

Original Research

Dietary Repletion with ω 3 Fatty Acid or with COX Inhibition Reverses Cognitive Effects in F3 ω 3 Fatty-Acid-Deficient Mice

Ahmad Hafandi,^{1,4,*} Denovan P Begg,^{1,3} Shirmila D Premaratna,¹ Andrew J Sinclair,³ Mark Jois,² and Richard S Weisinger¹

Dietary deficiency of ω 3 fatty acid during development leads to impaired cognitive function. However, the effects of multiple generations of ω 3 fatty-acid deficiency on cognitive impairment remain unclear. In addition, we sought to test the hypothesis that the cognitive impairments of ω 3 fatty-acid-deficient mice are mediated through the arachidonic acid-cyclooxygenase (COX) pathway. To address these issues, C57BL/6J mice were bred for 3 generations and fed diets either deficient (DEF) or sufficient (SUF) in ω 3 fatty acids. At postnatal day 21, the F3 offspring remained on the dam's diet or were switched to the opposite diet, creating 4 groups. In addition, 2 groups that remained on the dam's diet were treated with a COX inhibitor. At 19 wk of age, spatial-recognition memory was tested on a Y-maze. Results showed that 16 wk of SUF diet reversed the cognitive impairment of F3 DEF mice. However, 16 wk of ω 3 fatty-acid-deficient diet impaired the cognitive performance of the F3 SUF mice, which did not differ from that of the F3 DEF mice. These findings suggest that the cognitive deficits after multigenerational maintenance on ω 3 fatty-acid-deficient diet are not any greater than are those after deficiency during a single generation. In addition, treatment with a COX inhibitor prevented spatial-recognition deficits in F3 DEF mice. Therefore, cognitive impairment due to dietary ω 3 fatty-acid deficiency appears to be mediated by the arachidonic acid-COX pathway and can be prevented by 16 wk of dietary repletion with ω 3 fatty acids or COX inhibition.

Abbreviations: AA, arachidonic acid; COX, cyclooxygenase; DEF, ω 3 fatty-acid-deficient; DHA, docosahexaenoic acid; MWM, Morris water maze; SUF, ω 3 fatty-acid-sufficient.

Dietary deficiency of ω 3 fatty acid is associated with impaired cognitive function. For example, rats on ω 3 fatty-acid-deficient diet took significantly longer to locate the platform during the swimming test in the Morris water-maze (MWM) test.⁶ Previous studies report that dietary ω 3 fatty-acid deficiency led to significantly shorter latencies in the passive-avoidance test in rats³ and increased time in the Barnes circular test in mice.⁷ However, ω 3 fatty-acid-deficient diet increased the time and number of entries in the maze-learning task in a single generation of mice.²² These findings may suggest that ω 3 fatty-acid-deficient diet influences cognitive function in animals by impairing their performance in spatial-recognition memory tasks.

Previous studies have shown that rodents raised on an ω 3 fatty-acid-deficient diet over 2 or 3 generations have impaired learning performance in the MWM task.^{17,25} Dietary ω 3 fatty-acid deficiency in the F2 and F3 rats prolonged the escape latency and delayed acquisition of the MWM task compared with those of rats fed an ω 3 fatty-acid-sufficient diet for both generations.¹⁷ In a subsequent study,

F3 rats fed on ω 3 fatty-acid-deficient diet since birth or at weaning had a lower mean swimming speed to locate the platform during the MWM task.¹⁸ Previous studies show that feeding mice an ω 3 fatty-acid-deficient diet for 3 generations reduced swimming performance in the MWM test.²⁵ Interestingly, feeding rats suboptimal levels of docosahexaenoic acid (DHA) for four generations significantly prolonged latencies in the MWM task compared those of rats fed higher levels of DHA.¹² These results suggest that multigenerational feeding of an ω 3 fatty-acid-deficient diet impairs performance in tests of spatial-recognition memory.

Importantly, after several generations of ω 3 fatty-acid deficiency, switching rats to a sufficient diet at birth restored their performance on the spatial-recognition task to normal.¹⁸ Similarly, cognitive impairment in the brightness-discrimination test in mice after 2 generations of dietary ω 3 fatty-acid deficiency was reversed by providing ω 3 fatty-acid-sufficient diet after weaning.⁹ Cognitive performance in the MWM test did not differ in mice provided an ω 3 fatty-acid-sufficient diet only and those switched at 7 wk of age from an ω 3 fatty-acid-deficient diet to a sufficient diet.⁴ Overall, these findings indicate that the cognitive impairments due to ω 3 fatty-acid deficiency are reversed by providing a diet containing sufficient amounts of ω 3 fatty acids.

Previous studies have been shown that the cognitive and memory deficits of a transgenic mouse model are due to increased

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¹School of Psychological Science and ²School of Life Sciences, La Trobe University, Victoria, Australia; ³School of Exercise and Nutrition Sciences, Deakin University, Victoria, Australia; ⁴Department of Veterinary Preclinical Sciences, Faculty of Veterinary Medicine, University Putra Malaysia, Serdang, Selangor Malaysia.

*Corresponding author. Email: hafandi@upm.edu.my

prostaglandin activity from formation of cyclooxygenase (COX).¹³ Dietary ω 3 fatty acid deficiency has been suggested to increase prostaglandin activity in animals.¹⁶ Therefore, the administration of a COX inhibitor may protect against cognitive impairment in the elevated plus-maze task by inhibiting the synthesis of prostaglandin.¹¹ Treatment with a COX inhibitor improved open-field exploration in mice by inhibiting the synthesis of prostaglandin.²³ Similarly, COX inhibitors such as celecoxib inhibit prostaglandin E2 levels and consequently improve cognitive performance in rats as assessed by the elevated plus-maze test.⁵ In addition, the administration of naproxen, another COX inhibitor, was protective against motor and cognitive impairment in rats by decreasing oxidative stress.¹⁴ Moreover, naproxen reduced oxidative stress levels and prevented neurologic disorders, especially memory deficits, in an animal model of excitotoxic neuronal injury.²⁰ Clearly, these findings suggest that COX inhibitors may protect against cognitive and memory deficits in animals by inhibiting prostaglandin activity.

Clarifying the differences in cognitive function between the first and third generations of mice likely would improve our understanding of the factors contributing to differences in cognitive deficits due to dietary ω 3 fatty-acid deficiency. To this end, we raised and maintained third-generation mice on a diet either sufficient or deficient in ω 3 fatty acids or on a cross-over diet. Spatial-recognition memory in the F3 mice was tested by using the Y-maze. The aim of our transgenerational studies was to determine whether dietary ω 3 fatty-acid deficiency causes severe cognitive impairment in F3 mice. In addition, these studies examined the hypothesis that the cognitive impairment of F3 mice on an ω 3 fatty-acid deficient diet results from increased prostaglandin activity due to eicosanoid production from the arachidonic acid (AA)–COX pathway. Furthermore, we hypothesized that treatment with naproxen, a COX inhibitor, would improve cognitive function as a result of inhibiting prostaglandin activity.

Materials and Methods

Animals and diet. C57BL/6J breeder mice ($n = 16$, 8 male and 8 female; age, 10 wk) were purchased from the Australian Resource Centre (Western Australia). These mice were bred through 3 generations in the Central Animal House (La Trobe University, Victoria, Australia) on diets either deficient (DEF) or sufficient (SUF) in ω 3 fatty acids. All diets were made by Glen Forest Stock Feeders (Western Australia, Australia; Table 1). At postnatal day 21, male third-generation (F3) offspring were kept on the dam's diet or switched from dam's diet to the opposite diet, creating 4 groups (F3 SUF–SUF, F3 DEF–DEF, F3 SUF–DEF, and F3 DEF–SUF; $n = 15$ /group). In addition, 2 groups that remained on the dam's diet were treated with a COX inhibitor (naproxen, Sigma-Aldrich, St Louis, MO) at 0.07 mg/mL in drinking water (F3 SUF–SUF[+] and F3 DEF–DEF[+]; $n = 15$ /group). At 19 wk of age, spatial-recognition memory was tested in a Y-maze task. All procedures related to animal care and handling was approved by the Animal Ethics Committees of La Trobe University (approval no. AEC09-02-P).

Y-maze test. The spatial-recognition memory of the F3 mice was tested by using the Y-maze, which had 3 identical arms of equal size: the start arm, in which the mouse is first placed (always open); the familiar arm (always open); and the novel arm, which was blocked during the first trial but open during the second trial. Different visual cues were placed on the wall at the end of each

Table 1. Composition (g/100 g) of the treatment diets

	Sufficient ω 3 fatty acid diet	Deficient ω 3 fatty acid diet
Sucrose	10.692	10.692
Casein (acid)	20.000	20.000
Cellulose	5.000	5.000
Starch	39.750	39.750
Dextrinised starch	13.200	13.200
D,L methionine	0.300	0.300
Lime (fine calcium carbonate)	1.312	1.312
Salt (fine sodium chloride)	0.259	0.259
Potassium dihydrogen phosphate	0.686	0.686
Potassium sulphate	0.163	0.163
Potassium citrate	0.248	0.248
Choline chloride 50% w/w	0.250	0.250
AIN93G vitamins	1.000	1.000
AIN93G trace minerals	0.140	0.140
Safflower oil (high linoleic)	5.500	7.000
Flax oil	1.000	0
Tuna oil	0.500	0
Total	100	100

arm of the maze. Y-maze testing consisted of 2 trials separated by an interval of 1 h. The first trial was 10 min in duration and allowed the mouse to explore only 2 arms (the start and familiar arms) of the maze, with the third arm (novel arm) blocked. After 1 h, the second trial was conducted; mice were placed in the same starting arm as in trial 1, with free access to all 3 arms for 5 min. Trials were recorded by using a ceiling-mounted camera. Recordings were then watched to count the number of entries and the time spent in each arm. Y-maze performance was favorable when the number of entries and time spent in the novel arm were greater than those in the other arms. The total number of arm entries and time (in seconds) spent in the novel arm are indicator of spatial working memory.¹⁹

Statistical analysis. Two-way ANOVA with repeated measures on one variable followed by a post hoc least significant difference test (Statistica 7, StatSoft, Tulsa, OK) was used to assess performance in the Y-maze test between F3 groups. All data are reported as mean \pm SEM; statistical significance was defined as a P value of less than 0.05.

Results

Body weight and food and water intake. Differences in mean body weights and food and water intake between the 4 dietary groups were not significant (data not shown). Similarly, body weights and food and water intake did not differ between the naproxen-treated groups (data not shown).

Y-maze test. Effects of diet reversal on cognitive function. Regarding the effects of dietary ω 3 fatty-acid supplementation, two-way ANOVA indicated a significant ($F_{3,56} = 5.677$, $P < 0.05$) interaction between the preweaning and postweaning diets on the number of novel arm entries. The total number of novel arm entries was significantly ($P < 0.05$) higher in F3 SUF–SUF mice (15.6 ± 0.7 entries) than in F3 DEF–DEF mice (11.8 ± 0.8 entries). F3 SUF–DEF mice had significantly ($P < 0.05$) fewer novel arm

entries than did F3 SUF–SUF mice (9.6 ± 0.7 compared with 15.6 ± 0.7 entries). However, the number of novel arm entries mice was similar between of F3 DEF–SUF (14.7 ± 1.1 entries) and F3 SUF–SUF (15.6 ± 0.7 entries) mice.

Regarding the effects of dietary $\omega 3$ fatty-acid provision, two-way ANOVA indicated a significant ($F_{3,56} = 7.1163, P < 0.05$) interaction between preweaning and postweaning diets on time spent in the novel arm. F3 SUF–SUF mice spent more ($P < 0.05$) time in the novel arm compared with the F3 DEF–DEF mice (109.6 ± 4.7 s compared with 88.9 ± 4.9 s). F3 SUF–DEF mice spent less ($P < 0.05$) time in the novel arm than did F3 SUF–SUF mice (89.1 ± 3.3 s compared with 109.6 ± 4.7 s). However, the time spent in the novel arm was similar between the F3 DEF–SUF (111.9 ± 5.4 s) and F3 SUF–SUF (109.6 ± 4.7 s) mice.

COX-inhibition restores cognitive deficits in $\omega 3$ deficient mice. Two-way ANOVA indicated a significant ($F_{3,56} = 4.575, P < 0.05$) effect of postweaning diet (that is, with COX or without COX inhibitor) on the number of novel arm entries. F3 DEF–DEF(+) mice had significantly ($P < 0.05$) more novel arm entries than did F3 DEF–DEF mice (13.6 ± 0.8 compared with 11.8 ± 0.8 entries). However, the number of entries did not differ between F3 SUF–SUF(+) and F3 SUF–SUF mice (14.7 ± 0.7 compared with 15.6 ± 0.7 entries).

Likewise, 2-way ANOVA indicated a significant ($F_{3,56} = 2.8468, P < 0.05$) effect of postweaning diet (that is, with or without COX inhibitor) on the amount of time spent in the novel arm. F3 DEF–DEF(+) mice spent more time in the novel arm than did F3 DEF–DEF mice (109.5 ± 6.9 s compared with 88.9 ± 4.9 s). However, the time spent in the novel arm did not differ between F3 SUF–SUF(+) and F3 SUF–SUF mice (107.2 ± 6.6 s compared with 109.6 ± 4.7 s).

Discussion

The current study shows that the F3 DEF–SUF mice performed better than did F3 DEF–DEF mice in the Y-maze task. Accordingly, this study shows that the cognitive impairment caused by $\omega 3$ fatty-acid deficiency over 3 generations can be overcome by giving F3 DEF mice an $\omega 3$ fatty-acid–sufficient diet for 16 wk. These results are consistent with previous studies that have used different measures to assess visuospatial memory.^{9,17,18} In contrast, the spatial-recognition performance of the F3 SUF mice was impaired after 16 wk of $\omega 3$ fatty-acid deficiency and, interestingly, the cognitive performance of the F3 SUF–DEF mice did not differ from that of the F3 DEF–DEF mice. This finding suggests that the increased deficiency in $\omega 3$ fatty acids due to multigenerational maintenance on an $\omega 3$ fatty-acid–deficient diet does not cause any greater cognitive impairment than does deficiency for one generation.

The current study showed that naproxen treatment prevented spatial-recognition deficits in F3 DEF–DEF(+) mice. The decreases in the spatial-recognition memory of the F3 DEF–DEF mice may be due to the presence of high levels of prostaglandins. In a transgenic model, a COX inhibitor reportedly reduced memory deficits by reducing prostaglandin E2 production through AA and $\omega 6$ fatty acids;¹³ increased prostaglandin activity is a feature of $\omega 3$ fatty-acid deficiency.^{16,21} Therefore, the administration of COX inhibitors may protect against cognitive impairment in the elevated plus-maze task by inhibiting the synthesis of prostaglandins.¹¹ In the current study, the administration of naproxen for 16 wk increased the number of novel arm entries and times

spent in the novel arm compared with the familiar and start arms in the Y-maze task. In previous studies, antiinflammatory treatment with COX inhibitors decreased the risk of memory impairment and reduced the number of plaques in neurologic diseases such as Alzheimer disease.^{10,15} Together these findings suggest that treatment with a COX inhibitor may protect against memory impairment in F3 DEF–DEF(+) mice by inhibiting the production of prostaglandins.

However, spatial-recognition memory did not differ between F3 SUF–SUF(+) and F3 SUF–SUF mice. This result perhaps can be explained the fact that when $\omega 3$ fatty acid is sufficient, DHA levels accumulate rather than AA levels.⁸ For example, DHA supplementation in mice decreases brain AA levels.²⁴ Previous studies have suggested that the fish oil rich in DHA reduces the availability of AA.² Therefore, reduced levels of AA in membrane phospholipids leads to decreased prostaglandin E2 production and thus protects against deficits in spatial-recognition memory deficits even in F3 SUF–SUF(+) mice.

The current study showed that 16 wk of an $\omega 3$ fatty-acid–sufficient diet is sufficient to reestablish spatial-recognition memory in F3 DEF–SUF mice. This finding suggests that several weeks are needed for $\omega 3$ fatty acids, especially DHA, in brain membranes to recover to normal levels. For example, young rats on a DHA-deficient diet for 2 generations required 8 wk of feeding an $\omega 3$ -adequate diet for brain DHA levels to normalize.¹⁷ This result suggests that providing sufficient time is important to restore DHA levels in the brain and consequently improve cognitive function. Therefore, large amounts of $\omega 3$ fatty acids over prolonged periods are necessary for brain DHA levels to recover, given that the recovery of DHA levels in the brain nervous system is slow compared with that in other organs.^{1,26} Clearly, strategies to promote the recovery of brain DHA are important to prevent cognitive impairments, because as we showed in the current study, the F3 DEF–SUF mice demonstrated an improvement spatial-recognition–memory performance after 16 wk of receiving $\omega 3$ fatty-acid–sufficient diet.

Overall our current results suggest that the cognitive impairment caused by $\omega 3$ fatty-acid–deficient diet in the F3 DEF mice appears to be mediated by products of the AA–COX pathway and can be prevented by dietary repletion with $\omega 3$ fatty acids or by COX inhibition.

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The authors declare no conflict of interest.

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