Effects of Distinct n-6 to n-3 Polyunsaturated Fatty Acid Ratios on Insulin Resistant and AD-like Phenotypes in High-Fat Diets-Fed APP/PS1 Transgenic Mice

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Research Article

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Abstract

Background: Type 2 diabetes mellitus (T2DM) and Alzheimer's disease (AD) are two prevalent diseases with comparable pathophysiological features and genetic predisposition. Polyunsaturated fatty acids (PUFAs) are essential in maintaining normal brain function. However, little is known about the impact of dietary n-6/n-3 PUFA ratio on AD-like pathology, especially in high-fat diet (HFD)-fed AD model mice.

Methods: In the present study, the APP/PS1 mice were treated with 60% HFD for 3.5 months to induced insulin resistance. After that, 45% HFD with different n-6/n-3 PUFA ratios (n-6/n-3=1:1, 5:1 or 16:1) was applied for additional 3.5 months treatment. Following the dietary intervention, the behavior of mice was observed using the Water maze. Following behavioral testing, the animals were euthanized, and serum and tissue samples were collected for biochemical, histological and pathological analyses and evaluation. Cortical fatty acid profile was measured by gas chromatography. Western Blot and immunohistochemistry methods were used to detect protein expression of molecules related to AD pathology and insulin signaling pathway(s) in the brain sample tissues. Immunofluorescence assay was used to uncover the expression and migration of NF- κ B in the cortex. qPCR method was applied to determine the gene expression of cortical pro-inflammatory cytokines.

Results: HFD caused insulin resistance, increased serum IL-6 and TNF- α level, elevated cortical soluble A β ₁₋₄₀, A β ₁₋₄₂ content, and increased brain n-6/n-3 PUFAs ratio in APP/PS1 mice. Increased APP and BACE1 protein expression and p-IR/IR ratio, but decreased pro-inflammatory cytokines mRNA expression was observed in the cortex from 60% HFD-fed APP/PS1 mice. N-3 PUFAs rich diet (n-6/n-3=1:1) relieved insulin resistance and hyperlipidemia induced by 60% HFD. Cortical soluble A β ₁₋₄₀ and A β ₁₋₄₂ contents, the expression of cortical APP, GLUT3, insulin metabolism related molecules, and NF- κ B pathway downstream pro-inflammatory cytokines showed a dietary n-6/n-3 PUFAs ratio-dependent way, indicating that dietary n-6/n-3 PUFA ratio plays a critical role in modifying the responses of serum inflammatory cytokine, AD pathology, cortical n-6/n-3 PUFAs ratio, insulin signaling and neuroinflammation to HFD treatment.

Conclusion: Dietary n-6/n-3 PUFA ratio play an important role in modifying AD pathophysiology, insulin signaling pathway, and neuro-inflammation response to high fat diet treatment in brain.

Background

With accelerated aging of the world's population, dementia is undoubtedly becoming a growing global public health concern. Alzheimer's disease (AD) is the leading cause, accounting for about 70% of all dementia cases [1]. Although, tremendous efforts have been made in understanding the pathogenesis of AD, unfortunately, there still remains no available definitive or effective disease-modifying treatment approaches. Therefore, targeting AD at an earlier stage and mitigating or delaying the disease process seems to be promising strategies to help prevent AD.

Metabolic disturbances in the brain are reported to be principally involved in the onset and progression of AD [2]. Recent population-based epidemiological studies indicate that diabetic patient at an increased risk of developing AD [3]. Furthermore, about 80% of AD patients are also diagnosed with diabetes or glucose intolerance [4], and significantly reduced quantities of both insulin and insulin-like growth factor 1 (IGF-1) were observed in AD subjects' postmortem brains [5], suggesting that diabetes may play a causative role in the development of AD pathogenesis. Altered insulin signaling pathway in type 2 diabetes mellitus (T2DM) patients, including raised fasting blood glucose, glucose intolerance and insulin resistance, might form a coordinated link between AD and T2DM, and it was also speculated that AD could be a third form of diabetes (type $\times$ diabetes) [6]. Since AD and T2DM are the predominant underlying health conditions affecting the aging population, the largest volume of AD and T2DM can undoubtedly be anticipated, in low- and middle-income countries [7]. However, to date, the underlying mechanisms involved in diabetes-induced cognitive impairment and pathogenesis are not fully elucidated. It is of critical significance to illuminate the underlying molecular interactions between diabetes and AD, which would enable strategies for early prevention and/or intervention of dementia.

The long-chain polyunsaturated fatty acids (LCPUFAs) are important components of neuronal membranes, and essential for neuronal membrane integrity and function. The n-3 PUFAs, especially docosahexaenoic acid (DHA), are the major constituting acids of neurological and retinal membranes [8]. Data from population-based epidemiological studies consistently showed that the consumption of n-3 PUFAs-rich diets potentially reduces the risk of developing neurological disorders [9]. Reduced or depletion of n-3 PUFAs levels from erythrocyte membranes was observed in AD patients [10]. A retrospective study including 1,188 elderly American subjects reported that low levels of circulating DHA may pose a significant risk in the development of Alzheimer's dementia [11]. On the contrary, n-6 PUFAs and their conversion products favor immune and inflammatory reactions, which can contribute to the development of dementia. High erythrocyte total n-6 PUFA concentration was also suggested to negatively correlate with cognitive function [12]. Furthermore, diets enriched in n-6 PUFAs have also been reported to increase the production of arachidonic acid (ARA), causing islet inflammatory damages, ultimately contributing to insulin resistance and diabetes, which were proposed risk factors for dementia in aging subjects [13]. All these data demonstrate the important role of n-6 and n-3 PUFAs alterations in influencing the pathological process of dementia. A balanced intake of both n-6 and n-3 PUFA rations may be essential for reducing oxidative stress, inflammatory status, the incidence of diabetes, and thereby, decreasing the prevalence of AD. Data from the 2002 survey on nutrition and health status of Chinese residents reported that the values of n-6 to n-3 polyunsaturated fatty acid ratios (n-6/n-3) in urban and rural residents were 7.6 and 8.0 respectively [14], which are much higher than the recommended Chinese dietary n-6/n-3 ratio reference (4–6:1). In western countries, the dietary n-6/n-3 PUFA ratio was reported as 20:1, or even higher [15]. Currently, to our knowledge, few studies have explored the associated impact of dietary n-6/n-3 PUFA ratio on AD-like pathology and insulin metabolism in the brain, especially in HFD-treated AD model mice. The present study mainly focuses on clarifying the impacts of dietary n-3/n-6 PUFA ratio on AD-like and insulin resistant phenotypes in HFD-fed AD model mice. Our data highlight the significant role of dietary n-6/n-3 PUFAs ratio on affecting the

etiology of AD, as well as revealing the fundamental pathophysiological mechanisms of insulin resistant related dementia.

Methods

Animals and diet

A total of 36 male APP_{swe}/PS1_{dE9} transgenic mice and 6 sex-matched C57BL/6J control mice (all at 4 weeks old) were purchased from the experimental animal center of Capital Medical University. The mice were maintained on a 12:12 light-dark cycle and allowed access to water and food *ad libitum*. Experiment protocols were licensed according to Capital Medical University Animal Care and Use Committee regulations and associated guidelines (AEEI-2019-071). All diets were purchased from SYSE Bio-tech. Co. LTD, Changzhou, China. The normal diet (ND) and HFD differ in the composition of fat added to the diet. Full details of the diet are provided in Table 1.

Table 1
Composition of experimental diets.

Nutrients in diet(g/kg)	Normal control diet	60% HFD	45% HFD	45% HFD-N3	45% HFD-N6
Protein	192	260	240	240	240
Carbohydrate	673	260	410	410	410
Fat	350	350	240	240	240
kcal	3850	5240	4730	4730	4730
Ingredient					
Casein	200	200	200	200	200
L-Cystine	3	3	3	3	3
Corn Starch	315	0	72.8	72.8	72.8
Maltodextrin	35	125	100	100	100
Sucrose	350	68.8	172.8	172.8	172.8
Cellulose	50	50	50	50	50
Soybean oil	9.1	25	34.3	0	21
Lard	33.5	245	158	165.5	153.5
Perilla oil	0	0	0	37	0
Flaxseed oil	2.4	0	10.2	0	1.9
Corn oil	0	0	0	0	26.1
Mineral Mix	45	45	45	45	45
Vitamin Mix	10	10	10	10	10
Fatty acid					
SFA	12.4	105.4	71.6	71.3	70.6
MUFA	17.1	112.3	78.4	76.9	79.4
PUFA	12.4	52.5	52.6	54.5	52.5
n-6/n-3 PUFA	5.0	20.0	5.0	1.1	16.0
HFD: high fat diet; SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; IU: international unit.					

Treatment

According to baseline fasting blood glucose level, 36 male APP/PS1 mice were randomly split into 2 treatment groups: (1) ND group ($n = 6$): the mice were fed with a standard diet, in which 10% kcal are derived from fat chow (10 kcal%, Product code: D18091704); (2) HFD group ($n = 30$): in this group, the mice were fed with high-fat diet, in which 60% kcal are derived from fat (60 kcal%, Product code: D12492). Sucrose content per gram was equivalent between the two diets. Diet intakes of animals were monitored every 3 days, and body weights were assessed weekly. Plasma glucose levels of APP/PS1 mice treated with 60% HFD were monitored monthly. After 60% HFD dietary intervention for 3.5 months, glucose tolerance test (GTT) was performed to assess insulin resistance phenotype. Any mouse that did not show an increase in plasma glucose in comparison with control mice was excluded from the study. At the age of 4.5 months, the mice with increased fasting glucose level were further randomly divided into 4 subgroups (at least 6 mice for each group): (1) the high n-6/n-3 PUFA ratio diet group: the mice were provided with 45% HFD with n-6/n-3 = 16:1 (Product code: D18091701); (2) the medium n-6/n-3 PUFA ratio diet group: the mice were treated with 45% HFD with n-6/n-3 = 5:1 (Product code: D8091703); (3) the low n-6/n-3 PUFA ratio diet group: the mice were treated with 45% HFD with n-6/n-3 = 1:1 (Product code: D18091702); (4) the 60% HFD group: the mice were fed with their original 60% HFD diet. The control APP/PS1 mice and C57BL/6J mice were maintained on their original control diets. After 3.5 months under these dietary regimens, the mice were used for GTT and behavioral measurement. The whole schematic diagram of animal dietary intervention is shown in *Supplementary Fig. 1*.

Fasting blood glucose levels and GTT

Blood glucose levels were measured per month, using a standard glucometer (Accu-Chek, Roche) with blood obtained from the tail tip. GTT were performed at the time point of 60% HFD intervention for 14 weeks, and the end of the experiment.

Behavioral testing

At the end of the dietary intervention, the spatial learning and memory abilities of animals were analyzed by Morris water maze as described previously [16]. The escape latency, path length travelled to arrive at the platform, average swim speed, and platform site crossings were recorded with a video tracking system (Water Maze 2.6 Institute of Materia, Chinese Academy of Medical Sciences DMS-2, Beijing, China).

Tissue preparation

Following behavioral testing, all mice were euthanized and sacrificed. Their brains were removed and separated along the midsagittal sulcus. Half of each brain was immediately placed in a cold saline solution and then the cortical and hippocampal regions were dissected and stored at -80°C until used for biochemical measurements. The other half side of the brain was used for the histological studies. Other tissue samples including liver, muscle and adipose tissue were frozen at -80°C for biochemical assays or fixed in formalin for histological analyses.

Histology, immunofluorescent and immunohistochemical assay

The liver samples were embedded in 10% formaldehyde buffered solution, subsequently embedded in paraffin blocks, and sectioned into slices of 5 μ m, and then stained with hematoxylin-eosin (HE). Specimens were examined under a light microscope. Beta-amyloid plaque in the cortex and hippocampus was measured by using Congo red (KeyGEN BioTECH, Nanjing, China) staining kit.

Immunofluorescent (IF) analysis was applied to detect the expression and activation of cortical nuclear factor kappa-B p65 (NF- κ B p65). The 4',6-diamidino-2-phenylindole (DAPI) glows blue by UV excitation wavelength 330–380 nm and emission wavelength 420 nm, and NF- κ B p65 glows red by excitation wavelength 510–560 nm and emission wavelength 590 nm by using laser scanning confocal microscope (Leica, Solms, Germany).

Immunohistochemistry (IHC) assay was applied for measuring glucose transporters (GLUTs) in tissues. Briefly, brain, liver, skeletal muscle and omental fat tissue samples were fixed for 48 hours, embedded in paraffin, and then sectioned into slices of 5 μ m. IHC was performed on paraffin-embedded sections using antibodies directed against GLUT2, (1:100 dilution, Cell Signaling Technology, USA), GLUT3, (1:100 dilution, Cell Signaling Technology, USA), and GLUT4 (1:100 dilution, Proteintech, USA). Secondary DAB antibody was used for positive detection. Then, the section was observed and photographed by electric microscope (OLYMPUS BX61).

Cortical fatty acids profile measurement

The fatty acid concentration in the cortexes was determined according to the method described previously [17]. Fatty acid compositions of 3–4 brains per group were reported, and the fatty acids contents were expressed as g/100 g total fatty acids.

ELISA and biochemical assay

Serum TNF- α and IL-6 contents were measured using commercial ELISA kits (Immunoway, Suzhou, China) according to the manufacturer's instruction. Total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-c) level in the cortex was determined by ELISA kit (mlbio, Shanghai, China) according to manufacturer's instructions. Cortical insulin level was determined by ELISA kit (Elabscience Biotechnology Co., Ltd, Wuhan, China). The levels of soluble and aggregated A β _{1–40} and A β _{1–42} were measured by human β -Amyloid ELISA kits (Wako, Osaka, Japan) according to the manufacturer's instructions. Three independent measurements were performed for each sample.

Western blotting

A total of 20 μ g of protein lysis extracts were electrophoresed on Tris-tricine gradient gels as described previously [18]. And primary antibodies used in western blotting were shown in *Supplementary Table 1*.

RNA extraction and qRT-PCR

Cortical total RNA was extracted using Ultrapure RNA Kit (Cwbio, Jiangsu, China) according to the manufacturer's protocol. Reverse transcription and target gene amplification assays were performed as described previously [18]. The primers used for PCR were shown in *Supplementary Table 2*.

Statistical analysis

The results were reported as mean $\pm$ standard deviation (SD), and $P < 0.05$ was considered statistically significant. The data were calculated and analyzed by Prism (GraphPad Software Inc., San Diego, CA, USA). Comparisons between controls and treated groups were determined using one-way ANOVA followed by Bonferroni test.

Results

Bodyweight

Changes in body weight for the animals from different groups are depicted in Fig. 1A. During the first 3.5 months, 60% HFD-fed APP/PS1 mice showed significantly higher body weight than ND-fed C57BL/6J and APP/PS1 control mice. When changing the diet to 45% HFD, we found that the bodyweights of APP/PS1 mice decreased dramatically in comparison with 60% HFD-fed APP/PS1 mice, especially in mice treated with a low n-6/n-3 PUFA ratio diet; however, the bodyweights of these mice were continuously higher than ND-fed animals.

Serum glucose, insulin levels and lipid profile

Following 60% of HFD intervention for 3.5 months, the mice showed a significant increase of fasting blood glucose level and area of under glycemic curve (AUC) than normal diet-fed C57BL/6J and APP/PS1 mice ($P < 0.05$) (Fig. 1B). After changing 60% HFD to 45% HFD, a decrease in AUC value was observed in the APP/PS1 mice compared with the 60% HFD-fed animals, especially in the 45% HFD and 45% HFD-N3 diet-fed mice ($P < 0.05$) (Fig. 1D). Moreover, at the end of the experiment, 60% HFD-fed animals showed a significant increase in serum insulin level in comparison with 45% HFD-fed animals, and statistical significance was also observed between 60% HFD, 45% HFD and 45% HFD-N6 groups ($P < 0.05$) (Fig. 1E).

The serum lipid profile was presented in Table 2. We found that 60% HFD treatment significantly increased serum TC, LDL-c and high-density lipoprotein cholesterol (HDL-c) levels in APP/PS1 mice ($P < 0.05$). After changing the 60% HFD diet to 45% HFD, we observed a significant decrease in serum TC and HDL-c levels, and the mice fed with 45% HFD-N3 diet showed the lowest serum TC level ($P < 0.05$). Decrease in serum LDL-c level was only observed in 45% HFD-N3 diet-fed APP/PS1 mice ($P < 0.05$).

Table 2
Serum lipid levels in mice treated with different diet.

Parameters	C57BL/6J ND	APP/PS1 ND	APP/PS1 60% HFD	APP/PS1 45% HFD	APP/PS1 45% HFD- N3	APP/PS1 45% HFD- N6
TC(mmol/l)	2.84 ± 0.46	3.76 ± 0.33	5.64 ± 0.85 ^{ab}	4.65 ± 0.47 ^{abc}	3.67 ± 0.47 ^{acd}	4.32 ± 0.76 ^{ac}
TG(mmol/l)	0.41 ± 0.08	0.56 ± 0.09	0.38 ± 0.14	0.50 ± 0.08	0.58 ± 0.13 ^c	0.44 ± 0.06
HDL- c(mmol/l)	2.13 ± 0.44	2.49 ± 0.30	3.38 ± 0.60 ^{ab}	2.97 ± 0.20 ^a	2.58 ± 0.58 ^c	2.88 ± 0.37 ^{ac}
LDL- c(mmol/l)	0.27 ± 0.03	0.34 ± 0.03	0.50 ± 0.12 ^{ab}	0.47 ± 0.10 ^a	0.32 ± 0.07 ^{cd}	0.41 ± 0.14 ^a

Data were expressed as mean ± SD, n = 6 for each group. a: comparing with C57BL/6J ND group, $P < 0.05$. b: comparing with APP/PS1 ND group, $P < 0.05$. c: comparing with APP/PS1 60% HFD group, $P < 0.05$. d: comparing with APP/PS1 45% HFD group, $P < 0.05$. HFD: high fat diet; TC: total cholesterol; TG: triglyceride; HDL-c: high density lipoprotein cholesterol; LDL-c: low density lipoprotein cholesterol. C57BL/6J ND: C57BL/6J control mice treated with normal control diet; APP/PS1 ND: APP/PS1 mice treated with normal control diet; APP/PS1 60% HFD: APP/PS1 mice treated with 60% high fat diet for 7 months; APP/PS1 HFD-N3: APP/PS1 mice treated with mice 60% high fat diet for 3.5 months, then 45% high fat diet with n-3 PUFAs was supplied for another 3.5 months; APP/PS1 45% HFD: APP/PS1 mice were treated with 60% high fat diet for 3.5 months, then 45% high fat diet were provided for another 3.5 months; APP/PS1 45% HFD-N6: APP/PS1 mice were treated with 60% high fat diet for 3.5 months, then 45% high fat diet with n-6 PUFAs supplement were provided for another 3.5 months.

Hepatic histology

Compared with C57BL/6J control mice, the APP/PS1 control mice showed relatively higher lipid deposition in the livers. At the end of dietary intervention, the mice in the 60% HFD group showed a significant increase in hepatic lipid deposition. In contrast, hepatic lipid deposition was significantly lessened in mice treated with 45% HFD animals, especially in the n-3 PUFAs rich diet-treated mice (Fig. 1F).

Inflammatory factors in serum

As shown in Fig. 1G, 60% HFD-treated APP/PS1 mice showed higher serum IL-6 and TNF- α levels than ND-fed control mice, and the difference of TNF- α level reached statistical significance compared with control mice ($P < 0.05$). The 45% HFD decreased serum IL-6 and TNF- α level, and the APP/PS1 mice that received 45% HFD-N3 diet exhibited the lowest serum TNF- α level ($P < 0.05$), and the difference between 45% HFD-N3 and 45% HFD-N6 groups was also significant ($P < 0.05$).

Cortical n-3 and n-6 PUFAs level

Cortical n-6 and n-3 PUFA levels were shown in Table 3. ND-fed control mice showed lower cortical n-6 PUFAs than 60% HFD-fed APP/PS1 mice ($P < 0.05$). Compared with 45% HFD groups, mice from 45%

HFD-N6 group showed the highest cortical n-6 PUFAs levels ($P < 0.05$). The 60% HFD-fed mice showed a significant increase of n-6/n-3 PUFA ratio in the cortex than control mice ($P < 0.05$). Mice from 45% HFD and 45% HFD-N3 groups showed a significant decrease in n-6/n-3 PUFAs ratio the in cortex than mice from 60% HFD and 45% HFD-N6 groups ($P < 0.05$)

Table 3
The content of cortical fatty acids in mice treated with different diets.

Fatty acids (% of total cortical fatty acids)	C57BL/6J ND	APP/PS1 ND	APP/PS1 60% HFD	APP/PS1 45% HFD	APP/PS1 45% HFD-N3	APP/PS1 45% HFD-N6
PUFA	16.45 ± 1.64	17.76 ± 0.10	19.39 ± 1.14	18.16 ± 1.21	19.40 ± 0.37 ^a	19.46 ± 1.74 ^a
n-6 PUFA	6.75 ± 0.59	6.96 ± 0.03	7.88 ± 0.49 ^a	7.13 ± 0.39 ^c	7.58 ± 0.30 ^a	7.83 ± 0.63 ^{ad}
n-3 PUFA	10.63 ± 1.05	10.73 ± 0.06	11.34 ± 0.61	10.90 ± 0.80	11.64 ± 0.25	11.45 ± 1.11
DHA	10.19 ± 1.05	10.38 ± 0.08	10.95 ± 0.54	10.47 ± 0.81	11.18 ± 0.26	10.99 ± 1.07
EPA	0.27 ± 0.01	0.21 ± 0.02	0.23 ± 0.06	0.26 ± 0.06	0.28 ± 0.03	0.29 ± 0.06
n-6/n-3	0.63 ± 0.01	0.65 ± 0.01	0.69 ± 0.01 ^{ab}	0.65 ± 0.02 ^c	0.65 ± 0.03 ^c	0.68 ± 0.02 ^{abde}

Data were expressed as mean ± SD, $n = 6$ for each group. ^a: comparing with C57 ND group, $P < 0.05$. ^b: comparing with APP/PS1 ND group, $P < 0.05$. ^c: comparing with APP/PS1 60% HFD group, $P < 0.05$. ^d: comparing with APP/PS1 45% HFD group, $P < 0.05$. ^e: comparing with APP/PS1 45% HFD-N3 group, $P < 0.05$. HFD: high fat diet; PUFA: polyunsaturated fatty acid; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid. C57 ND: C57BL/6J control mice treated with normal control diet; APP/PS1 ND: APP/PS1 mice treated with normal control diet; APP/PS1 60% HFD: APP/PS1 mice treated with 60% high fat diet for 7 months; APP/PS1 HFD-N3: APP/PS1 mice treated with mice 60% high fat diet for 3.5 months, then 45% high fat diet with n-3 PUFAs was supplied for another 3.5 months; APP/PS1 45% HFD: APP/PS1 mice were treated with 60% high fat diet for 3.5 months, then 45% high fat diet were provided for another 3.5 months; APP/PS1 45% HFD-N6: APP/PS1 mice were treated with 60% high fat diet for 3.5 months, then 45% high fat diet with n-6 PUFAs supplement were provided for another 3.5 months.

Behavior

As showed in Fig. 2A, after 4 days of training, both control mice and HFD-fed APP/PS1 mice showed a significant decrease in escape latency. On day 5, the mice treated with n-3 PUFA rich 45% HFD showed increased escape latency than mice from other groups, however, we did not observe any statistical significance between the groups ($P > 0.05$). Additionally, the APP/PS1 mice treated with 60% HFD and n-3 PUFA rich diet showed relatively shorter platform crossing times on day 5 than mice from other groups, but the differences between groups are not statistically significant ($P > 0.05$).

AD pathology

As shown in Fig. 2B, in comparison with C57BL/6J control mice, typical A β plaque deposition was found in the cortexes and hippocampi of APP/PS1 control mice. The treatment of 60% HFD seemed to have no significant effect on the deposition of A β plaque in cortexes and hippocampi of the APP/PS1 mice. Diets with different n-6/n-3 PUFA ratios also had no significant impact on cortical and hippocampal A β plaque deposition in the APP/PS1 mice.

As shown in Fig. 2C, treatment of 60% HFD caused a significant increase of soluble A β_{1-40} and A β_{1-42} contents in the cortex ($P < 0.05$). The mice treated with n-3 and n-6 PUFAs rich 45% HFD showed lower cortical soluble A β_{1-40} and A β_{1-42} content than mice-fed with 60% HFD ($P < 0.05$). We also found that diets with different n-6/n-3 PUFAs ratio showed discrepant effects on cortical soluble A β_{1-40} and A β_{1-42} contents, which was indicated by a much lower cortical A β_{1-40} and A β_{1-42} contents in mice that received n-6 PUFAs rich 45% HFD than mice treated with n-3 PUFAs rich 45% HFD ($P < 0.05$). The 60% HFD-fed mice showed lower cortical aggregated A β_{1-40} and A β_{1-42} than control mice ($P < 0.05$). The 45% HFD treatment increased cortical aggregated A β_{1-40} and A β_{1-42} content dramatically in comparison with mice from control and 60% HFD groups ($P < 0.05$). The n-3 and n-6 PUFAs rich 45% HFD significantly reduced cortical aggregated A β_{1-40} and A β_{1-42} content, and the n-6 PUFAs rich 45% HFD showed the strongest impact on reducing cortical aggregated A β_{1-40} and A β_{1-42} content ($P < 0.05$). We also found that the mice treated with n-3 and n-6 PUFAs rich 45% HFD exhibited a lower aggregated A β_{1-40} to A β_{1-42} ratio than mice from other treatment groups ($P < 0.05$).

Cortical LDL-c and TC levels are shown in Fig. 2D. Compared with the control mice, the mice fed with 60% HFD showed a significant increase of cortical LDL-c content ($P < 0.05$). In contrast, 45% HFD-fed mice showed lower cortical LDL-c levels as compared with mice from 60% HFD-fed group, and the n-6 PUFAs rich 45% HFD showed the lowest cortical LDL-c content than mice in other HFD-fed groups ($P < 0.05$). HFD treatment did not affect cortical TC levels, and no statistical significance was observed between the groups ($P > 0.05$).

Cortical amyloid precursor protein (APP) and β -secretase-1 (BACE1) protein expression were shown in Fig. 2E. APP/PS1 control mice showed higher cortical APP protein expression than C57BL/6J control mice. The treatment of 60% HFD further up-regulated the cortical APP protein expression in APP/PS1 mice. After changing 60% HFD diet to 45% HFD diet, the decreased APP protein expression was observed in the cerebral cortex of APP/PS1 mice, especially in the mice from 45% HFD and 45% HFD-N6 groups. The dietary intervention did not affect cortical BACE1 protein expression, and no difference was observed between the groups ($P > 0.05$).

GLUT3 and insulin metabolism-related molecular in the cortex

Cortical GLUT3 protein expression was shown in Fig. 3A. APP/PS1 control mice displayed lower cortical GLUT3 protein expression in C57BL/6J control mice. The treatment of 60% HFD significantly increased the cortical GLUT3 protein expression in APP/PS1 mice. After replacing 60% HFD with 45% HFD, cortical GLUT3 protein expression was down-regulated and the mice from n-3 PUFAs rich 45% HFD group showed relatively lower cortical GLUT3 expression than mice treated with 45% HFD and n-6 PUFAs rich 45% HFD.

Cortical insulin level was shown in Fig. 3B. The APP/PS1 control mice showed similar cortical insulin level as the C57BL/6J control mice and the treatment of HFDs did not affect cortical insulin level in APP/PS1 mice, although the mice from the 45% HFD group showed decreases in cortical insulin levels, no statistical significance was observed between the groups ($P > 0.05$).

High-fat diet treatment did not affect cortical insulin receptor (IR), insulin receptor substrate 1 (IRS-1), insulin degrading enzyme (IDE) and phosphorylated-insulin receptors (p-IR) protein expressions in APP/PS1 mice. The n-6 and n-3 rich 45% HFD-fed mice showed a significant decrease of cortical p-IR/IR ratio, and the mice fed with n-6 PUFA rich 45% HFD showed the lowest cortical p-IR/IR ratio than mice from the other groups ($P < 0.05$) (Fig. 3C & 3D).

As shown in Fig. 3E, APP/PS1 control mice showed relatively higher cortical glycogen synthase kinase-3 β (GSK-3 β), but lower p-GSK3 β /GSK-3 β ratio than C57BL/6J control mice. The 60% HFD treatment caused a decrease of cortical phosphorylated-glycogen synthase kinase-3 β (p-GSK3 β) protein expression in APP/PS1 mice, but no effect on cortical GSK3 β protein expression. The n-6 and n-3 rich 45% HFD caused a significant decrease of cortical GSK3 β protein expression, and the lowest p-GSK3 β protein expression was observed in n-6 rich 45% HFD-fed mice ($P < 0.05$). The ratio of p-GSK3 β to GSK3 β showed a decreasing trend in HFD-treated animals, and the mice from the 45% HFD-N6 group exhibited the lowest cortical p-GSK3 β /GSK-3 β ratio ($P < 0.05$).

Expression of cortical NF- κ B p65 and the mRNA of inflammatory cytokines

The expression of cortical NF- κ B p65 protein expression was shown in Fig. 4A. The red fluorescence was observed in the cortices of all mice groups, indicating that NF- κ B was abundantly expressed in the cortex. Meanwhile, mice from 60% HFD-fed group and n-6 PUFAs rich 45% HFD-fed group exhibited higher NF- κ B p65 expression in the nuclei than mice in other groups, indicating a migration of NF- κ B p65 from the cytoplasm to nuclei.

We further examined the mRNA expression of pro-inflammatory cytokines of the NF- κ B downstream pathway. As illustrated in Fig. 4B, the APP/PS1 control mice displayed higher cortical mRNA expression of TNF- α , COX2, IL-6 and IL-1 β , but lower iNOS mRNA than C57BL/6J control mice ($P < 0.05$). Compared with APP/PS1 control group, the expression of TNF- α , COX2, and IL-6 were down-regulated in APP/PS1 mice receiving either 60% HFD or 45% HFD intervention ($P < 0.05$). The same alterations were observed in

n-6 PUFAs rich 45% HFD group and n-3 PUFAs rich 45% HFD group ($P < 0.05$). In addition, we found that n-3 PUFAs rich 45% HFD could potentially elevate the mRNA expression of IL-1 β in APP/PS1 mice compared to the C57BL/6J control group, the APP/PS1 60% HFD-fed group and APP/PS1 45% HFD-fed group ($P < 0.05$). N-6 rich 45% HFD caused a significant decrease of cortical IL-1 β mRNA expression compared to 45% HFD-N3 group ($P < 0.05$).

Discussion

In the current study, AD model mice (APP/PS1) were treated with a HFD to induce insulin resistance and the effect of diet with different n-6/n-3 PUFAs ratios on insulin resistance phenotype and AD-like pathology was explored. Our data showed that HFD efficiently induced insulin resistance in APP/PS1 mice, which was indicated by the dramatic increase in body weight, blood glucose, lipids and insulin levels, glucose tolerance, and severe hepatic lipidosis as compared with the normal diet-fed control mice. We also found that a reduction of fat-derived energy (as demonstrated with a 45% HFD) was efficient in reversing the insulin resistant phenotype that was initially caused by the 60% HFD in APP/PS1 mice.

Dietary n-6/n-3 PUFA ratio-dependent regulatory effect of HFD on insulin resistance has been previously described, in which the author reported that rats fed with HFD containing n-3 PUFAs (n-6/n-3 = 1:1) showed significantly higher insulin sensitivity than rats treated with high n-6 PUFA HFD (n-6/n-3 = 4:1) [19], suggesting that an increase in dietary n-3 PUFAs proportion could improve insulin sensitivity and prevent HFD-induced insulin resistance. It has also been postulated that the visceral adipose tissue (e.g., fatty liver) induced increased circulating free fatty acids (FFAs) and triglycerides, released a significant amount of pro-inflammatory cytokines, and these cytokines disrupt the insulin action, consequently leading to decreased insulin sensitivity and resistance [20]. Consistent with these studies, our data indicated that diets with different n-6/n-3 PUFAs ratios discrepantly affected insulin resistance phenotype in AD model mice, which was demonstrated by the significant decrease in body weight, serum TC, LDL-c levels and hepatic lipid deposition in the initial HFD-induced APP/PS1 mice later treated with high n-3 PUFA diet (n-6/n-3 = 1:1). These results align well with the previous report, which showed that n-6 PUFA rich diet-fed animals were more likely to manifest liver steatosis [21], while n-3 PUFAs intervention significantly reduced serum TC and LDL-c levels [22], and significantly relieved the signs of HFD-mediated hepatic steatosis. Unexpectedly, in our study, we did not observe the different reflection of serum glucose and insulin levels to diets with different n-6/n-3 PUFA ratios, demonstrating that the regulation of lipids metabolism may partly account for the discrepant insulin resistance relieving effect of the high n-3 PUFA diet (n-6/n-3 = 1:1) in AD model mice.

A study has shown that an HFD resulted in a significant increase in plasma IL-6 and TNF- α levels in APP/PS1 mice [23]. Consistent with this report, we observed significantly increased levels of serum IL-6 and TNF- α in 60% HFD-fed mice, and the elevation of serum pro-inflammatory markers were dramatically lowered following treatment with 45% HFD. Moreover, dietary n-6/n-3 ratio-dependent inhibition of serum TNF- α level was also observed, which was indicated by the lowest serum TNF- α level in n-3 PUFA rich diet-fed APP/PS1 mice. High dietary n-6/n-3 PUFAs ratio has been reported to correlate with elevated plasma

concentration of IL-6 and TNF- α in rats [24]. High n-6/n-3 PUFAs ratio (4:1) HFD-fed SD rats also displayed higher serum IL-6 and TNF- α than low n-6/n-3 ratio (1:1) HFD-fed animals [19]. Reduced IL-6 and TNF- α gene expression have been reported in skeletal muscle tissue of diabetic rat-supplemented with n-3 PUFA [25]. All these results suggested that n-3 PUFA enriched diet might antagonize HFD-induced pro-inflammatory response, and dietary n-6/n-3 PUFAs ratio might have potent modulatory effects on inflammatory reaction in experimental animals.

Reduced n-3 PUFA levels have been found in the brain (mainly in the hippocampus) of AD patients, and the increase of n-6/n-3 PUFAs ratio in the brain has been considered to be associated with the increased risk of AD [26]. In the current study, we did not observe a significant difference in cortical PUFA between C57BL/6J control mice and APP/PS1 control mice, indicating that the mutation of APP gene did not alter the cortical PUFAs profile in the AD model mice. Of note, the treatment of HFD caused an increased trend in cortical PUFAs profile in APP/PS1 mice, although the difference was not statistically significant when compared with control mice. Importantly, our data indicated the potential effect of n-6 PUFA rich diet on elevating cortical n-6 PUFA level and n-6/n-3 PUFAs ratio in APP/PS1 mice. Moreover, this increase of cortical n-6 PUFA level and n-6/n-3 PUFAs ratio effect could be antagonized by 45% HFD (n-6/n-3 = 5:1) and 45% HFD-N3 (n-6/n-3 = 1:1) diets. All these results indicated a strong correlation between cerebral PUFAs profile and dietary fatty acids status, indicating a potential modifying role of dietary n-6/n-3 PUFAs ratio on cerebral n-6 and n-3 PUFA levels.

The senile plaque remains the pathological hallmark sign in AD process, and results from clinical studies have also shown that n-3 PUFA interventions can alleviate AD pathological changes and prevent synaptic degeneration [27]. Data from experimental animals also found that n-3 PUFAs rich diet has been effective in inhibiting the formation of amyloid plaque in the hippocampus and cortex in APP transgenic mice [28]. In our study, although the number of plaques in 45% HFD-N3 diet-fed APP/PS1 mice showed a slightly lesser number of senile plaques than mice treated with control diet, 60% HFD and 45% HFD-N6 diet, no statistical significance was observed between groups. We further detected cortical soluble and insoluble A β_{1-40} and A β_{1-42} levels. Consistent with a previous report [29], we found that HFD treatment increased cortical soluble A β_{1-40} and A β_{1-42} levels in APP/PS1 mice, indicating that HFD might promote the generation of neurotoxic soluble A β_{1-42} and potentially accelerate synaptic degeneration and AD pathological process. It was reported that the excessive increase of lipids (especially cholesterol) in the brain could modify the composition and function of lipid raft in neuronal membranes and enhance β -secretase-mediated APP processing, ultimately promoting over-generation of A β [30]. Thus, we further examined cortical cholesterol levels in mice treated with different diets. The dramatic increase in cortical LDL-c was observed in 60% HFD-fed APP/PS1 mice, but HFD fortified with PUFAs significantly reduced cortical LDL-c levels, especially in HFD-N6 diet-fed group. This finding is consistent with a previous study, that identified n-6 PUFA as a potent down-regulator of LDL-c level and alleviating depressed LDL receptor activity while promoting the diminishment of circulating LDL-c [31]. We, therefore, speculated that the disturbance of cerebral lipid hemostasis in HFD-fed animals might contribute to the increased cortical soluble A β content observed in the AD model mice. Ettcheto *et al.* demonstrated that HFD-fed APP/PS1

mice showed significantly higher cerebral insoluble A β_{1-42} content than ND-fed control animals [32]. On the contrary, we found that 60% HFD treatment decreased cortical insoluble A β_{1-40} and A β_{1-42} levels. A study conducted in FAT-1 mice has found that n-3 PUFA enriched diet could promote the clearance of A β from the brain through the cerebral lymphatic system [33]. In our study, we observed an n-6/n-3 ratio-dependent effect of HFD on regulating cortical soluble and insoluble A β levels, which was demonstrated by lower soluble and insoluble A β levels in mice treated with 45% HFD-N3 and 45% HFD-N6 diets. Also, consistent with the change in LDL-c level as documented earlier, we found that the diet with the highest dietary n-6/n-3 ratio caused the most significant decrease in soluble and insoluble A β levels in the cortex, suggesting the important role of n-6/n-3 PUFA ratio in modulating cerebral A β_{1-40} and A β_{1-42} levels. Our findings also imply that specific PUFAs (n-3 or n-6) may be critically required by cerebral components to execute optimal brain function(s) and that could account for the observed discrepant levels of cortical A β and LDL-c in response to HFD fortified with high n-6/n-3 PUFAs ratio. Further studies are needed to uncover the underlying mechanisms behind the observed discrepant response of A β levels to dietary n-6/n-3 PUFA ratio.

It is well known that BACE1 catalyzes the formation of the amyloid-beta peptide from the APP. An increase in BACE1 activity enhanced the production of A β and triggered the deposition of A β plaque in the brain [34]. In our study, we only observed a decreased trend of APP in mice treated with different n-6/n-3 PUFA ratios without affecting the protein expression of BACE1. This finding suggests that the APP was modulated by PUFAs in HFD, and this modulatory effect was exclusive to BACE1 gene activity. The mismatched changes in APP and BACE1 protein expressions and cerebral A β content suggest the involvement of other potential molecular mechanisms in the homeostasis of A β in the brain.

The IR is a kind of receptor from the tyrosine kinase family. After binding with insulin, IR activates intracellular tyrosine kinase and initiates intracellular signaling pathways involving a series of physiological responses. Reduced IR sensitivity and hyperphosphorylation of IR have been reported in AD patients [35], potentially suggesting that impaired insulin responsiveness in the brain may be intrinsic to AD pathogenesis. In the current study, we found that the mice treated with n-3 and n-6 PUFA rich diets showed a significant decrease in cortical p-IR/IR ratio. The decrease of p-IR/IR ratio indicates the inhibition of insulin metabolic pathway, which in turn inhibits the glucose uptake. Vishal Kothari *et al.* also reported that the HFD-fed C57BL/6J mice exhibited a lower p-IR/IR ratio than the normal diet-fed control animals [36]. Contrary to that finding, in our study, 60% and 45% HFD did not cause a significant decrease of cortical p-IR/IR ratio, however, a decrease of cortical p-IR/IR ratio was observed, especially in mice treated with n-6 PUFA rich 45% HFD, suggesting that dietary PUFAs have the potential in regulating phosphorylation of IR in HFD-fed APP/PS1 mice. It has been reported that increasing pro-inflammatory cytokines (such as TNF- α and IL-6) can potentially disorient insulin metabolic action(s) and receptor functioning [37]. Excessively high n-6 PUFAs was reported to accelerate oxidative stress and oxidized LDL-c [38]. All, these results could partially explain the decrease of cortical p-IR/IR ratio which was especially observed in mice treated with n-6 PUFA rich 45% HFD.

Insulin mRNA expression has been found in both human and mouse brain samples. Rhea *et al.* also reported that insulin can transport across the blood-brain barrier (BBB) *in vivo* with either loss or inhibition of the signaling-related insulin receptor, but the binding of insulin to the brain endothelial cells would be decreased [39]. In our study, APP/PS1 mice showed similar cortical insulin levels with C57BL/6J control mice, indicating that the AD pathological changes did not affect the insulin content in the AD model mice. Also, we did not observe any alteration in cortical insulin level in mice treated with HFD. Stanley *et al.* reported that peripheral hyperinsulinemia could not affect cerebral insulin signaling and insulin levels [40]. Moreover, a study has proved that cells from the hippocampus and olfactory bulb can produce insulin [41]. Our study further confirms that the cerebral insulin homeostasis cannot be affected by peripheral hyperinsulinemia in APP/PS1 mice, and we reasonably hypothesized that this restriction to the hyper-permeability of insulin to the cortex may be tightly regulated by the BBB. The inconsistent changes of serum and cortical insulin level in response to HFD treatment indicated a discrepant regulating mechanism of peripheral and central insulin metabolism. Further investigations are needed to elucidate the underlying mechanism attributing to the discrepant peripheral and central insulin metabolism.

Glucose uptake in the brain is dependent on IR-stimulated translocation of glucose transporter in the plasma membrane. GLUT3 is extensively expressed in the cortex and plays an important role in neuronal glucose uptake. Evidence suggests that HFD has the potential of reducing cerebral GLUT3 protein expression and causing impaired cerebral glucose uptake [36]. Conversely, Kothari and colleagues' study reported a compensatory overexpression of GLUT3 in the brains of diabetic rats [42]. In the present study, APP/PS1 mice showed lower cortical expression of GLUT3 protein than C57BL/6J control mice, which indicated an AD-like pathology-mediated decrease of cerebral glucose uptake in the AD-model mouse. We also found that 60% HFD-fed APP/PS1 mice displayed a significant increase of cortical GLUT3 expression, suggesting that HFD was efficient in reversing the downregulated glucose uptake in the brain in AD model mice. These results indicated that the increased cortical expression of GLUT3 protein expression could be a compensatory genetic positive-feed response to the insulin resistance initially induced by the HFD, and potentially due to a decreased threshold to cortical insulin sensitivity (since the primary energy source to the brain is obtained from glucose). Prolonged exposure to HFD not only induces pro-inflammatory cytokines and insulin resistance but may also be involved in adaptive genetic feedback response to engage essential proteins and transporters to compensate and improve glucose or nutrient uptake into cells as we have observed with GLUT3 in these HFD-induced insulin resistant APP/PS1 mice. Relative lower cortical GLUT3 expression was found in APP/PS1 mice from 45% HFD-N3 group as compared with mice treated with 45% HFD and 45% HFD-N6 diets, indicating a dietary PUFAs-dependent optimal response of cerebral GLUT3 expression. On the contrary, we detected higher GLUT3 expression in the liver and GLUT4 expression in muscle in APP/PS1 control mice in comparison with C57BL/6J mice (*Supplementary Fig. 2A&B*). This observation suggests that there may be an overlap of peripheral (hepatic and skeletal) insulin resistance phenotype and AD-like pathological cascade in these ND-fed APP/PS1 mice, implying that AD pathologic process (particularly, APP gene dysfunction) may be a risk factor for peripheral insulin resistance. Further study is required to ascertain this observation. We, however, observed a significant decrease of these GLUTs in 60% HFD-fed APP/PS1 mice in liver and

muscle tissues (potentially due to a slightly increased threshold to peripheral insulin sensitivity) further indicating a discrepant peripheral and central glucose metabolism in response to HFD intervention in APP/PS1 mice. Additionally, the divergent sensitivities of liver or muscle GLUT3/4 expression in mice treated with the different diets also hint the role of dietary PUFA rations on glucose uptake in peripheral tissues.

IRSs are known to be major substrates of receptor kinases, mediating insulin-like growth factors (IGFs)/insulin signals to direct anabolic bioactivities, such as energy and glucose homeostasis, growth and adipocyte differentiation [43]. Postmortem studies have described a reduction in brain expression of IR adapter protein IRS-1 in AD subjects [44]. In comparison with C57BL/6J mice, significantly upregulated IRS-1 protein expression was found in the hippocampus of APP/PS1 mice [45]. The IDE is responsible for insulin breakdown in various tissues. Results from other studies have indicated that HFD-fed APP/PS1 mice showed significantly lower brain IDE protein expression than normal diet-fed control animals [46]. We found that the IRS-1 and IDE cortical protein expression remained unchanged in APP/PS1 mice treated with different diets. These results indicate that the effect of n-6/n-3 PUFA ratios in HFD on cerebral glucose homeostasis and insulin signaling pathway in APP/PS1 mice are not entirely attributable to the regulation of IRS-1 and IDE expression. The other mechanisms, such as the regulation of enzyme activity, interactions or binding between substrates and enzymes, might contribute to the HFD-mediated changes of central insulin metabolism, warranting further exploration.

The GSK3 β is a key molecule involving in neural progenitor cell proliferation, neuronal polarity, and neuroplasticity [47]. Insulin could stimulate phosphorylation of GSK3 β and consequently result in bidirectional (phosphorylation and inactivation) effect on glycogen synthase, an enzyme responsible for glycogenesis. Elevated total GSK3 β protein expression indicates hyper-activity of GSK3 β signaling [48]. In the pathology of AD, GSK3 β has been shown to promote Tau phosphorylation through the insulin/phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) signaling pathway [49]. In our study, APP/PS1 mice showed a relatively higher cortical GSK3 β expression but lower p-GSK3 β /GSK3 β ratio than C57BL/6J control mice, indicating a potential activation of the GSK3 β signaling pathway in this AD model mice. HFD treatment has been reported to cause a decrease in cortical p-GSK3 β expression and p-GSK3 β /GSK3 β ratio in APP/PS1 mice, suggesting a regulatory effect of HFD on the phosphorylation of GSK3 β and in modifying the activity of GSK3 β . Moreover, a decreased trend of cortical GSK3 β expression was found in 45% HFD-N3 and 45% HFD-N6 diets treated mice. Additionally, significant decreases of cortical p-GSK3 β and p-GSK3 β /GSK3 β ratio were also found in 45% HFD-N6 diet-fed APP/PS1 mice, indicating hyper-activation of GSK3 β in these high n-6/n-3 PUFA ratio diet-fed APP/PS1 mice. Together with the relatively high cortical GLUT3 protein expression observed in these animals, we speculate that diet with a high n-6/n-3 PUFA ratio might affect brain glucose metabolism through regulating the phosphorylation of GSK3 β , therefore affecting the uptake of glucose in the brain. Altogether, our findings indicated that the discrepant regulation of GSK3 β activation might contribute to the dietary n-6/n-3 PUFA ratio-associated modifying effect in insulin signaling pathway in the brain of APP/PS1 mice.

NF- κ B is a nuclear transcription factor that belongs to the Rel protein family, and accumulating evidence suggests its critical role in the pathophysiology of AD. Activated NF- κ B p65 in the brain could regulate the expression of many genes, most of which encode immune proteins with pro-inflammatory activities [50]. Consistent with a previous study [51], we detected that cortical NF- κ B p65 translocated from the cytoplasm to nuclei in the cortex in mice-treated with 60% HFD, indicating the activation of NF- κ B p65 signaling pathway. While, the treatment of PUFAs rich HFD treatment inhibited the intracellular translocation of NF- κ B p65, especially in n-3 PUFAs rich HFD-fed animals. We also found that APP/PS1 mice showed significantly up-regulated cortical TNF- α , COX2, IL-1 β and IL-6 mRNA expressions, but lower iNOS mRNA expression than C57BL/6J mice, demonstrating an enhanced neuro-inflammatory response in these AD model mice. Anthony Pinçon *et al.* reported that administration of a high-cholesterol diet caused 38% increase of cortical TNF- α mRNA expression in APP/PS1 mice as compared with control mice without affecting IL-6 and IL-1 β mRNA expressions [52]. Inconsistent with this report, we observed a significant decrease in cortical TNF- α , COX2 and IL-6 mRNA expression, but an increase in iNOS mRNA expression in HFD-fed APP/PS1 mice. The discrepancy between studies might attribute to the diversity of intervention diet or the study design. Moreover, we observed that the 60% and 45% HFDs did not affect cortical IL-1 β mRNA expression. In contrast, n-3 PUFAs-rich 45% HFD significantly increased cortical IL-1 β mRNA level, and a decrease expression was observed in mice treated with n-6 PUFAs-rich 45% HFD. Although it is commonly recognized that n-6 PUFA has pro-inflammatory functions as prostaglandin and leukotriene precursors, n-6 PUFA can also be converted into anti-inflammatory mediators or modulate the gene expression of the pro-inflammatory cytokine to exert anti-inflammatory effects. Alashmali *et al.* reported that adequate dietary n-6 PUFA intake restored hippocampal inflammation cytokine gene expression caused by intra-cerebroventricular lipopolysaccharide (LPS) injection [53]. We, therefore, speculate that the AD-like pathology in APP/PS1 mice might have caused hyperactivated neuroinflammation in the brain, which could be antagonized by the treatment with HFD. The inconsistent expressional pattern of pro-inflammatory cytokines in the cortexes of HFD-fed mice further indicated the potentially varied regulatory effect(s) of dietary fatty acids on the cerebral inflammatory response. Thus, further studies are needed to elucidate the relationship between HFD (especially with different PUFAs ratios) and neuroinflammation in modulating AD pathology process.

Limitations

A few limitations remain in this study. First, due to the young age of the mice and the short duration of the intervention, no significant differences were found in learning and memory abilities. Therefore, a much longer period of dietary intervention was required of to explore the persistent effects of dietary PUFAs on these behavioral indices. Second, we only used male APP/PS1 mice for experiment, while recent studies have shown that APP/PS1 transgenic mice showed a gender difference in behavior and AD-like pathology in brain [54]. Thus, it is essential to further investigate the sex-based effects of dietary PUFAs on APP/PS1 mice. Also, due to the limited brain species, we fail to detect the level of pro-inflammatory cytokine in the brain, thus, we could not provide a more accurate cerebral neuro-inflammatory profile in the experimental animals.

Conclusion

In conclusion, HFD induced insulin resistance phenotype in peripheral tissues and increased circulating pro-inflammatory cytokine levels in APP/PS1 mice. Dietary n-6/n-3 PUFA ratio plays a critical role in modifying AD pathological process, insulin signaling pathway, and neuroinflammation in the cortex in response to high-fat diet treatment. In preventing AD, optimal dietary n-6/n-3 PUFAs ratio should be taken into consideration in PUFA-fortified diet interventional strategy. Extensive studies are essentially needed to further uncover the relevant underlying molecular mechanisms and bridge the knowledge gap associated with the potential effects of dietary PUFAs on insulin resistance and cortical degeneration in AD.

Abbreviations

T2DM

Type 2 diabetes mellitus; AD:Alzheimer's disease; PUFAs:Polyunsaturated fatty acids; HFD:high-fat diet; ND:normal diet; DHA:Docosahexaenoic acid; ARA:arachidonic acid; GTT:glucose tolerance test; AUC:under glycemic curve; NF- κ B p65:Nuclear factor kappa-B p65; GLUTs:Glucose transporters; TNF- α :Tumor Necrosis Factor- α ; IL-1 β :Interleukin-1 β ; IL-6:Interleukin-6; COX2:Cyclooxygenase 2; iNOS:inducible nitric oxide synthase; TC:Total cholesterol; LDL-c:Low-density lipoprotein cholesterol; HDL-c:High-density lipoprotein cholesterol; IR:Insulin receptor; IRS-1:insulin receptor substrate 1; IDE:insulin degrading enzyme; p-IR:phosphorylated-insulin receptors; GSK-3 β :glycogen synthase kinase-3 β ; p-GSK3 β :phosphorylated-glycogen synthase kinase-3 β

Declarations

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interest

The authors declare that they have no competing interests.

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Authors' contributors

Linhong Yuan designed the work; Xiaojun Ma, Yujie Guo, Pengfei Li and Jingjing Xu performed most of the experiments; Shengqi Dong, Pengfei Li and Yanyan Gao carried out other lab works; Linhong Yuan, Nicholas Van Halm-Lutterodt and Xiaojun Ma contributed to the data interpretation and drafting the manuscript. All authors approved the final version of the manuscript.

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Figures

Figure 1

Mice insulin resistance phenotype, hepatic steatosis and peripheral inflammatory cytokine levels. A: Body weight monitoring of C57BL/6J and APP/PS1 mice during the dietary intervention (from the age of 1 month). B: Fasting blood glucose level and glucose tolerance test (GTT) after HFD intervention for 3.5 months. C: Baseline GTT and area under the curve (AUC) analysis after grouping the animals into different diet-treated groups. D: GTT and AUC analysis at the end of the intervention phase. E: Serum

insulin level after dietary intervention. F: Histological evaluation of hepatic steatosis in mice treated with different diets. G: Serum IL-6 and TNF- α level in mice treated with different diets. Data were represented as mean $\pm$ SD, n = 6 in each group. a: comparing with C57BL/6J ND group, P< 0.05. b: comparing with APP/PS1 ND group, P< 0.05. c: comparing with APP/PS1 60% HFD group, P< 0.05. e: comparing with APP/PS1 45% HFD-N3 group, P< 0.05. One-way ANOVA was used for statistical tests followed by Bonferroni post-test.

Figure 2

Behavior and AD pathological changes. A: Results of spatial learning and memory function after dietary intervention. B: Congo red staining of mice hippocampus and cortex. C: Soluble and Aggregated A β in cortex of mice fed with different diets. D: Cortical total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-c) levels of mice fed with different diets. E: Immunoblots of APP and BACE1 in cortex of mice fed with different diets. Actin was served as the internal control. Data were represented as mean $\pm$ SD, n = 6 in each group. a: comparing with C57BL/6J ND group, $P < 0.05$. b: comparing with APP/PS1 ND group, $P < 0.05$. c: comparing with APP/PS1 60% HFD group, $P < 0.05$. d: comparing with APP/PS1 45% HFD group, $P < 0.05$. e: comparing with APP/PS1 45% HFD-N3 group, $P < 0.05$. One-way ANOVA was used for statistical tests followed by Bonferroni post-test.

A

B

C

D

E

Figure 3

Glucose transporter and insulin signaling pathway related molecules expression. A: GLUT3 protein expression in cortex of mice fed with different diets. B: Cortical insulin level in mice treated with different diets. C-E: Immunoblots of insulin signaling proteins in cortex of mice fed with different diets. Actin was served as the internal control. Data were represented as mean $\pm$ SD, $n = 6$ in each group. a: comparing with C57BL/6J ND group, $P < 0.05$. b: comparing with APP/PS1 ND group, $P < 0.05$. c: comparing with

APP/PS1 60% HFD group, $P < 0.05$. d: comparing with APP/PS1 45% HFD group, $P < 0.05$. e: comparing with APP/PS1 45% HFD-N3 group, $P < 0.05$. One-way ANOVA was used for statistical tests followed by Bonferroni post-test.

A

B

Figure 4

NF-κB p65 expression and its downstream inflammatory gene mRNA expression.A:NF-κBp65 immunofluorescence staining in cortexof mice fed with different diets.B: Cortical mRNA expression levels

of iNOS, TNF- α , COX2, IL-1 β and IL-6. Data were represented as mean $\pm$ SD, n = 6 in each group. a: comparing with C57BL/6J ND group, P < 0.05. b: comparing with APP/PS1 ND group, P < 0.05. c: comparing with APP/PS1 60% HFD group, P < 0.05. d: comparing with APP/PS1 45% HFD group, P < 0.05. e: comparing with APP/PS1 45% HFD-N3 group, P < 0.05. One-way ANOVA was used for statistical tests followed by Bonferroni post-test.

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