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Effects of Different Proportions of n-3 and n-6 Polyunsaturated Fatty Acids on Cognitive Function in Mice

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ABSTRACT: The n-3 and n-6 polyunsaturated fatty acids play an important role in nerve development and cognitive function. However, the effects of docosahexaenoic acid (DHA)/arachidonic acid (ARA) and linolenic acid (ALA)/linoleic acid (LOA) supplementation on cognitive function in mice have not been fully clarified. The purpose of this study is to explore the mechanism of different proportions of n-3 and n-6 fatty acids on cognitive function and hippocampus-related protein expression in mice, and to provide theoretical support for improving brain development and cognitive function in mice. After mice were given different proportions of ALA/LOA and DHA/ARA, the learning and memory abilities of mice were evaluated by Y-maze, open field and Morris water maze, and brain samples were collected to detect the morphology of hippocampal neurons and the expression of synaptic plasticity-related proteins. The results of Golgi Staining showed that the density of dendritic spines in ALA/LOA=1:1 and DHA/ARA=1:1 groups was significantly higher than that in the control group, which affected the synaptic structure of mouse neurons. DHA/ARA=1:4 and 1:1 groups up-regulated the expression of BDNF, TrkB, CREB and synaptic plasticity related proteins (PSD95, Syn, Snap) in hippocampus, increased the number of normal neurons in CA1 and CA3 areas of hippocampus, and significantly improved the cognition of mice. This study confirmed that DHA/ARA ratio of 1:4 and 1:1 can activate BDNF-TrkB-CREB signal axis, significantly enhance the learning ability of mice, and improve spatial memory (manifested by the increase of crossing times of Morris water maze platform) and working memory (manifested by the increase of the autonomous alternation rate of Y-maze), while the ratio of 4:1 will lead to metabolic disorder. It provides an important theoretical basis for optimizing the ratio of dietary fatty acids to promote cognitive function.

Keywords: Cognitive function; n-3 PUFA; n-6 PUFA; Hippocampus

1. Introduction

Early life represented a critical period for brain development and immune system maturation, during which long-chain polyunsaturated fatty acids (LCPUFAs) played a pivotal role. Specifically, α -linolenic acid (ALA) and docosahexaenoic acid (DHA) from the n-3 PUFA, as well as linoleic acid (LOA) and arachidonic acid (ARA) from the n-6 PUFA, exhibited profound impacts on cognitive function and long-term health through their intake ratios and metabolic balance⁰⁰. ALA, a fundamental nutrient for brain neurons, promoted neural network development, thereby enhancing intelligence and memory⁰. Adequate LOA intake reduced the

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incidence of neuroinflammation⁰. ALA and LOA could be converted to DHA and ARA via biochemical pathways involving desaturases and elongases in the liver. However, endogenous conversion efficiency was limited, making direct DHA/ARA intake more effective for meeting the high demands of brain development.

DHA is one of the most abundant fatty acids in brain gray matter, particularly accumulated in the central nervous system⁰⁰. The rapid accumulation of brain DHA occurs primarily from the third trimester of human pregnancy to two years of age, or within the first three postnatal weeks in rodents⁰⁰. Studies have shown that DHA promotes synaptic plasticity and enhances interneuronal signal transduction⁰⁰, thereby exerting positive effects on cognitive function, memory, and learning ability in early life⁰. ARA, as a key precursor of intra-brain signaling molecules⁰, participates in regulating inflammatory responses and neuroprotection, and is closely associated with infant brain development. It also influences neuronal excitability and synaptic transmission by modulating neuronal membrane potential and ion channels⁰⁰.

Recent studies further confirmed that sufficient intake of n-3 PUFA (ALA, DHA) and n-6 PUFA (LOA, ARA) during brain development significantly improves long-term neurodevelopmental outcomes in offspring, particularly in language ability, visual perception, and attention⁰⁰. Deficiency in either fatty acid may lead to irreversible impairment of intellectual and cognitive functions, and even increase the risk of neurodegenerative diseases in adulthood⁰. Thus, rational supplementation of n-3 and n-6 fatty acids in early life is crucial for maintaining brain health and cognitive function. However, previous research predominantly focused on the mechanisms of individual fatty acids or their absolute intake, while overlooking the potential synergistic or antagonistic effects of the n-3/n-6 ratio in neurodevelopment. For instance, an excessively high n-6/n-3 ratio may increase pro-inflammatory mediators, thereby exerting adverse effects on neural development⁰⁰. Meanwhile, a consensus on the optimal range of the n-3/n-6 ratio has not yet been reached. A study in infants showed that a DHA:ARA ratio of 1:1 significantly improved cognitive function and visual development⁰, whereas another study in older adults found that DHA:ARA ratio of 2:1 notably enhanced cognitive functions, particularly memory and attention⁰. Additionally, animal experiments have demonstrated that a DHA:ARA ratio of 2:1 improved learning-memory ability and spatial cognition in mice⁰. However, this animal study lasted only two weeks, which hinders the comprehensive evaluation of a potential effects of early fatty acid intake.

In summary, although extensive research has explored the roles of n-3 and n-6, the mechanisms underlying how their different ratios affect cognitive function remain unclear. Specifically, how the n-3/n-6 ratio influences learning-memory ability, spatial cognition, and expression of related neural proteins in animal models requires further investigation. This study aims to observe changes in cognitive function and explore the underlying molecular mechanisms in mice fed with varying n-3/n-6 fatty acid ratios, thereby providing new experimental evidence for the effects of n-3 and n-6 fatty acids on cognitive function.

2. Materials and methods

2.1 Materials

Olive oil and Soybean oil were procured from Yihai Kerry Food Marketing Co., Ltd. The contents of linoleic acid and linolenic acid in soybean oil were 48.32% and 6.75%, respectively. Linseed oil was obtained from Ningxia Junxingfang Co., Ltd., with linoleic acid and linolenic acid contents of 16.52% and 60.42%, respectively. DHA was supplied by Shaanxi Haoyun Pharmaceutical Technology Co., Ltd. (China) at a purity of 91%, and ARA was provided by Shaanxi Haise Biotechnology Co., Ltd. at a purity of 60%. These oils were esterified with methanol sulfate and toluene at 70 °C for 2 h, dissolved again after nitrogen blowing, and quantified by gas chromatography. The interventions were administered in the form of ethyl esters. The fatty acid composition of each intervention is shown in Supplementary Table S1.

To ensure the stability of DHA and ARA, α -tocopherol (vitamin E) was added as an antioxidant into the lipids to prevent oxidation throughout the experimental period. Furthermore, stringent handling protocols were implemented: individual aliquots of the oil preparations were retrieved from storage immediately prior to each gavage procedure to minimize exposure to room temperature and air. All bulk and aliquoted oil stocks were consistently stored in light-protected containers at -20°C to maintain chemical stability until use.

2.2 Animals Experiments

C57BL/6J mice of 3 weeks old were produced by mating in the laboratory, and were kept in the experimental animal room of Qingdao University in strict accordance with the standard: the animals were exposed to day and night cycles for 12 h and temperature of 24±2 °C.

70 three-week-old C57BL/6J male mice were randomly divided into 7 groups (10 mice per group), including 1 control group and 6 intervention groups. The control group was intragastrically administered olive oil (0.1 mL/20 g/d). The other six groups, namely, ALA:LOA=1:4, ALA:LOA=1:1, ALA:LOA=4:1, DHA:ARA=1:4, DHA:ARA=1:1 and DHA:ARA=4:1, were given different kinds of lipids: ALA:LOA=1:4 (80 mg/kg/d ALA, 320 mg/kg/d LOA), ALA:LOA=1:1 (200 mg/kg/d ALA, 200 mg/kg/d LOA), ALA:LOA=4:1 (320 mg/kg/d ALA, 80 mg/kg/d LOA), DHA:ARA=1:4 (80 mg/kg/d DHA, 320 mg/kg/d ARA), DHA:ARA=1:1 (200 mg/kg/d DHA, 200 mg/kg/d ARA), DHA:ARA=4:1 (320 mg/kg/d DHA, 80 mg/kg/d ARA). Olive oil was used to make up for the dose less than 0.1 mL, so as to ensure the same energy intake.

2.3 Behavioral tests

Behavioral tests, including the Y-maze (Supplementary Figure S1), Open field test (Supplementary Figure S2), and Morris water maze (Supplementary Figure S3). Animal behavior during these tests was recorded and analyzed using video-tracking software (Panlab). This software automatically tracked the movement and interactions of the animals with objects and the environment, measuring parameters such as distance moved, time spent exploring, and object touches. The data processing and analysis software is SMARTv3.0.

Behavioral experiments were carried out in the eighth week and completed within one week, with execution following a 12-h fast. The specific order was as follows: open field test, Y-maze, and Morris water maze. The same cohort of animals sequentially underwent all three behavioral assessments.

For data such as escape latency in the Morris water maze, repeated-measures ANOVA was used for assessment, with training days treated as a within-subjects factor. For single-measure comparisons across multiple groups, one-way ANOVA followed by Tukey post-hoc comparison was used. All statistical analyses were performed using SPSS 27.0 software.

2.4 Measurement of serum and hepatic biochemical parameters

Total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL-C) and low density lipoprotein (LDL-C) in serum and liver of mice were determined by enzyme-linked immunosorbent assay (ELISA) kits (Catalog Nos.: A111-1-1, A110-1-1, A112-1-1, A113-1-1, respectively) purchased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.5 Quantification of fatty acid levels in the liver and cerebral cortex

For serum and liver biochemical index detection: weighed tissues were homogenized in 1 mL chloroform:methanol (1:1, v/v) twice at 60 Hz for 30 s using a KZ-II grinder (Servicebio, Wuhan), then extracted with 4 mL of the same solvent for 24 h. The mixture was transferred to a 100 mL separatory funnel, mixed with 4 mL 0.9% NaCl, shaken, and left to stand for 2 h. The lower chloroform phase was collected, rotary-evaporated at 30°C, and reacted with 3 mL 5% sulfuric acid-methanol (1:20, v/v) and 1 mL toluene in a water bath for 2 h (vortexed every 30 min). After adding 2 mL n-hexane and 1 mL saline, the mixture was vortexed, centrifuged at 2000 rpm for 10 min, and the upper phase was purified through a column with 4 mL 5% ether/petroleum ether. The eluate was nitrogen-dried, redissolved in n-hexane, and analyzed by GC with 1 µL injection. GC parameters: H₂ carrier gas (75 kPa), air (50 kPa), temperature program (140°C×2 min, 3°C/min to 200°C×2 min, 1°C/min to 204°C×8 min), injector/detector at 270°C. Fatty acids were identified by comparing with 37-standard retention times and quantified via peak area normalization, expressed as mass % of total fatty acids.

2.6 Histological Analysis of Brain Tissue Sections

(1) HE Staining of Brain Tissue: Briefly, tissues were cut, washed by saline and then fixed in 4% paraformaldehyde for 24 h. Following dehydration, paraffin embedding, and sectioning, the tissue sections were subjected to H&E staining.

(2) Golgi Staining of Brain Tissue: The brain tissues were immersed in the Golgi-Cox solution (Servicebio Technology, Wuhan, China) for 14 days. Following staining completion, longitudinal sections with a thickness of 60 µm were prepared using a freezing microtome. Microscopic examination was conducted with a Nikon Eclipse E100 microscope (Japan), while image capture and subsequent analysis were carried out using a Nikon DS-U3 imaging system (Japan), and to obtain the panoramic images of the brain tissue, panoramic multi-layer scanning was performed.

2.7 Lipidomics analysis

The 10 mg brain tissue was add two 2 mm zirconium beads and 100 uL ultrapure water and then let the homogenizer homogenize it for three times (60 Hz, 60 s each time, 10 s between homogenates). After taking

it out of the homogenizer, add the extraction solvent (dichloromethane: methanol=1:1) into the homogenizer tube, and mix the supernatant: extraction solvent at a ratio of 1:10 by vortex for 30 s. Take out the sample and centrifuge it fully (4 °C, 13000 rpm, 15 min), and take the supernatant and store it at -20 °C for testing. Centrifuge the supernatant once again (4 °C, 13000 rpm, 15 min) before using the machine, and then put it into a liquid vial with a glass cannula for testing.

Lipid analysis was performed using a reversed-phase liquid chromatography-tandem mass spectrometry (RPLC-MS/MS) system with electrospray ionization (ESI) in positive/negative switching mode (Agilent Technologies Inc., USA). The analytical conditions were set as follows: an ACQUITY UPLC BEH C18 column (2.1 × 100 mm, 1.7 µm; Waters, Milford, MA, USA) was employed with a constant flow rate of 0.4 mL/min and a column temperature maintained at 50 °C. The mobile phase consisted of solvent A (acetonitrile/water, 60:40, v/v) containing 5 mmol/L ammonium formate and 0.2% ammonia, and solvent B (isopropanol/acetonitrile, 90:10, v/v). The gradient elution program was optimized as follows: initial 5% B held for 1 min, increased linearly to 95% B over 15 min and maintained for 2 min, then reduced to 5% B within 17.1 min, followed by a 5-min equilibration period. Mobile Phase C was water containing 5 mmol L⁻¹ ammonium formate, and Phase D was acetonitrile with 5 mmol L⁻¹ ammonium acetate. Similarly, for solvent D (isopropanol/acetonitrile, 90:10, v/v), the gradient started at 2% D, held for 1 min, increased to 99% D over 17 min, held for 2 min, and returned to 5% D at 19.1 min, with a 5-min re-equilibration step.

The Q-TOF mass spectrometer was operated in both positive and negative electrospray ionization (ESI) modes with the following parameters: capillary voltage ±3.5 kV, nozzle voltage 500 V, drying gas temperature 300 °C, sheath gas temperature 350 °C, and nebulizer pressure 35 psi. Mass calibration was performed using standard reference masses prior to analysis. Data acquisition included both MS¹ (m/z 50-1000) and data-dependent MS² scans with collision energies optimized between 10-40 eV.

Quality control measures included: System suitability testing using reference standards; Injection of pooled quality control samples every 10 injections to monitor instrument stability; Continuous mass accuracy verification using reference ions during analysis; Regular column performance evaluation using test mixtures.

Significantly altered lipids were selected based on a *Q*-value < 0.05 and a log₂fold change (log₂FC) > 1, using linear modeling implemented in the R package MaAsLin.

2.8 Quantitative PCR

The total RNA of mouse brain tissue was extracted by TRIzol method, and the specific steps were as follows: the tissue lysate was transferred into an EP tube for 15min. Detecting the concentration (A₂₆₀/A₂₈₀=1.8-2.1), and synthesizing cDNA; by reverse transcriptase; Then, according to the designed primers, the amplification efficiency (90-110%) and the specificity were verified by the standard curve. Then, the reaction system (including SYBRGreenMasterMix, primers and template cDNA) was configured, and the two-step amplification program was set up. Finally, the relative/absolute quantitative analysis was carried out by $\Delta\Delta C_t$ method or standard curve, and GAPDH was used as the reference gene correction data $2^{\Delta\Delta C_t}=2^{\Delta C_t}$

target gene- $\Delta\text{Ct}(\text{GAPDH})$). The primer sequences for the qPCR experiments are provided in the Supplementary Table S2.

2.9 Western blotting analysis

Firstly, cells or tissues were lysed with RIPA lysate (Beyotime, Shanghai) containing protease/phosphatase inhibitor, and the supernatant was centrifuged to determine the protein concentration by bicinchoninic acid (BCA) method and adjust it to the same amount. The sample was mixed with loading buffer, boiled and denatured, and the protein was transferred onto PVDF membranes after SDS-PAGE and incubated on the corresponding primary and secondary antibodies, respectively. The images were finally acquired in a gel imaging system and quantified by ImageLab software. Specific primary antibodies used were as follows: BDNF (1:1000; DF6387; Affinity Biosciences), NGF (1:1000; DF6061; Affinity Biosciences), TrkB (1:1000; AF6461; Affinity Biosciences), and β -actin (1:1000; AF7018; Affinity Biosciences).

2.10 Data analysis

SPSS27.0 and Origin2021 were used for statistical analysis. The weekly weight changes, biochemical indexes, protein determination and escape latency changes of mice were analyzed by multivariate analysis of variance. $P < 0.05$ thought the difference was statistically significant.

3. Results

3.1 Effects of Supplementation with Different Ratios of n-3 and n-6 PUFA on the body weight and food intake of Mice

The results showed that compared with the control group, there were no significant differences in body weight and food intake among ALA:LOA=1:4, ALA:LOA=1:1, ALA:LOA=1:4, DHA:ARA=1:4, DHA:ARA=1:1 and DHA:ARA=4:1 (Figure 1).

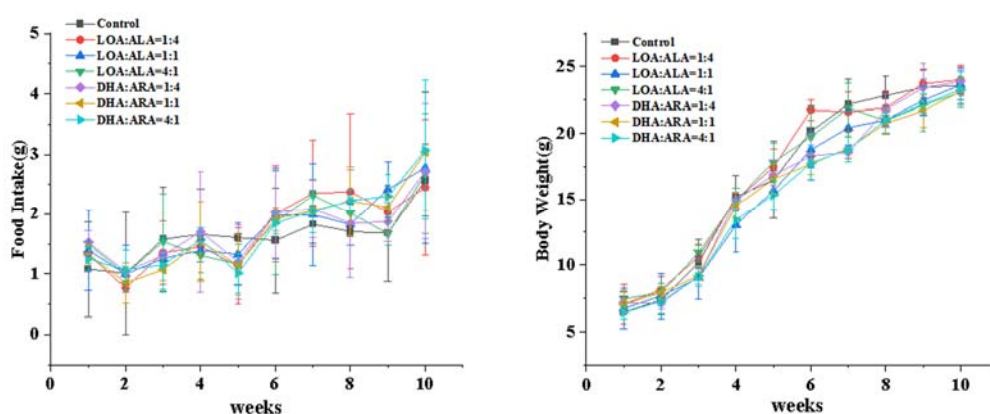


Fig 1. Effects of different proportions of n-3 and n-6 PUFA supplementation on food intake and body weight of mice. A: changes in food intake; B: weight changes.

3.2 Effects of Supplementation with Different Ratios of n-3 and n-6 PUFA on the serum biochemical indexes of Mice

After 8 weeks of intervention, the TC levels in DHA:ARA=1:4 group and DHA:ARA=1:1 group were significantly lower than those in the control group (Figure 2A), but there was no statistical difference in TG

levels among the groups (Figure 2B). The HDL-C of DHA:ARA=1:1 group and DHA:ARA=1:4 group was significantly lower than that of the control group, and there was no significant difference between the other intervention groups and the control group (Figure 2C). The level of LDL-C in DHA:ARA=1:1 group was significantly lower than that in the control group, and there was no significant difference between the other groups and the control group (Figure 2D).

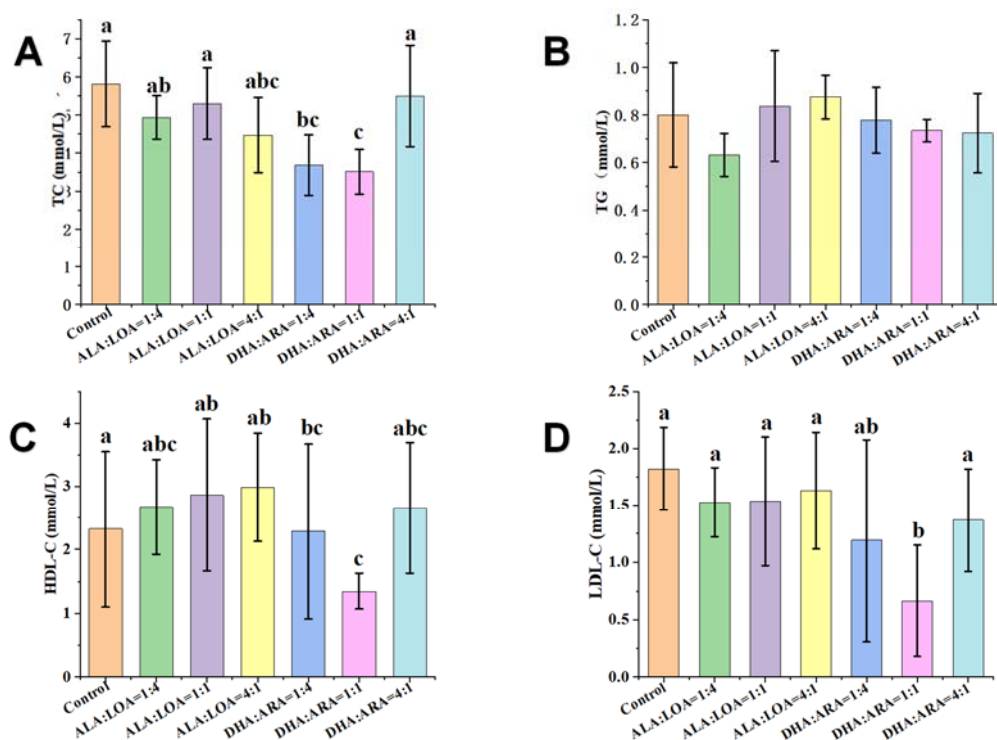


Fig 2. Effects of different proportions of n-3 and n-6 PUFA supplementation on serum biochemical indexes. A: total cholesterol (TC); B: triglyceride (TG); C: high-density lipoprotein (HDL-C); D: low-density lipoprotein (LDL-C), and the differences indicated by different letters were statistically significant ($P < 0.05$).

3.3 Effects of different proportions of n-3 and n-6 PUFA supplementation on cognitive function in mice

After 8 weeks of intervention, the open field test, Y-maze test and Morris water maze test were carried out on each group of mice. In the open field test, the total distance of mice moving in the open field, the distance and time of mice moving in the central area were measured. Compared with the control group, the total distance of ALA:LOA=1:1 and ALA:LOA=4:1 in the open field increased, but there was no difference in the other intervention groups. Compared with the control group, ALA:LOA=4:1, DHA:ARA=1:4 and DHA:ARA=1:1 all move longer in the central area. In addition, compared with the control group, the DHA:ARA=1:1 group stayed in the central area longer (Figure 3).

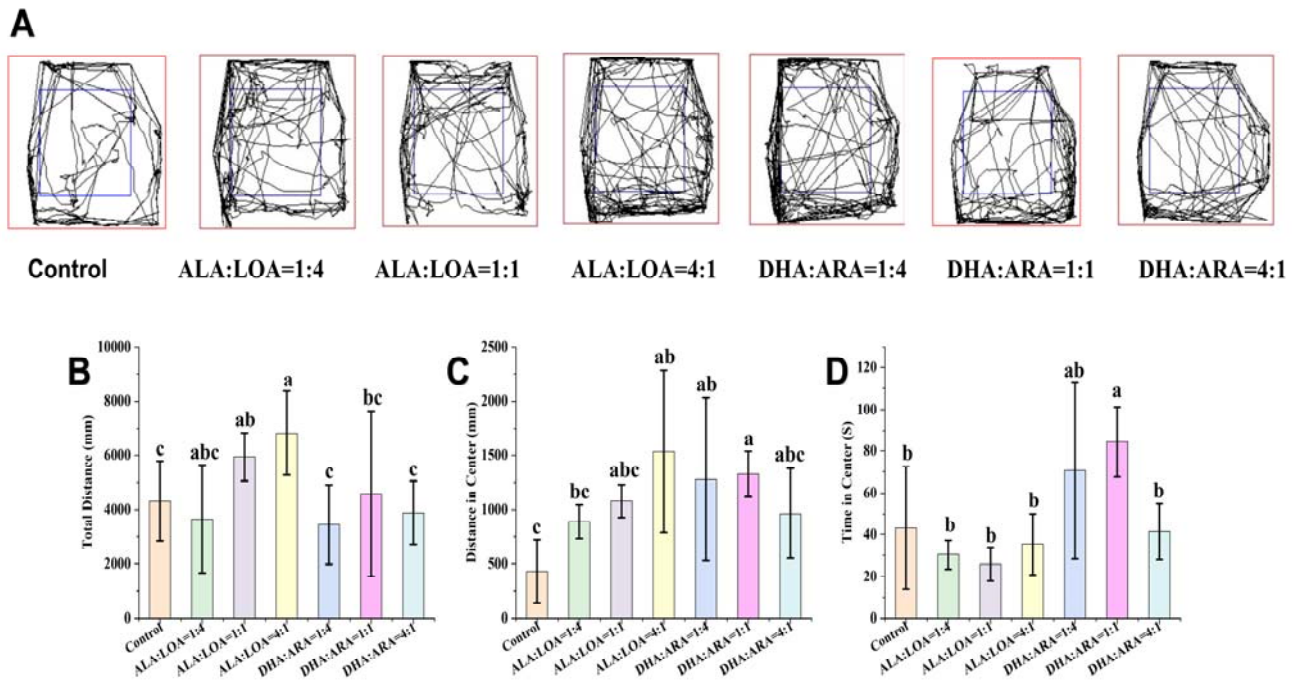


Fig. 3 Effects of different proportions of n-3 and n-6 PUFA supplementation on behavior of mice. In the open field test, a: open field trajectory diagram; B: total moving distance; C: the moving distance of the central area; D: residence time in the central area. Note: Data are expressed as mean standard deviation ($n=6/\text{group}$); Different letters indicate differences with statistical significance ($P < 0.05$).

In the Y-maze test, the number of mice entering the total arm and the voluntary alternation rate were measured. It was found that there was no difference in the number of mice entering each arm, but the voluntary alternation rate of mice in DHA:ARA=1:1 group was significantly higher than that in the control group (Figure

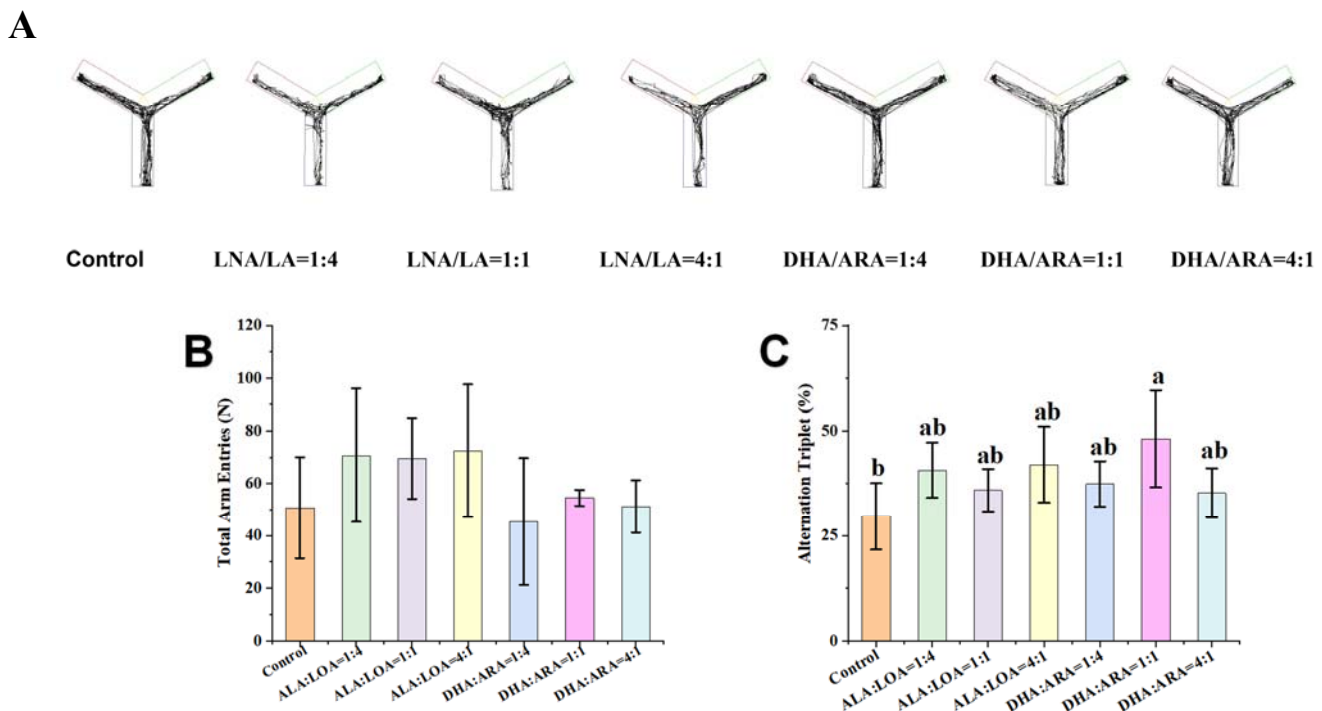


Fig. 4 Effects of different proportions of n-3 and n-6 PUFA supplementation on behavior of mice. In y maze experiment, A: Y maze trajectory diagram; B: total arm feeding times C: autonomous alternation rate; Note: Data are expressed as mean standard deviation ($n=6/\text{group}$); Different letters indicate differences with statistical significance ($P < 0.05$).

In the Morris water maze test, the hidden platform period of the Morris water maze showed that there was no significant difference in escape latency or distance to the target among the mice in each group, indicating comparable hidden-platform performance across groups (Figure 5A,B). Figure 5C showed the representative trajectory diagram in the Morris water maze test. The test of Morris water maze space exploration showed that there was no significant difference between the intervention group and the control group in the residence time of the target quadrant (Figure 5D), but compared with the control group, the number of platform crossing in DHA:ARA=1:4 group was significantly increased (Figure 5F).

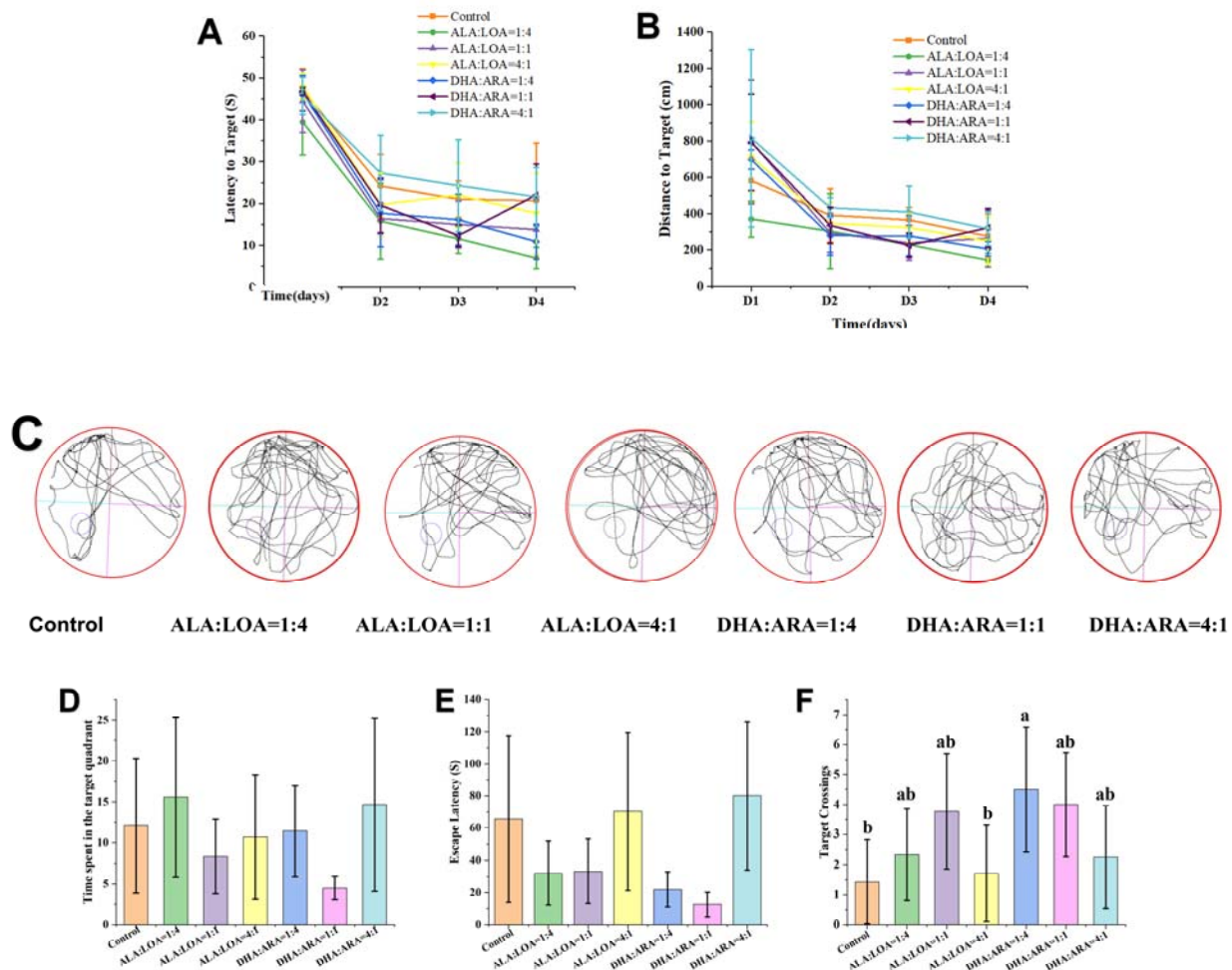


Fig. 5 Effects of different proportions of n-3 and n-6 PUFA supplementation on behavior of mice. A: escape the change of incubation period; B: the distance from the training period to the movement of the target quadrant; C: water maze trajectory diagram; D: target quadrant residence time; E: escape the incubation period; F: platform crossing times; Note: Data are expressed as mean standard deviation (n=6/group); Different letters indicate differences with statistical significance ($P < 0.05$).

3.4 Effects of different proportions of n-3 and n-6 PUFA supplementation on liver biochemical indexes and fatty acid composition in mice

TC levels in DHA:ARA=1:4 and DHA:ARA=1:1 groups were significantly lower than those in the control group, while TG levels in DHA:ARA=4:1 groups were significantly higher than those in the control group. There was no significant difference in HDL-C and LDL-C levels between each group and the control group (Figure 6).

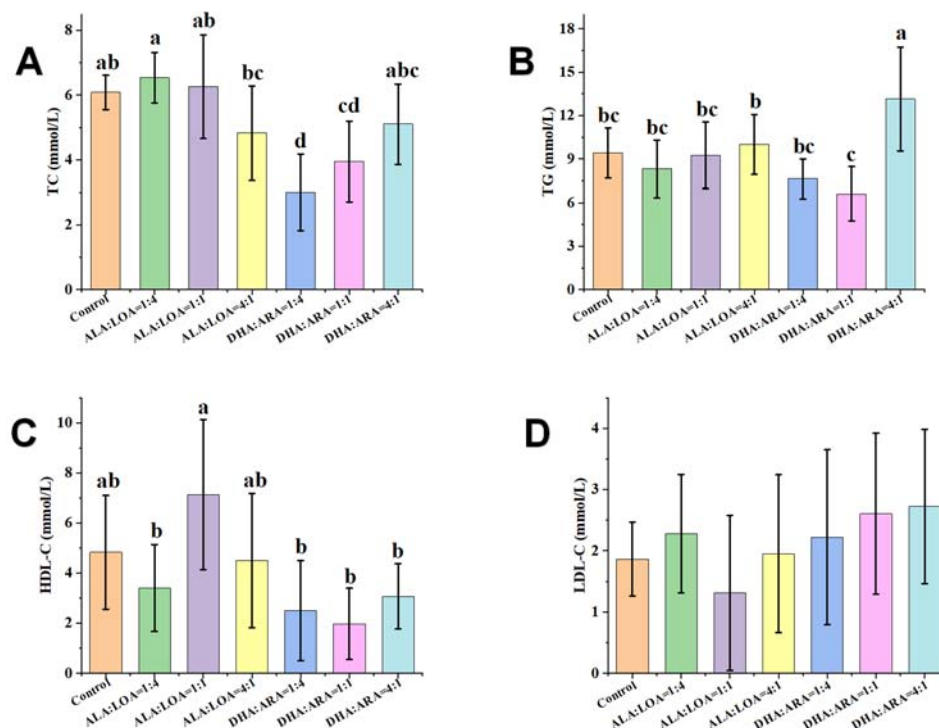


Fig. 6 Effects of different proportions of n-3 and n-6 PUFA supplementation on liver biochemical indexes. A: total cholesterol (TC); B: triglyceride (TG); C: high-density lipoprotein (HDL-C); D: low-density lipoprotein (LDL-C), and the differences indicated by different letters were statistically significant ($P < 0.05$).

The fatty acid composition analysis of liver tissue found (Table 1) that the contents of n-3 and n-6 PUFA fatty acids in ALA:LOA groups increased with the increase of the contents of n-3 and n-6 PUFA in the intervention, but this trend was not found in DHA:ARA group. When the ratio of DHA:ARA was 4:1, even if the dosage of DHA in the intervention increased, the DHA in liver tissue would not continue to increase. The DHA content in DHA:ARA=1:4, DHA:ARA=1:1 and DHA:ARA=4:1 reached 13.87%, 14.18% and 13.65% respectively.

3.5 Effects of different proportions of n-3 and n-6 PUFA supplementation on the synthesis of polyunsaturated fatty acids and the expression of elongation-related proteins in mouse liver

The n-3 and n-6 PUFAs cannot be directly synthesized in the brain, but the liver is the main organ for fatty acid synthesis. Therefore, it is speculated that the contents of n-3 and n-6 PUFA in mouse tissues are related to the synthesis of proteins extending n-3 and n-6 PUFA in the liver, and fatty acid desaturase (FattyAcidDesaturase1/2, Fads1) and long-chain fatty acid elongase (Elovl5). As shown in Figure 7A, western blot was used to detect the expression of Fads1 and Elovl5 in the liver. The protein expression of Fads1 in DHA:ARA=1:1 group was significantly higher than that in the control group ($P < 0.05$, Figure 7B), and the protein expression of Elovl5 in the groups of ALA:LOA=1:4, ALA:LOA=1:1, DHA:ARA=1:4 and DHA:ARA=1:1 was significantly higher than that in the control group ($P < 0.05$, Figure 7C). The results showed that the ratios of n-3 to n-6 PUFA were 1:1 and 1:4, which promoted the protein expression of elongation-related enzymes. Dietary supplementation of n-3 and n-6 PUFA could increase the contents of DHA and ARA in the liver and brain of mice.

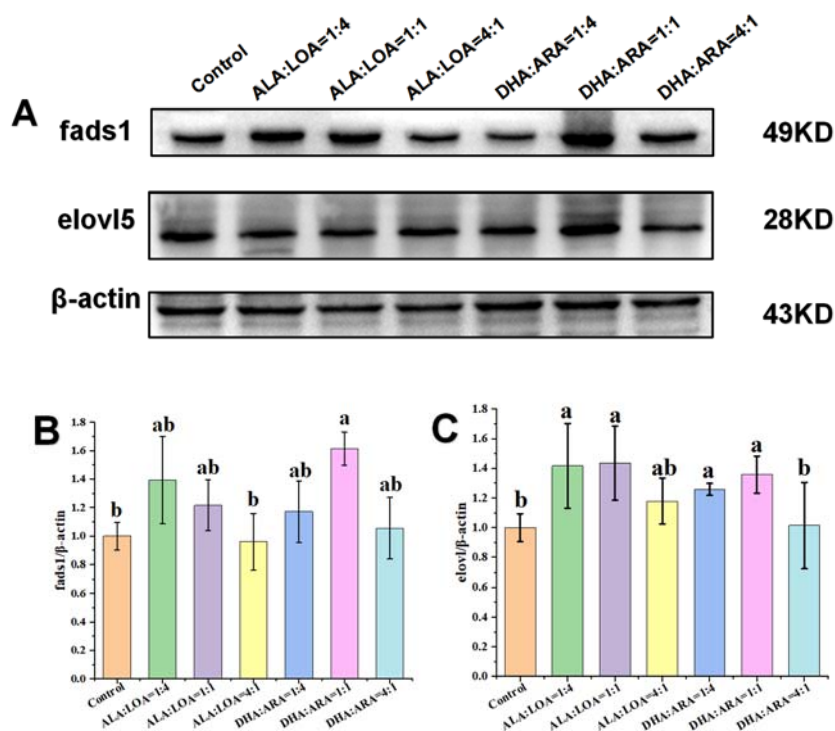


Fig. 7 Effects of different proportions of n-3 and n-6 PUFA on the protein related to metabolic transformation in the liver. A: western blot was used to detect the expression of Fads1 and Elovl5 in liver; B: expression intensity of B:Fads1; The expression intensity of C:Elovl5, with different letters, was statistically significant ($P < 0.05$).

3.6 Effects of different proportions of n-3 and n-6 PUFA supplementation on fatty acids in the brain of mice

The fatty acid composition analysis of mouse brain tissue found (Table 2) that the contents of n-3 and n-6 fatty acids in ALA:LOA groups increased with the increase of the contents of n-3 and n-6 PUFA in the intervention, but this trend was not found in DHA:ARA group. When the ratio of DHA:ARA was 4:1, even if the dosage of DHA in the intervention increased, the DHA content in brain tissue would not continue to increase. The DHA content in DHA:ARA=1:4, DHA:ARA=1:1 and DHA:ARA=4:1 reached 17.08%, 18.16% and 17.76% respectively. Different from the liver, the DHA content in the brain of mice in each group is higher.

3.7 Effects of different proportions of n-3 and n-6 PUFA supplementation on lipid metabolisms in the brain of mice

Volcano plot construction based on brain tissue lipid metabolites is shown in Figure 8. Six kinds of significantly different lipids were screened between the control group and ALA:LOA=1:4 group (Figure 8A), and three kinds of down-regulated substances were found. A total of 338 significantly different lipids were screened out between the control group and the ALA:LOA=1:1 group (Figure 8B), of which 75 were up-regulated and 263 were down-regulated. A total of 246 significantly different lipids were screened between the control group and ALA:LOA=4:1 group (Figure 8C), of which 94 were up-regulated and 152 were down-regulated.

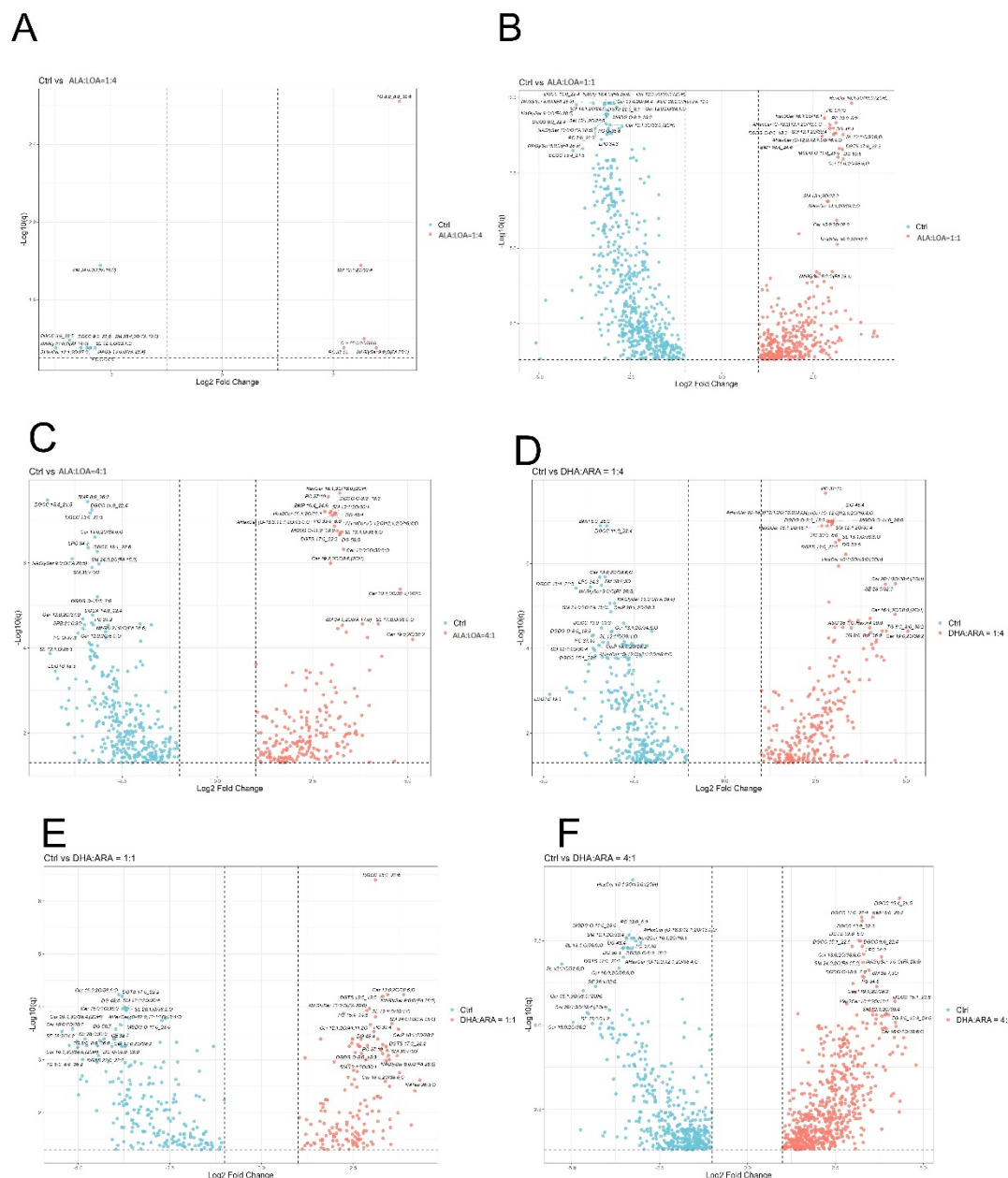


Fig. 8 Comparison of volcanic maps in pairs, control group and ALA:LOA=1:4 group (Figure A), control group and ALA:LOA=1:1 group (Figure B), control group and ALA:LOA=4:1 group (Figure C), control group and DHA:ARA=1:4 group (Figure D), control group and DHA:ARA=1:1 group (Figure E), control group and DHA:ARA=4:1 group (Figure F).

A total of 263 significantly different lipids were screened between the control group and the DHA:ARA=1:4 group (Figure 8D). Compared with the control group, there were 127 up-regulated substances in the DHA:ARA=1:4 group, including 23 diacylglycerols (DG), 58 ceramides (Cer), 11 sphingomyelins (SM) and triglycerides (TG). There are 136 down-regulated substances, 27 DG substances and 15 saturated DG substances (such as DGO-24:0_28:0 and DGO-16:0_24:0), and their excessive accumulation will aggravate cognitive impairment. There are 43 kinds of CERs, among which 22 kinds of CERs with 16-24 carbons or more are down-regulated, which are the key factors of cognitive impairment. There are 11 kinds of sphingolipids (SL), 10 kinds of NAGly and phosphatidylcholine (PC), 2 kinds of TG, 1 kind of SL and 1 kind of CE.

A total of 221 kinds of significantly different lipids were screened between the control group and the DHA:ARA=1:1 group (Figure 8E). Compared with the control group, there were 82 kinds of up-regulated substances in the DHA:ARA=1:1 group, including 20 kinds of DG, 24 kinds of Cer, 6 kinds of PC, 6 kinds of N- acyl glycyserine (NAGlySer), 1 kind of TG and SM. There are 139 kinds of down-regulated substances, 25 kinds of DG, 18 kinds of saturated DG and 48 kinds of CERs, among which 32 kinds of CERs with 16-24 carbons or more are down-regulated, 12 kinds of SL, 17 kinds of TG and 6 kinds of NAGlySer.

A total of 375 significantly different lipids were screened between the control group and the DHA:ARA=4:1 group (Figure 8F). Compared with the control group, there were 230 substances up-regulated, including 40 DG, 4 Cer, 87 NAGlySer, 10 PC, 17 SL, 11 SM and 9 TG. O) there is one kind; There are 145 down-regulated substances, 32 DG, 48 Cer, 5 SE, 16 TG, 13 SL, 16 TG, 7 SM and 7 NAGly.

3.8 Effects of different proportions of n-3 and n-6 PUFA supplementation on morphological development of neurons in mouse brain

The results of HE staining in the brain tissue of mice showed that compared with the control group, neurons in CA1 and DG areas of hippocampus in DHA:ARA=4:1 were loosely arranged and disordered, and chromatin was concentrated and compact, and nuclei were condensed (Figure 9A). Compared with the control group, the number of normal neurons in DG area in DHA:ARA=4:1 group decreased significantly (Figure 9B), while there was no significant difference between DHA:ARA=1:4 group and the control group. For CA1 and CA3 regions, compared with the control group, the number of normal neurons in DHA:ARA=1:4 group and DHA:ARA=1:1 group increased significantly (Figure 9C,D), but there was no significant change in DHA:ARA=4:1 group.

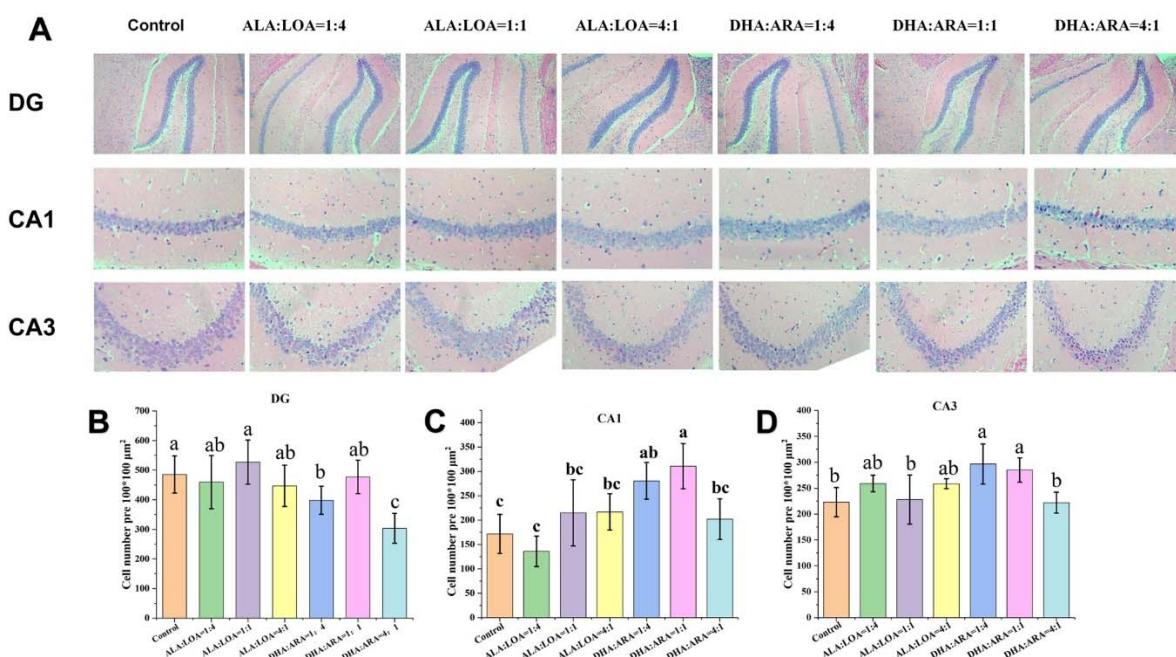


Fig. 9 Effects of different proportions of n-3 and n-6 PUFA supplementation on the morphology of DG, CA1 and CA3 regions of hippocampal neurons in mice. B: the number of normal neurons in B:DG area; C: the number of normal neurons in C:CA1 area; D: the number of normal neurons in D: CA3 area; Different letters indicate differences with statistical significance ($P < 0.05$).

3.9 Effects of different proportions of n-3 and n-6 PUFA supplementation on synaptic plasticity in mice brain

In order to determine whether the intervention of different proportions of n-3 and n-6 PUFA will affect the synaptic structure of mouse neurons, Golgi staining was performed on the brain tissues of mice in each group. The results showed that compared with the control group, the distribution of dendritic spines in DHA:ARA=1:1 group was more similar, and the distribution of dendritic spines was more abundant and uniform. However, the distribution of dendritic spines in other groups is sparse and uneven (Figure 10A). The analysis of dendritic length and density of dendritic spines in CA1, CA2 and CA3 areas of hippocampus showed that compared with the control group, the density of dendritic spines in CA1 area in ALA:LOA=1:1 group increased significantly (Figure 10B), while the dendritic length in ALA:LOA=4:1 group and DHA:ARA=4:1 group decreased significantly (Figure 10E-G). The analysis of CA2 region showed that there was no significant difference in dendritic spine density and dendritic length between the intervention groups and the control group (Figure 10C,F). The density of dendritic spines in CA3 region was analyzed, and it was found that the DHA:ARA=1:4 group was significantly higher than the control group (Figure 10D).

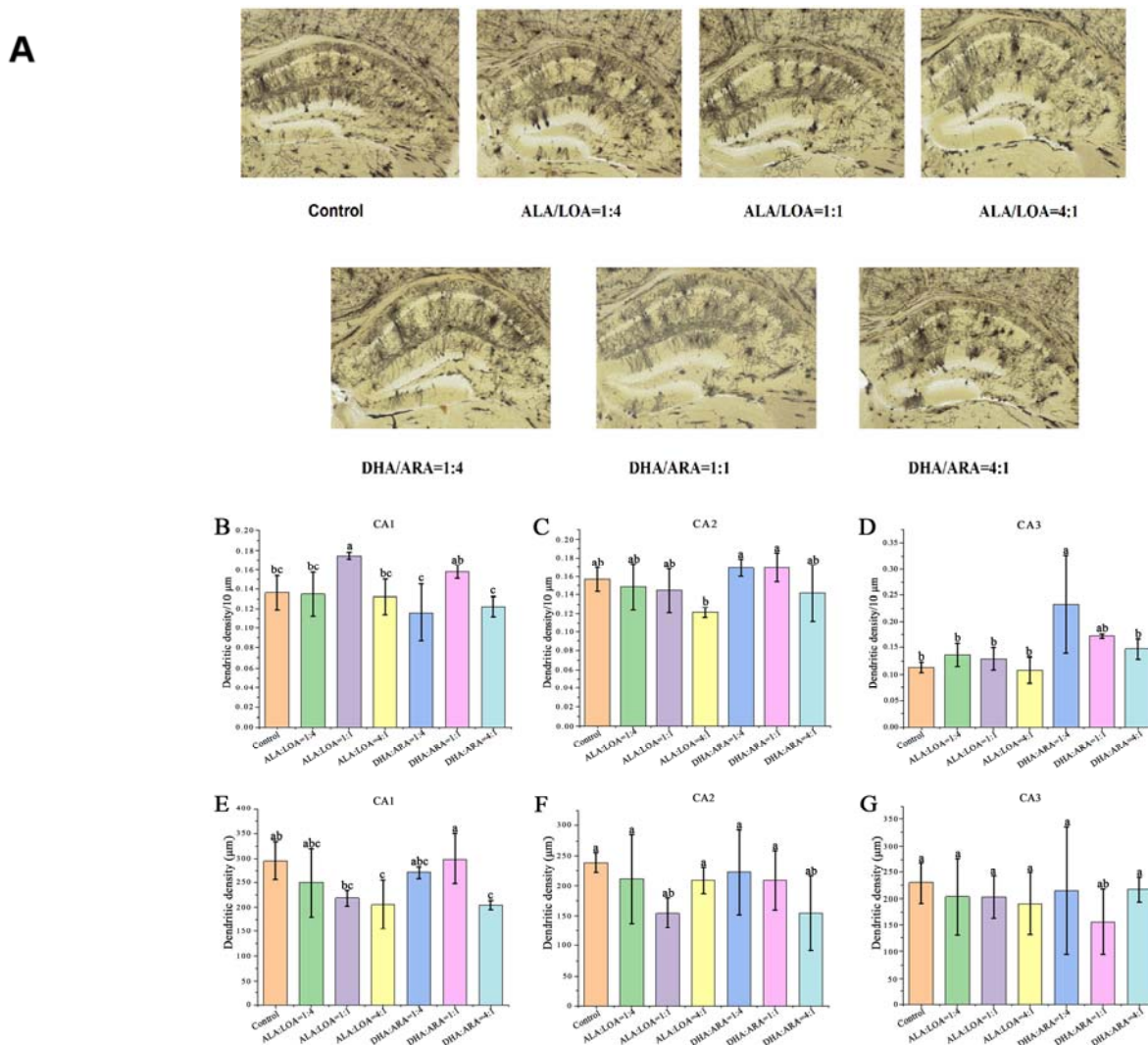


Fig. 10 Effects of different proportions of n-3 and n-6 PUFA supplementation on synaptic morphology in brain A: Golgi staining was performed on brain sections of mice in each group; B: Dendritic spines density analysis in B:CA1 region; C: Dendritic spines density analysis in C:CA2 region; D: Dendritic spines density analysis in D:CA3 area; E: analysis of dendritic length in E:CA1 region; F: Dendritic length analysis of Ca2 region; G: Dendritic length analysis of G:CA3 region; Different letters indicate differences with statistical significance ($P < 0.05$).

3.10 Effects of different proportions of n-3 and n-6 PUFA supplementation on cognitive related genes in hippocampus of mice

The results of behavioral experiments show that the cognitive function of mice in DHA:ARA=1:4 group and DHA:ARA=1:1 group is significantly improved compared with the control group. Brain-derived neurotrophic factor (BDNF) and NGF are members of neurotrophic factor family, which not only participate in the development and survival of neurons, but also participate in various physiological functions and pathological processes of the nervous system. The analysis of the results of the expression of genes involved in cognition in the hippocampus of mice showed that the expression of BDNF gene in DHA:ARA=1:4 group and DHA:ARA=1:1 group was significantly higher than that in the control group (Figure 11A), but there was no significant difference in NGF gene among the groups (Figure 11B). The expression of DLG4 gene (PSD-95) in DHA:ARA=1:4 group was significantly higher than that in the control group (Figure 11C).

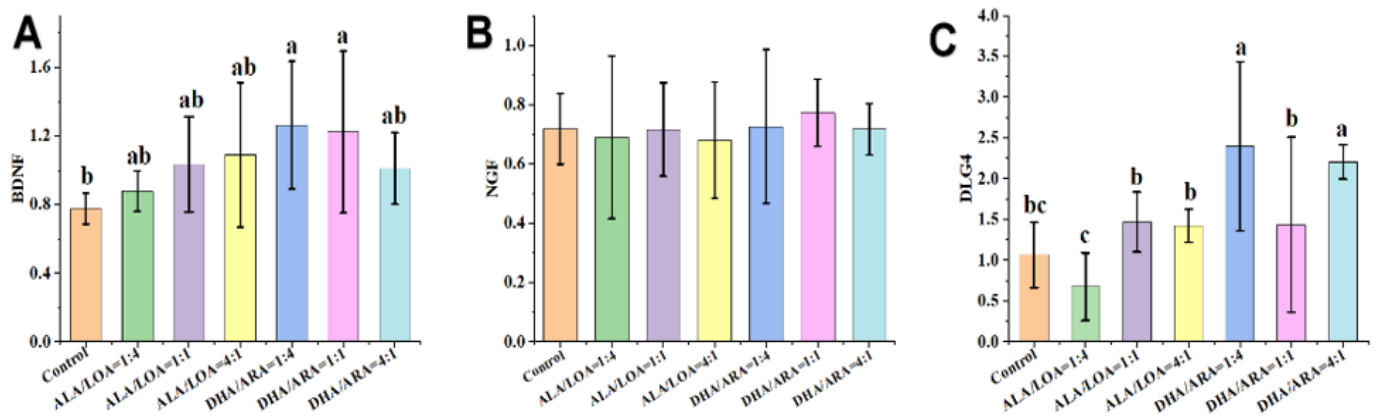


Fig. 11 Effects of different proportions of n-3 and n-6PUFA supplementation on cognitive related genes in hippocampus A: BDNF; B:NGF; C:PSD95; Different letters indicate differences with statistical significance ($P < 0.05$).

3.12 Effects of different proportions of n-3 and n-6 PUFA supplementation on cognitive related proteins in cerebral cortex and hippocampus of mice

The protein expressions of BDNF, NGF and receptor TrkB of BDNF in cerebral cortex and hippocampus were detected (Figure 12). The results showed that the protein expressions of BDNF in DHA:ARA=1:4 group and DHA:ARA=1:1 group in cerebral cortex were significantly higher than those in control group, but there was no significant difference in NGF protein expression among the groups. The protein expression of TrkB in DHA:ARA=1:1 group was significantly higher than that in control group.

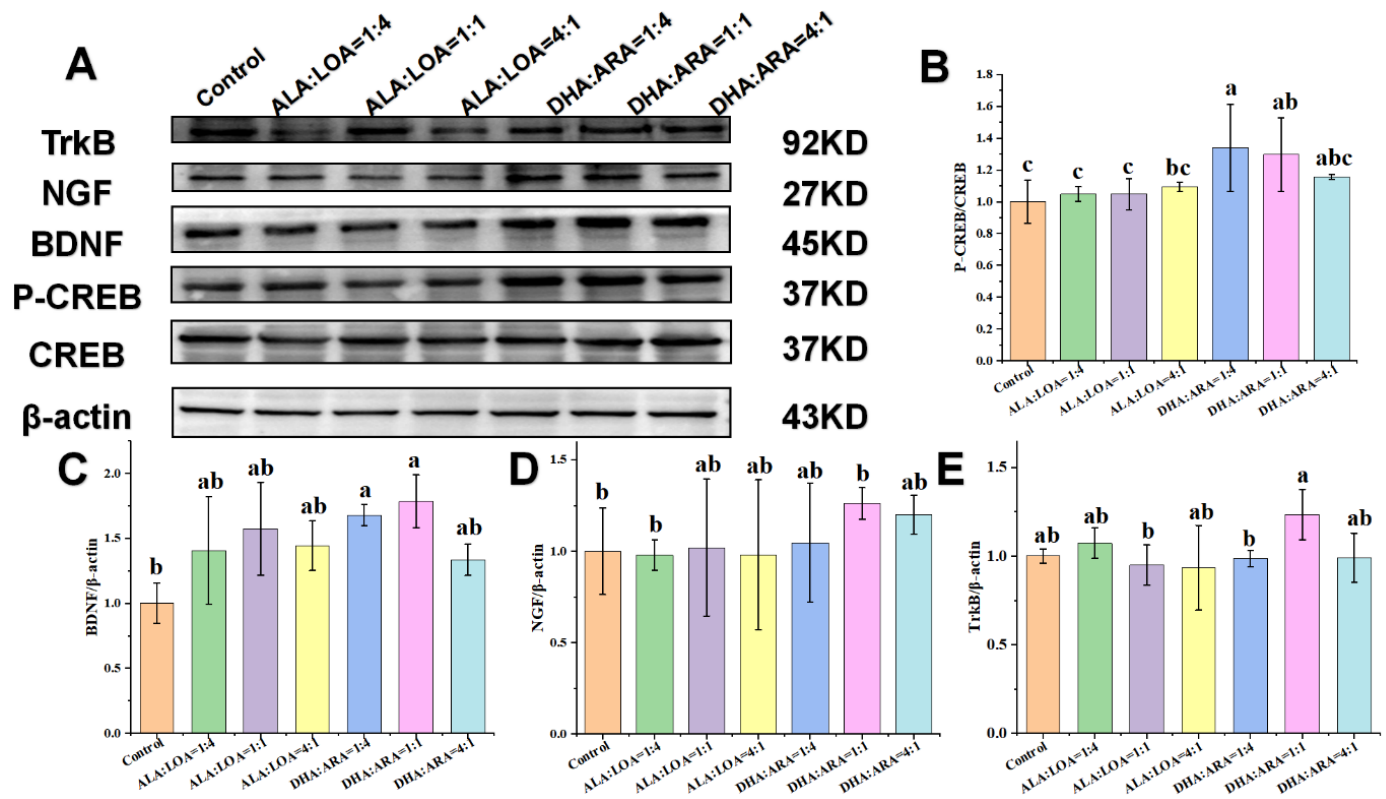


Fig. 12 Effects of different proportions of n-3 and n-6 PUFA supplementation on cognitive function-related proteins in mouse cerebral cortex. A: the expression of trkb, NGF and BDNF in cerebral cortex was detected by western blot; B: the expression intensity of P-CREB/CREB C: the expression intensity of C:BDNF; D: the expression intensity of D:NGF; The expression intensity of E:TrkB, with different letters, was statistically significant ($P < 0.05$).

The results of protein determination in hippocampus showed that the expression of BDNF protein in DHA:ARA=1:1 group was significantly higher than that in the control group, and there was no significant difference in NGF expression among the groups, but the expression of TrkB in DHA:ARA=1:4 group and DHA:ARA=1:1 group was significantly higher than that in the control group (Figure 13). In addition, the results in cerebral cortex and hippocampus showed that the CREB and p-CREB proteins related to memory formation in DHA:ARA=1:1 and DHA:ARA=1:4 groups were significantly higher than those in the control group (Figure 12B and Figure 13B).

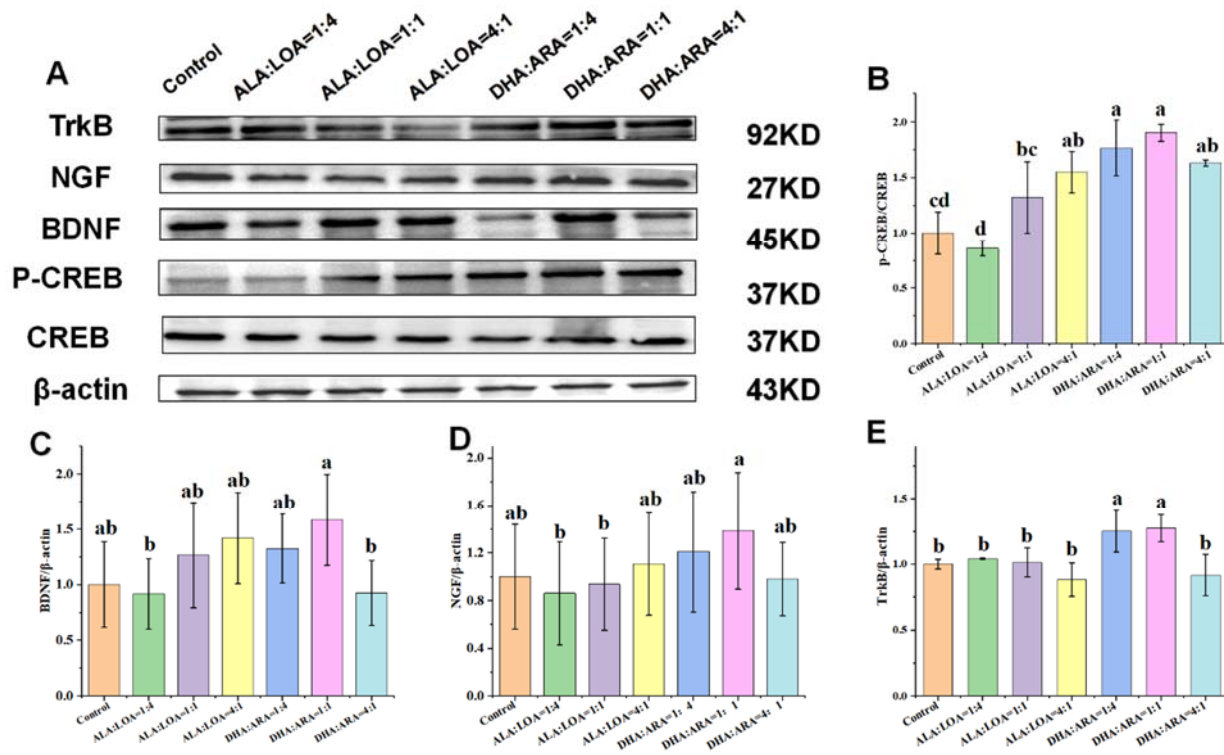


Fig. 13 Effects of different proportions of n-3 and n-6PUFA supplementation on proteins related to cognitive function in hippocampus of mice. A: the expression of trkb, NGF and BDNF in hippocampus was detected by western blot; B: the expression intensity of P-CREB/CREB C: the expression intensity of C:BDNF; D: the expression intensity of D:NGF; The expression intensity of E:TrkB, with different letters, was statistically significant ($P < 0.05$).

3.13 Effects of different proportions of n-3 and n-6 PUFA supplementation on synaptic plasticity-related proteins in mice

The expressions of synaptic plasticity related proteins Soluble NSF Attachment Protein (Snap), Synapsin (Syn) and Postsynaptic Density Protein 95 (PSD95) in cerebral cortex and hippocampus were detected (Figure 14,15). It was found that the Snap and Syn proteins in DHA:ARA=1:4 and DHA:ARA=1:1 groups in cerebral cortex were significantly higher than those in control group, but there was no significant difference between DHA:ARA=4:1 group and control group, and the expression of PSD95 was significantly higher only in DHA:ARA=1:1 group. The expressions of Snap, Syn and PSD95 proteins in hippocampus were significantly higher in DHA:ARA=1:4 and DHA:ARA=1:1 groups than those in control group.

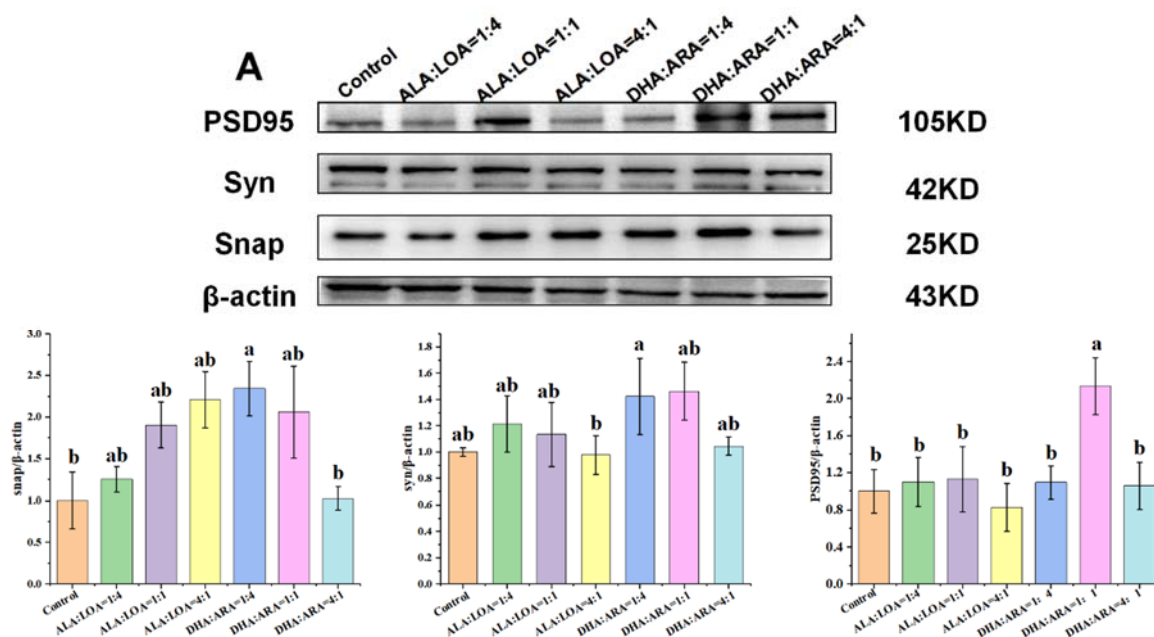


Fig. 14 Effects of different proportions of n-3 and n-6 PUFA supplementation on synaptic plasticity related proteins in mouse cerebral cortex. A: the expression of SNAP, syn and PSD95 in cerebral cortex was detected by western blot; B: the expression intensity of SNAP in cerebral cortex; C: the expression intensity of syn in cerebral cortex; D: the expression intensity of PSD95 in cerebral cortex; Different letters indicate differences with statistical significance ($P < 0.05$).

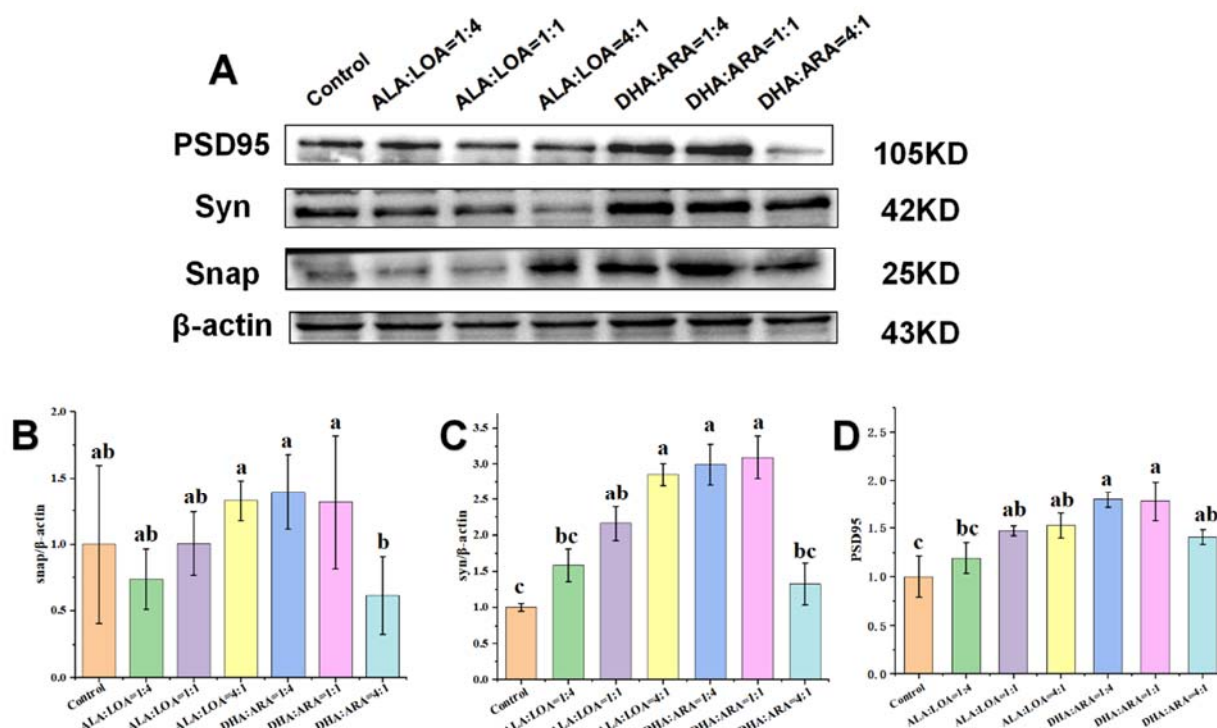


Fig. 15 Effects of different proportions of n-3 and n-6 PUFA supplementation on synaptic plasticity related proteins in hippocampus of mice. A: the expression of SNAP, syn and PSD95 in hippocampus was detected by western blot; B: the expression intensity of SNAP in hippocampus; C: the expression intensity of syn in hippocampus; D: the expression intensity of PSD95 in hippocampus; Different letters indicate differences with statistical significance ($P < 0.05$).

4. Discussion

Previous studies had shown that n-3 and n-6 PUFAs could reduce the levels of TC and TG in human serum⁰. In the mouse model, however, when the ratio of DHA to ARA was 1:4 and 1:1, the TC levels were

significantly decreased, while no similar effects were observed in the group with a DHA:ARA ratio of 4:1 and all groups with different ratios of alpha-linolenic acid (ALA) to linoleic acid (LOA)⁰. This phenomenon suggested that specific ratios of DHA to ARA (such as 1:4 to 1:1) could improve lipid metabolism by regulating liver cholesterol-synthesizing genes (such as HMG-CoA reductase) and bile acid excretion pathways, and played a key role in regulating cholesterol metabolism in mice⁰⁰. However, high DHA ratio (such as 4:1) or ALA:LOA ratio might lead to TG accumulation by promoting fatty acid re-esterification or inhibiting lipoprotein lipase activity, which was not sufficient to activate relevant metabolic pathways⁰. It was worth noting that although the content of liver n-3 and n-6 PUFAs in the ALA:LOA groups increased with the increase of intervention dose, the content of n-3 and n-6 PUFAs in the DHA:ARA=4:1 group did not continue to increase after reaching a certain ratio⁰. This phenomenon might be related to the negative-feedback regulation of liver fatty acid metabolism: high-dose DHA might limit the synthesis of ARA by inhibiting the activity of desaturase and elongase, and excessive ARA would competitively inhibit the esterification and storage of DHA. In addition, the DHA:ARA=1:1 group showed a significant effect in reducing LDL-C, which supported the potential regulatory role of the balanced ratio of DHA to ARA in cholesterol transport or the expression of liver LDL receptors. In contrast, the decrease in HDL-C was only observed in the DHA:ARA=1:1 and 4:1 groups, which might be related to the complex effects of increased DHA proportion on the structure or function of HDL. Moreover, all intervention groups had no significant effect on TG. Overall, the ratio of DHA to ARA (rather than the ALA:LOA ratio) was more specific for improving cholesterol metabolism. Especially, the 1:1 ratio was outstanding in reducing TC and LDL-C, which might be achieved by regulating the expression of genes and proteins related to cholesterol synthesis and absorption.

In addition, previous studies on the intervention of n-6 and n-3 fatty acids in human populations had yielded inconsistent results, and some studies had suggested that they have a beneficial effect on cognitive function. This study systematically evaluated this through an intervention on a mouse model. Given the particular importance of early-life nutrition for brain development, and considering that the digestive physiology of specific populations such as infants may influence the efficiency of digestion and absorption of key fatty acids such as DHA and ARA⁰, it is especially important to investigate their optimal supply ratios. Concurrently, a thorough understanding of the spatial distribution and dynamic metabolic processes of nutrients in the organism is key to elucidating the mechanisms by which they improve cognitive function⁰. The results showed that the adjustment of the ALA:LOA and DHA:ARA ratios had an impact on the exploratory behavior and spatial memory of mice. In the open field test, the total movement distances of the ALA:LOA=1:1 and 4:1 groups increased significantly, while there were no significant changes in the movement distance and time in the central area, suggesting that a high ratio of ALA to LOA might enhance spontaneous motor activity by regulating the dopamine system or energy-metabolism pathways (glycolipid metabolism processes)⁰. However, the increase in total movement distance did not reflect an improvement in cognitive function. It was worth noting that the DHA:ARA=1:1 group showed a significant increase in the exploration distance and time in the central area, which might indicate that this ratio could promote the

environmental adaptability of mice. This result might be related to the mechanism by which DHA relieves anxiety by enhancing the expression of hippocampal BDNF⁰. However, the synergistic or antagonistic effects of ARA in this process still need to be further verified. The Y-maze could be used to evaluate the working memory of mice⁰. In this test, there was no significant difference in the total number of arm entries among groups, but the spontaneous alternation rate of the DHA:ARA=1:1 group was significantly increased, suggesting that this ratio could improve the working memory of mice. The Morris water maze spatial exploration test further revealed the potential effect of the DHA:ARA ratio on spatial memory. The number of platform crossings in the DHA:ARA=1:4 group increased significantly, which might be related to its promotion of hippocampal neurogenesis or enhancement of LTP effect⁰. Although there were no inter-group differences in escape latency and residence time in the target quadrant, the significant increase in the number of platform crossings in the DHA:ARA=1:4 group of mice indicated that this ratio might improve spatial memory by regulating the neural coding efficiency during the memory consolidation stage⁰. In conclusion, the DHA:ARA ratio (especially 1:1 and 1:4) was more prominent in the regulation of cognitive function. This difference might be related to the different distributions and metabolic pathways of the two types of fatty acids in the central nervous system: ALA:LOA regulated behavior more by affecting monoamine neurotransmitters, while DHA:ARA was directly involved in synaptic membrane structure and signal transduction⁰.

In this study, it was observed that although the contents of n-3 and n-6 PUFAs in the cerebral cortex of the ALA:LOA intervention groups had increased with the increase in dietary supplementation dose, when the DHA:ARA ratio was 4:1, the concentrations of DHA and ARA in the brain had stabilized without further increase after reaching a certain level. This phenomenon had indicated that there had existed a dynamic balance mechanism in the uptake and storage of DHA and ARA in brain tissues, which might have been related to the active transport regulation of the blood-brain barrier and the feedback inhibition of intracellular metabolism⁰. Firstly, as metabolic products of long-chain PUFAs, the concentrations of DHA and ARA in the brain might have been limited by transport proteins (such as Mfsd2a). Mfsd2a, as a DHA-specific transporter, had had a direct correlation between its expression level and transport efficiency⁰. When the supplementation of exogenous DHA and ARA reaches a certain threshold, the transport capacity of the blood-brain barrier might have approached saturation, resulting in the inability of brain concentrations to increase further⁰. In addition, Δ -6 desaturase, a key enzyme in the endogenous synthesis of PUFAs, may be subject to negative feedback inhibition under high-dose DHA and ARA intervention, thereby limiting the *de novo* synthesis of ARA⁰.

The groups with DHA:ARA=1:1 group and DHA:ARA=4:1 group demonstrated superior cognitive improvement compared to the control group. This benefit is primarily attributable to their neuroprotective effects mediated by modulating key lipid metabolic pathways. Specifically, these ratios significantly downregulated long-chain ceramides (C16–C24 Cer) (22 and 32 species reduced in the 1:4 and 1:1 groups, respectively), thereby suppressing neuroinflammatory processes (e.g., NLRP3 inflammasome activation)⁰. In contrast, the DHA:ARA=4:1 group exhibited insufficient ceramide clearance (with only 48 species

downregulated but 87 species upregulated), which may contribute to exacerbated cognitive impairment. Furthermore, an imbalanced DHA:ARA=4:1 led to excess DHA interfering with ARA metabolism and induced sphingolipid metabolic dysregulation (17 sphingolipid species increased)⁰, thereby compromising anti-inflammatory and reparative capacity. In summary, the 1:4 and 1:1 groups more effectively improved cognitive function by coordinately regulating ceramides, DG, and TG, and by maintaining a balanced metabolic state.

Sun et al revealed that excessive DHA could enhance the susceptibility of hippocampal neurons to lipid peroxidation products, thereby inducing DNA damage and cell apoptosis⁰. This explained the results of HE staining and neuronal quantitative analysis, which revealed the effects of different DHA:ARA ratios on the neuronal structure and quantity in hippocampal subregions (DG, CA1, CA3) of mice. In the DHA:ARA=4:1 group, neurons in the DG and CA1 regions showed disordered arrangement, nuclear pyknosis, and a significant reduction in cell number. The deficiency of ARA may further exacerbate the process of DHA-induced oxidative stress and synaptic dysfunction, as ARA plays a vital anti-inflammatory and antioxidant role in neural protection. Specifically, ARA can regulate the synthesis of epoxy eicosatrienoic acids (EETs), which are known to attenuate oxidative damage and inflammation in the brain. This is supported by the findings in the previous study, which demonstrates in a rat cerebral ischemia-reperfusion model that ARA administration significantly reduced oxidative stress markers, including decreased malondialdehyde (MDA) levels and increased activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), while also lowering pro-inflammatory cytokines such as tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6)⁰. These results confirm the antioxidant and anti-inflammatory capacities of ARA in vivo. Therefore, insufficient ARA may impair such protective mechanisms, thereby aggravating oxidative stress and compromising neuronal integrity, particularly under conditions of imbalanced DHA/ARA ratios. Notably, the number of neurons in the CA1 and CA3 regions was significantly increased in the DHA:ARA=1:4 and 1:1 groups, indicating that an appropriate ratio of n-3 and n-6 PUFAs may enhance neurogenesis by activating BDNF or promoting synaptic plasticity⁰.

In the cerebral cortex, the upregulation of Snap and Syn may be associated with the balanced DHA and ARA promoting the efficiency of synaptic vesicle release. Syn is a key marker protein of presynaptic vesicles, and increased expression of Syn usually reflect enhanced synaptic transmission activity⁰. Snap proteins (such as SNAP-25), as core components of the SNARE complex, directly regulate the process of vesicle fusion with the plasma membrane⁰. The upregulation of Syn caused by the synergistic effect of DHA and ARA in the DHA:ARA=1:1 and 1:4 groups indicated that an appropriate ratio of n-3 and n-6 PUFAs may optimize vesicle recycling by enhancing membrane phospholipid fluidity (especially the presynaptic membrane rich in DHA)⁰. However, PSD95 was significantly increased only in the DHA:ARA=1:1 group, suggesting that the remodeling of postsynaptic structures requires a more stringent balance between DHA and ARA, which may depend on the TrkB-BDNF signaling pathway activated by DHA⁰. Meanwhile, BDNF has been confirmed to specifically induce PSD95 expression and promote dendritic spine maturation⁰. The comprehensive

upregulation of PSD95, Syn, and Snap in the hippocampus further highlights the sensitivity of this region to the ratio of n-3 and n-6 PUFAs. Hippocampal neurons are highly dependent on PUFAs to maintain synaptic plasticity, and the expression of PSD95 in them is closely related to LTP⁰. The DHA:ARA=1:1 and 1:4 groups may enhance presynaptic vesicle release and postsynaptic receptors by synergistically regulating lipid mediators derived from n-3 and n-6 PUFAs (such as neuroprotectin D1 and prostaglandin E2) while inhibiting inflammatory signaling pathways (such as NF- κ B)⁰. However, the insignificant effect in the DHA:ARA=4:1 group may be related to the imbalance of cyclooxygenase (COX) metabolism caused by insufficient ARA. The deficiency of anti-inflammatory mediators derived from ARA (such as lipoxin A4) may offset the synapse-promoting effect of DHA⁰.

Although this study demonstrates that DHA:ARA=1:1 group and DHA:ARA=1:4 group upregulate BDNF-TrkB-CREB signaling and synaptic proteins in the brain, correlating with improved cognitive function, The necessity of this pathway was not directly verified through application of TrkB receptor antagonists or CREB gene knockdown techniques. Consequently, the findings presented here remain indicative of a strong association, and whether the BDNF-TrkB-CREB axis acts as the sole pathway requires further investigation through more direct interventional approaches in future studies. It is worth noting that the BDNF-TrkB-CREB axis is not the only pathway mediating the cognitive effects of n-3/n-6 ratio imbalance. Neuroinflammation and oxidative stress also play non-negligible roles in this process. The lipidomics results showed that the DHA:ARA=1:4 and 1:1 groups significantly downregulated 22 and 32 species of C16-C24 ceramides respectively, key metabolites that can activate the NLRP3 inflammasome and induce microglial-mediated neuroinflammation. The histological analysis revealed disordered neuronal arrangement in the hippocampal CA1 and CA3 regions in the DHA:ARA = 4:1 group. This morphological alteration is likely associated with an imbalance in the omega-6/omega-3 ratio, which is linked to pro-inflammatory states and has been implicated in oxidative stress pathways as shown in a prior studies⁰. In addition, appropriate ARA intake can be metabolized into anti-inflammatory mediators such as lipoxin A4 and EETs, while ARA deficiency under the 4:1 ratio may weaken this protective effect.

Future research should combine targeted intervention of neuroinflammatory and oxidative stress pathways to clarify the interaction between these mechanisms and the BDNF-TrkB-CREB axis, so as to fully elucidate the regulatory network of n-3/n-6 ratios on cognitive function.

In summary, the differences between the hippocampus and cerebral cortex may arise from their distinct mechanisms in PUFA metabolism and synaptic plasticity. Hippocampal neurons are rich in the DHA transporter Mfsd2a, enabling the hippocampus to more efficiently uptake DHA and integrate it into synaptic membranes⁰, whereas the cerebral cortex may rely more on ARA metabolism to regulate postsynaptic signaling. Overall, the DHA:ARA ratios of 1:1 and 1:4 protect synaptic plasticity by regulating the expression of presynaptic/postsynaptic proteins, while a high DHA ratio (4:1) may impair the synergistic effect due to ARA deficiency⁰.

5. Conclusion

When the ratio of DHA to ARA is 1:4 and 1:1, it can significantly enhance the performance of mice in open field test, Y-maze and Morris water maze. These ratios also improved the learning and memory ability of mice, significantly increased the number of normal neurons in hippocampal CA1 and CA3 areas, and enhanced synaptic plasticity. The cognitive improvements observed in the DHA:ARA=1:1 and 1:4 groups were closely associated with the activation of the BDNF-TrkB-CREB signaling axis, which up-regulated synaptic plasticity-related proteins (PSD95, Syn, Snap), promoted synaptic remodeling of hippocampal neurons, and significantly improved spatial memory (increased crossing times of Morris water maze) and working memory (spontaneous alternation rate of Y-maze). This study may provide critical theoretical rationale and dosage references for optimizing dietary fatty acid ratios to enhance cognitive function and brain development, particularly in the domain of infant formula products.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Ethical statement

The project was approved by the Ethics Committee of Qingdao University (No.QDU-AEC-2023380).

References

- S. M. Innis, "The role of dietary n-6 and n-3 fatty acids in the developing brain," *Developmental Neuroscience* 22(5-6) (2000) 474-480. <https://doi.org/10.1159/000017478>.
- G. Schmitz, J. Ecker, "The opposing effects of n-3 and n-6 fatty acids," *Progress in Lipid Research* 47(2) (2008) 147-155. <https://doi.org/10.1016/j.plipres.2007.12.004>.
- K. Tanaka, A.A. Farooqui, N.J. Siddiqi, et al., "Effects of docosahexaenoic Acid on neurotransmission," *Biomolecules & therapeutics* 20(2) (2012) 152-157. doi:10.4062/biomolther.2012.20.2.152.
- H. Tallima, R. El Ridi, "Arachidonic acid: Physiological roles and potential health benefits-A review," *Journal of Advanced Research* 11 (2017) 33-41. doi:10.1016/j.jare.2017.11.004.
- L. Lauritzen, P. Brambilla, A. Mazzocchi, et al., "DHA Effects in Brain Development and Function," *Nutrients* 8(1) (2016) 6. <https://doi.org/10.3390/nu8010006>.
- A. Wu, Z. Ying, F. Gomez-Pinilla, et al., "Docosahexaenoic acid dietary supplementation enhances the effects of exercise on synaptic plasticity and cognition," *euroscience* 155(3) (2008) 751-759. doi:10.1016/j.neuroscience.2008.05.061.
- F. Echeverría, R. Valenzuela, M. Hernandez-Rodas, et al., "Docosahexaenoic acid (DHA), a fundamental fatty acid for the brain: New dietary sources," *Prostaglandins, Leukotrienes, and Essential Fatty Acids* 124 (2017) 1-10. doi:10.1016/j.plefa.2017.08.001.
- K. Tanaka, A.A. Farooqui, N.J. Siddiqi, et al., "Effects of docosahexaenoic Acid on neurotransmission," *Biomolecules & therapeutics* 20(2) (2012) 152-157. doi:10.4062/biomolther.2012.20.2.152.
- R.J. Wurtman, "Synapse formation and cognitive brain development: effect of docosahexaenoic acid and other dietary constituents," *Metabolism: Clinical and Experimental* 57(Suppl 2) (2008) S6-S10. doi:10.1016/j.metabol.2008.07.007.

- M.C. Morris, D.A. Evans, J.L. Bienias, et al., "Consumption of fish and n-3 fatty acids and risk of incident Alzheimer disease," *Archives of Neurology* 60(7) (2003) 940-946. doi:10.1001/archneur.60.7.940.
- L. Lauritzen, "DHA Effects in Brain Development and Function," *Nutrients* 8(1) (2016) 6. <https://doi.org/10.3390/nu8010006>.
- R.S. Chapkin, S.L. Carmichael, "Effects of dietary n-3 and n-6 polyunsaturated fatty acids on macrophage phospholipid classes and subclasses," *Lipids* 25(12) (1990) 827-834. doi:10.1007/BF02535905.
- H.C. Lee-Okada, C. Xue, T. Yokomizo, et al., "Recent advances on the physiological and pathophysiological roles of polyunsaturated fatty acids and their biosynthetic pathway," *Biochimica et Biophysica Acta. Molecular and Cell Biology of Lipids* 1870(1) (2025) 159564. doi:10.1016/j.bbalip.2024.159564.
- B. Wang, L. Wu, J. Chen, et al., "Metabolism pathways of arachidonic acids: mechanisms and potential therapeutic targets," *Signal Transduction and Targeted Therapy* 6(1) (2021) 94. doi:10.1038/s41392-020-00443-w.
- SM. Innis, "The role of dietary n-6 and n-3 fatty acids in the developing brain," *Developmental Neuroscience* 22(5-6) (2000) 474-480. doi:10.1159/000017478.
- A. Gázquez, E. Larqué, "Towards an Optimized Fetal DHA Accretion: Differences on Maternal DHA Supplementation Using Phospholipids vs. Triglycerides during Pregnancy in Different Models," *Nutrients* 13(2) (2021) 511. doi:10.3390/nu13020511.
- C.P. Duggan, L. Brennan, P. Christian, et al., "Knowledge and debate in the American Journal of Clinical Nutrition: new sections, new science, and looking forward and outward," *The American Journal of Clinical Nutrition* 111(1) (2020) 1-3. doi:10.1093/ajcn/nqz267.
- A. Poli, C. Agostoni, F. Visioli, et al., "Dietary Fatty Acids and Inflammation: Focus on the n-6 Series," *International Journal of Molecular Sciences* 24(5) (2023) 4567. doi:10.3390/ijms24054567.
- E. Patterson, R. Wall, G.F. Fitzgerald, et al., "Health implications of high dietary omega-6 polyunsaturated Fatty acids," *Journal of Nutrition and Metabolism* 2012 (2012) 539426. doi:10.1155/2012/539426.
- E.E. Birch, S. Garfield, D.R. Hoffman, et al., "A randomized controlled trial of early dietary supply of long-chain polyunsaturated fatty acids and mental development in term infants," *Developmental Medicine and Child Neurology* 42(3) (2000) 174-181. doi:10.1017/s0012162200000311.
- E.J. Johnson, K. McDonald, SM. Caldarella, et al., "Cognitive findings of an exploratory trial of docosahexaenoic acid and lutein supplementation in older women," *Nutritional Neuroscience* 11(2) (2008) 75-83. doi:10.1179/147683008X301450.
- R.S. Chapkin, S.L. Carmichael, "Effects of dietary n-3 and n-6 polyunsaturated fatty acids on macrophage phospholipid classes and subclasses," *Lipids* 25(12) (1990) 827-834. doi:10.1007/BF02535905.
- A. Alshweki, AP. Muñozuri, AM. Baña, et al., "Effects of different arachidonic acid supplementation on psychomotor development in very preterm infants; a randomized controlled trial," *Nutrition Journal* 14 (2015) 101. doi:10.1186/s12937-015-0091-3.
- S. Gamoh, M. Hashimoto, S. Hossain, et al., "Chronic administration of docosahexaenoic acid improves the performance of radial arm maze task in aged rats," *Clinical and Experimental pharmacology & physiology* 28(4) (2001) 266-270. doi:10.1046/j.1440-1681.2001.03437.x.
- K.A. Volcik, J.A. Nettleton, CM. Ballantyne, et al., "Peroxisome proliferator-activated receptor [alpha] genetic variation interacts with n-6 and long-chain n-3 fatty acid intake to affect total cholesterol and LDL-cholesterol concentrations in the Atherosclerosis Risk in Communities Study," *The American Journal of Clinical Nutrition* 87(6) (2008) 1926-1931. doi:10.1093/ajcn/87.6.1926.
- H. Zou, Y. Gong, H. Ye, et al., "Dietary regulation of peroxisome proliferator-activated receptors in metabolic syndrome," *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology* 116 (2023) 154904. doi:10.1016/j.phymed.2023.154904.
- DB. Jump, D. Botolin, Y. Wang, et al., "Fatty acid regulation of hepatic gene transcription," *The Journal of Nutrition* 135(11) (2005) 2503-2506. doi:10.1093/jn/135.11.2503.
- JT. Brenna, GY. Diau - Prostaglandins, "The influence of dietary docosahexaenoic acid and arachidonic acid on central nervous system polyunsaturated fatty acid composition," *Prostaglandins, Leukotrienes, and Essential Fatty Acids* 77(5-6) (2007) 247-250. doi:10.1016/j.plefa.2007.10.016.
- P. Wu, SX. Pu. MS. Jiang, et al., "Enhancing food digestion and nutrient absorption through pre-digestion: Tailored strategies for special populations.," *Food & Medicine Homology* 4(2) (2025) 9420069. doi:10.26599/FMH.2025.9420069

- MZ. Jia, XY. Hu, FJ. Ning, et al., "Dietary Fiber Deprivation Disrupts Colonic Homeostasis to Drive Anxiety-Like Behavior via the Gut-Brain Axis.," *J Agric Food Chem* 73(51) (2025) 32685-32699. doi:10.1021/acs.jafc.5c12112
- L. Prut, C. Belzung, "The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review," *European Journal of Pharmacology* 463(1-3) (2003) 3-33. doi:10.1016/s0014-2999(03)01272-x.
- CIF. Janssen, AJ. Kiliaan, "Long-chain polyunsaturated fatty acids (LCPUFA) from genesis to senescence: the influence of LCPUFA on neural development, aging, and neurodegeneration," *Progress in Lipid Research* 53 (2014) 1-17. doi:10.1016/j.plipres.2013.10.002.
- RN. Hughes, "The value of spontaneous alternation behavior (SAB) as a test of retention in pharmacological investigations of memory," *Neuroscience and Biobehavioral Reviews* 28(5) (2004) 497-505. doi:10.1016/j.neubiorev.2004.06.006.
- A. Borsini, D. Stangl, AR. Jeffries, et al., "The role of omega-3 fatty acids in preventing glucocorticoid-induced reduction in human hippocampal neurogenesis and increase in apoptosis," *Translational Psychiatry* 10(1) (2020) 219. doi:10.1038/s41398-020-00908-0.
- CV. Vorhees, MT. Williams, "Assessing spatial learning and memory in rodents," *ILAR Journal* 55(2) (2014) 310-332. doi:10.1093/ilar/ilu013.
- T. Larrieu, S. Lay , "Food for Mood: Relevance of Nutritional Omega-3 Fatty Acids for Depression and Anxiety," *Frontiers in Physiology* 9 (2018) 1047. doi:10.3389/fphys.2018.01047.
- SB. Gustafsson, A. Wallenius, H. Zackrisson, et al., "Effects of cannabinoids and related fatty acids upon the viability of P19 embryonal carcinoma cells," *Archives of Toxicology* 87(11) (2013) 1939-1951. doi:10.1007/s00204-013-1051-3.
- LN. Nguyen, D. Ma, G. Shui, et al., "Mfsd2a is a transporter for the essential omega-3 fatty acid docosahexaenoic acid," *Nature* 509(7501) (2014) 503-506. doi:10.1038/nature13241.
- J. Colombo, DJ. Shaddy, EH. Kerling, et al., "Docosahexaenoic acid (DHA) and arachidonic acid (ARA) balance in developmental outcomes," *Prostaglandins, Leukotrienes, and Essential Fatty Acids* 121 (2017) 52-56. doi:10.1016/j.plefa.2017.05.005.
- MT. Nakamura, HP. Cho, SD. Clarke, et al., "Regulation of hepatic delta-6 desaturase expression and its role in the polyunsaturated fatty acid inhibition of fatty acid synthase gene expression in mice," *The Journal of Nutrition* 130(6) (2000) 1561-1565. doi:10.1093/jn/130.6.1561.
- M. Neuringer, S. Reisbick, J. Janowsky, et al., "The role of n-3 fatty acids in visual and cognitive development: current evidence and methods of assessment." *The Journal of pediatrics* vol. 125(5Pt2) (1994) S39-47. doi:10.1016/s0022-3476(06)80735-3
- Uauy. R, D. Alan, et al., "Nutrition in brain development and aging: role of essential fatty acids." *Nutrition reviews* vol. 64(5Pt2) (2006) S24-91. doi:10.1301/nr.2006.may.s24-s33
- GY. Sun, A. Simonyi, KL. Fritsche, et al., "Docosahexaenoic acid (DHA): An essential nutrient and a nutraceutical for brain health and diseases," *Prostaglandins, Leukotrienes, and Essential Fatty Acids* 136 (2018) 3-13. doi:10.1016/j.plefa.2017.03.006.
- Y. Qu, HL. Zhang, XP. Zhang, et al., "Arachidonic acid attenuates brain damage in a rat model of ischemia/reperfusion by inhibiting inflammatory response and oxidative stress," *Human & Experimental toxicology* 37(2) (2018) 135-141. doi:10.1177/0960327117692134.
- PR. Kumar, MM. Essa, S. Al-Adawi, et al., "Omega-3 Fatty acids could alleviate the risks of traumatic brain injury - a mini review," *Journal of Traditional and Complementary Medicine* 4(2) (2014) 89-92. doi:10.4103/2225-4110.130374.
- P. Greengard, F. Valtorta, AJ. Czernik, et al., "Synaptic vesicle phosphoproteins and regulation of synaptic function," *Science (New York, N.Y.)* 259(5096) (1993) 780-785. doi:10.1126/science.8430330.
- TC. S dhof, JE. Rothman, "Membrane fusion: grappling with SNARE and SM proteins," *Science (New York, N.Y.)* 323(5913) (2009) 474-477. doi:10.1126/science.1161748.
- F. Darios, C. Wasser, A. Shakirzyanova, et al., "Sphingosine facilitates SNARE complex assembly and activates synaptic vesicle exocytosis," *Neuron* 62(5) (2009) 683-694. doi:10.1016/j.neuron.2009.04.024.
- F. Calderon, HY. Kim, "Docosahexaenoic acid promotes neurite growth in hippocampal neurons," *Journal of Neurochemistry* 90(4) (2004) 979-988. doi:10.1111/j.1471-4159.2004.02520.x.
- A. Yoshii, M. Constantine-Paton, "BDNF induces transport of PSD-95 to dendrites through PI3K-AKT signaling after NMDA receptor activation," *Nature Neuroscience* 10(6) (2007) 702-711. doi:10.1038/nn1903.

- M. Migaud, P. Charlesworth, M. Dempster, et al., "Enhanced long-term potentiation and impaired learning in mice with mutant postsynaptic density-95 protein," *Nature* 396(6710) (1998) 433-439. doi:10.1038/24790.
- NG. Bazan, MF. Molina, WC. Gordon, et al., "Docosahexaenoic acid signalolipidomics in nutrition: significance in aging, neuroinflammation, macular degeneration, Alzheimer's, and other neurodegenerative diseases," *Annual Review of Nutrition* 31 (2011) 321-351. doi:10.1146/annurev.nutr.012809.104635.
- CN. Serhan, N. Chiang, J. Dalli, "The resolution code of acute inflammation: Novel pro-resolving lipid mediators in resolution," *Seminars in Immunology* 27(3) (2015) 200-215. doi:10.1016/j.smim.2015.03.004.
- Z. Khabir, A. Abdelhafez, F. Camponovo, et al., "Role of the EPA: DHA dosing ratio in omega-3 supplements on blood fatty acid profiles and inflammation: a systematic review and meta-analysis," *Critical reviews in food science and nutrition* 22 (2026) 1-22. doi:10.1080/10408398.2026.2615693
- LN. Nguyen, D. Ma, G. Shui, et al., "Mfsd2a is a transporter for the essential omega-3 fatty acid docosahexaenoic acid," *Nature* 509(7501) (2014) 503-506. doi:10.1038/nature13241.
- JA. Allen, RA. Halverson-Tamboli, MM. Rasenick, et al., "Lipid raft microdomains and neurotransmitter signalling," *Nature Reviews. Neuroscience* 8(2) (2007) 128-140. doi:10.1038/nrn2059.