ± SD) | EPA-PE (Mean ± SD) |
|---------------------------|---------------|-------------------|-------------------|
| Lauric acid (C12:0)       | 0.16 ± 0.02   | 0.15 ± 0.01       | 0.15 ± 0.01       |
| Myristic acid (C14:0)     | 1.04 ± 0.12   | 0.98 ± 0.08       | 0.98 ± 0.02       |
| Palmitic acid (C16:0)     | 21.26 ± 1.64  | 21.50 ± 1.36      | 21.48 ± 1.50      |
| Palmitoleic acid (C16:1)  | 2.20 ± 0.49   | 2.05 ± 0.13       | 2.05 ± 0.10       |
| Stearic acid (C18:0)      | 11.12 ± 0.94  | 11.16 ± 0.63      | 11.15 ± 0.53      |
| Oleic acid (C18:1)        | 43.22 ± 3.73  | 41.23 ± 3.48      | 41.22 ± 2.82      |
| Linoleic acid (C18:2)     | 19.44 ± 1.31  | 19.51 ± 0.26      | 19.51 ± 0.23      |
| Linolenic acid (C18:3)    | 1.10 ± 0.13   | 1.06 ± 0.03       | 1.10 ± 0.03       |
| EPA (C20:5)               | -             | 1.67 ± 0.29       | 1.67 ± 0.23       |
| DHA (C22:6)               | -             | 0.32 ± 0.13       | 0.33 ± 0.10       |
| Other fatty acid          | 0.46 ± 0.12   | 0.37 ± 0.10       | 0.39 ± 0.16       |

Note: "-", not detected.
2.3 Tissue sample preparation and lipidomic analysis

After eight weeks of intervention, the mice were anesthetized by an intraperitoneal injection of sodium chloride solution containing 1% pentobarbital sodium, and then all the mice were decapitated. Cortex was quickly separated from the brain. Cortex was crushed in phosphate buffer using tissue homogenizer and then was extracted with chloroform:methanol (2:1, v/v). NP-HPLC was used to separate the polar lipids from total lipids using Phenomenex Luna 3 μm micron silica column (150 x 2.0 mm). Mobile phase A was chloroform:methanol:ammonia water at the ratio of 89.5:10:0.5, and mobile phase B was chloroform:ethanol:ammonia water:water at the ratio of 55:39:0.5:5.5. The flow rate was 0.25 mL/min. The gradient of mobile phase A was maintained at 95% for 5 min, then decreased linearly to 60% within 7 min and kept for 4 min. The gradient of mobile phase A was further increased to 70% and kept for 15 minutes. Finally, re-equilibration was performed from 33 min to 38 min with 95% A. All experiments were carried out by Exion UPLC-QTRAP 6500 PLUS (Sciex) liquid chromatography-mass spectrometry, and the analyses were performed in the electrospray ionization (ESI) mode. The conditions were: curtain gas = 20 psi, ion spray voltage = 5500 V, temperature = 400°C, ion source gas 1 = 35 psi, ion source gas 2 = 35 psi.

2.4 Statistical analysis

Statistical analyses were performed using MATLAB R2019b (MathWorks, USA). The differences between groups were tested by one-way ANOVA (Turkey’s test). P < 0.05 was considered to indicate a statistically significant difference.

3 Results and Discussion

3.1 Alterations of glycerophospholipids

There was no significant difference in body weight, food intake and brain weight among the HF group, EPA-PC group and EPA-PE group (Table 4). Brain is the most lipid-rich organ except for the adipose tissue, and brain lipids account for at least 50% of the dry brain weight. Brain lipids are comprised of 50% phospholipids, 40% glycolipids, 10% cholesterol, cholesterol ester and traces of triacylglycerols. Glycerophospholipid, such as phosphatidylcholine (PC) and phosphatidylethanolamine (PE), is one of the most important lipids in cerebral cortex. Destruction of lipid homeostasis is related to neurodegenerative diseases, such as Alzheimer’s disease. It is of vital importance to illustrate the changes of lipid profile in brain after dietary intervention.

In order to illustrate the effects of EPA-PC and EPA-PE on AD brain lipid profiles in SAMP8 mice fed with high-fat diet, the lipid composition in cerebral cortex was detected by lipidomic analysis. We mainly identified 9 classes of glycerophospholipids comprised of 170 molecular species. After 8 weeks of EPA-PC and EPA-PE intervention, the changes of brain glycerophospholipid in AD model mice were shown in Fig. 1. There were only small changes without significance in the levels of the main glycerophospholipid classes. PC and PE, accounting for more than 60% of the total glycerophospholipid, were the primary ingredients in the cerebral cortex, which were not easily affected by exogenous phospholipid intake (Fig. 1). Dietary

| Table 4 | Growth parameters of SAMP8 mice. |
|---------|----------------------------------|
|         | HF                          | EPA-PC               | EPA-PE               |
|         | Mean ± SD                   | Mean ± SD            | Mean ± SD            |
| Food intake (g day⁻¹) | 2.09 ± 0.06 | 2.02 ± 0.12 | 2.04 ± 0.12 |
| Final BW (g)        | 23.88 ± 0.69 | 24.60 ± 1.55 | 23.94 ± 1.70 |
| Brain weight (g)    | 0.27 ± 0.01 | 0.27 ± 0.03 | 0.27 ± 0.01 |
EPA could not affect the molecular species of brain phospholipids, especially PC, phosphatidylserine (PS), PE and lyso-phosphatidylcholine (LPC), which was consistent with the present study. Notably, dietary supplementation with EPA-PE and EPA-PC for 8 weeks significantly increased the amount of choline plasmalogen (pPC) and lyso-phosphatidylethanolamine (LPE) in the cerebral cortex although the content was relatively low. Several studies showed a decrease of pPC content in AD brains, and the increase of pPC content after dietary intervention suggested the therapeutic effect of EPA-PE and EPA-PC on AD.

### 3.2 The fatty acid composition of each glycerophospholipid

As for PC molecular species, 16:0/16:0, 16:0/18:1, 16:0/22:6 and 18:0/22:6 were the main fatty acid species as shown in Table 5. A metabonomics study found that patients with AD and mild cognitive impairment (MCI) had lower PC (16:0/22:6) and PC (18:0/22:6) compared with the control group. In the present study, supplementation with EPA-PC and EPA-PE did not reverse the decrease of PC (16:0/22:6) and PC (18:0/22:6). Zhao et al. also reported that different dietary conditions did not change the fatty acid composition of PC in the cerebral cortex of dementia mice, even if DHA-PC and DHA-enriched phosphatidylserine (DHA-PS) were supplemented for 8 weeks.

In Table 6, 18:0/22:6, 18:1/22:5, 18:0/18:1, 18:1/20:3, 18:0/20:4, 18:1/22:6, 16:0/22:6 and 18:1/18:2 were the main fatty acids of PE molecular species, in which 18:0/20:4 and 18:1/20:3 exhibited significant difference between the three dietary groups. High fat diet could reduce the level of 18:0/20:4, but the content of 18:0/20:4 could be restored after the intervention of EPA-PC and EPA-PE. The change of 18:1/20:3 was consistent with that of 18:0/20:4.

Plasmalogens, a kind of ether phospholipids, are characterized by vinyl ether bond at sn-1 position and ester bond at sn-2 position. Plasmalogens are essential for human health as endogenous antioxidants, and play an important role in neuronal development and the immune response. Ethanolamine plasmalogen (pPE) and choline plasmalogen (pPC) are the main components of plasmalogen. Among pPE molecular species, 16:0p/22:6, 18:0p/18:1, 18:0p/20:3, 18:0p/22:6, 18:1p/18:1, 18:1p/22:5 and 18:1p/22:6 are the main fatty acids, as shown in Table 7, but the main fatty acids, including 18:0p/18:1, 18:0p/20:3, 18:0p/22:6, 18:1p/18:1, 18:1p/22:5 and 18:1p/22:6 did not significantly change after intervention with EPA-PC and EPA-PE for 8 weeks. Notably, supplementation with EPA-PC and EPA-PE significantly increased the level of 16:0p/22:6.

Among PS molecular species, 18:0/18:1, 18:0/20:3,
| Fatty Acid (%) | HF  | EPA-PC | EPA-PE |
|---------------|-----|--------|--------|
| 16:0/20:4     | 6.17 ± 1.12 | 5.82 ± 0.47 | 5.93 ± 0.52 |
| 16:0/22:1     | 0.20 ± 0.03a | 0.20 ± 0.03a | 0.25 ± 0.03b |
| 16:0/22:6     | 11.66 ± 1.24 | 11.13 ± 0.88 | 11.32 ± 1.22 |
| 16:0/24:2     | 0.01 ± 0.00ab | 0.01 ± 0.00ab | 0.02 ± 0.00b |
| 16:1/18:2     | 0.01 ± 0.00ab | 0.01 ± 0.00ab | 0.01 ± 0.00a |
| 16:1/19:1     | 0.01 ± 0.00a | 0.01 ± 0.00a | 0.01 ± 0.00a |
| 16:1/20:4     | 0.01 ± 0.00a | 0.01 ± 0.00b | 0.01 ± 0.00b |
| 16:1/21:2     | 0.01 ± 0.00b | 0.01 ± 0.00b | 0.01 ± 0.00b |
| 16:1/22:6     | 0.04 ± 0.00ab | 0.04 ± 0.00a | 0.04 ± 0.00b |
| 16:1/24:7     | 0.04 ± 0.01 | 0.04 ± 0.00 | 0.04 ± 0.00 |
| 17:0/20:4     | 0.02 ± 0.00b | 0.08 ± 0.01a | 0.02 ± 0.00b |
| 17:1/18:1     | 0.01 ± 0.00a | 0.01 ± 0.00a | 0.01 ± 0.00a |
| 17:1/20:4     | 0.05 ± 0.01a | 0.07 ± 0.01b | 0.09 ± 0.01b |
| 18:0/13:0     | 0.01 ± 0.00ab | 0.01 ± 0.00a | 0.01 ± 0.00ab |
| 18:0/16:0     | 1.23 ± 0.16 | 1.22 ± 0.15 | 1.12 ± 0.08 |
| 18:0/17:1     | 0.01 ± 0.00a | 0.01 ± 0.00b | 0.01 ± 0.00b |
| 18:0/18:1     | 1.90 ± 0.18 | 2.02 ± 0.24 | 2.00 ± 0.23 |
| 18:0/18:2     | 0.60 ± 0.05a | 0.71 ± 0.14b | 1.06 ± 0.12b |
| 18:0/19:1     | 0.01 ± 0.00a | 0.01 ± 0.00a | 0.01 ± 0.00b |
| 18:0/20:4     | 0.16 ± 0.01b | 0.17 ± 0.01b | 0.31 ± 0.02b |
| 18:0/22:1     | 0.01 ± 0.00ab | 0.01 ± 0.00a | 0.01 ± 0.00b |
| 18:0/22:6     | 7.78 ± 0.62 | 7.21 ± 0.71 | 7.73 ± 0.74 |
| 18:0/24:1     | 0.01 ± 0.00ab | 0.01 ± 0.00a | 0.01 ± 0.00ab |
| 18:0/24:2     | 0.01 ± 0.00a | 0.01 ± 0.00ab | 0.01 ± 0.00b |
| 18:1/18:2     | 0.06 ± 0.00b | 0.06 ± 0.01a | 0.06 ± 0.01b |
| 18:1/18:3     | 0.35 ± 0.04 | 0.38 ± 0.05 | 0.33 ± 0.04 |
| 18:1/20:4     | 3.90 ± 0.35 | 3.95 ± 0.52 | 4.02 ± 0.90 |
| 18:1/22:6     | 0.40 ± 0.07 | 0.47 ± 0.06 | 0.43 ± 0.05 |
| 18:2/19:1     | 0.00 ± 0.00b | 0.01 ± 0.00b | 0.01 ± 0.00b |
| 20:0/16:0     | 0.02 ± 0.00b | 0.02 ± 0.00b | 0.02 ± 0.00b |
| 20:0/18:2     | 0.25 ± 0.02a | 0.24 ± 0.03a | 0.31 ± 0.03b |
| 20:0/19:1     | 0.14 ± 0.02 | 0.16 ± 0.02 | 0.17 ± 0.02 |
| 20:0/20:4     | 1.52 ± 0.12b | 1.66 ± 0.33ab | 1.75 ± 0.09b |
| 20:1/14:0     | 6.44 ± 0.81 | 6.83 ± 0.59 | 6.43 ± 1.06 |
| 20:1/15:0     | 0.01 ± 0.00a | 0.01 ± 0.00ab | 0.01 ± 0.00a |
| 20:1/20:4     | 0.05 ± 0.01a | 0.02 ± 0.00b | 0.01 ± 0.00a |
| 20:1/22:6     | 0.02 ± 0.00b | 0.03 ± 0.00b | 0.03 ± 0.00b |
| 20:4/22:6     | 0.08 ± 0.01 | 0.09 ± 0.01 | 0.08 ± 0.02 |
| 22:4/22:6     | 0.01 ± 0.00b | 0.01 ± 0.00b | 0.01 ± 0.00b |
| 22:6/22:6     | 0.02 ± 0.00b | 0.02 ± 0.00b | 0.02 ± 0.00b |
Table 6  The lipid profile of PE in cerebral cortex after dietary intake of EPA enriched phospholipids.

| Fatty Acid (%) | HF Mean ± SD  | EPA-PC Mean ± SD  | EPA-PE Mean ± SD |
|----------------|---------------|--------------------|------------------|
| 16:0/18:1      | 1.42 ± 0.17   | 1.43 ± 0.13        | 1.62 ± 0.34      |
| 16:0/18:2      | 0.13 ± 0.01a  | 0.18 ± 0.03b       | 0.21 ± 0.03b     |
| 16:0/20:2      | 1.19 ± 0.12b  | 0.17 ± 0.02a       | 0.15 ± 0.02a     |
| 16:0/20:4      | 1.28 ± 0.11   | 1.41 ± 0.28        | 1.38 ± 0.12      |
| 16:0/22:1      | 1.51 ± 0.11   | 1.62 ± 0.16        | 1.49 ± 0.10      |
| 16:0/22:4      | 0.35 ± 0.02a  | 0.92 ± 0.05b       | 0.35 ± 0.02a     |
| 16:0/22:5      | 2.75 ± 0.36b  | 1.80 ± 0.25a       | 1.75 ± 0.24a     |
| 16:0/22:6      | 5.27 ± 0.38   | 4.91 ± 0.45        | 5.01 ± 0.38      |
| 16:1/20:4      | 0.09 ± 0.01   | 0.08 ± 0.01        | 0.09 ± 0.01      |
| 16:1/22:6      | 0.03 ± 0.00b  | 0.05 ± 0.00b       | 0.05 ± 0.01b     |
| 18:0/16:0      | 0.14 ± 0.01b  | 0.11 ± 0.01a       | 0.13 ± 0.03b     |
| 18:0/18:1      | 9.54 ± 1.34   | 11.14 ± 1.52       | 10.10 ± 1.47     |
| 18:0/18:2      | 2.34 ± 0.26b  | 1.46 ± 0.20a       | 1.56 ± 0.21a     |
| 18:0/20:1      | 1.51 ± 0.35   | 1.63 ± 0.17        | 1.49 ± 0.17      |
| 18:0/20:4      | 7.13 ± 0.61a  | 9.01 ± 0.88b       | 9.96 ± 1.16b     |
| 18:0/22:4      | 1.50 ± 0.27   | 1.62 ± 0.18        | 1.53 ± 0.17      |
| 18:0/22:6      | 17.68 ± 2.54  | 17.25 ± 1.37       | 17.31 ± 1.82     |
| 18:1/18:1      | 3.93 ± 0.49b  | 3.08 ± 0.44a       | 3.15 ± 0.27a     |
| 18:1/18:2      | 5.10 ± 0.48   | 4.72 ± 0.59        | 4.27 ± 0.62      |
| 18:1/20:3      | 7.86 ± 1.01a  | 9.69 ± 0.83b       | 10.02 ± 0.92b    |
| 18:1/20:4      | 1.67 ± 0.24   | 1.65 ± 0.21        | 1.67 ± 0.25      |
| 18:1/20:5      | 4.33 ± 0.43   | 3.89 ± 0.36        | 4.71 ± 0.38      |
| 18:1/22:4      | 0.11 ± 0.01   | 0.11 ± 0.01        | 0.12 ± 0.01      |
| 18:1/22:5      | 16.50 ± 2.50  | 15.81 ± 1.67       | 15.34 ± 1.98     |
| 18:1/22:6      | 5.73 ± 0.48   | 5.42 ± 0.65        | 5.61 ± 1.08      |
| 20:4/22:6      | 0.64 ± 0.05   | 0.55 ± 0.06        | 0.64 ± 0.07      |
| 22:4/22:6      | 0.09 ± 0.02b  | 0.07 ± 0.01a       | 0.11 ± 0.00b     |
| 22:6/22:6      | 0.20 ± 0.03   | 0.18 ± 0.02        | 0.19 ± 0.01      |

18:0/22:6 and 20:1/18:2 were the main fatty acids, as shown in Table 8. In line with previous studies, 18:0/22:6 exhibited the highest abundance in PS with a content of 27%[^20]. Although the total PS level had no significant change after eight weeks of dietary intervention (Fig. 1), EPA-PC and EPA-PE group could significantly change the fatty acid composition of PS. It has been reported that the relative content of PS (18:0/22:6) was significantly decreased by intervention with high fat diet[^20], while the relative content of PS (18:0/22:6) could be reversed by supplementation with EPA-enriched phospholipids.

Compared with glycerophospholipid, ether-linked phospholipid has more potent biological activity of brain function, but the possible underlying mechanism has not been elucidated. Among alkyl ether analog of phosphatidylethanolamine (ePE) molecular species, 16:0e/22:4 and 18:0e/20:4 were the main fatty acids, as shown in Table 9, and there was no obvious change. What’s more, the main fatty acids, such as 16:0e/16:0, 16:0e/16:1, 16:0e/18:1, 16:0e/22:1, and 18:0e/22:6, in alkyl ether analog of phosphatidylincholine (ePC) molecular species had no significant change as shown in Table 10.

Compared with the high-fat group, EPA-PC and EPA-PE diet reduced the relative content of DHA-containing pPC (Table 11). The potential mechanisms have not been illustrated yet and require further study.
3.3 PUFA content in glycerophospholipids

The main long chain fatty acids, including DHA, AA, docosapentaenoic acid (DPA) and EPA, in glycerophospholipids from cerebral cortex are important components for brain growth and function. DHA is mainly deposited in lipids from cerebral cortex are important components for brain growth and function. DHA is mainly deposited in lipids from cerebral cortex are important components for brain growth and function.

Table 7 The lipid profile of pPE in cerebral cortex after dietary intake of EPA enriched phospholipids.

| Fatty Acid (%) | HF Mean ± SD | EPA-PC Mean ± SD | EPA-PE Mean ± SD |
|----------------|-------------|------------------|------------------|
| 16:0p/16:0     | 0.05 ± 0.00 | 0.05 ± 0.00      | 0.05 ± 0.01      |
| 16:0p/18:1     | 1.98 ± 0.17 | 1.81 ± 0.18      | 2.09 ± 0.21      |
| 16:0p/20:4     | 0.91 ± 0.08 | 0.96 ± 0.08      | 0.93 ± 0.09      |
| 16:0p/22:4     | 0.87 ± 0.10 | 0.96 ± 0.09      | 0.84 ± 0.13      |
| 16:0p/22:5     | 0.56 ± 0.15b| 0.08 ± 0.01a     | 0.07 ± 0.02a     |
| 16:0p/22:6     | 9.11 ± 0.48b| 9.93 ± 0.63b     | 9.44 ± 0.42b     |
| 16:0p/24:7     | 0.16 ± 0.02a| 0.20 ± 0.02b     | 0.16 ± 0.04ab    |
| 18:0p/16:0     | 0.13 ± 0.01 | 0.11 ± 0.00      | 0.13 ± 0.03      |
| 18:0p/18:1     | 10.75 ± 1.30| 11.37 ± 0.87     | 11.25 ± 1.06     |
| 18:0p/20:1     | 0.240 ± 0.02a| 0.26 ± 0.03a     | 0.32 ± 0.03b     |
| 18:0p/20:3     | 9.09 ± 0.96 | 9.13 ± 0.78      | 8.83 ± 0.52      |
| 18:0p/20:4     | 1.52 ± 0.22 | 1.56 ± 0.20      | 1.52 ± 0.17      |
| 18:0p/22:4     | 2.30 ± 0.25 | 2.59 ± 0.35      | 2.46 ± 0.24      |
| 18:0p/22:5     | 0.16 ± 0.02b| 0.08 ± 0.02a     | 0.07 ± 0.01a     |
| 18:0p/22:6     | 14.96 ± 1.24| 14.11 ± 1.58     | 14.50 ± 1.72     |
| 18:1p/16:0     | 0.90 ± 0.10 | 0.82 ± 0.09      | 0.95 ± 0.10      |
| 18:1p/18:1     | 10.71 ± 1.18| 11.13 ± 1.24     | 11.62 ± 1.27     |
| 18:1p/20:1     | 0.43 ± 0.10b| 0.58 ± 0.05b     | 0.65 ± 0.04b     |
| 18:1p/20:4     | 1.64 ± 0.14b| 1.86 ± 0.11b     | 1.65 ± 0.06b     |
| 18:1p/20:5     | 3.94 ± 0.11 | 4.12 ± 0.46      | 4.08 ± 0.75      |
| 18:1p/22:1     | 0.06 ± 0.01b| 0.08 ± 0.01b     | 0.08 ± 0.01b     |
| 18:1p/22:4     | 0.94 ± 0.16b| 1.14 ± 0.14ab    | 1.18 ± 0.09b     |
| 18:1p/22:5     | 5.78 ± 0.70 | 5.20 ± 0.55      | 5.29 ± 0.37      |
| 18:1p/22:6     | 22.58 ± 3.83| 21.55 ± 1.99     | 21.56 ± 1.73     |
| 18:1p/24:1     | 0.17 ± 0.02c| 0.27 ± 0.03c     | 0.21 ± 0.02b     |
| 18:2p/20:4     | 0.05 ± 0.01b| 0.00 ± 0.00b     | 0.04 ± 0.00b     |
| 18:2p/22:6     | 0.03 ± 0.00b| 0.02 ± 0.00a     | 0.03 ± 0.00b     |

As an important source of n-3 PUFA, EPA has a lot of nutritional functions, such as reducing blood lipids, anti-atherosclerosis, anti-thrombosis and supplement essential nutrients for the brain. In cerebral cortex of AD model mice, most of EPA was combined with PE and pPE (Figs. 2B and C), which might be due to the responsibility of PE and pPE for the membrane fluidity. Although no significant changes were found in EPA-PC and EPA-PE groups compared with the HF group, notably, there was significant difference between EPA-PC and EPA-PE groups. Valentini et al. also reported that fish oil supplementation did not change the DHA and EPA levels in the brain.

AA exists in the phospholipids of cell membrane in the form of esterification, and can be released from phospholipids through a variety of enzyme pathways. AA and its metabolites have strong physiological activity in the nervous and immune system. The levels of AA in PC, PE, pPE and PS in Fig. 2 were significantly higher than those in other glycerophospholipids.
Table 8  The lipid profile of PS in cerebral cortex after dietary intake of EPA enriched phospholipids.

| Fatty Acid (%) | HF               | EPA-PC           | EPA-PE           |
|----------------|------------------|------------------|------------------|
|                | Mean ± SD        | Mean ± SD        | Mean ± SD        |
| 18:0/18:1      | 8.67 ± 0.86<sup>b</sup> | 6.61 ± 0.64<sup>a</sup> | 6.68 ± 1.03<sup>a</sup> |
| 18:0/20:1      | 2.44 ± 0.22<sup>b</sup> | 2.07 ± 0.22<sup>a</sup> | 1.92 ± 0.14<sup>a</sup> |
| 18:0/20:3      | 25.02 ± 1.49     | 26.57 ± 1.92     | 26.89 ± 3.32     |
| 18:0/20:4      | 1.59 ± 0.19<sup>a</sup> | 2.16 ± 0.20<sup>b</sup> | 7.52 ± 0.29<sup>a</sup> |
| 18:0/22:4      | 2.51 ± 0.30<sup>b</sup> | 2.21 ± 0.29<sup>ab</sup> | 1.96 ± 0.15<sup>a</sup> |
| 18:0/22:5      | 2.66 ± 0.35<sup>b</sup> | 1.46 ± 0.15<sup>b</sup> | 1.24 ± 0.11<sup>a</sup> |
| 18:0/22:6      | 26.99 ± 2.02<sup>b</sup> | 28.42 ± 1.92<sup>b</sup> | 29.11 ± 1.80<sup>a</sup> |
| 18:1/18:1      | 1.91 ± 0.27<sup>ab</sup> | 1.76 ± 0.19<sup>b</sup> | 1.11 ± 0.10<sup>a</sup> |
| 18:1/20:3      | 0.77 ± 0.06<sup>b</sup> | 0.46 ± 0.09<sup>a</sup> | 0.55 ± 0.07<sup>a</sup> |
| 18:1/22:6      | 0.77 ± 0.08<sup>b</sup> | 0.17 ± 0.03<sup>a</sup> | 0.56 ± 0.07<sup>a</sup> |
| 20:1/18:2      | 25.03 ± 1.67<sup>ab</sup> | 26.57 ± 5.19<sup>ab</sup> | 21.32 ± 2.18<sup>a</sup> |
| 22:4/22:6      | 1.65 ± 0.25<sup>b</sup> | 1.51 ± 0.20<sup>b</sup> | 1.15 ± 0.14<sup>a</sup> |

Table 9  The lipid profile of ePE in cerebral cortex after dietary intake of EPA enriched phospholipids.

| Fatty Acid (%) | HF               | EPA-PC           | EPA-PE           |
|----------------|------------------|------------------|------------------|
|                | Mean ± SD        | Mean ± SD        | Mean ± SD        |
| 16:0e/22:4     | 49.28 ± 4.749    | 48.90 ± 9.333    | 49.02 ± 5.057    |
| 18:0e/18:2     | 0.499 ± 0.051<sup>a</sup> | 0.736 ± 0.055<sup>b</sup> | 0.791 ± 0.061<sup>b</sup> |
| 18:0e/20:4     | 49.28 ± 3.210    | 48.90 ± 5.616    | 49.02 ± 9.480    |
| 18:0e/22:5     | 0.938 ± 0.110<sup>b</sup> | 1.451 ± 0.245<sup>b</sup> | 1.153 ± 0.189<sup>b</sup> |

Table 10  The lipid profile of ePC in cerebral cortex after dietary intake of EPA enriched phospholipids.

| Fatty Acid (%) | HF               | EPA-PC           | EPA-PE           |
|----------------|------------------|------------------|------------------|
|                | Mean ± SD        | Mean ± SD        | Mean ± SD        |
| 16:0e/16:0     | 8.73 ± 0.87      | 8.45 ± 0.86      | 8.07 ± 1.04      |
| 16:0e/16:1     | 6.64 ± 0.70      | 7.06 ± 1.06      | 6.63 ± 0.72      |
| 16:0e/18:1     | 53.61 ± 9.54     | 54.20 ± 3.72     | 53.66 ± 4.45     |
| 16:0e/18:2     | 2.59 ± 0.28      | 2.73 ± 0.27      | 2.81 ± 0.24      |
| 16:0e/19:1     | 0.44 ± 0.06<sup>b</sup> | 0.34 ± 0.04<sup>b</sup> | 0.27 ± 0.06<sup>a</sup> |
| 16:0e/22:1     | 12.88 ± 1.06     | 11.63 ± 1.41     | 12.84 ± 1.74     |
| 18:0e/16:0     | 3.54 ± 0.42      | 3.41 ± 0.50      | 3.14 ± 0.27      |
| 18:0e/18:1     | 1.07 ± 0.13<sup>b</sup> | 1.23 ± 0.17<sup>ab</sup> | 1.44 ± 0.12<sup>b</sup> |
| 18:0e/18:2     | 1.48 ± 0.11<sup>b</sup> | 1.91 ± 0.29<sup>b</sup> | 1.97 ± 0.21<sup>b</sup> |
| 18:0e/22:1     | 0.67 ± 0.06      | 0.65 ± 0.05      | 0.75 ± 0.15      |
| 18:0e/22:6     | 4.95 ± 0.50      | 5.04 ± 0.46      | 4.87 ± 1.19      |
| 18:0e/23:2     | 0.54 ± 0.15<sup>ab</sup> | 0.57 ± 0.05<sup>b</sup> | 0.50 ± 0.03<sup>a</sup> |
| 20:0e/19:1     | 2.85 ± 0.19      | 2.75 ± 0.27      | 3.05 ± 0.35      |
Table 11  The lipid profile of pPC in cerebral cortex after dietary intake of EPA enriched phospholipids.

| Fatty Acid  | HF          | EPA-PC        | EPA-PE        |
|------------|-------------|---------------|---------------|
| (%)        | Mean ± SD   | Mean ± SD     | Mean ± SD     |
| 14:0/20:4  | 11.31 ± 1.48 | 13.76 ± 2.26  | 13.90 ± 1.65  |
| 14:0/22:6  | 7.78 ± 0.98a | 6.02 ± 0.25a  | 5.84 ± 0.57a  |
| 16:0/20:4  | 9.33 ± 0.10  | 8.60 ± 0.84   | 9.65 ± 1.26   |
| 16:1/21:2  | 9.49 ± 1.07  | 9.79 ± 1.20   | 9.67 ± 0.65   |
| 18:2p/19:1 | 1.95 ± 0.16a | 4.90 ± 0.41b  | 4.83 ± 0.43b  |
| 20:0p/19:1 | 60.12 ± 9.08 | 56.92 ± 3.45  | 56.11 ± 9.19  |

Fig. 2  The relative abundance of polyunsaturated fatty acid (PUFA) including DHA, AA, DPA and EPA attached glycerophospholipid following different dietary interventions within eight weeks. A The relative percentage of each PUFA attached phosphatidylcholine (PC) occupied in total PC of cerebral cortex. B The relative percentage of each PUFA attached phosphatidylethanolamine (PE) occupied in total PE of cerebral cortex. C The relative percentage of each PUFA attached phosphatidylethanolamine plasmalogen (pPE) occupied in total pPE of cerebral cortex. D The relative percentage of each PUFA attached phosphatidylserine (PS) occupied in total PS of cerebral cortex. E The relative percentage of each PUFA attached ether-linked phosphatidylethanolamine (ePE) occupied in total ePE of cerebral cortex. ND meant that substance was not detected. Results were presented as mean and standard deviation (n = 8/group). Different letters among HF, EPA-PC and EPA-PE groups represented significant difference at p < 0.05 determined by ANOVA (Tukey’s test).
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The intake of EPA-PE could significantly increase the levels of AA-containing PE and PS in cerebral cortex, while EPA-PC supplementation could only enhance the level of DHA-containing PS. In addition, the administration with EPA-PC and EPA-PE could significantly reduce the levels of AA-containing ePE in cerebral cortex.

DPA is the most abundant n-3 long-chain polyunsaturated fatty acid in the brain after DHA, which has special benefits for the neuro-protection. DPA was mainly concentrated in PE, pPE and PS, as shown in Fig. 2. EPA-PE intake could significantly reduce the level of DPA-containing pPE and PS caused by high fat diet in cerebral cortex, while EPA-PC only reduced DPA-containing PS. It has been reported that DHA-PS supplementation could remarkably decrease the level of DPA-containing PS compared with the HF group.

In the present study, no significant changes were observed in the major brain glycerophospholipids (PC, PE, pPE, PS and ePE) of SAMP8 mice after administration of EPA-enriched phospholipids for 8 weeks. Despite the relatively low abundance of LPE, HF diet reduced LPE content in the cerebral cortex compared with low-fat fed rats. In the present study, dietary EPA-PC and EPA-PE significantly increased the LPE content in the cerebral cortex of high-fat fed mice. The administration with EPA-PC and EPA-PE could not significantly change the levels of DHA and EPA in cerebral cortex, which might be attributed to the low dose of EPA-enriched phospholipids (1%, w/w) in diet (Tables 2 and 3). In our previous study, 2% (w/w) EPA-enriched phospholipids intervention significantly reduced the pathological symptoms of male SAMP8 mice, and changed the whole brain DHA and EPA level. Compared with the low-fat group, high-fat diet could significantly decrease the levels of DHA-containing PS/pPE as well as AA-containing PS and ePE in the cerebral cortex of SAMP8 mice fed with high fat diet. Meanwhile, administration with EPA-PC and EPA-PE for 8 weeks significantly increased the amount of pPC and LPE in the cerebral cortex of SAMP8 mice fed with high fat diet. Our results demonstrated that dietary EPA-PE could significantly reduce the level of DPA-containing pPE and PS caused by high fat diet in cerebral cortex, while EPA-PC reduced DPA-containing PS. Previous studies have shown that high consumption of butter or saturated fatty acids could aggravate the severity of age-related senile amyloidosis, while dietary 20% (w/w) soybean oil rich in n-6 polyunsaturated fatty acids for 21 days enhanced learning ability. Moreover, compared with 5% (w/w) lard in diet, SAMP8 mice fed with 5% (w/w) soybean oil had better memory, greater longevity and higher concentrations of C16:0 and C18:2n-6 in the brain.

The digestion and absorption of triacylglycerol (TG) and phospholipid (PL) in small intestine are different. PL is mainly hydrolyzed by pancreatic Phospholipase A2, which releases fatty acids from sn-2 position to form 1-lyso-PC. After the released fatty acids and lyso-phospholipids enter enterocyte, a part of lyso-phospholipids is further hydrolyzed into fatty acids and glycerophospholipids by an enterocyte-derived lysophospholipase. Moreover, the metabolism of lyso-phospholipids includes the re-esterification to TG (2-monoacylglycerol pathway) as well as PL (a-glycerophosphate pathway) and the generation of chylomicrons for further transport. The digestion of choline phospholipids is of great significance for choline homeostasis, lipid signaling, postprandial lipid and energy metabolism, and interaction with intestinal bacteria. A previous study found that dietary PC was mainly hydrolyzed to 1-lyso-PC, which was effectively absorbed and reacylated to PC or degraded to glycerophosphocholine (GPC), glycerophosphate and free choline. Although dietary EPA-PC and EPA-PE can change the composition of fatty acids in the blood, the brain is a closed system isolated by the blood-brain barrier, and supplementation of exogenous lipids may not cause significant changes in the composition of brain lipids.

4 Conclusion

Supplementation with EPA-PC and EPA-PE could improve brain function, and lipid homeostasis was closely related to human health. In the present study, the lipid profile in cerebral cortex was measured by lipidomics after treatment with EPA-PC and EPA-PE for 8 weeks in high-fat diet induced SAMP8 mice with cognitive deficiency. Dietary supplementation with EPA-PE and EPA-PC for 8 weeks significantly increased the amount of pPC and LPE in the cerebral cortex of SAMP8 mice fed with high fat diet. Meanwhile, administration with EPA-PE and EPA-PC could significantly decrease the level of DPA-containing PS and increase the levels of AA-containing PE as well as PS in cerebral cortex. These results might provide a potential new treatment strategy and dietary intervention direction for patients with cognitive impairment.

Acknowledgements

This work was supported by National Natural Science Foundation of China (31901688).

References

1) Wang, C.C.; Guo, Y.; Zhou, M.M.; Xue, C.H.; Chang, Y.G.; Zhang, T.T.; Wang, Y.M. Comparative studies of DHA-ethyl ester with egg phosphatidylcholine on ameliorating memory and cognitive deficiency in SAMP8 mice. Food Funct. 10, 938-950 (2019).
2) Livingston, G.; Sommerlad, A.; Orgeta, V.; Costafreda, S.G.; Huntley, J.; Ames, D.; Ballard, C.; Banerjee, S.;
Burns, A.; Cohen-Mansfield, J.; Cooper, C.; Fox, N.; Gitlin, L.N.; Howard, R.; Kales, H.C.; Larson, E.B.; Ritchie, K.; Rockwood, K.; Sampson, E.L.; Samus, Q.; Schneider, L.S.; Selbaek, G.; Teri, L.; Mukadam, N. Dementia prevention, intervention, and care. *Lancet* **390**, 2673-2734 (2017).

3) van Gelder, B.M.; Tijhuis, M.; Kalmin, S.; Kromhout, D. Fish consumption, n-3 fatty acids, and subsequent 5-y cognitive decline in elderly men: the Zutphen Elderly Study. *Am. J. Clin. Nutr.* **85**, 1142-1147 (2007).

4) Mehta, J.; Chauhan, B.C.; Chauhan, N.B. Experimental induction of type 2 diabetes in aging-accelerated mice triggered Alzheimer-like pathology and memory deficits. *J. Alzheimers Dis.* **39**, 145-162 (2014).

5) Zhou, M.M.; Ding, L.; Wen, M.; Che, H.X.; Huang, J.Q.; Zhang, T.T.; Xue, C.H.; Mao, X.Z.; Wang, Y.M. Mechanisms of DHA-enriched phospholipids in improving cognitive deficits in aged SAMP8 mice with high-fat diet. *J. Nutr. Biochem.* **59**, 64-75 (2018).

6) Elhaik Goldman, S.; Goez, D.; Last, D.; Naor, S.; Piraz, Zaltsman, S.; Sharvit-Ginon, I.; Atrakchi-Baranes, D.; Shemesh, C.; Twitto-Greenberg, R.; Tsach, S.; Lotan, R.; Leikin-Frenkel, A.; Shish, A.; Mardor, Y.; Schneider Beeri, M.; Cooper, I. High-fat diet protects the blood-brain barrier in an Alzheimer’s disease mouse model. *Aging Cell* **17**, el2818 (2018).

7) Yang, X.; Sheng, W.; Sun, G.Y.; Lee, J.C. Effects of fatty acid unsaturation numbers on membrane fluidity and α-secretase-dependent amyloid precursor protein processing. *Neurochem. Int.* **58**, 321-329 (2010).

8) Ding, N.; Xue, Y.; Tang, X.; Sun, Z.M.; Yanagita, T.; Xue, C.H.; Wang, Y.M. Short-term effects of different fish oil formulations on tissue absorption of docosahexaenoic acid in mice fed high- and low-fat diets. *J. Oleo Sci.* **62**, 883-891 (2013).

9) Wen, M.; Ding, L.; Zhang, L.; Zhang, T.; Teruyoshi, Y.; Wang, Y.; Xue, C. Eicosapentaenoic acid-enriched phosphatidylcholine mitigated Aβ42-induced neurotoxicity via autophagy-inflammasome pathway. *J. Agric. Food Chem.* **67**, 13767-13774 (2019).

10) Che, H.; Zhou, M.; Zhang, T.; Zhang, L.; Ding, L.; Yanagita, T.; Xu, J.; Xue, C.; Wang, Y. Comparative study of the effects of phosphatidylcholine rich in DHA and EPA on Alzheimer’s disease and the possible mechanisms in CHO-APP/PS1 cells and SAMP8 mice. *Food Funct.* **9**, 643-654 (2018).

11) Che, H.; Zhang, L.; Ding, L.; Xie, W.; Jiang, X.; Xue, C.; Zhang, T.; Wang, Y. EPA-enriched ethanolamine plasmalogen and EPA-enriched phosphatidylethanolamine enhance BDNF/TrkB/CREB signaling and inhibit neuronal apoptosis in vitro and in vivo. *Food Funct.* **11**, 1729-1739 (2020).

12) Che, H.; Li, Q.; Zhang, T.; Ding, L.; Zhang, L.; Shi, H.; Yanagita, T.; Xue, C.; Chang, Y.; Wang, Y. A comparative study of EPA-enriched ethanolamine plasmalogen and EPA-enriched phosphatidylethanolamine on Aβ (42) induced cognitive deficiency in a rat model of Alzheimer’s disease. *Food Funct.* **9**, 3008-3017 (2018).

13) Liu, B.; Liu, J.; Shi, J.S. SAMP8 mice as a model of age-related cognition decline with underlying mechanisms in Alzheimer’s disease. *J. Alzheimers Dis.* **75**, 385-395 (2020).

14) Folch, J.; Ascoli, I.; Lees, M.; Meath, J.A.; Le, B.N. Preparation of lipide extracts from brain tissue. *J. Biol. Chem.* **191**, 833-841 (1951).

15) Carl, J.; Cynthia, T.; Aili, P.; Line, B.; Vincent, E.; Pierre, J.; Frédéric, C. High-fat diet aggravates amyloid-beta and tau pathologies in the 3xTg-AD mouse. *Neurobiol. Aging* **31**, 1516-1531 (2010).

16) Kao, Y.C.; Ho, P.C.; Tu, Y.K.; Jou, I.M.; Tsai, K.J. Lipothypoxia and Alzheimer’s disease. *Int. J. Mol. Sci.* **21**, 1505 (2020).

17) Cha, J.Y.; Cho, Y.S. Effects of alpha-linolenic, eicosapentaenoic and docosahexaenoic acids on the content and fatty acid composition of brain phospholipid in rats. *Agricultural Chemistry and Biotechnology* **42**, 75-80 (1999).

18) Grinn, M.O.W.; Michaelson, D.M.; Hartmann, T. Omega-3 fatty acids, lipids, and apoE lipification in Alzheimer’s disease: a rationale for multi-nutrient dementia prevention. *J. Lipid Res.* **58**, 2083-2101 (2017).

19) Luke, W.; Arundhuti, S.; James, H.; Petroula, P.; Diego, G.; Rufina, L.; Norman, S.; Madhav, T.; Iwona, K.; Patricia, M.; Hilika, S.; Magda, T.; Bruno, V.; Simon, L.; Cristina, L.; AddNeuroMed, Consortium. Evidence of altered phosphatidylcholine metabolism in Alzheimer’s disease. *Neurobiol. Aging* **35**, 271-278 (2014).

20) Zhao, Y.C.; Zhou, M.M.; Zhang, L.Y.; Cong, P.X.; Xu, J.; Xue, C.H.; Yanagita, T.; Chi, N.; Zhang, T.T.; Liu, F.H.; Wang, Y.M. Recovery of brain DHA-containing phosphatidylserine and ethanolamine plasmalogen after dietary DHA-enriched phosphatidylcholine and phosphatidylserine in SAMP8 mice fed with high-fat diet. *Lipids Health Dis.* **19**, 104 (2020).

21) Paul, S.; Lancaster, G.I.; Meikle, P.J. Plasmalogens: A potential therapeutic target for neurodegenerative and cardiometabolic disease. *Prog. Lipid Res.* **74**, 186-195 (2019).

22) Kim, H.Y.; Akbar, M.; Kim, Y.S. Phosphatidylserine-dependent neuroprotective signaling promoted by docosahexaenoic acid. *Prostaglandins Leukot. Essent. Fatty Acids* **82**(4-6), 165-172 (2010).

23) Zhang, T.T.; Xu, J.; Wang, Y.M.; Xue, C.H. Health benefits of dietary marine DHA/EPA-enriched glycerophospholipids. *Prog. Lipid Res.* **75**, 100997 (2019).

24) Brenna, J.T.; Carlson, S.E. Docosahexaenoic acid and human brain development: evidence that a dietary supply is needed for optimal development. *J. Hum.*
Evol. 77, 99-106 (2014).

25) Valenti, K.J.; Pickens, C.A.; Wiesinger, J.A.; Fenton, J.I. The effect of fish oil supplementation on brain DHA and EPA content and fatty acid profile in mice. Int. J. Food Sci. Nutr. 69, 705-717 (2018).

26) Drouin, G.; Rioux, V.; Legrand, P. The n-3 docosapentaenoic acid (DPA): A new player in the n-3 long chain polyunsaturated fatty acid family. Biochimie 159, 36-48 (2019).

27) Zhou, M.M.; Che, H.X.; Huang, J.Q.; Zhang, T.T.; Xu, J.; Xue, C.H.; Wang, Y.M. Comparative study of different polar groups of EPA-enriched phospholipids on ameliorating memory loss and cognitive deficiency in aged SAMP8 mice. Mol. Nutr. Food Res. 62, e1700637 (2018).

28) Umezawa, M.; Tatematsu, K.; Korenaga, T.; Fu, X.; Matsushita, T.; Okuyama, H.; Hosokawa, M.; Takeda, T.; Higuchi, K. Dietary fat modulation of apoA-II metabolism and prevention of senile amyloidosis in the senescence-accelerated mouse. J. Lipid Res. 44, 762-769 (2003).

29) Coscina, D.V.; Yehuda, S.; Dixon, L.M.; Kish, S.J.; Leprohon-Greenwood, C.E. Learning is improved by a soybean oil diet in rats. Life Sci. 38, 1789-1794 (1986).

30) Ueda, Y.; Wang, M.F.; Irei, A.V.; Sarukura, N.; Sakai, T.; Hsu, T.F. Effect of dietary lipids on longevity and memory in the SAMP8 mice. J. Nutr. Sci. Vitaminol. (Tokyo) 57, 36-41 (2011).

31) Galli, C.; Sirtori, C.R.; Mosconi, C.; Medini, L.; Gianfranceschi, G.; Vaccarino, V.; Scolastico, C. Prolonged retention of doubly labeled phosphatidylcholine in human plasma and erythrocytes after oral administration. Lipids 27, 1005-1012 (1992).

32) Schuchardt, J.P.; Schneider, I.; Meyer, H.; Neubronner, J.; von Schacky, C.; Hahn, A. Incorporation of EPA and DHA into plasma phospholipids in response to different omega-3 fatty acid formulations—a comparative bioavailability study of fish oil vs. krill oil. Lipids Health Dis. 10, 145 (2011).

33) Tsushima, T.; Ohkubo, T.; Onoyama, K.; Linder, M.; Takahashi, K. Lysophosphatidylserine form DHA may be the most effective as substrate for brain DHA accretion. Biocatal. Agric. Biotechnol. 3, 303-309 (2014).

34) Nilsson, A.; Duan, R.D. Pancreatic and mucosal enzymes in choline phospholipid digestion. Am. J. Physiol. Gastrointest. Liver Physiol. 316, G425-G445 (2019).

35) Nilsson, A. Intestinal absorption of lecithin and lysophosphatidylethanolamine ameliorate lipid accumulation and insulin resistance via activation of PPARα/γ in mice. Food Funct. 11, 8248-8258 (2020).