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Dietary omega-3 polyunsaturated fatty acid supplementation in an animal model of anxiety

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ABSTRACT

A large body of evidence suggests that dietary supplementation with omega-3 fatty acids may ameliorate depressed mood. The magnitude of the effect varies between studies, however, ranging from none at all to being of clinical significance. Given that substantial comorbidity occurs between mood and anxiety disorders, suggesting that they have one or more pathophysiological mechanisms in common, we hypothesized that omega-3 fatty acids may be acting primarily to reduce anxiety rather than depression *per se*, a possibility which could underlie their variable effects on mood. To test this hypothesis rats were fed for 8 weeks with diets containing one of three types of omega-3 fatty acids, alpha-linolenic acid, eicosapentaenoic acid and docosahexaenoic acid, as well as a low omega-3 fatty acid control diet. Although brain omega-3 fatty acid concentrations were altered by dietary supplementation with eicospentaenoic acid and docosahexaenoic acid, no significant change in anxiety related behaviors were observed compared to the control group as assessed by the elevated-plus maze test. Our data therefore do not support an anxiolytic effect of omega-3 fatty acids and suggest that any effect of these lipids on mood likely occurs by a mechanism unrelated to reducing anxiety.

1. Introduction

Omega-3 polyunsaturated fatty acids have been argued to have psychoactive properties [1,2]. The best evidence for such an effect is their proposed antidepressant action in mood disorders [3]. This includes findings that those with major depressive disorder (a type of mood disorder) have lower concentrations of omega-3 fatty acids in blood cells, and that a variety of clinical trials have shown a beneficial effect of dietary supplementation with these lipids on the mood of depressed patients. Such a mood elevating action is of great potential benefit however not all studies report a positive effect (reviewed in references 3–5), and, even in those that do find benefit, the magnitude of response varies widely. The explanation for such variability is unclear but has been suggested either to be that the true effect size is low and unlikely to be of any clinical relevance [5], or that not all omega-3 fatty acids have the same level of efficacy, with some evidence suggesting that of the most three most biologically abundant examples of the type, eicosapentaenoic acid (EPA), is of greater benefit than either alpha-linoleic acid (ALA) or docosahexaenoic acid (DHA) [3,4,6]. An alternative explanation lies in the fact that mood disorders are highly comorbid with anxiety disorders such as generalized anxiety disorder [7] and are amenable to treatment with the same types of pharmacotherapies [8,9]. Mood and anxiety disorders may therefore share some pathophysiological mechanisms with a body evidence indicating that chronic anxiety can lead to a higher risk of a depressive episode occuring [10–13]. We hypothesized that omega-3 fatty acids may be actually reducing anxiety in some patients as their primary mechanims of action, and that it was this that was causing the antidepressant effect. Given that this potential confound was not accounted for in the clinical trials, and that the degree of comorbidity varies depending on the characteristics of the population studied [14,15], a primary effect upon anxiety could explain the variability of response noted in the literature [16,17]. The possibility that omega-3 fatty acids have anxiolytic effects is one which has not been extensively examined although social phobia is associated with reduced cellular omega-3 PUFA concentrations, patients with comorbid anxiety and depression exhibit a greater depletion in cellular omega-3 PUFA concentration than those with depression alone, and omega-3 PUFA supplementation reduced anxiety in both test-takers, and in substance users [18–21]. To investigate the role of omega-3 fatty acids in anxiety we have used an animal model, the elevated plus maze test, a model which is responsive to existing anxiolytic medications [22]. The test makes use of the conflict between the animal's fear of being in open areas versus their desire to explore novel environments. The elevated

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plus maze has both enclosed arms which provide security and open arms which have exploratory value. When more anxious animals prefer the enclosed areas, whereas when they are less anxious they will enter the open arms more often. We therefore observed animals tested using the elevated plus maze after being fed with a diet supplemented with either ALA, EPA, or DHA, or an omega-3 fatty acid free control diet.

2. Materials and Methods

2.1. Dietary intervention

Animal experimentation was carried according to an animal utilization protocol approved by the Lakehead University animal care committee. Male Wistar rats were allowed to acclimatize to the lab environment for 2 weeks after arrival. They were randomly assigned into pairs and placed into cages with free access to food and water. Rats were fed for 8 weeks on supplementation diets containing 90% (by weight) rat chow containing all required nutrients except for fats (TD99159 basal mix, Harlan Laboratories) plus 10% added fat. The fat component for the control group was exclusively palm oil (New Directions Aromatics, Canada), containing (as per our own analysis) 1.2% (mole %) 14:0, 42.2% 16:0, 3.5% 18:0, 41.9% 18:1, and 11.2% 18:2, n-6, while other groups received palm oil plus omega-3 fatty acids ("95% purity" preparations from Equatech, UK which contained as per our own analysis over 97% of the described fatty acid) in the ratio 9 parts palm oil to 1 part omega-3 fatty acid as described in Table 1. Food was prepared ahead of time and stored in canisters at −20 °C until use. Body weight was monitored throughout the experiment and did not differ significantly (ANOVA; $P > 0.05$) between the dietary groups.

2.2. Elevated plus maze

The maze was purchased from Harvard Apparatus (Massachusetts, USA). It consists of a cross, lifted 660 mm above the ground, with a central portion surrounded by 4 black coloured arms of length 450 mm and width 100 mm. Two arms have 500 mm high opaque grey walls (closed arms) enclosed at one end and open to the central area at the other, while the other two arms have 100 mm clear walls (to prevent falls) and are open at each end. A video camera was suspended above the apparatus to record activity. The movement of animals was analysed using the SMART video analysis software provided by Harvard Apparatus to produce the following measures: relative time spent in the open arms, closed arms and central areas of the maze, and the total number of entries by animals into these three areas. On the day of testing animals were transferred from their housing area to the procedure room and left in the room for 30 min prior to the start of the experiment. Each animal was then transferred from their cage into the central area of the apparatus and the investigator left the room. Rats were recorded for 10 min with the first two minutes being an acclimatisation period followed by data recording for a further 8 min.

2.3. Fatty acid analysis

One day after completion of the intervention the rats were euthanized by first anaesthetizing with isoflurane gas followed by decapitation using a small animal guillotine. The brain and liver were rapidly removed and tissue stored in a −80 °C freezer until required. Fatty acids were analysed essentially as described previously [23]. Briefly, the entire organ was ground in liquid nitrogen using a mortar and pestle and approximately 300 mg of tissue powder was homogenized in ice cold water using a Polytron homogenizer. Lipids were then extracted from the samples according to the method of Bligh & Dyer [24] in the presence of the internal standard tritridecanoin. Fatty acid methyl esters were prepared using boron trichloride in methanol, and heating the methylation tubes in a boiling water bath. The resulting fatty acid methyl esters were analysed on a Varian 3400 gas-liquid chromatograph, with a 60-m DB-23 capillary column (0.32 mm internal diameter), and quantified using flame ionisation. Results are expressed as the relative concentration of each fatty acid species.

3. Results

3.1. Brain lipids

Animal were fed a diet which was either omega-3 fatty acid free or supplemented with one of ALA, EPA or DHA. The results of the fatty acid analysis of brain conducted after the intervention have previously been described in detail [25] and are summarized in Table 2 with liver fatty acids analysis being shown for the purpose of comparison. Omega-3 supplementation resulted in much larger changes in liver fatty acid composition than that in brain. Nevertheless, supplementation with DHA resulted in higher brain DHA, total omega-3 fatty acids concentrations and omega-3/omega-6 fatty acids ration, and lower arachidonic acid and total omega-6 fatty acid concentrations compared to the control group. The brain contained very little ALA and EPA with ALA supplementation having no effect on brain ALA or arachidonic acid concentrations. EPA supplementation did result in small increase in brain EPA concentration, and a somewhat larger increase in total omega-3 fatty acids, omega-3/omega-6 ratio, and a decrease in arachidonic acid and total omega-6 fatty acid concentration compared to the control diet, although the magnitude of response (except for decreased arachidonic acid concentration) was less than that observed

Table 1
Composition of test diet.

Component	Weight per kg
Added fat[a]	100 g
Caesin	181.9 g
DL-Methionine	2.84 g
Sucrose	636.11 g
Cellulose	47.37 g
Calcium monohydrogen phosphate	3.32 g
Choline bitartrate	2.38 g
Calcium carbonate	11.84 g
Potassium dihydrogen phosphate	6.5 g
Potassium citrate monohydrate	2.35 g
Sodium chloride	2.45 g
Potassium sulphate	1.55 g
Magnesium oxide	0.81 g
Ferric citrate	0.2 g
Zinc carbonate	54.72 mg
Manganese carbonate	20.89 mg
Copper carbonate	10.28 mg
Potassium iodate	0.33 mg
Sodium selenate	0.34 mg
Ammonium paramolybdate, tetrahydrate	0.27 mg
Sodium meta-silicate, nonahydrate	48.09 mg
Chromium potassium sulphate, dodecahydrate	9.12 mg
Lithiun chloride	0.58 mg
Boric acid	2.7 mg
Sodium fluoride	2.11 mg
Nickel carbonate hydroxide, tetrahydrate	1.05 mg
Ammonium meta-vanadate	0.22 mg
Niacin	28.43 mg
Calcium pantothenate	15.16 mg
Pyroxidine hydrochloride	6.63 mg
Thiamin hydrochloride	5.69 mg
Riboflavin	5.69 mg
Folic acid	1.9 mg
Biotin	0.19 mg
Vitamin B12 (0.1% in mannitol)	23.69 mg
Vitamin E, *DL*-alpha tocopheryl acetate (500 IU/g)	142.16 mg
Vitamin A palmitate (500,000 IU/g)	7.58 mg
Vitamin D3, cholecalciferol (500,000 IU/g)	1.9 mg
Vitamin K1, phylloquinone	0.71 mg

[a] The added fat contained either 100 g palm oil or 90 g palm oil plus 10 g omega-3 fatty acid.