British Journal of Nutrition

cambridge.org/bjn

Oral supplementation of fucoxanthin regulates gene expression in the brain of middle-aged rats

Han-A Park[1,2,3], Emma Amjad[1], Garrett Burnett[1], Khondoker Adeba Ferdous[1], Madison Scott[1], Joseph Jansen[1], Sydni Bannerman[1], Megan Scott[1], Robert N. Correll[3,4], Lukasz Ciesla[3,4] and Amy Ellis[1,2]

[1]Department of Human Nutrition, The University of Alabama, Tuscaloosa, AL, USA; [2]Alabama Research Institute on Aging, The University of Alabama, Tuscaloosa, AL, USA; [3]Center for Convergent Bioscience and Medicine, The University of Alabama, Tuscaloosa, AL, USA and [4]Department of Biological Sciences, The University of Alabama, Tuscaloosa, AL, USA

Research Article

Cite this article: Park H-A, Amjad E, Burnett G, Ferdous KA, Scott M, Jansen J, Bannerman S, Scott M, Correll RN, Ciesla L, and Ellis A (2025) Oral supplementation of fucoxanthin regulates gene expression in the brain of middle-aged rats. *British Journal of Nutrition* **134**: 451–461. doi: 10.1017/S0007114525104996

Received: 11 February 2025
Revised: 11 August 2025
Accepted: 15 August 2025
First published online: 3 September 2025

Keywords:
Fucoxanthin; Ageing; Neurodegeneration; Antioxidant

Abbreviations:
Aβ, amyloid-β; GO, gene ontology; LDH, lactate dehydrogenase; MDA, malondialdehyde; OHDA, 6-hydroxydopamine; ROS, reactive oxygen species

Corresponding author:
Han-A Park; Email: hpark36@ches.ua.edu

Abstract

Age is the main risk factor for many neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and frontotemporal dementia. Despite our limited understanding of cellular mechanisms of ageing-associated neuronal loss, an increasing number of studies demonstrate that oxidative stress and inflammation are key drivers. Epidemiological studies indicate that diet during middle adulthood can influence the risk of developing neurodegenerative diseases later in life, so it is important to investigate dietary interventions to combat oxidative stress and inflammation. In this study, we hypothesised that treatment with fucoxanthin, a marine carotenoid with strong antioxidant properties, prevents ageing-associated oxidative stress that is known to be related to natural brain ageing. Treatment with fucoxanthin protected rat primary hippocampal neurons against oxidative stress and ageing *in vitro*. In our *in vivo* study, middle-aged male Sprague-Dawley rats were gavaged with fucoxanthin (1 mg/kg, 5 d/week, *n* 6) or vehicle (*n* 6) for 4 weeks. After supplementation was completed, brain samples were harvested and subjected to quantitative and bioinformatic analyses. Fucoxanthin was detected and shown to decrease lipid peroxidation in the brains of the animals supplemented with fucoxanthin. Microarray analysis showed that treatment with fucoxanthin changed 5602 genes. Together, our results suggest that treatment with fucoxanthin prevents ageing-associated oxidative stress and is capable of regulating genes that potentially ameliorate age-related changes to the brain.

The risk of developing neurodegenerative diseases increases with age[1]. More than one in ten (10·9 %) people age 65 and older have Alzheimer's disease, and the prevalence increases to approximately one-third of those age 85 and older[2]. Likewise, the risk of Parkinson's disease increases with age, and the WHO estimates that the prevalence of Parkinson's disease has doubled over the past two decades[3,4]. Considering the percentage of the global population age 65 and older is growing faster than any other age group, the burden of these age-related neurodegenerative diseases will intensify[5], especially as widely consumed dietary factors such as fructose, alcohol and high-fat diets have been implicated in promoting oxidative stress, lipid peroxidation, mitochondrial dysfunction and possibly ageing[6–8]. Therefore, nonpharmacological targets for prevention are warranted. In particular, a neuroprotective dietary strategy that can be implemented in daily life may be ideal. An increasing number of studies have demonstrated the protective effects of brown seaweed for brain health[9,10]. A diet rich in brown seaweed is inversely associated with cerebrovascular disease among centenarians[11,12]. Seaweed contains bioactive compounds with antioxidant and anti-inflammatory effects that could mediate ageing processes[9,13]. Notably, brown seaweed is rich in fucoxanthin, a carotenoid pigment that is a strong antioxidant with the ability to scavenge reactive oxygen species (ROS) including hydroxyl radical, peroxyl radical and hypochlorous acid[14–16].

Fucoxanthin is reported to exhibit neuroprotective effects against neurodegenerative diseases such as Alzheimer's and Parkinson's disease. Studies have shown that treatment with fucoxanthin prevented amyloid-β (Aβ)-induced oxidative stress and apoptosis *in vitro*[17], and oral supplementation with fucoxanthin in mice improved cognitive behaviours against Aβ challenges[18]. In addition, fucoxanthin protected 6-hydroxydopamine (OHDA)-challenged PC12 cells by regulating the nuclear factor erythroid 2-related factor 2 pathway via binding to the hydrophobic regions of Keap1[19]. A 6-OHDA-induced mouse Parkinson's disease model also demonstrated that oral supplementation with fucoxanthin-enhanced motor function[20]. Despite these encouraging results that have demonstrated protective effects of fucoxanthin in neurodegenerative disease models, the neurodegenerative processes that underlie these diseases begin decades before clinical symptoms appear[21]. Currently, the role of fucoxanthin in the non-

pathological ageing brain is less known. In this study, we hypothesised that treatment with fucoxanthin prevents ageing-associated oxidative stress and thus supports natural brain ageing. We tested the role of fucoxanthin against ROS and ageing *in vitro*. Additionally, this is the first test to examine whether orally administered fucoxanthin is found in the brain and exhibits a direct antioxidant effect in healthy middle-aged rats, and we further explored genes that are sensitive to fucoxanthin treatment.

Materials and methods

Culture of primary neurons

Primary rat hippocampal neurons were prepared from rat feti (Sprague-Dawley, day 18 of gestation; Envigo) as described previously[22–25]. Briefly, neurons ($0{\cdot}3 \times 10^6$ cells/35 mm plate) were seeded on poly-L-lysine-coated plates and grown in neurobasal medium supplemented with B-27, glutamine and antibiotics (Invitrogen Gibco Life Technologies) for 3 or 6 weeks *in vitro*. *Fucoxanthin*: Neurons were either treated with fucoxanthin (100 nM, Cayman Chemical) or a vehicle (DMSO). *Hydrogen peroxide treatment*: 25 μM of hydrogen peroxide (Sigma-Aldrich) were freshly prepared in sterile PBS and added to the cell culture medium. The vehicle control for hydrogen peroxide experiments was sterile PBS. All protocols were approved by the Institutional Animal Care Committee (IACUC) of University of Alabama, Tuscaloosa, AL (23-11-7123).

Reactive oxygen species measurement

2′,7′- dichlorodihydrofluorescein diacetate staining

Primary hippocampal neurons were treated with 10 μM of dichlorofluorescein (DCF) (Invitrogen) solution prepared in a light protected vessel, then incubated for 30 min at 37°C in the dark[26] and processed as the manufacturer's protocol. After incubation, neurons were carefully washed with pre-warmed Hanks' Balanced Salt Solution (HBSS). Intracellular fluorescence was measured using a fluorescent microplate reader (CLARIOstar, BMG Labtech) at excitation and emission wavelengths of 470–15 and 515–20 nm, respectively.

mitoSOX staining

Production of mitochondrial ROS was analysed using MitoSOX Red (Invitrogen). The MitoSOX Red dye is oxidised by superoxide in the mitochondria, emitting red fluorescence. After treatment of neurons as described in relevant figure legends, 1·25 μM of MitoSOX was added to the cell culture medium. Cultures were incubated for 30 min at 37°C and washed twice with warm HBSS. Fluorescent images were taken with a Zeiss AxioVert.A1 microscope and analysed using AxioVision 4·9.

Viability measurement

Lactate dehydrogenase assay

The level of cytotoxicity in primary neurons was assayed by measuring leakage of lactate dehydrogenase (LDH) using an in vitro toxicology assay kit (Sigma-Aldrich) as previously described[27]. In brief, the culture media and lysed cells were collected after 24-h treatment. The LDH assay mixture was added to each sample. After 20-min incubation, the reaction was terminated by adding 1N HCl. LDH activity was spectrophotometrically measured with a Clariostar microplate reader (BMG Labtech) with absorbance set at 490 nm. Data were calculated by finding the activity of LDH which leaked into the medium from damaged cells/total LDH activity in the culture.

Propidium iodide (PI) staining

Dead cells were stained with PI as previously described[22,25,28]. After treatment, 0·5 μM PI (Invitrogen) was added into the culture medium for 30 min at 37°C in the dark. Images were taken using a Zeiss AxioVert.A1 microscope using consistent exposure time. The number of PI positive neurons was analysed using AxioVision 4·9.

Experimental animals and study design

Male Sprague-Dawley rats (*n* 12, 17 months old, Envigo) were randomly divided into two groups using 'sort by random' tool in Excel: control (*n* 6) and supplemented (*n* 6) groups after a one-week acclimation period. To test the effect of dietary fucoxanthin in the brain, the supplemented group was orally gavaged with fucoxanthin (Algatechnologies Ltd) in olive oil at a dosage of 1 mg/kg body weight, 5 days per week for 4 weeks as described previously[29]. The control group was orally gavaged with olive oil with volume matching the mean volume of the supplemented group. After supplementation was completed, and tissue from control and supplemented animals were harvested. The exclusion criteria include animals with clinical and behavioural changes such as hunching, abnormal vocalisation, nosy breathing and hair loss. Animals were maintained under standard condition at 22 ± 2°C with 12:12 dark: light cycles in the Animal Care Facility in the University of Alabama. The sample size was determined by a previous report with rats orally supplemented with fucoxanthin[30]. All animal protocols were approved by the Institutional Animal Care and Use Committee of the University of Alabama (20-07-3739).

Thiobarbituric acid reactive substance assay

The level of lipid oxidation was assessed by measuring malondialdehyde (MDA) using thiobarbituric acid reactive substance assay kit per the manufacturer's protocol (Cayman ChemicalMI). In brief, the samples were homogenised in 1X radioimmunoprecipitation assay buffer containing protease inhibitors, and protein concentrations were determined using the bicinchoninic acid assay. Protein samples, sodium dodecyl sulphate and the colour reagent containing thiobarbituric acid, acetic acid and sodium hydroxide were mixed and boiled for 1 h. After 1 h, the samples were cooled in ice for 10 min followed by centrifugation. The MDA level was measured with a CLARIOstar microplate reader (BMG Labtech) with an excitation of 530 nm and an emission of 550 nm.

Quantification of fucoxanthin in the brain

The brain extraction procedure was performed in accordance with a protocol adapted from Vishwanathan *et al*[31]. Approximately 40 mg of brain tissue was homogenised with 60 μl saline and 0·1 ml ethanol in a 50 ml glass Dounce homogeniser. About 10 μl of internal standard (1 mg/ml Astaxanthin, Sigma) and 0·4 ml of ethanol were added to the homogenate and mixed well. The mixture was then incubated in a 70°C water bath for 2 min, followed by the addition of 25 % sodium ascorbate (0·1 ml) and 5 % sodium hydroxide (0·2 ml) and incubated again in a 60°C water bath for 20 min (saponification to remove esters, as carotenoids tend to be esterified with fatty acids)[32]. Following incubation, 0·1 ml distilled water was added, and the mixture was cooled for 5 min. Subsequently, 1 ml of hexane was added, and the mixture was

vortexed vigorously for 2 min. The mixture was then centrifuged at 1000 × g for 10 min at 4°C. The upper hexane layer was removed and evaporated to dryness under nitrogen. The extraction was repeated with 1 ml of hexane, and the mixture was processed as described above. The second hexane layer was combined with the first extract and evaporated to dryness under nitrogen. The dry residue was reconstituted in 20 µl of ethanol, vortexed, sonicated (30 s) and transferred to HPLC inserts. The inserts were centrifuged at 2000 × g for 3 min, and the pellet was discarded. The supernatant was then transferred to clean inserts, and 10 µl was injected into the HPLC-DAD-MS system comprising of C30 carotenoid column (3 µm, 150 × 4·6 mm, Acclaim, ThermoFisher)[33] and DAD and MS detectors. Fucoxanthin was identified and quantified in the analysed samples by comparing their retention times, absorbance spectra and mass-to-charge ratio (m/z) to those of the external standards (Sigma Aldrich).

RNA extraction

Total RNA was isolated from brains of fucoxanthin-treated and control (vehicle) treated rats, and a NanoDrop ND-1000 (Thermo Fisher Scientific) was used to quantify RNA and evaluate quality. Standard denaturing agarose gel electrophoresis was used to assess RNA integrity.

Microarray analysis

Microarray analysis was performed to systematically profile expression changes in protein-coding mRNAs using the Arraystar Rat lncRNA Microarray v3.0 (Arraystar), which can detect 10 333 lncRNAs and 28 287 coding transcripts. The Agilent One-Color Microarray-Based Gene Expression Analysis protocol (Agilent Technology) was used to perform sample labelling and array hybridisation with minor modifications. Briefly, the Arraystar Flash RNA Labeling Kit (Arraystar) was used to amplify and transcribe each RNA sample into fluorescent cRNA along the entire length of the transcripts without 3' bias utilising a random priming method. The labelled cRNAs were purified using an RNeasy Mini Kit (Qiagen, Hilden, DE). A NanoDrop ND-1000 (Thermo Fisher Scientific) was used to measure the concentration and specific activity of the labelled cRNAs (pmol Cy3/µg cRNA). 1 µg of each labeled cRNA was fragmented by adding 5 µl 10× blocking agent and 1 µl of 25× fragmentation buffer. The mixture was then heated at 60°C for 30 min, and 25 µl 2× GE hybridisation buffer was then added to dilute the labelled cRNA. 50 µl of hybridisation solution was dispensed into the gasket slide and assembled to the lncRNA expression microarray slide. The slides were incubated for 17 h at 65°C in an Agilent Hybridisation Oven (Agilent Technology). The hybridised arrays were washed, fixed and scanned using the Agilent DNA Microarray Scanner, part number G2505C (Agilent Technology).

Bioinformatics analysis

Array images were analysed using Agilent Feature Extraction software, version 11.0.1.1 (Agilent Technology), and the GeneSpring GX v12·1 software package (Agilent Technology) was used for quantile normalisation and subsequent data processing. After quantile normalisation of the raw data, lncRNAs and mRNAs where at least four out of eight samples had present or marginal flags in 'All Targets Value' were chosen for further data analysis. Fold change filtering or volcano filtering was used to identify statistically significant and differentially expressed lncRNAs and mRNAs between the fucoxanthin and control (vehicle) samples. Hierarchical clustering was performed using the R software (R Core Team, R Foundation for Statistical Computing). Gene ontology (GO) analysis was performed using the topGO package in the R environment for statistical computing and graphics, and pathway analysis was calculated by Fisher's exact test.

Statistical analysis

Data are reported as the mean (SD) of at least three independent samples. Differences in means were tested using one-way ANOVA with Tukey's test or Student's *t* test in GraphPad Prism software (San Diego, CA) and $P < 0{\cdot}05$ was considered statistically significant. *P* values are provided in figure legends.

Results

Treatment with fucoxanthin prevents oxidative stress-mediated neuronal death

To test neuroprotective properties of fucoxanthin against oxidative stress, we quantified LDH release (Figure 1(a)) in primary hippocampal neurons 24 h after treatment with hydrogen peroxide (25 µM) and fucoxanthin (0, 10, 100 and 1000 nM). We have previously shown that 25 µM hydrogen peroxide exhibits a moderate cytotoxicity that is sensitive to carotenoid treatment[34]. Consistently, 25 µM hydrogen peroxide increased LDH release in primary hippocampal neurons (control 1·00 (SD 0·01) *v.* hydrogen peroxide 1·37 (SD 0·1), $P < 0{\cdot}0001$), whereas treatment with 100 and 1000 nM fucoxanthin prevented the effect of hydrogen peroxide (100 nM fucoxanthin 1·23 (SD 0·05) *v.* hydrogen peroxide 1·37 (SD 0·1), $P = 0{\cdot}002$; 1000 nM fucoxanthin 1·14 (SD 0·08) *v.* hydrogen peroxide 1·37 (SD 0·1), $P = 0{\cdot}01$). We further measured mitochondrial superoxide using mitoSOX staining (Figure 1(b)). Primary hippocampal neurons treated with hydrogen peroxide significantly increased mitoSOX fluorescence (control 1·00 (SD 0·51) *v.* hydrogen peroxide 1·59 (SD 0·93), $P < 0{\cdot}0001$). However, co-treatment with 100 nM fucoxanthin attenuated hydrogen peroxide-induced mitoSOX signal (hydrogen peroxide 1·59 (SD 0·93) *v.* fucoxanthin cotreatment 1·15 (SD 0·65), $P = 0{\cdot}0001$). Additionally, 100 nM fucoxanthin lowered PI-positive cells (Figure 1(c)), indicating a decreased late apoptotic and necrotic population, against hydrogen peroxide challenge (control 1·60 (SD 2·44) *v.* hydrogen peroxide 6·33 (SD 7·68), $P = 0{\cdot}0004$; hydrogen peroxide 6·33 (SD 7·68) *v.* fucoxanthin cotreatment 2·83 (SD 3·4), $P = 0{\cdot}02$).

Treatment with fucoxanthin protects primary neurons against ageing in vitro

To test whether *in vitro* ageing changes intracellular oxidative stress level, we labelled 3 and 6 weeks old primary hippocampal neurons with DCF (Figure 2(a)). Six-week-old primary hippocampal neurons significantly increased DCF fluorescent intensity compared to the 3 weeks old group indicating increased intracellular ROS (3-week 1·00 (SD 0·09) *v.* 6-week 1·34 (SD 0·17), $P = 0{\cdot}01$). Although fucoxanthin did not show any significance in the 3-week-old group, treatment with fucoxanthin significantly prevented mitochondrial superoxide production (6-week without fucoxanthin 1·59 (SD 0·63) *v.* 6-week with fucoxanthin 1·28 (SD 0·58), $P = 0{\cdot}02$, Figure 2(b)) and PI-positive dead cells in the 6 weeks group (6-week without fucoxanthin 6·20

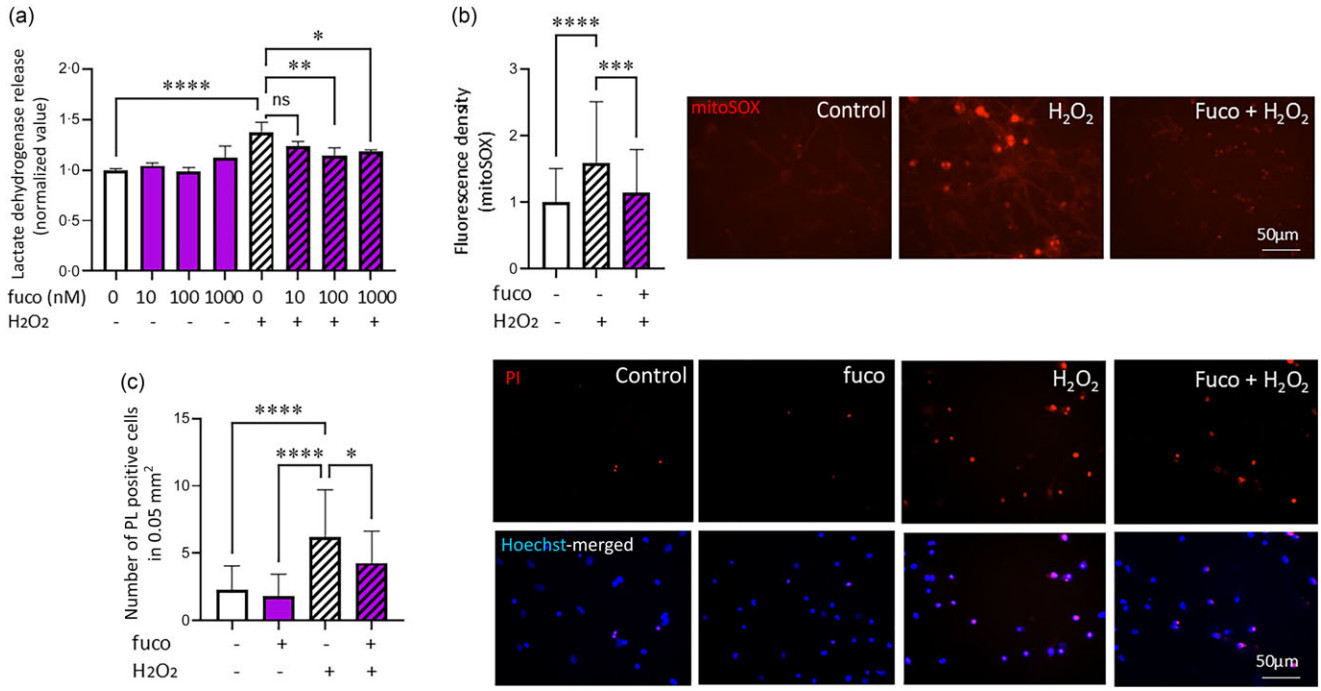

Figure 1. Fucoxanthin attenuates hydrogen peroxide-induced oxidative stress in primary hippocampal neurons. Primary hippocampal neurons were treated with fucoxanthin (10, 100 and 1000 nM) and hydrogen peroxide (25 μM) for 24 h. Quantified cytotoxicity was measured by lactate dehydrogenase (LDH) release (a), *n* 4 from three independent cultures. Primary hippocampal neurons were treated with hydrogen peroxide (25 μM) or a combination of hydrogen peroxide and fucoxanthin (100 nM) for 6 h. Mitochondrial superoxide was measured by mitoSOX staining (b), *n* 90 micrographs per group. Neurons were treated with fucoxanthin (100 nM), hydrogen peroxide (25 μM) or a combination of both for 24 h. Cell death was measured by quantifying PI-positive cells (c), *n* 30 micrographs per group. Scale bar = 50 μm. Red: PI; blue: 4′,6-diamidino-2-phenylindole (DAPI). $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$ and $^{***}P < 0{\cdot}001$, one-way ANOVA with a Tukey's *post hoc* analysis. PI, propidium iodide.

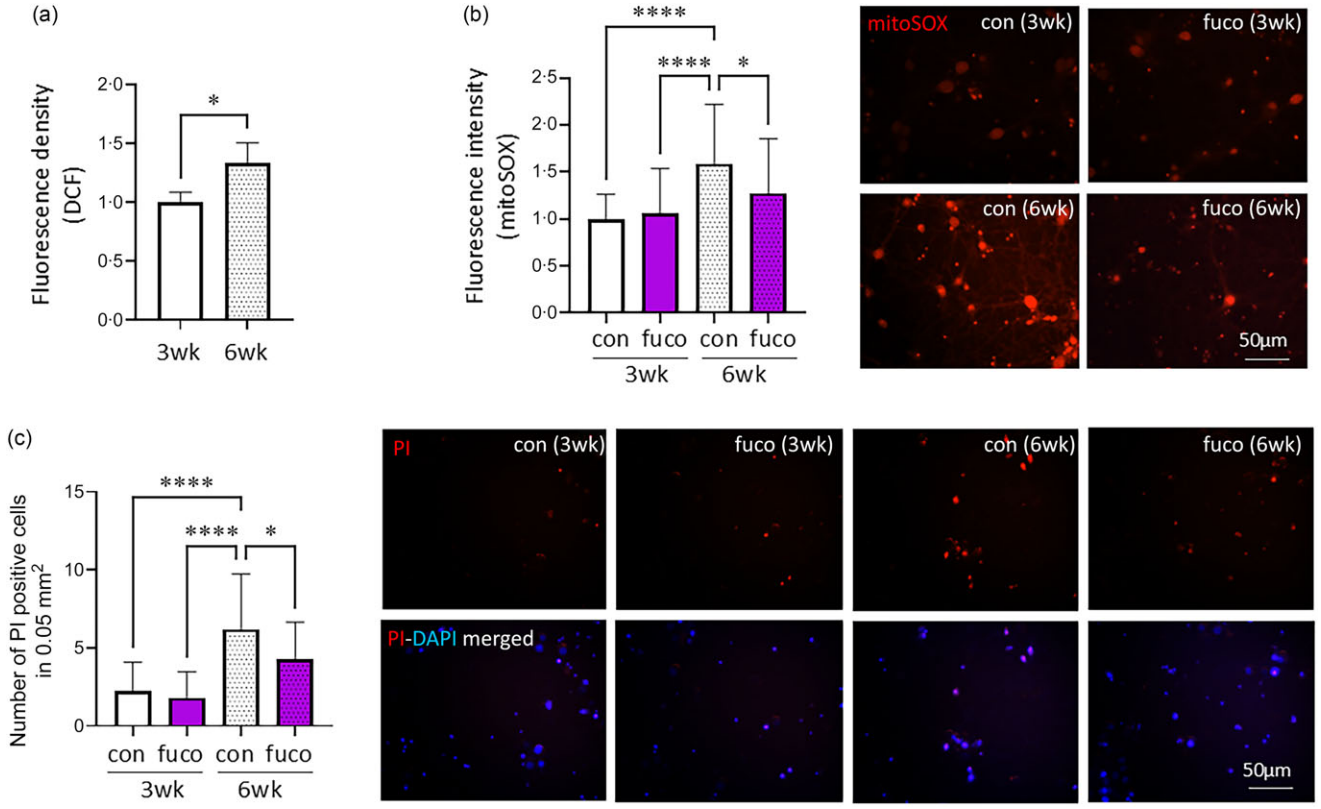

Figure 2. Fucoxanthin protects primary hippocampal neurons against *in vitro* ageing. Primary hippocampal neurons were grown in neurobasal media for 3 or 6 weeks. Intracellular hydrogen peroxide levels were assayed by measuring DCF (a), *n* 4 from three independent cultures. $^{*}P < 0{\cdot}05$, Student's *t* test. Three or 6-week-old primary hippocampal neurons were treated with or without fucoxanthin (100 nM) for 24 h, and mitochondrial oxidative stress was measured by mitoSOX staining (b), *n* 45 micrographs per group. Scale bar = 50 μm. Red: mitoSOX. Cell death was measured by quantifying PI-positive cells (c), *n* 30 micrographs per group. Scale bar = 50 μm. Red: PI; blue: DAPI. $^{*}P < 0{\cdot}05$ and $^{****}P < 0{\cdot}0001$, one-way ANOVA with a Tukey's *post hoc* analysis. PI, propidium iodide.

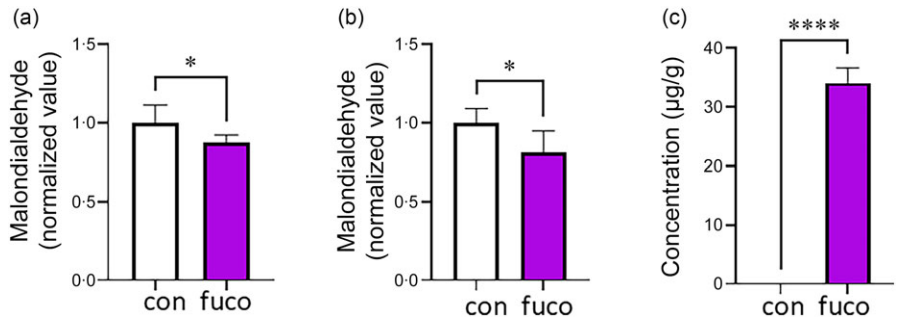

Figure 3. Fucoxanthin is found in the brain of rats after oral supplementation. Oral supplementation of fucoxanthin significantly decreases malondialdehyde levels in blood (a) and the brain (b) collected from rats (n 6). (c) Oral supplementation of fucoxanthin increased fucoxanthin in the brain (n 3). *$P < 0·05$ and ****$P < 0·0001$, Student's t test.

(SD 3·53) *v.* 6-week with fucoxanthin 4·30 (SD 2·35), $P = 0·02$, Figure 2(c)), suggesting neuroprotective effect during *in vitro* ageing.

Fucoxanthin is found in the brain after oral supplementation

Previous studies in animals that underwent oral supplementation with fucoxanthin were reported to demonstrate improved cognitive behaviours against Alzheimer's disease and acute brain injury[18,35]. Similarly, intragastric administration of fucoxanthin alleviated behaviours associated with depression and ischaemia[36,37]. However, it is still unclear whether orally administered fucoxanthin is delivered to the brain and exhibits a direct protective effect. In this study, male Sprague-Dawley rats were orally gavaged with 1 mg/kg fucoxanthin or the same volume of vehicle for 4 weeks. The concentration of fucoxanthin was determined based on the estimated habitual intake of fucoxanthin in Japanese women, which ranges from 0 to 5·66 mg/d, corresponding to approximately 0–0·12 mg/kg body weight[38]. The animal dose equivalence of which is around 0–0·7 mg/kg in rats[39]. Therefore, a dose of 1 mg/kg was chosen to ensure a physiologically relevant exposure. To evaluate the direct antioxidant effects of fucoxanthin, we measured MDA to determine the level of lipid peroxidation (Figure 3 (a) and (b)). MDA is a final product of oxidation of PUFA that are abundant in the brain. The MDA level in the fucoxanthin group significantly decreased, indicating that oral supplementation with fucoxanthin exhibits an antioxidant effect in blood (control 1·00 (SD 0·12) *v.* fucoxanthin 0·88 (SD 0·05), $P = 0·04$) and the brain (control 1·00 (SD 0·09) *v.* fucoxanthin 0·70 (SD 0·14), $P = 0·02$). Quantitative analysis showed that oral supplementation with fucoxanthin significantly increased the concentration of fucoxanthin in the brain compared with control groups (control 0·00 (SD 0·00) *v.* fucoxanthin 34·11 (SD 2·56), $P < 0·0001$, Figure 3(c)).

Oral supplementation of fucoxanthin regulates gene expression in the brain

To determine whether oral administration of fucoxanthin resulted in changes in gene expression, we submitted whole brain tissue for rat lncRNA microarray (Arraystar) (heat map and hierarchical clustering shown in Figure 4(a)). Using this assay, we identified 5602 mRNAs that were significantly and differentially expressed (2666 upregulated, 2936 downregulated and 22252 unchanged) via volcano filtering with fold change cut-off of 2·0 and P value cut-off of 0·05 (Figure 4(b)). To determine the major biological processes, cellular compartments and molecular processes regulated by fucoxanthin administration, GO analysis using the topGO package was performed. The GO terms most highly enriched in upregulated genes were detection of stimulus (GO:0051606, biological processes), cell periphery (GO:0071944, cellular compartments) and olfactory receptor activity (GO:0004964, molecular processes). The ten most high upregulated GO terms are shown in Figure 5(a). The GO terms most highly enriched in downregulated genes were ion transport (GO:0006811, BP), extracellular space (GO:0005615, cellular compartments) and signalling receptor activator activity (GO:0030546, molecular processes). The ten most highly downregulated GO terms are shown in Figure 5(b).

Pathway analysis of rats treated with fucoxanthin compared with vehicle

To determine biological pathways represented by differentially expressed mRNAs following fucoxanthin administration, pathway analysis based on the Kyoto Encyclopedia of Genes and Genomes database was performed. Compared with vehicle control, fucoxanthin administration resulted in twenty-two upregulated pathways and forty-eight downregulated pathways. Treatment with fucoxanthin highly upregulated olfactory transduction, Ca signalling pathway, focal adhesion and ECM–receptor interaction (Figure 6(a)), whereas cytokine–cytokine receptor interaction, inflammatory mediator regulation of TRP channels, protein digestion and absorption and neuroactive ligand–receptor interaction were highly downregulated (Figure 6(b)).

Included in the significantly downregulated pathways such as thyroid cancer (rno05216), pathways in cancer (rno05200), transcriptional misregulation in cancer (rno05202) and PI3K-Akt signaling pathway (rno04151) were numerous genes involved in cell death processes such as apoptosis, including Bak1 encoding Bak (2·90-fold downregulated) and Bcl2l11 encoding Bim (9·17-fold downregulated and among the 50 most downregulated genes, Table 1). Given the prior known neuroprotective properties of fucoxanthin, we also found it interesting that the cytokine–cytokine receptor interaction pathway (rno04060) included Tnfrsf 9 gene (2·11-fold downregulated), a deletion of which was recently associated with a juvenile-onset Parkinson's disease phenotype[40]. An expanded analysis of candidate genes associated with neurodegenerative diseases revealed downregulation of Snca encoding α-synuclein (3·16-fold downregulated), Park7 encoding DJ1 (2·01-fold downregulated), Aplp2 encoding amyloid precursor-like protein 2 (2·36-fold downregulated) and Apbdlip encoding amyloid beta precursor protein binding family B member 1 interacting protein (2·54-fold downregulated).

Discussion

In this study, we showed neuroprotective roles of fucoxanthin against oxidative stress and *in vitro* ageing in primary hippocampal neurons. We further quantified fucoxanthin in the brain after oral

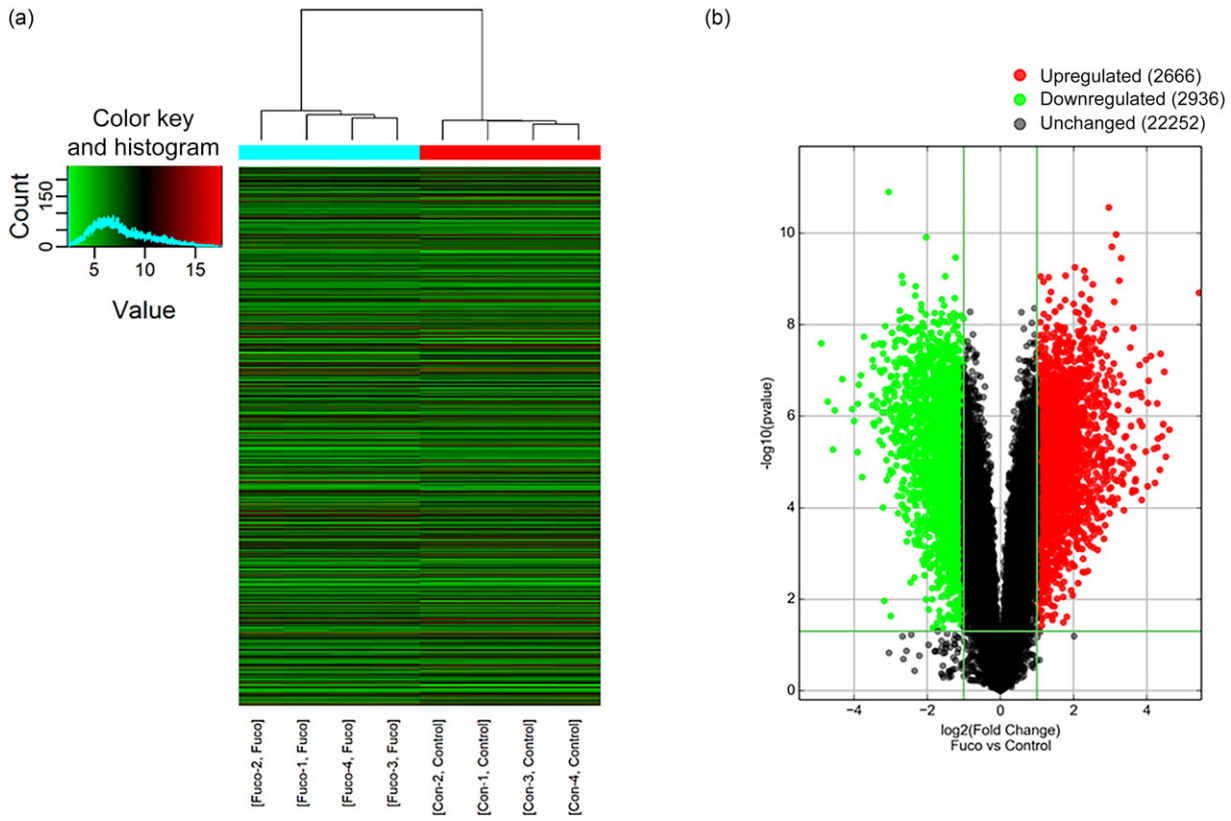

Figure 4. Differentially expressed mRNAs between rats treated with fucoxanthin or vehicle. Hierarchical clustering (a) of differentially expressed mRNAs and volcano plots (b) of mRNA expression in the brain of rats. In this plot, genes with high relative expression are colored in red, while genes with low expression are coloured in green. Data were generated from four rats receiving fucoxanthin treatment (Fuco-1 through Fuco-4) and four rats receiving vehicle treatment (Con-1 through Con-4).

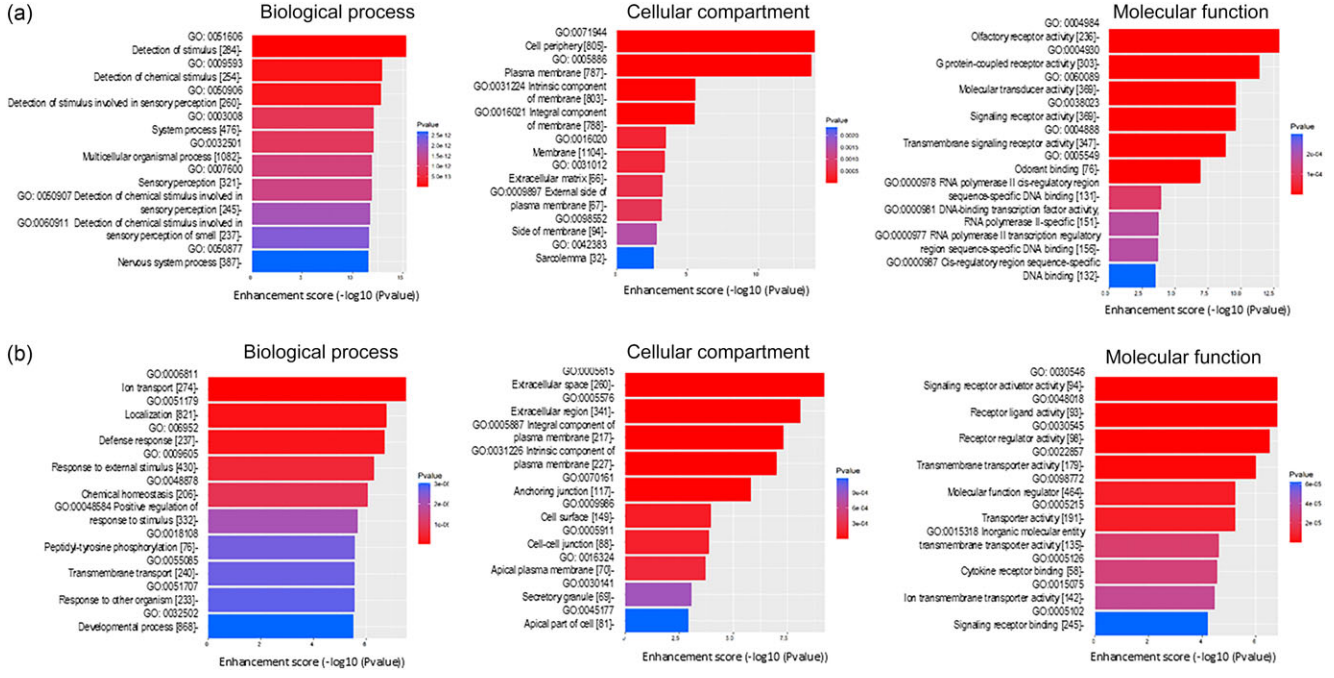

Figure 5. GO analysis of rats treated with fucoxanthin compared with vehicle. The ten most enriched GO categories containing mRNAs that are upregulated (a) and the ten most enriched GO categories containing mRNAs that are downregulated (b) by fucoxanthin treatment grouped by biological process, cellular compartment and molecular function. GO, gene ontology.

Table 1. Top 50 differentially expressed mRNAs in fucoxanthin-treated rats *v.* vehicle-treated rats

Upregulated mRNAs			
Transcript ID	Gene	Fold change	FDR
NM_001106440	Naxe	43·14	0·00000256
ENSRNOT00000037133	Stk39	24·62	0·000027076
ENSRNOT00000079433	Tbce	23·01	0·000061601
ENSRNOT00000083275	Bach1	22·37	0·000006647
NM_001007636	S100a1	21·91	0·00002334
ENSRNOT00000054966	Hgs	21·36	0·000033146
NM_012954	Fosl2	20·83	0·000004763
ENSRNOT00000025879	Trpm8	20·55	0·000095758
ENSRNOT00000084304	Wnk2	19·81	0·000035569
ENSRNOT00000050792	Pan2	19·61	0·00004531
ENSRNOT00000060210	Cyp4f37	19·53	0·000013661
ENSRNOT00000057971	AABR07034739·1	18·73	0·000150562
ENSRNOT00000042046	Myog	18·39	0·000047504
ENSRNOT00000001197	Rxfp2	17·32	0·000004932
ENSRNOT00000019018	Ccdc169	16·60	0·0000081
ENSRNOT00000093103	Fbxo10	16·39	0·000013541
ENSRNOT00000042952	Ano5	16·30	0·000169924
ENSRNOT00000060868	Bmp2k	15·77	0·00000517
ENSRNOT00000021167	LOC103690120	15·71	0·000052025
ENSRNOT00000020516	RGD1561662	14·69	0·000024389
ENSRNOT00000004032	Itpkb	14·57	0·000282446
ENSRNOT00000002151	Bach1	14·48	0·000011635
ENSRNOT00000027237	Flnc	14·17	0·000010303
ENSRNOT00000085688	AC111734·2	14·02	0·000021631
ENSRNOT00000083188	Clec2g	14·00	0·000005763
ENSRNOT00000059997	AABR07070046·1	13·16	0·000010678
ENSRNOT00000011374	Krt85	12·97	0·00019912
ENSRNOT00000080155	Mtss1	12·96	0·00013263
ENSRNOT00000073487	AABR07049320·2	12·86	0·000142599
ENSRNOT00000002191	Cep97	12·83	0·0001995
ENSRNOT00000010521	Reln	12·54	0·000044786
ENSRNOT00000009960	Sorcs2	12·48	0·000201803
ENSRNOT00000011213	Hoxb2	12·45	0·000003625
ENSRNOT00000002111	Agps	12·31	0·000077854
ENSRNOT00000021970	Uox	12·27	0·000196462
ENSRNOT00000026271	Mpv17l2	12·15	0·000211988
ENSRNOT00000012600	Tbc1d4	12·15	0·000010968
ENSRNOT00000003466	Serpinb7	11·89	0·000024648
ENSRNOT00000030881	LOC500567	11·85	0·000072453
ENSRNOT00000089221	Olr1664	11·73	0·000004337
ENSRNOT00000091079	AABR07011857·2	11·36	0·000091647
ENSRNOT00000073233	Gde1	11·33	0·000015953
ENSRNOT00000083977	AC135822·2	11·20	0·000061582
ENSRNOT00000034817	Qrich2	10·78	0·000033057
ENSRNOT00000088536	Zp2	10·62	0·000078034
ENSRNOT00000055124	Calca	10·55	0·000034085
ENSRNOT00000027487	Tnni2	10·39	0·000420397
ENSRNOT00000031275	Ppp3r1	10·30	0·000109288
ENSRNOT00000087073	LOC102557137	10·27	0·000008375
NM_001271093	Ndrg4	10·22	0·000157325
Downregulated mRNAs			
Transcript ID	Gene	Fold Change	FDR
ENSRNOT00000007932	Irak4	29·81	0·000004174
ENSRNOT00000060427	Olr1645	26·39	0·000013086
ENSRNOT00000090332	Terb1	23·89	0·000048704
NM_001108957	Nutm1	23·07	0·000015998
ENSRNOT00000039776	AABR07064102·1	19·99	0·000007758
ENSRNOT00000048860	AABR07007399·1	16·57	0·000015506
ENSRNOT00000045375	Slc5a4b	15·99	0·000021549
ENSRNOT00000008045	Rmdn2	14·96	0·000052879
ENSRNOT00000007724	Oxtr	14·85	0·000013765
ENSRNOT00000074738	AABR07018078·1	14·63	0·000008579
ENSRNOT00000058414	Tbc1d14	14·03	0·000007107
ENSRNOT00000081345	Hist1h1t	13·71	0·00012276
ENSRNOT00000076673	Optc	13·25	0·000003927
ENSRNOT00000077259	Bin3	11·47	0·000011136
ENSRNOT00000092560	AABR07020966·1	11·29	0·000014132
ENSRNOT00000023657	Mylk3	11·25	0·00000517
ENSRNOT00000078082	Fancg	11·04	0·000022748
ENSRNOT00000087154	Setd2	11·03	0·000006093
ENSRNOT00000066218	Ntng1	10·94	0·000004223
NM_001246283	Wfdc6a	10·88	0·000024069
ENSRNOT00000028693	Anxa9	10·41	0·000012797
ENSRNOT00000044417	Jsrp1	10·32	0·000015067
ENSRNOT00000017972	Casp9	9·94	0·000021311
ENSRNOT00000031889	Chodl	9·88	0·00003794
ENSRNOT00000046890	Retnlb	9·80	0·000004174
ENSRNOT00000075038	Smim24	9·77	0·000006505
ENSRNOT00000011509	Csf3	9·55	0·00000673
ENSRNOT00000091822	Fchsd2	9·36	0·000016748
ENSRNOT00000013956	Rcan2	9·33	0·000004219
ENSRNOT00000039983	Krtap3–3	9·31	0·000028394
ENSRNOT00000087182	Clvs1	9·30	0·00004254
ENSRNOT00000034487	Esf1	9·25	0·000377264
ENSRNOT00000016781	Actrt2	9·22	0·000013661

Table 1. (*Continued*)

Downregulated mRNAs			
Transcript ID	Gene	Fold Change	FDR
ENSRNOT00000003954	Ccnb3	9·20	0·000004763
ENSRNOT00000022596	Bcl2l11	9·17	0·000004174
NM_032613	Lasp1	9·12	0·000013762
ENSRNOT00000093640	Sh3d19	9·04	0·000006441
ENSRNOT00000093066	Clasp1	9·02	0·018294211
NM_053649	Kremen1	8·86	0·000003625
ENSRNOT00000026680	Ankrd28	8·84	0·000009734
ENSRNOT00000022074	Fxyd2	8·78	0·000010968
ENSRNOT00000082271	Plec	8·67	0·000016748
ENSRNOT00000073930	Plin5	8·64	0·000069243
ENSRNOT00000080931	Sema3e	8·61	0·000012708
ENSRNOT00000064580	Swsap1	8·59	0·000137225
ENSRNOT00000058079	Dgkh	8·52	0·000026859
NM_001110336	Vegfa	8·51	0·00000517
ENSRNOT00000003989	Bmp15	8·31	0·000000351
NM_001047956	Ier3ip1	8·24	0·000009569
ENSRNOT00000024374	Slc26a2	8·21	0·000114582

FDR, false discovery rate.
Fold change is fucoxanthin *v.* vehicle (control).

supplementation and demonstrated its delivery to the brain. Fucoxanthin exhibited direct effects in the brain as an antioxidant and a gene regulator. We identified over 5000 genes that are sensitive to fucoxanthin treatment, including genes that are associated with neurodegeneration.

While the pharmacokinetics of fucoxanthin in the blood and other tissues, including liver, lung, heart and adipose tissue, have been reported in rodents[30,41], no prior studies have demonstrated the delivery of fucoxanthin to the brain. Our data are the first to provide a quantitative analysis of fucoxanthin in the brain after oral administration. Although a single administration may be inadequate for the distribution of fucoxanthin in tissue, as fucoxanthin can be converted to fucoxanthionol and amarouciaxanthin A via hydrolysis and dehydrogenation, respectively[42]. Hashimoto *et al.* showed that fucoxanthin was detectable in various tissues after daily oral administration of 160 nM fucoxanthin for 1 week[41]. We successfully detected fucoxanthin in the brain after the administration of fucoxanthin (1 mg/kg) for 4 weeks, and thus, habitual intake may favour the accumulation of fucoxanthin in the target tissues. Although we did not perform quantitative analysis on fucoxanthin's metabolites in this study, the mechanisms of fucoxanthin and metabolites may be distinctive[43]. To understand the effect of the habitual intake of fucoxanthin, future quantitative analysis with metabolites following the prolonged administration of fucoxanthin can elucidate the metabolism, kinetics and bioavailability of fucoxanthin in the brain.

Although it is still unclear whether administration of fucoxanthin in a range similar to 1 mg/kg is neuroprotective in humans, a single oral administration of 31 mg fucoxanthin to humans with a mean body weight of 60 kg (which is approximately 0·5 mg/kg) demonstrated pharmacokinetic profiles in plasma[44]. In addition, oral administration of a high concentration of fucoxanthin (500 and 1000 mg/kg) in mice for 30 d did not alter mortality and the growth rate[45]. Although clinical trials investigating fucoxanthin for neuroprotection are lacking, the few that have investigated fucoxanthin supplementation for obesity and other cardiometabolic disorders have not shown adverse effects[46]. Thus, a milligram range in concentrations may be feasible to investigate the translational aspects of neuroprotective effects of fucoxanthin in the future.

We selected middle-aged healthy rats to eliminate the effects of ageing-associated pathology, thus our *in vivo* data may not directly represent neuroprotective function of fucoxanthin for specific disease states. Rather, we investigated the role of fucoxanthin in natural ageing. We found that oral supplementation with fucoxanthin alters numerous genes that control biological processes, cellular components and molecular function. In particular, pathway analysis based upon the Kyoto Encyclopedia of Genes and Genomes database revealed that fucoxanthin inhibits signaling pathways responsible for inflammation, such as cytokine–cytokine receptor interaction and inflammatory mediator regulation of TRP channels. Unexpectedly, we also found that

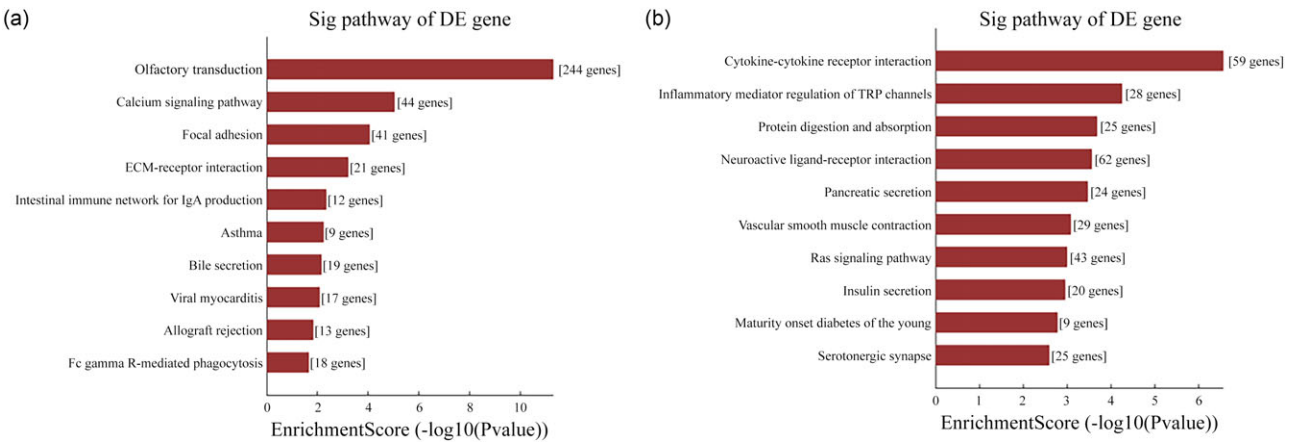

Figure 6. Pathway analysis of rats treated with fucoxanthin compared with vehicle. KEGG pathway analysis was used to identify the ten most enriched pathways containing mRNAs that are upregulated (a) and the ten most enriched pathways containing mRNAs that are downregulated (b) by fucoxanthin treatment. KEGG, Kyoto Encyclopedia of Genes and Genomes.

treatment with fucoxanthin upregulated 244 genes in olfactory transduction. The results of this pathway analysis are interesting because preclinical processes of neurodegeneration are known to occur decades before the diagnosis of neurodegenerative diseases, and inflammation and olfactory dysfunction are important predictors[47,48] and biomarkers of neurodegeneration[49,50]. Approximately 90 % of individuals with early-stage Parkinson's disease and 85 % of those with early-stage Alzheimer's disease exhibit olfactory dysfunction[51]. When the Movement Disorder Society's Research Criteria for Prodromal Parkinson's Disease were updated in 2019, olfactory dysfunction was identified as a strong marker with a 6·4 likelihood ratio of risk[52]. Evidence suggests that accumulation of misfolded α-synuclein in the olfactory bulb precedes accumulation in other brain regions[53].

Neuroprotective properties of fucoxanthin have been reported in models of neurodegenerative diseases. Treatment with fucoxanthin prevents 6-OHDA-mediated neurotoxicity both *in vitro* and *in vivo*[19,20]. 6-OHDA is taken up by dopaminergic neurons via dopamine receptors and releases hydrogen peroxide; thus, it is commonly used to mimic oxidative stress in Parkinson's disease pathology. Wu *et al.* showed that fucoxanthin directly binds to Keap1 and increases expression of the downstream antioxidant enzymes of the Keap1/nuclear factor erythroid 2-related factor 2-ARE pathway such as nicotinamide heme oxygenase-1, glutamate-cysteine ligase modifier subunit and glutamate cysteine ligase catalytic subunit[19]. Further, fucoxanthin has a concentration-dependent agonist effect on dopamine D3 and D4 receptors[54]. Previously, fucoxanthin was reported to prevent the loss of mitochondrial membrane potential against 6-OHDA and levodopa challenges[20]. Although the exact mechanism is unknown, we found that fucoxanthin downregulated the Ntrk3 gene, which encodes tropomyosin receptor kinase C. Tropomyosin receptor kinase C has been shown to inhibit DJ1 degradation[55], and thus, treatment with fucoxanthin may help enhance DJ1's stability.

Fucoxanthin has been tested in models of Alzheimer's disease. Treatment with fucoxanthin was found to alleviate Aβ-mediated cytotoxicity *in vitro* and cognitive impairments *in vivo*[17,18,56,57]. Notably, fucoxanthin showed a concentration-dependent Bax/Bcl-2 ratio reduction and prevented the loss of mitochondrial membrane potential against Aβ challenge, indicating mitochondrial protection[17]. Fucoxanthin binds to Aβ directly via hydrophobic interactions and prevents Aβ oligomerisation[18]. Similarly, a molecular docking simulation study identified fucoxanthin's inhibitory effects on Aβ aggregation by its interaction with 16–20, 24–28 and 33–37 residues of Aβ[58]. In addition to functional inhibition, fucoxanthin may prevent the production of Aβ. In addition, as an antioxidant, fucoxanthin may prevent cleavage of APP to Aβ by blocking oxidative stress-mediated secretase activation.

Because our pathway analysis revealed that fucoxanthin downregulates fifty-nine genes that regulate cytokine–cytokine receptor interaction and twenty-eight genes that regulate inflammatory mediator regulation of TRP channels, it is not surprising to find that fucoxanthin's anti-inflammatory effects have been reported in the literature. Microglial cells challenged with lipopolysaccharide increased mRNA and protein levels of pro-inflammatory cytokines such as TNF-α and IL-6, while treatment with fucoxanthin exhibited a concentration-dependent inhibitory effect. The authors further showed that fucoxanthin inhibits Akt/nuclear factor-kappa B and mitogen-activated protein kinase/activator protein-1 pathways[59]. Similarly, treatment with fucoxanthin attenuated the production of pro-inflammatory cytokines against Aβ challenge in microglial cells[60].

Although our study identifies fucoxanthin-sensitive genes and pathways in the brain, some limitations exist. In this study, we used whole-brain samples for *in vivo* analysis; therefore, our findings are insufficient for explaining the heterogeneity of the brain. For example, because the olfactory transduction is the major pathway affected by fucoxanthin, future analysis of the olfactory bulb can help explain the region-specific role of fucoxanthin. Given that current studies primarily focus on the role of fucoxanthin in natural ageing, introducing neuropathology with functional and behavioural analysis in future projects can bridge the gap between healthy brain ageing and neurodegenerative disease and can help elucidate the therapeutic potential of fucoxanthin in the brain.

Conclusion

In summary, treatment with fucoxanthin protects neurons against ageing-associated damage *in vitro*. We also show that orally administered fucoxanthin is detected in the brain and exhibits an antioxidant effect. We demonstrated that oral supplementation with fucoxanthin changes genes that are associated with neuronal damage in middle-aged rats without a phenotype of neurodegenerative diseases. Combined with evidence from reported epidemiological studies with populations with frequent dietary consumption of brown seaweed[61], our data suggest that fucoxanthin is a potential dietary strategy to benefit natural brain ageing.

Acknowledgements. We would like to thank Algatechnologies Ltd (Israel) for donating fucoxanthin.

This study was supported by The University of Alabama Joint Institute Pandemic Pilot Project (H-A. P. and A. E.), and R. N. C. was supported by a contribution from the Dale/Roy family.

H-A. P. was involved in conceptualization, formal analysis, funding acquisition, investigation, resources, supervision, and writing; E. A. was involved in investigation and formal analysis; G. B. was involved in investigation and formal analysis; K. A. F. was involved in investigation and formal analysis; M. S. was involved in investigation and formal analysis; J. J. was involved in investigation and formal analysis; S. B. was involved in investigation and formal analysis; M.S. was involved in investigation and formal analysis; R. N. C. was involved in formal analysis, funding acquisition, investigation, and writing; L. C. was involved in formal analysis, investigation, resources, and writing; A. E. was involved in funding acquisition, supervision, and writing.

The authors declare no conflict of interest.

References

1. Hou Y, Dan X, Babbar M, *et al.* (2019) Ageing as a risk factor for neurodegenerative disease. *Nat Rev Neurol* **15**, 565–581.
2. Dement A (2024) 2024 Alzheimer's disease facts and figures. *Alzheimers Dement* **20**, 3708–3821.
3. Willis AW, Roberts E, Beck JC, *et al.* (2022) Incidence of Parkinson disease in North America. *NPJ Parkinsons Dis* **8**, 170.
4. WHO (2022) Launch of WHO's Parkinson Disease Technical Brief. https://www.who.int/news/item/14–06–2022-launch-of-who-s-parkinson-disease-technical-brief (accessed June 2022).
5. Collaborators GBDN (2019) Global, regional, and national burden of neurological disorders, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol* **18**, 459–480.
6. Alwahsh SM & Gebhardt R (2017) Dietary fructose as a risk factor for non-alcoholic fatty liver disease (NAFLD). *Arch Toxicol* **91**, 1545–1563.
7. Alwahsh SM, Xu M, Schultze FC, *et al.* (2014) Combination of alcohol and fructose exacerbates metabolic imbalance in terms of hepatic damage, dyslipidemia, and insulin resistance in rats. *PLoS One* **9**, e104220.

8. Song BJ, Akbar M, Abdelmegeed MA, *et al.* (2014) Mitochondrial dysfunction and tissue injury by alcohol, high fat, nonalcoholic substances and pathological conditions through post-translational protein modifications. *Redox Biol* **3**, 109–123.
9. Ferdous KA, Sarihi S, Ellis AC, *et al.* (2023) Neuroprotective potential of brown seaweed phytochemicals in rodent models of cerebral ischemia. *J Med Food* **26**, 436–444.
10. Park HA, Crowe-White KM, Ciesla L, *et al.* (2022) Alpha-tocotrienol enhances arborization of primary hippocampal neurons via upregulation of Bcl-xL. *Nutr Res* **101**, 31–42.
11. Willcox DC, Willcox BJ, Shimajiri S, *et al.* (2007) Aging gracefully: a retrospective analysis of functional status in Okinawan centenarians. *Am J Geriatr Psychiatry* **15**, 252–256.
12. Willcox DC, Scapagnini G & Willcox BJ (2014) Healthy aging diets other than the Mediterranean: a focus on the Okinawan diet. *Mech Ageing Dev* **136–137**, 148–162.
13. Peng J, Yuan JP, Wu CF, *et al.* (2011) Fucoxanthin, a marine carotenoid present in brown seaweeds and diatoms: metabolism and bioactivities relevant to human health. *Mar Drugs* **9**, 1806–1828.
14. Sachindra NM, Sato E, Maeda H, *et al.* (2007) Radical scavenging and singlet oxygen quenching activity of marine carotenoid fucoxanthin and its metabolites. *J Agric Food Chem* **55**, 8516–8522.
15. Rodrigues E, Mariutti LR & Mercadante AZ (2012) Scavenging capacity of marine carotenoids against reactive oxygen and nitrogen species in a membrane-mimicking system. *Mar Drugs* **10**, 1784–1798.
16. Takashima M, Shichiri M, Hagihara Y, *et al.* (2012) Capacity of fucoxanthin for scavenging peroxyl radicals and inhibition of lipid peroxidation in model systems. *Free Radic Res* **46**, 1406–1412.
17. Lee N, Youn K, Yoon JH, *et al.* (2023) The role of fucoxanthin as a potent Nrf2 activator via Akt/GSK-3beta/Fyn axis against amyloid-beta peptide-induced oxidative damage. *Antioxidants (Basel)* **12**, 629.
18. Xiang S, Liu F, Lin J, *et al.* (2017) Fucoxanthin inhibits beta-amyloid assembly and attenuates beta-amyloid oligomer-induced cognitive impairments. *J Agric Food Chem* **65**, 4092–4102.
19. Wu W, Han H, Liu J, *et al.* (2021) Fucoxanthin prevents 6-OHDA-induced neurotoxicity by targeting Keap1. *Oxid Med Cell Longev* **2021**, 6688708.
20. Liu J, Lu Y, Tang M, *et al.* (2022) Fucoxanthin prevents long-term administration l-DOPA-induced neurotoxicity through the ERK/JNK-c-Jun system in 6-OHDA-lesioned mice and PC12 cells. *Mar Drugs* **20**, 245.
21. Wilson DM 3rd, Cookson MR, Van Den Bosch L, *et al.* (2023) Hallmarks of neurodegenerative diseases. *Cell* **186**, 693–714.
22. Park HA, Licznerski P, Alavian KN, *et al.* (2015) Bcl-xL is necessary for neurite outgrowth in hippocampal neurons. *Antioxid Redox Signal* **22**, 93–108.
23. Beaudoin GM 3rd, Lee SH, Singh D, *et al.* (2012) Culturing pyramidal neurons from the early postnatal mouse hippocampus and cortex. *Nat Protoc* **7**, 1741–1754.
24. Kaech S & Banker G (2006) Culturing hippocampal neurons. *Nat Protoc* **1**, 2406–2415.
25. Park HA, Licznerski P, Mnatsakanyan N, *et al.* (2017) Inhibition of Bcl-xL prevents pro-death actions of DeltaN-Bcl-xL at the mitochondrial inner membrane during glutamate excitotoxicity. *Cell Death Differ* **24**, 1963–1974.
26. Duan Y, Gross RA & Sheu SS (2007) Ca2+-dependent generation of mitochondrial reactive oxygen species serves as a signal for poly (ADP-ribose) polymerase-1 activation during glutamate excitotoxicity. *J Physiol* **585**, 741–758.
27. Park HA, Mnatsakanyan N, Broman K, *et al.* (2019) Alpha-tocotrienol prevents oxidative stress-mediated post-translational cleavage of Bcl-xL in primary hippocampal neurons. *Int J Mol Sci* **21**, 220.
28. Alavian KN, Beutner G, Lazrove E, *et al.* (2014) An uncoupling channel within the c-subunit ring of the F1FO ATP synthase is the mitochondrial permeability transition pore. *Proc Natl Acad Sci USA* **111**, 10580–10585.
29. Zhang L, Wang H, Fan Y, *et al.* (2017) Fucoxanthin provides neuroprotection in models of traumatic brain injury via the Nrf2-ARE and Nrf2-autophagy pathways. *Sci Rep* **7**, 46763.
30. Zhang Y, Wu H, Wen H, *et al.* (2015) Simultaneous determination of fucoxanthin and its deacetylated metabolite fucoxanthinol in rat plasma by liquid chromatography-tandem mass spectrometry. *Mar Drugs* **13**, 6521–6536.
31. Vishwanathan R, Neuringer M, Snodderly DM, *et al.* (2013) Macular lutein and zeaxanthin are related to brain lutein and zeaxanthin in primates. *Nutr Neurosci* **16**, 21–29.
32. Toomey MB & McGraw KJ (2007) Modified saponification and HPLC methods for analyzing carotenoids from the retina of quail: implications for its use as a nonprimate model species. *Invest Ophthalmol Vis Sci* **48**, 3976–3982.
33. Waksmundzka-Hajnos M & Sherma J (2011) *High Performance Liquid Chromatography in Phytochemical Analysis. Chromatographic Science Series v 102*, p. 1 online resource (xx, 975 pages). Boca Raton, FL: CRC Press.
34. Park HA, Stumpf A, Broman K, *et al.* (2021) Role of lycopene in mitochondrial protection during differential levels of oxidative stress in primary cortical neurons. *Brain Disorders* **3**, 100016.
35. Zhang XS, Lu Y, Tao T, *et al.* (2020) Fucoxanthin mitigates subarachnoid hemorrhage-induced oxidative damage via sirtuin 1-dependent pathway. *Mol Neurobiol* **57**, 5286–5298.
36. Jiang X, Wang G, Lin Q, *et al.* (2019) Fucoxanthin prevents lipopolysaccharide-induced depressive-like behavior in mice via AMPK- NF-kappaB pathway. *Metab Brain Dis* **34**, 431–442.
37. Hu L, Chen W, Tian F, *et al.* (2018) Neuroprotective role of fucoxanthin against cerebral ischemic/reperfusion injury through activation of Nrf2/HO-1 signaling. *Biomed Pharmacother* **106**, 1484–1489.
38. Fukushima Y, Taguchi C, Kishimoto Y, *et al.* (2023) Japanese carotenoid database with α- and beta-carotene, beta-cryptoxanthin, lutein, zeaxanthin, lycopene, and fucoxanthin and intake in adult women. *Int J Vitam Nutr Res* **93**, 42–53.
39. Nair AB & Jacob S (2016) A simple practice guide for dose conversion between animals and human. *J Basic Clin Pharm* **7**, 27–31.
40. Guler S, Gul T, Guler S, *et al.* (2021) Early-onset Parkinson's disease: a novel deletion comprising the DJ-1 and TNFRSF9 genes. *Mov Disord* **36**, 2973–2976.
41. Hashimoto T, Ozaki Y, Taminato M, *et al.* (2009) The distribution and accumulation of fucoxanthin and its metabolites after oral administration in mice. *Br J Nutr* **102**, 242–248.
42. Asai A, Sugawara T, Ono H, *et al.* (2004) Biotransformation of fucoxanthinol into amarouciaxanthin A in mice and HepG2 cells: formation and cytotoxicity of fucoxanthin metabolites. *Drug Metab Dispos* **32**, 205–211.
43. Pruccoli L, Balducci M, Pagliarani B, *et al.* (2024) Antioxidant and neuroprotective effects of fucoxanthin and its metabolite fucoxanthinol: a comparative *in vitro* study. *Curr Issues Mol Biol* **46**, 5984–5998.
44. Hashimoto T, Ozaki Y, Mizuno M, *et al.* (2012) Pharmacokinetics of fucoxanthinol in human plasma after the oral administration of kombu extract. *Br J Nutr* **107**, 1566–1569.
45. Beppu F, Niwano Y, Tsukui T, *et al.* (2009) Single and repeated oral dose toxicity study of fucoxanthin (FX), a marine carotenoid, in mice. *J Toxicol Sci* **34**, 501–510.
46. Kumarasinghe HS & Gunathilaka MD (2024) A systematic review of fucoxanthin as a promising bioactive compound in drug development. *Phytochem Lett* **61**, 52–65.
47. Lafaille-Magnan ME, Poirier J, Etienne P, *et al.* (2017) Odor identification as a biomarker of preclinical AD in older adults at risk. *Neurology* **89**, 327–335.
48. Alcolea D, Martinez-Lage P, Sanchez-Juan P, *et al.* (2015) Amyloid precursor protein metabolism and inflammation markers in preclinical Alzheimer disease. *Neurology* **85**, 626–633.
49. Tian Q, Bilgel M, Moghekar AR, *et al.* (2022) Olfaction, cognitive impairment, and PET biomarkers in community-dwelling older adults. *J Alzheimers Dis* **86**, 1275–1285.
50. Morgan AR, Touchard S, Leckey C, *et al.* (2019) Inflammatory biomarkers in Alzheimer's disease plasma. *Alzheimers Dement* **15**, 776–787.
51. Dan X, Wechter N, Gray S, *et al.* (2021) Olfactory dysfunction in aging and neurodegenerative diseases. *Ageing Res Rev* **70**, 101416.
52. Heinzel S, Berg D, Gasser T, *et al.* (2019) Update of the MDS research criteria for prodromal Parkinson's disease. *Mov Disord* **34**, 1464–1470.

53. Saramago I & Franceschi AM (2021) Olfactory dysfunction in neurodegenerative disease. *Top Magn Reson Imaging* **30**, 167–172.
54. Paudel P, Seong SH, Jung HA, *et al.* (2019) Characterizing fucoxanthin as a selective dopamine D(3)/D(4) receptor agonist: relevance to Parkinson's disease. *Chem Biol Interact* **310**, 108757.
55. Kim MS, Lee WS, Park Y, *et al.* (2022) TrkC-mediated inhibition of DJ-1 degradation is essential for direct regulation of pathogenesis of hepatocellular carcinoma. *Cell Death Dis* **13**, 850.
56. Alghazwi M, Smid S, Musgrave I, *et al.* (2019) *In vitro* studies of the neuroprotective activities of astaxanthin and fucoxanthin against amyloid beta (Abeta(1–42)) toxicity and aggregation. *Neurochem Int* **124**, 215–224.
57. Lin J, Yu J, Zhao J, *et al.* (2017) Fucoxanthin, a marine carotenoid, attenuates beta-amyloid oligomer-induced neurotoxicity possibly via regulating the PI3K/Akt and the ERK pathways in SH-SY5Y cells. *Oxid Med Cell Longev* **2017**, 6792543.
58. Lakey-Beitia J, Kumar DJ, Hegde ML, *et al.* (2019) Carotenoids as novel therapeutic molecules against neurodegenerative disorders: chemistry and molecular docking analysis. *Int J Mol Sci* **20**, 5553.
59. Zhao D, Kwon SH, Chun YS, *et al.* (2017) Anti-neuroinflammatory effects of fucoxanthin via inhibition of Akt/NF-kappaB and MAPKs/AP-1 pathways and activation of PKA/CREB pathway in lipopolysaccharide-activated BV-2 microglial cells. *Neurochem Res* **42**, 667–677.
60. Pangestuti R, Vo TS, Ngo DH, *et al.* (2013) Fucoxanthin ameliorates inflammation and oxidative reponses in microglia. *J Agric Food Chem* **61**, 3876–3883.
61. Brown ES, Allsopp PJ, Magee PJ, *et al.* (2014) Seaweed and human health. *Nutr Rev* **72**, 205–216.