Fructo-oligosaccharide improved brain β-amyloid, β-secretase, cognitive function, and plasma antioxidant levels in D-galactose-treated Balb/cJ mice

Chi-Hua Yen[1,2,3,†], Cheng-Hsin Wang[4], Wen-Tzu Wu[5], Hsiao-Ling Chen[5,6]

[1]Department of Family and Community Medicine, Chung Shan Medical University Hospital, Taichung, Taiwan, [2]School of Medicine, Chung Shan Medical University, Taichung, Taiwan, [3]Center for Education and Research on Geriatrics and Gerontology, Chung Shan Medical University, Taichung, Taiwan, [4]Department of Food and Nutrition, Providence University, Taichung, Taiwan, [5]Department of Health and Nutrition Biotechnology, Asia University, Taichung, Taiwan, [6]Department of Medical Research, China Medical University Hospital, China Medical University, Taichung, Taiwan

Objectives: Long-term D-galactose injection induces accelerated aging in experimental rodent models. The aim of this study was to determine the effects of dietary fructo-oligosaccharide (FO) on the brain β-amyloid (Aβ), amyloid-associated enzymes, cognitive function, and plasma antioxidant levels in D-galactose-treated Balb/c mice.

Methods: The subcutaneous (s.c.) injection and the dietary treatment were conducted simultaneously for 49 days. Mice (12 weeks of age) were divided into five groups ($n = 14$/group): control (s.c. saline, control diet) serving as a young control, DG (s.c. 1.2 g D-galactose/kg body weight, control diet), DG + LFO (2.5% w/w FO, low-dose FO diet), DG + HFO (5% w/w FO, high-dose FO diet), and DG + E (α-tocopherol 0.2% w/w, vitamin E diet) as an antioxidant reference group. Another group of older mice (64 weeks of age) without any injection served as a natural aging (NA) group.

Results: The DG and NA groups had greater Aβ levels in the cortex, hippocampus, and the whole brain. High-dose FO, similar to α-tocopherol, attenuated the D-galactose-induced Aβ density in the cortex and hippocampus. In addition, FO attenuated the D-galactose-induced protein expression of Aβ and beta-site amyloid precursor cleaving enzyme of the whole brain in a dose–response manner. Either dose of FO supplementation, similar to α-tocopherol, attenuated the D-galactose-induced cognitive dysfunction. In addition, FO improved the plasma ascorbic acid level in a dose–response manner.

Conclusion: Dietary FO (2.5–5% w/w diet) could attenuate the development of Alzheimer's disease, which was likely to be associated with its systematic antioxidant effects.

Keywords: Fructo-oligosaccharides, D-Galactose, β-Amyloid, Alzheimer, Amyloid precursor cleaving enzyme

Introduction

Aging is commonly associated with chronic oxidative stress, mild inflammation, and progressive loss of cognitive function and even severe dementia.[1] Alzheimer's disease is the most common cause of dementia in the elderly and is characterized by extensive oxidative stress and inflammation.[1] Supplementation of antioxidants such as vitamin C and E has been shown to reduce the vascular dementia in men.[2] However, it remained unclear the effect of antioxidant supplementation on the incidence of Alzheimer's dementia.[2–4] Another characteristics of Alzheimer's disease is the amyloid plaques.[5] The extracellular amyloid plaques is derived from the larger amyloid precursor protein (APP) bound in the cell membrane.[5] The cleavage of APP by β-secretase, such as beta-site amyloid precursor cleaving enzyme 1 (BACE1) in neurons, leads to the formation of soluble APP-β, and together with γ-secretase leads to the generation of β-amyloid peptides (Aβ) 1-40 and Aβ 1-42.[5,6] These β-amyloid peptides have the ability to aggregate and generate the β-amyloid plaques found in the Alzheimer's disease. The β-amyloid plaques are also sites of chronic inflammation and cause oxidative stress due to the release of superoxide and nitric oxide, which

†Equal contribution to the first author.
Correspondence to: Hsiao-Ling Chen, College of Medical and Health Science, Department of Health and Nutrition Biotechnology, Asia University, No. 500, Lioufeng Rd., Taichung, Taiwan.
Email: hlchen908@asia.edu.tw

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could further facilitate the development of Alzheimer's disease.[7–9] On the other hand, neprilysin (NEP) is the dominant Aβ-degrading enzyme in the brain; NEP is found to be inactivated and down-regulated during both the early stage of Alzheimer's disease and aging.[10–11] Previous studies have shown that the elevation of NEP expression reduced the accumulation of both soluble and fibrillary Aβ in APP transgenic mice.[12] Therefore, the current potential therapy of Alzheimer's disease is to block the β-secretase activity and/or to restore the NEP activity of the patient.[11,13,14]

It was demonstrated that a chronic overdose of D-galactose induces oxidative stress in mouse brain and ultimately resulted in neurological inflammation and degeneration.[15–18] However, it has not been fully demonstrated that this animal model resembles the cognitive dysfunction, and amyloid deposition observed in Alzheimer's disease. In addition, it is necessary to determine the expression of β-secretase and NEP activities in this D-galactose-treated mouse model and older mice in order to explore innovative prevention strategy for the Alzheimer's disease.

The impact of phytochemicals on brain health is increasingly appreciated.[19,20] However, the role of dietary fiber in the brain health has not been studied. Fructo-oligosaccharides (FO), oligomers of D-fructose linked by β (2→1) bonds with a terminal α (1→2) linked glucose, is a prebiotic fiber with a well-recognized role in stimulating the growth of bifidobacteria.[21,22] It has also been demonstrated that supplementation of FO in nursing-home resident beneficially relieve constipation.[23] Therefore, FO is a soluble fiber that is easily incorporated into fluid and ordinary diet of the elderly to help improve their bowel function. Besides its beneficial effects locally in the intestine, authors have demonstrated that FO systemically reduced the lipid peroxide in the plasma in the nursing-home residents.[22] Furthermore, it has been demonstrated that dietary FO systemically ameliorated the D-galactose-induced malonaldehyde dimethyl acetal (MDA) in the cerebral cortex and protein carbonyls in the hippocampus and various tissues, DNA oxidation in the hepatic mitochondria, and lung pro-inflammatory status in Balb/cJ mice.[15] Therefore, the function of FO in the intestine could systematically promote antioxidant status in the brain. There are two goals of the present study: (1) to compare the Aβ deposition, Aβ metabolism-associated proteins (BACE1 and NEP), cognitive function, and plasma antioxidants (ascorbic acid and α-tocopherol) between D-galactose-treated young Balb/cJ mice and their natural aging counterparts (aged control); (2) to determine the modulatory effects of FO and α-tocopherol (antioxidant control) on these D-galactose-induced alterations.

Methods

Animals and diets

Male Balb/cJ mice (BioLASCO Taiwan Company Limited, Taipei city, Taiwan) were housed in solid-bottomed plastic cages with wood shavings for bedding in a room maintained on a 12-hours light–dark cycle (08:00–20:00) at 24 ± 1 °C and 50% humidity in the Experimental Animal Center of Chung Shan Medical University. After a week of adaptation, the subcutaneous injection and the diet treatment were conducted simultaneously for 49 days according to the method used in a previous study.[24] Young Balb/cJ mice (12 weeks of age, n = 14 animals per group) were subcutaneously injected with saline (vehicle) or D-galactose (1.2 g/kg body weight) for 46 days so that abnormal Aβ deposition could develop by the D-galactose treatment.[24] During the period of injection, the saline-treated group was fed the control diet (control group); the D-galactose-treated mice were fed either the control diet (DG group), or that containing low-level of FO (25 g active ingredients/kg diet; DG + LFO group), high-level of FO (50 g active ingredients/kg diet; DG + HFO group), or vitamin E (2 g α-tocopherol/kg diet; DG + E group). The vitamin E was used as an antioxidant reference. The control diet (kg^{-1}) consisted of 953.0 g ground rodent chow (Lab 5001, Purina Mills, St Louis, MO, USA) and 47.0 g sucrose, in which chow was partially substituted by FO or vitamin E in experimental diets. The FO was provided by the Institute of Microbial Resources (Taichung city, Taiwan). Another untreated older mice (age of 64 weeks) served as a natural aging control (NA group) in order to determine whether D-galactose treatment could mimic the aging effect on Aβ metabolism and cognitive function. All animals were allowed to have free access to water and food during the study. The Morris water maze experiment was conducted 1 week prior to the end of the experiment in order to evaluate the spatial learning/ memory function. At the end of the experiment, mice starved for18 hours before they were anesthetized with CO_2. After blood samples collected from the right atrium, the mice were transcardially perfused with ice-cold normal saline for 15 minutes, followed by 4% (w/w) paraformaldehyde for 5 minutes in order to fix the brain. The brains (n = 8 animals per group) were then removed and stored at −80°C for the western blotting of Aβ, BACE 1, and NEP. The remaining brains (n = 6 animals per group) were processed for further histological procedure and immunohistochemical staining of Aβ 1-42. Animal care followed the guideline of the National Research Council was approved by the Institutional Animal

Care and Use Committee in the Chung Shan Medical University.[25]

Histology and immunohistochemical staining of Aβ

The paraformaldehyde (4% w/w)-fixed brain was dehydrated through a graded sucrose series, frozen on dry-ice and sliced coronally into 30-μm sections with a cryostat (Leica, Inc., Wetzlar, German). The sections 1.6–2.0-mm distal to the bregma, with clear cortex and hippocampal structure based on the mouse atlas, were processed for *in situ* detection of Aβ.[26] Slices were blocked with 5% (w/w) goat serum in 0.1 M PBS containing 0.1% (w/v) tween-20 for 30 minutes, incubated with a Aβ 1-42 mouse monoclone antibody (Chemicon, Temecula, CA, USA) at a dilution of 1 : 100 for 2 hours, followed by biotinylated anti-mouse IgG (Chemicon) at a dilution of 1:100 for 1 hour, and streptavidin-horse radish peroxidase (Immunoperoxide secondary detection system, Chemicon) for 30 minutes. The slides were then incubated with 3,3′-diaminobenzidine for 10 minutes and counter-stained with hematoxylin and eosin with the standard procedure. All reactions were conducted at room temperature. The Aβ density (Aβ-positive plaque/focus) was determined with 200 magnification examined under a light microscope (Nikon Eclipse E400, Tokyo, Japan) equipped with a digital camera (Evolution™ VF, Media Cybernetics, Inc., Rockville, MD, USA) from seven animals in each group with five sections from each mouse.

Western blotting of Aβ metabolism-associated proteins

The protein expression of Aβ, BACE, and NEP was determined with the western blotting. The brain was homogenized with 50 mM Tris–HCl buffer (pH 7.5) containing 150 mM NaCl, 1% (w/w) Tween 20, 0.1% (w/w) SDS, and 1% (w/w) PMSF protease inhibitor cocktail (Sigma, St Louis, MO, USA). The lysate was sonicated on ice for 1 minute followed by centrifugation at 12 000×*g* for 10 minutes at 4°C to collect the supernatant. An aliquot of the sample was adjusted to contain 28 μg protein and then was resolved by 10% (w/w) SDS–PAGE and transferred to nitrocellulose membrane using 0.013 M carbonate buffer (pH 9.9) containing 20% (v/v) methanol. After blocking with 20 mM Tris–HCl buffer (pH 7.5) containing 150 mM NaCl and 1% (w/w) non-fat milk, the membrane was immunoblotted. The primary antibodies (dilution of 1:1000 in PBS) for Aβ, BACE, and NEP were mouse antibody against human Aβ 1-42 (Chemicon), rabbit antibodies against human BACE (Chemicon), and rabbit antibodies against human NEP (Chemicon), respectively. The β-actin was used as a loading control and immunoblotted with mouse anti-actin monoclonal antibody (dilution of 1:1000 in PBS, Chemicon). These proteins were detected with appropriate secondary antibody conjugated with horseradish peroxidase (Chemicon) at a dilution of 1:5000 in PBS and visualized using an enhanced chemiluminescent reagent provided with the ECL detection kit (Visual Protein Biotechnology Co., Taipei, Taiwan). The images were quantified with an acquisition system (Fusion SL2, Vilber Lourmat, Marne-la-Vallée, France). The intensity of blot was quantified with a densitometry (Gel-Pro Analyzer 4.0, Media Cybernetics, Inc., Bethesda, MD, USA).

Assessment of cognitive function with Morris water maze

The learning/memory ability of mice (seven per group) was evaluated on days 42–44 using a water maze behavior test modified from the procedure described previously.[27] A circular pool (80 cm in diameter) with wall and floor painted black was filled with pool water that was maintained at 23 ± 2°C and mixed with milk to obscure the platform. An escape platform (9.5 cm in diameter) submerged 1.0 cm below the surface of pool water was located in the center of northwest quadrant throughout the test. On each day, mouse was trained before the trial; it was put on the platform for 15 seconds and then was given 30-second free swim and then assisted to the platform where it was allowed for another 15-second rest. Then the test began, the mouse was released into the water facing the wall of the pool, in turn of north, south, east, and west for each trial. If the mouse did not reach the platform within 60 seconds, the mouse could be assisted to the platform and the latency was recorded as 60 seconds. No matter when the mouse reaches the platform, it was maintained on the platform for 15 seconds before the next swim. After the mouse finished the test from each quadrant, he was dried and placed in a cage resting for 1 hour for each trial. Latencies to escape from the water maze (to find the submerged platform) and the swimming distance were collected, and the swim speed of each mouse was then calculated for each trial. Three trials were conducted on each mouse each day for three consecutive days.

Plasma ascorbic acid and α-tocopherol

Plasma ascorbic acid was determined according to the method described previously with a HPLC system (Jasco, Tokyo, Japan) equipped with a C18 reverse phase column (LiChroCART 250-4, Merck, Darmstadt, Germany) capped with a guard column (LiChrospher 100 RP-18e, Merck).[28] The sample was eluted with potassium phosphate buffer (0.1 M, pH 3.5) with a flow rate of 0.8 ml/min. The ascorbic acid was detected at 245 nm

and quantified with a standard curve. Plasma α-tocopherol was extracted and analyzed according to the method described by Catignani and Bieri[29] with the HPLC system described above. In brief, an aliquot (0.2 ml) of plasma was mixed with an internal standard (0.1 ml of α-tocopheryl acetate, 105.7 μM, Sigma) and extracted with *n*-hexane twice. After removal of the hexane under vacuum, samples were dissolved in methanol (99%, v/v). The sample was eluted with 98% (v/v) methanol (1.2 ml/min) in a C18 reverse phase column (LiChroCART 250-4, Merck). The α-tocopherol was detected at 290 nm and quantified with a standard curve.

Statistical analysis

Data are presented as means ± standard deviation and analyzed using SPSS (version 12.0, SPSS, Inc., Chicago, IL, USA). All data except the water maze test were analyzed using one-way ANOVA followed by the *post hoc* analysis using Tukey's test. The behavior parameters were determined with two-way ANOVA, with group and day factors. The difference in water maze parameter, such as latency to platform, distance to platform and swimming speed, among groups, was determined using *post hoc* Tukey's test on individual days. *P*-value <0.05 was considered to be statistically significant.

Results

The growth and the physical activity of all mice throughout the experimental period were normal. The weight gain (mean ± SD) of the DG group during the experiment was 4.0 ± 1.0 g, which was significantly greater than that in the control (3.3 ± 1.5 g), DG + LFO (3.3 ± 0.8 g), DG + HFO (3.6 ± 1.1 g), and DG + E (3.3 ± 0.9 g) group, respectively. The mean caloric intake (KJ/d) for control DG, DG + LFO, DG + HFO, and DG + E was 74.6 ± 9.9, 73.9 ± 10.4, 67.1 ± 7.9, 64.2 ± 4.5, and 74.7 ± 11.3, respectively. Supplementation of either dose of FO decreased the caloric intake as compared to that of DG counterpart ($P < 0.05$ vs. DG). However, the feed efficiency (weight gain (g/d)/feed intake (kJ/d)) was similar among control- and DG-treated groups. The untreated NA mice weighed 34.6 ± 0.3 g when they were sacrificed at the age of 64 weeks. The mean caloric intake (KJ/d) of NA mice during the last week was 68.6 kJ/d.

The representative immunohistochemical staining of Aβ 1-42 in the cortex and hippocampus is shown in Fig. 1A and B, respectively, and the Aβ density (shown as fold of control) is shown in Fig. 1C. The cortex Aβ density in the DG group was similar to that in the NA group, and was about six-folds ($P < 0.001$ vs. control) than that in the control group. Supplementation of low and high dose of FO reduced the cortex Aβ density for ~40% ($P < 0.01$) and 60% ($P < 0.01$) as compared to that in the DG group, respectively. Vitamin E, similar to the high-dose FO, significantly ($P < 0.01$) reduced the D-galactose-induced cortex Aβ density. The Aβ density in the hippocampus of NA and DG groups was similar, and about five-folds than that in the control group ($P < 0.001$ vs. control). Supplementation of either dose of FO and vitamin E significantly attenuated the D-galactose-induced Aβ density by 40–60% ($P < 0.05$).

A representative western blotting of Aβ 1-42, BACE, and NEP in the whole brain is shown in Fig. 2A–C, respectively, and the quantification of these proteins is shown in Fig. 2D. The expression of brain Aβ was the greatest in the NA group, almost doubled the level in the control group ($P < 0.01$ vs. control). The Aβ expression in the DG group was significantly greater ($P < 0.05$) than that in the control group. The low-dose FO did not affect the Aβ expression determined from the whole brain. However, both the high-dose FO and vitamin E supplementation diminished the effect of D-galactose treatment on the brain Aβ expression. The expression of brain BACE was increased in the NA and DG groups to two-fold level ($P < 0.01$ vs. control) of that in the control group, respectively. The low-dose FO did not significantly affect the Aβ expression. However, both high-dose FO ($P < 0.01$) and vitamin E ($P < 0.01$) supplementation significantly reduced the D-galactose-induced brain BACE expression. The protein expression of brain NEP did not differ between NA, control, and DG groups. Neither did any dietary supplement affect the brain NEP expression as compared to the DG group.

The spatial learning/memory ability of mice is determined with the water maze test in 3 days (Fig. 3). Two-way ANOVA indicated that the mean latency to platform was significantly affected by group ($P < 0.05$) and day ($P < 0.05$) effect, respectively (Fig. 3A). On the first day, the latency to platform was not significantly different between NA, control, and DG groups. However, supplementation of high-dose FO and vitamin E significantly reduced the time to reach the platform compared to that in the DG group ($P < 0.05$). On the second and third days of the water maze test, NA and DG groups spent more time to reach the platform than did the control group, respectively, and all dietary supplements reversed this effect of D-galactose.

The mean swimming distance taken to reach the platform also indicated group ($P < 0.05$) and day ($P < 0.05$) effects, without significant interaction between these two factors. The swimming distance taken to reach the platform was not different among NA, control, and DG groups on the first day (Fig. 3B). None of the supplement affected the swimming distance as compared to that of the DG group.

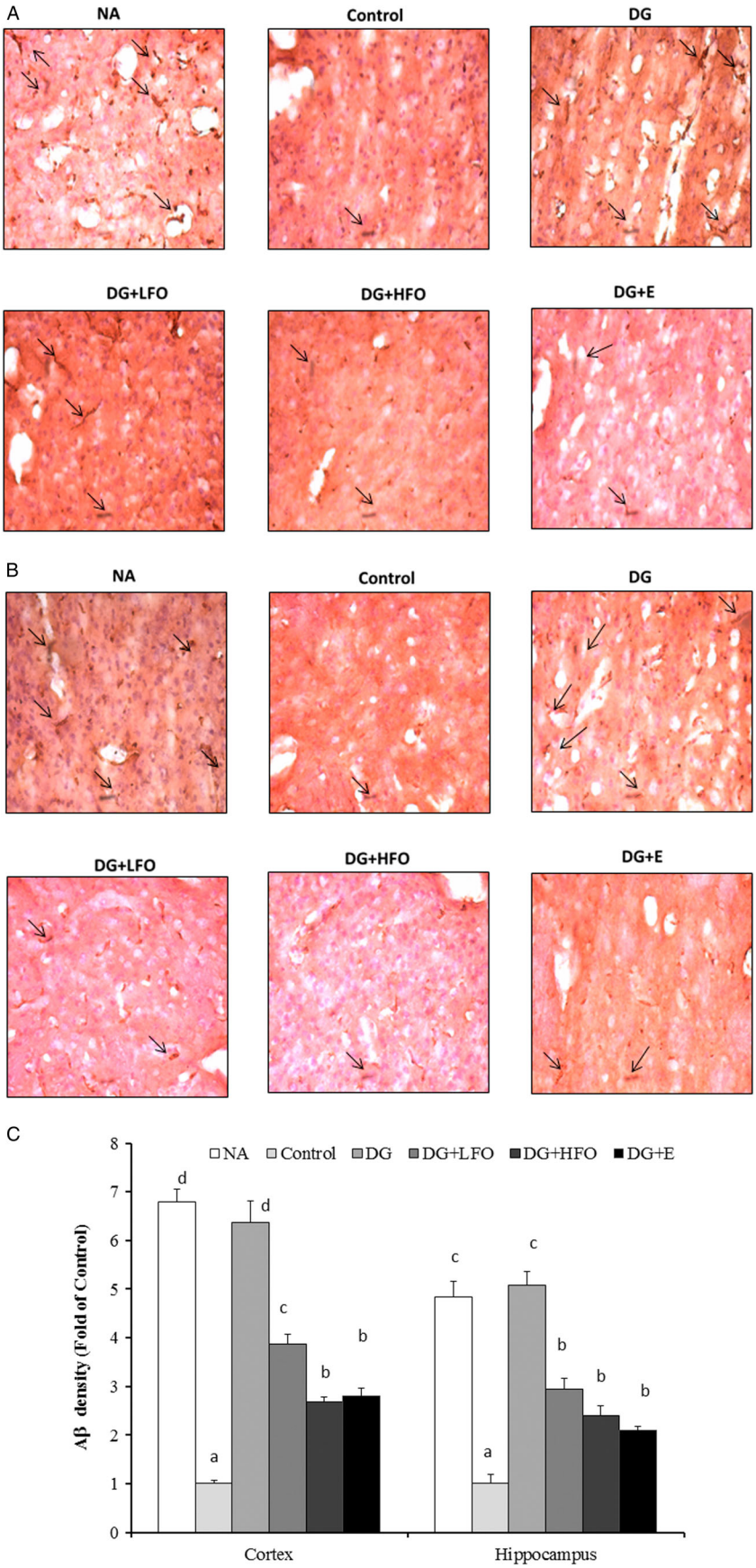

Figure 1 Immunohistology of Aβ in the (A) cortex and (B) hippocampus; (C) Aβ density (shown as fold of control) in these two regions. Male Balb/cJ mice (12 weeks old) were subcutaneously injected with saline (control group) or D-galactose (1.2 g/kg BW) for 49 days during which mice were fed control diet (DG group) or control diet containing fructo-oligosaccharide of low dose (25 g active ingredients/kg diet; DG + LFO group), high dose (50 g active ingredients/kg basal diet; DG + HFO group), or vitamin E (2 g α-tocopherol/kg diet; DG + E group). NA: natural aging mice (64 weeks old) without any subcutaneous injection were fed the control diet. Values are means with their standard deviation ($n = 6$ animals per group, three slices per animal). Mean values with unlike superscript letters were significantly different ($P < 0.05$).

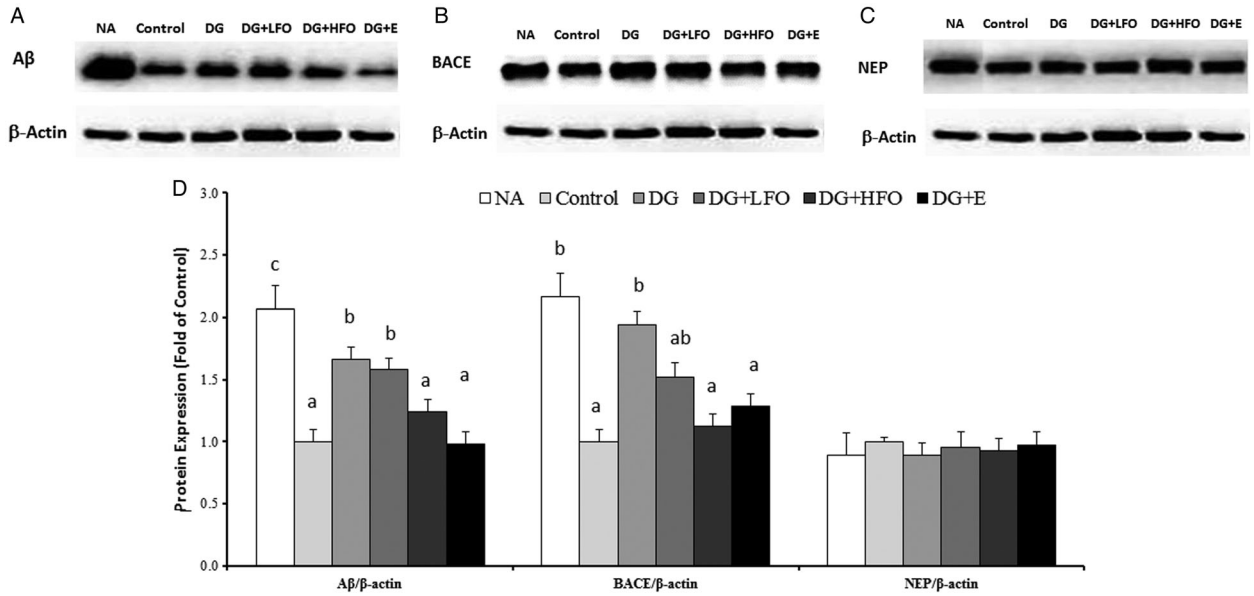

Figure 2 Immunoblotting of brain (A) Aβ, (B) BACE, and (C) NEP; (D) protein expression (fold of control). Male Balb/cJ mice (12 weeks old) were subcutaneously injected with saline (control group) or D-galactose (1.2 g/kg BW) for 49 days during which mice were fed control diet (DG group) or supplemented with fructo-oligosaccharide at low dose (25 g active ingredients/kg diet; DG + LFO group), high dose (50 g active ingredients/kg diet; DG + HFO group), or vitamin E (2 g α-tocopherol/kg diet; DG + E group). NA: natural aging mice (64 weeks old) without any subcutaneous injection. Values are means with their standard deviation ($n = 8$ animals per group). Mean values with unlike superscript letters were significantly different ($P < 0.05$).

On the second and third days, the distance taken to reach the platform in the NA and DG groups were similar; both were longer ($P < 0.05$) than that in the control group. All dietary supplements successfully diminished the effect of D-galactose treatment. The swimming speed did not indicate group or day effect. The swimming speed was similar among NA, control, and DG groups (Fig. 3C). Neither did any supplement significantly affect the swimming speed.

NA and D-galactose treatment significantly reduced the plasma ascorbic acid concentration compared to that in the control group, respectively ($P < 0.05$, respectively) (Fig. 4). The low-dose FO ($P = 0.06$) and vitamin E ($P = 0.06$) only tended to attenuate the D-galactose effect. However, the high-dose FO almost diminished the effect of D-galactose on the plasma ascorbic acid concentration ($P < 0.05$ vs. DG). Similarly, the plasma α-tocopherol concentrations were lower in the NA and DG groups compared to that in the control group, respectively ($P < 0.05$). Either dose of FO successfully normalized the plasma α-tocopherol concentrations. The DG + E group, an antioxidant reference group, had the greatest plasma α-tocopherol level among groups ($P < 0.001$ vs. DG).

Discussion

In agreement with a previous observation in the Kumning strain mice, we found that D-galactose treatment in Balb/cJ mice increases the neurotoxic Aβ 1→42 level specifically in the cortex and hippocampus, the brain region that is associated with the learning/memory function.[30] We particularly indicated that D-galactose treatment generally mimicked the effects of NA on most of parameters determined in this study, such as the Aβ density in the cortex and hippocampus, brain BACE expression, spatial learning/memory ability, and plasma antioxidant levels. In addition, this study was the first to indicate that daily supplementation of 5% (w/w diet) FO could ameliorate the alteration in the brain Aβ level and cognitive function caused by the D-galactose treatment. Furthermore, effects of high-level FO diet were generally similar to those of vitamin E diet in this study, which suggested that the underlying mechanism of FO was partially mediated by its systemic antioxidant effects.[15,22]

Aging is closely related to chronic oxidative stress and low-grade inflammation.[1,31] It is considered that metabolism of high level of D-galactose causes the accumulation of reactive oxygen species and advanced glycation end products (AGEs), which in turn bind to the AGE receptors in various cells to induce pathological cascades, including ROS generation and pro-inflammatory responses.[32] Previous studies indeed have shown that this D-galactose treatment induced oxidative molecule damages such as MDA, protein carbonyls, and mitochondrial 8-oxo-deoxyguanosine in various organs including the brain, liver, and plasma.[15,33] The D-galactose treatment also alters antioxidant enzyme activities in the brain, liver, and erythrocyte of various strains of rodent models.[15,33] The present study further indicated that the D-galactose treatment, similar to NA, reduced the plasma ascorbic

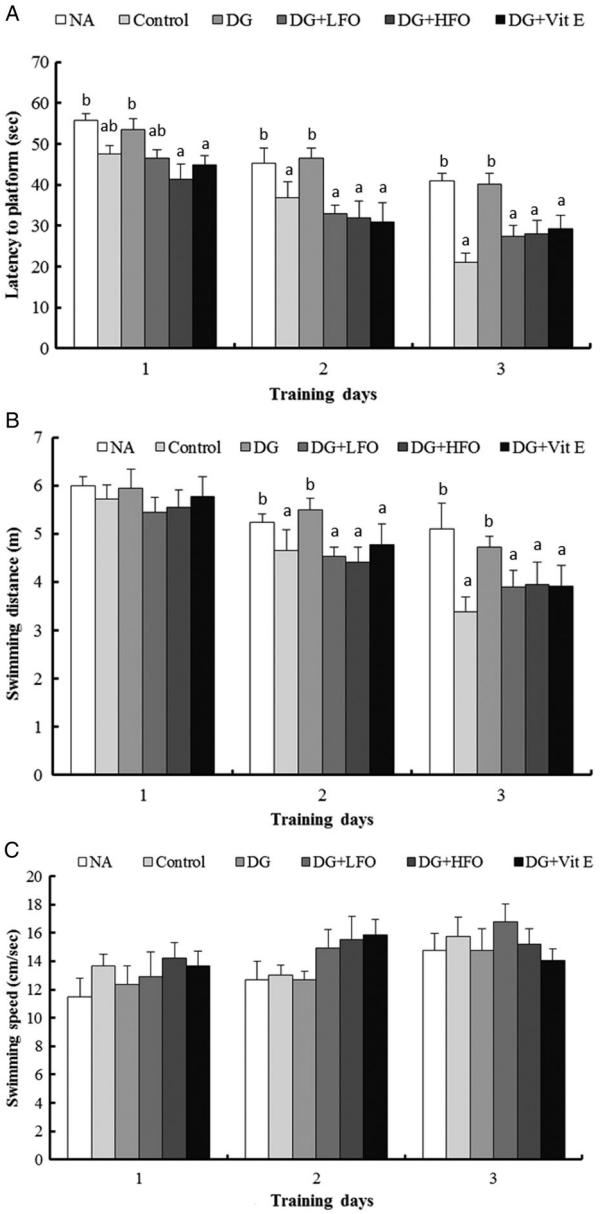

Figure 3 Morris water maze test (A), latencies to platform (B), swimming distance (C), swimming speed. Male Balb/cJ mice (12 weeks old) were subcutaneously injected with saline (control group) or D-galactose (1.2 g/kg BW) for 49 days during which mice were fed control diet (DG group) or supplemented with fructo-oligosaccharide at low dose (25 g active ingredients/kg diet; DG + LFO group), high dose (50 g active ingredients/kg diet; DG + HFO group), or vitamin E (2 g α-tocopherol/kg diet; DG + E group). NA: natural aging mice (64 weeks old) without any subcutaneous injection. Values are means with their standard deviation ($n = 7$ animals per group). Mean values with unlike superscript letters were significantly different ($P < 0.05$).

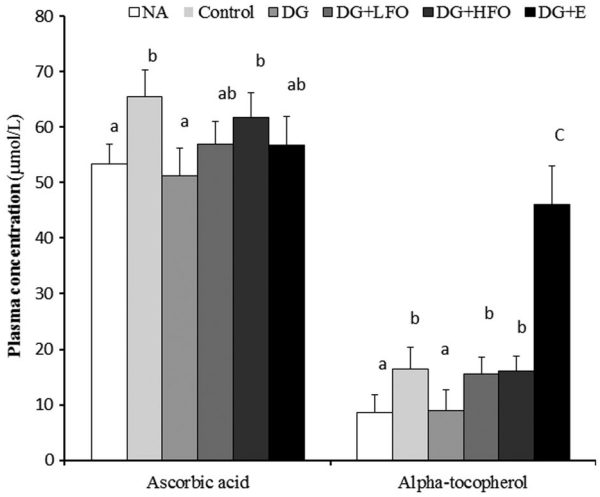

Figure 4 Plasma ascorbic acid and α-tocopherol levels. Male Balb/cJ mice (12 weeks old) were subcutaneously injected with saline (control group) or D-galactose (1.2 g/kg BW) for 49 days during which mice were fed control diet (DG group) or supplemented with fructo-oligosaccharide at low dose (25 g active ingredients/kg diet; DG + LFO group), high dose (50 g active ingredients/kg diet; DG + HFO group), or vitamin E (2 g α-tocopherol/kg diet; DG + E group). NA: natural aging mice (64 weeks old) without any subcutaneous injection. Values are means with their standard deviation ($n = 14$ animals per group). Mean values with unlike superscript letters were significantly different ($P < 0.05$).

acid and α-tocopherol levels. Therefore, it is reasonable to suggest that oxidative stress was one of the underlying mechanism for D-galactose-induced senility observed in the present study.

Enhanced brain APP and Aβ aggregation, observed in facilitated aging subjects, are considered closely associated with the development of Alzheimer's disease.[34] The over-expression of APP and the increasing cleavage of this protein by β-secretase could contribute to the Alzheimer's disease.[34,35] Enhanced Aβ aggregation and fibrillogenesis could hamper the neural synaptic function, activate the neurotoxic cascade, and eventually cause dementia.[34,35] In addition, intracellular Aβ protein binds to mitochondrial membranes, alters the electron flow through the respiratory chain, and further leads to ROS cascade.[36] The present study confirmed that the NA increased the neurotoxic Aβ 1→42 density in the cortex and hippocampus, and brain Aβ and BACE protein levels. In addition, age is found to be reversely correlated with NEP, the Aβ-degrading enzyme, level in specific human brain region, such as temporal and frontal cortex.[11] The present study determined the NEP level in the whole brain instead of specific brain region and did not agree with the finding in the previous study.[11] Therefore, the present results suggest that the elevated Aβ level in NA group might be mainly due to the elevated BACE level. This study further indicated that D-galactose treatment exerted similar effects on these measurements as did NA. Therefore, the chronic D-galactose treatment induced the development of Alzheimer's disease in Balb/cJ mice through increases in both the Aβ 1→42 deposition in specific brain regions and brain BACE protein expression.

Our study is the first to determine effects of dietary oligosaccharides on the brain Aβ metabolism. The expression of BACE in the whole brain was reduced

with FO in a dose–response manner. In agreement with that, FO reduced the cortex and hippocampus Aβ densities. In addition, this study found that the efficacy of high-dose FO was equal to that of 0.2% (w/w) α-tocopherol in reducing the Aβ formation and BACE protein expression. These results suggest that the antioxidant pathway could be a mechanism of FO's effects. This hypothesis was supported by previous studies that showed FO supplementation attenuated the activation of the JNK/Jun pathway in the lungs of D-galactose-treated mice, and JNK and c-jun was associated with BACE1 expression.[37,38] Therefore, the present study suggests that FO ameliorated the oxidative/inflammation stress in the brain, which might further reduce the brain Aβ deposition and BACE1 expression. However, the intracellular pathways that mediate this effect of FO in the brain remained to be further investigated.

To our best knowledge, the present study was the first to determine the role of FO in the learning/memory function. We indicate that the supplementation of 2.5–5% (w/w) FO was sufficient to improve the spatial learning/memory function after 1 day of training. The capability of 2.5–5% (w/w) FO was similar to 0.2% (w/w) α-tocopherol, again supporting that the systemic antioxidant ability of FO was likely to be the underlying mechanism. In addition, FO supplement reduced the BACE expression, which was likely to reduce the formation of N-APP.[39] In contrast, neither does of FO further modulated the normal (similar to control) level of NEP in the D-galactose-treated mice. The N-APP acts as a death receptor 6 (DR6) ligand to trigger neuron degeneration via a widespread caspase-dependent pathways.[40] Therefore, FO may ameliorate D-galactose-induced senescence via DR6-downstream pathways. In agreement with that, the histological observation indicated that FO reduced Aβ formation in the cortex and hippocampus, areas of memory and learning function. Therefore, FO reduced the memory/learning dysfunction induced by the D-galactose treatment was likely to be mediated by the reduced BACE protein expression and subsequently the formation of APP-β, Aβ, and N-APP.

The present results indicated that FO feeding improved the plasma antioxidant levels. Vitamin E has been postulated to prevent or minimize oxidative stress-dependent brain damage.[41] In addition, a meta-analysis study indicates that dietary intakes of vitamin E, vitamin C, and β-carotene can lower the risk of Alzheimer's disease.[42] Therefore, we suggested that the preventive effects of FO on the development of Alzheimer's disease biomarkers were partly due to the increased plasma antioxidant levels. Furthermore, previous studies reported that FO normalized the erythrocyte antioxidant enzyme activities and decreased oxidative damages in the liver and brain in the D-galactose-treated mice.[15] These observations suggest that FO supplementation could exert systemic anti-oxidative capacity. Therefore, the present study suggests that FO supplementation prevented the D-galactose-induced decreases in the plasma antioxidants, and further ameliorated the cascades of AGE formation, Aβ formation, and oxidative damages in the brain.

How FO, a non-digestible fiber, diminished D-galactose-induced oxidative stress and subsequently Alzheimer's disease parameters remained to be investigated. Several studies suggest that anti-oxidative effects of soluble dietary fibers are related to their prebiotic effect, since several *in vitro* studies have indicated anti-oxidative capacities of lactic acid bacteria.[22,43–45] In addition, we have shown that dietary FO reduced plasma lipid peroxide levels in the nursing-home elderly and this effect was associated with the increase in fecal bifidobacteria.[22] Therefore, we suggest that FO exerts systemic antioxidant effects, partially by stimulating the proliferation of colonic prebiotic bacteria. Other mediators for the effects of FO in this study are hydrogen gas and short chain fatty acids, fermentation products of FO by colonic bacteria.[46] These fermentation products are absorbed by the colonocytes and are carried to organs and tissues via blood circulation. Previous studies show that exogenously administered hydrogen gas prevented the progression of severe oxidative stress in ischemia animal models.[47,48] It is likely that the hydrogen gas produced in the colon from fermentation of FO transferred via the blood stream to organs distal to the intestine, including the brain. The hydrogen gas acts as an antioxidant agent to reduce the oxidative stress in the brain and subsequently reduces the Aβ plaque and the neuron damage related to the learning and memory dysfunction.

In conclusion, the present study demonstrated that the D-galactose treatment, similar to the NA process, increased the formation of Aβ, especially in the cortex and hippocampus, decreased the circulating antioxidants, and jeopardized the cognition function. FO supplementation attenuated these parameters, likely through systemic antioxidant effects, its prebiotic effects and fermentation products.

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Disclaimer statements

Contributors

C.-H.Y. co-designed and analyzed the data; C.-H.W. conducted the study and co-wrote the manuscript; W.-T. W. conducted the study; H.-L.C. designed and carried out the study, and wrote the manuscript. All

authors read and approved the findings of the study. All authors contributed equally.

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Conflict-of-interest

There are no conflicts-of-interest.

Ethics Approval

Animal care followed the guideline of the National Research Council was approved by the Institutional Animal Care and Use Committee in the Chung Shan Medical University.

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