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Original Research

Dietary Repletion with ω3 Fatty Acid or with COX Inhibition Reverses Cognitive Effects in F3 ω3 Fatty-Acid–Deficient Mice

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Dietary deficiency of ω3 fatty acid during development leads to impaired cognitive function. However, the effects of multiple generations of ω3 fatty-acid deficiency on cognitive impairment remain unclear. In addition, we sought to test the hypothesis that the cognitive impairments of ω3 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 ω3 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 ω3 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 ω3 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 ω3 fatty-acid deficiency appears to be mediated by the arachidonic acid–COX pathway and can be prevented by 16 wk of dietary repletion with ω3 fatty acids or COX inhibition.

Abbreviations: AA, arachidonic acid; COX, cyclooxygenase; DEF, ω3 fatty-acid–deficient; DHA, docosahexaenoic acid; MWM, Morris water maze; SUF, ω3 fatty-acid–sufficient.

Dietary deficiency of ω3 fatty acid is associated with impaired cognitive function. For example, rats on ω3 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 ω3 fatty-acid deficiency led to significantly shorter latencies in the passive-avoidance test in rats[3] and increased time in the Barnes circular test in mice.[7] However, ω3 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 ω3 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 ω3 fatty-acid–deficient diet over 2 or 3 generations have impaired learning performance in the MWM task.[17,25] Dietary ω3 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 ω3 fatty-acid–sufficient diet for both generations.[17] In a subsequent study, F3 rats fed on ω3 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 ω3 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 ω3 fatty-acid–deficient diet impairs performance in tests of spatial-recognition memory.

Importantly, after several generations of ω3 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 ω3 fatty-acid deficiency was reversed by providing ω3 fatty-acid–sufficient diet after weaning.[9] Cognitive performance in the MWM test did not differ in mice provided an ω3 fatty-acid–sufficient diet only and those switched at 7 wk of age from an ω3 fatty-acid–deficient diet to a sufficient diet.[4] Overall, these findings indicate that the cognitive impairments due to ω3 fatty-acid deficiency are reversed by providing a diet containing sufficient amounts of ω3 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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prostaglandin activity from formation of cyclooxygenase (COX).[13] Dietary ω3 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 ω3 fatty-acid deficiency. To this end, we raised and maintained third-generation mice on a diet either sufficient or deficient in ω3 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 ω3 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 ω3 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 ω3 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 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]

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

	Sufficient ω3 fatty acid diet	Deficient ω3 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

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 ± 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 ω3 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 ω3 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 ω3 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 ω3 fatty-acid deficiency over 3 generations can be overcome by giving F3 DEF mice an ω3 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 ω3 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 ω3 fatty acids due to multigenerational maintenance on an ω3 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 ω6 fatty acids;[13] increased prostaglandin activity is a feature of ω3 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 ω3 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 in 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 ω3 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 ω3 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 ω3-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 ω3 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 ω3 fatty-acid–sufficient diet.

Overall our current results suggest that the cognitive impairment caused by ω3 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 ω3 fatty acids or by COX inhibition.

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References

1. **Bourre JM, Pascal G, Durand G, Masson M, Dumont O, Piciotti M.** 1984. Alterations in the fatty acid composition of rat brain cells (neurons, astrocytes, and oligodendrocytes) and of subcellular fractions (myelin and synaptosomes) induced by a diet devoid of n3 fatty acids. J Neurochem **43:**342–348.
2. **Carlson SE, Werkman SH, Peeples JM, Cooke RJ, Tolley EA.** 1993. Arachidonic acid status correlates with first-year growth in preterm infants. Proc Natl Acad Sci USA **90:**1073–1077.
3. **Carrie I, Clement M, De Javel D, Frances H, Bourre JM.** 1999. Learning deficits in first generation OF1 mice deficient in (n3) poly-

unsaturated fatty acids do not result from visual alteration. Neurosci Lett **266:**69–72.

4. **Carrie I, Clement M, de Javel D, Frances H, Bourre JM.** 2000. Phospholipid supplementation reverses behavioral and biochemical alterations induced by n3 polyunsaturated fatty acid deficiency in mice. J Lipid Res **41:**473–480.
5. **Casolini P, Catalani A, Zuena AR, Angelucci L.** 2002. Inhibition of COX2 reduces the age-dependent increase of hippocampal inflammatory markers, corticosterone secretion, and behavioral impairments in the rat. J Neurosci Res **68:**337–343.
6. **Chung WL, Chen JJ, Su HM.** 2008. Fish oil supplementation of control and (n3) fatty acid-deficient male rats enhances reference and working memory performance and increases brain regional docosahexaenoic acid levels. J Nutr **138:**1165–1171.
7. **Fedorova I, Hussein N, Martino CD, Moriguchi T, Hoshiba J, Majchrzak S, Salem N Jr.** 2007. An n3 fatty-acid-deficient diet affects mouse spatial learning in the Barnes circular maze. Prostaglandins Leukot Essent Fatty Acids **77:**269–277.
8. **Guesnet P, Alasnier C, Alessandri JM, Durand G.** 1997. Modifying the n3 fatty-acid content of the maternal diet to determine the requirements of the fetal and suckling rat. Lipids **32:**527–534.
9. **Ikemoto A, Ohishi M, Sato Y, Hata N, Misawa Y, Fujii Y, Okuyama H.** 2001. Reversibility of n3 fatty-acid–deficiency-induced alterations of learning behavior in the rat: level of n6 fatty acids as another critical factor. J Lipid Res **42:**1655–1663.
10. **in t' Veld BA, Ruitenberg A, Hofman A, Launer LJ, van Duijn CM, Stijnen T, Breteler MM, Stricker BH.** 2001. Nonsteroidal antiinflammatory drugs and the risk of Alzheimer disease. N Engl J Med **345:**1515–1521.
11. **Jain NK, Patil CS, Kulkarni SK, Singh A.** 2002. Modulatory role of cyclooxygenase inhibitors in aging- and scopolamine- or lipopolysaccharide-induced cognitive dysfunction in mice. Behav Brain Res **133:**369–376.
12. **Jensen MM, Skarsfeldt T, Hey CE.** 1996. Correlation between level of (n3) polyunsaturated fatty acids in brain phospholipids and learning ability in rats. A multiple-generation study. Biochim Biophys Acta **1300:**203–209.
13. **Kotilinek LA, Westerman MA, Wang Q, Panizzon K, Lim GP, Simonyi A, Lesne S, Falinska A, Younkin LH, Younkin SG, Rowan M, Cleary J, Wallis RA, Sun GY, Cole G, Frautschy S, Anwyl R, Ashe KH.** 2008. Cyclooxygenase-2 inhibition improves amyloid-β-mediated suppression of memory and synaptic plasticity. Brain **131:**651–664.
14. **Kumar P, Padi SSV, Naidu PS, Kumar A.** 2007. Cyclooxygenase inhibition attenuates 3-nitropropionic acid-induced neurotoxicity in rats: possible antioxidant mechanisms. Fundam Clin Pharmacol **21:**297–306.
15. **Liang X, Wang Q, Hand T, Wu L, Breyer RM, Montine TJ, Andreasson K.** 2005. Deletion of the prostaglandin E2 EP2 receptor reduces oxidative damage and amyloid burden in a model of Alzheimer disease. J Neurosci **25:**10180–10187.
16. **Mollard RC, Gillam ME, Wood TM, Taylor CG, Weiler HA.** 2005. (n3) fatty acids reduce the release of prostaglandin E2 from bone but do not affect bone mass in obese (fa/fa) and lean Zucker rats. J Nutr **135:**499–504.
17. **Moriguchi T, Greiner RS, Salem N Jr.** 2000. Behavioral deficits associated with dietary induction of decreased brain docosahexaenoic acid concentration. J Neurochem **75:**2563–2573.
18. **Moriguchi T, Salem JN.** 2003. Recovery of brain docosahexaenoate leads to recovery of spatial task performance. J Neurochem **87:**297–309.
19. **Onaolapo OJ, Onaolapo AY, Mosaku TJ, Akanji OO, Abiodun OR.** 2012. Elevated plus maze and y-maze behavioral effects of subchronic, oral low dose monosodium glutamate in Swiss albino mice. J Pharmacy Biol Sc **3:**21–27.
20. **Silakova JM, Hewett JA, Hewett SJ.** 2004. Naproxen reduces excitotoxic neurodegeneration in vivo with an extended therapeutic window. J Pharmacol Exp Ther **309:**1060–1066.
21. **Song C, Manku MS, Horrobin DF.** 2008. Long-chain polyunsaturated fatty acids modulate interleukin-1β-induced changes in behavior, monoaminergic neurotransmitters, and brain inflammation in rats. J Nutr **138:**954–963.
22. **Suzuki H, Park SJ, Tamura M, Ando S.** 1998. Effect of the long-term feeding of dietary lipids on the learning ability, fatty acid composition of brain stem phospholipids and synaptic membrane fluidity in adult mice: a comparison of sardine oil diet with palm oil diet. Mech Ageing Dev **101:**119–128.
23. **Teeling JL, Cunningham C, Newmana TA, Perry VH.** 2010. The effect of nonsteroidal antiinflammatory agents on behavioural changes and cytokine production following systemic inflammation: implications for a role of COX1. Brain Behav Immun **24:**409–419.
24. **Wainwright PE, Xing HC, Mutsaers L, McCutcheon D, Kyle D.** 1997. Arachidonic acid offsets the effects on mouse brain and behavior of a diet with a low (n6):(n3) ratio and very high levels of docosahexaenoic acid. J Nutr **127:**184–193.
25. **Wainwright PE, Huang YS, Coscina DV, Levesque S, McCutcheon D.** 1994. Brain and behavioral effects of dietarv n3 deficiencv in mice: a 3-generational study. Dev Psychobiol **27:**467–487.
26. **Youyou A, Durand G, Pascal G, Piciotti M, Dumont O, Bourre JM.** 1986. Recovery of altered fatty acid composition induced by a diet devoid of n3 fatty acids in myelin, synaptosomes, mitochondria, and microsomes of developing rat brain. J Neurochem **46:**224–228.