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Randomised open-label trial of docosahexaenoic acid -enriched fish oil and fish meal on cognitive and behavioural functioning in Omani children

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Authorship:

SSA: Recruited, enrolled and followed up the children, liaised with teachers and parents, oversaw data collection, and reviewed the final manuscript.

SA: Assisted with the study protocol and implementation, and drafted the manuscript.

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Highlights:

- Cognitive and behavioural functioning was assessed in school children before and after intervention with DHA-enriched fish oil and fish meal
- Fish oil and fish meal improved executive functioning with significantly better results with fish oil but not verbal ability and memory
- Fish oil ameliorated ADHD-related symptoms but fish meal did not.

Abstract (231 words)

Objective: Examine the effect of docosahexaenoic acid (DHA) enriched fish oil supplement and fish meal on cognitive and behavioural functioning manifested as attention-deficit/hyperactivity disorder in primary school students (9-10 years old) in Muscat, Oman.

Methods: Randomised open-label trial involving two types of intervention: fish oil supplement or one serving (100 gram) of fish per weekday for 12 weeks. Red cell total lipid docosahexaenoic acid levels were assessed. *Verbal Fluency Test*, *Buschke Selective Reminding Test*, and *Trail Making Test* were used to measure cognitive functioning. Behavioural functioning was assessed using a standardised Arabic version of the *National Initiative for Children's Health Quality Vanderbilt Assessment Scales*. All measurements were carried out before and after intervention.

Results: DHA levels increased by 72% and 64% in fish oil (Mean: 3.6% to 6.2%) and fish meal (Mean: 3.4% to 5.6%) group, respectively (p=0.000). The *Trail Making Test* was the only cognitive test that demonstrated marked differences between groups: median inter-quartile range difference between pre and post intervention in the *Trail Making Part B* score was 61.5 (SE: 19.3, 103.2) in the fish oil vs. Fish meal group, 24.5 (SE: -15.2, 74.7, p=0.005). The *Vanderbilt Assessment Scales* also showed significant differences between groups ($p<0.001$).

Conclusion: This study contributes to available evidence on the cognitive and behavioural benefits of DHA in healthy school children. Consideration should be made to expand

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fortification programme of food with DHA-enriched fish oil as part of broader policy to improve child health.

Introduction

The Sultanate of Oman, a country on the southeast corner of the Arabian peninsula with a long coastline overlooking the Arabian Sea, the Sea of Oman and the Indian Ocean, is known for its rich fish resources [1]. Although fish is a major national commodity, population intake is minimal [2]. Due to its multiple health benefits, the Ministry of Health is promoting the consumption of fish in its efforts to improve the dietary intake of the population [2].

The abundance of long-chain omega-3 fatty acids in fish, such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), is necessary for human growth and development. They not only benefit cardiovascular and immune systems but are also necessary for the development of the brain and nervous system [3-6]. These are particularly important during the brain growth spurts during infancy, early childhood and adolescence [7]. Promoting consumption of omega-3 fatty acids could be part of a multi-prong approach to address concerns raised by parents, teachers and child welfare advocates about children's school performance and their emotional well-being.

Studies have found that omega-3 fatty acid supplementation benefits cognition (thinking, reasoning, memory), behaviour and school performance among healthy children [7-10]. Supplementation in healthy but underperforming children aged 7 to 9 years in the UK appears to improve reading and behaviour [8] while supplementation among 200 healthy children aged 8 and 16 years in Mauritius resulted in the reduction of behavioural problems [9]. A review of 15 observational and randomised controlled trials in healthy children aged 4 to 18 years reported improvements in learning ability, reading and spelling as well as in

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behaviour [10]. Studies have also demonstrated their efficacy in reducing soft neurological sign, enhancing cognitive functioning, attenuating externalization- internalization disorders and improving behaviour [11]. Moreover, several studies have reported that omega-3 oil supplementation alleviated some attention-deficit/hyperactivity disorder (ADHD) related symptoms [9, 10, 12, 13].

The benefits of fish consumption on cognitive development is less substantive. A cross-sectional study of Swedish adolescents reported significantly higher grades among participants with more frequent fish intake [3]. A six-country European study of elementary school children reported negative associations of fatty fish consumption with emotional and behavioural problems [14]. The Cape Code Health Study have also found high childhood intake of fish associated with some cognitive benefits in adulthood [15].

Research on the associations of omega-3 fatty acids supplementation and fish consumption and learning and behaviour among healthy school children has largely been limited to populations in the West; little is known about other parts of the world. Based on two cross-sectional studies, estimates on the prevalence of ADHD symptoms among Omani schoolgirls and schoolboys are 5.1% and 7.8% respectively [16, 17]. In this study, we examined the effects of fish and DHA-enriched fish oil supplement on cognitive (verbal ability, learning and remembering and executive functioning) and behavioural functioning in primary school students living in Muscat, Oman.

Methods

This study was a randomised open-label trial and part of a larger study assessing nutritional status of children and the effect of DHA intake on children's health.

The study was approved by the Research Ethics Committee of the Ministry of Health, Sultanate of Oman (Ref. MH/DGP/R&S/Proposal Approved/8/2012) and the National

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Research Ethics Committee North West – Haydock, UK (REC reference no. 12/NW/0760). The trial was registered with the ISRCTN Register (Reg. No. ISRCTN93233285). Informed and signed consent was obtained from the parents or guardians of the children. The study was conducted in accordance with the provisions of the ethical approval of the two ethics committees and the principles of the Helsinki Declaration.

Participants and intervention

Participants were healthy children aged 9 and 10 years old attending Grade 4 in primary schools in Muscat, the capital of Oman. Children with a known hereditary or chronic medical condition which requires medication or suffer from fish or shell fish allergy were excluded.

DHA was provided either in the form of DHA-enriched fish oil capsule or grilled fish meal. Children in the fish meal group were given a daily mid-day lunch snack comprising 100g of lightly grilled fish sandwich with some vegetables. Five types of fish, namely Grouper, Sea bream, Kingfish, Emperor and Snapper were used. It was estimated that 100g of grilled fish provides 150 to 200 mg of DHA. The meals were prepared tastefully by professional chefs at the Intercontinental City Hotel, Muscat to enhance compliance. Children in the fish oil group were given a capsule containing 403 mg DHA daily during the mid-day break. The intervention took place for 12 weeks during weekdays when school was in session.

Sample size

The sample size for this sub-study was based on the Oxford-Durham study, a randomised control trial using omega-3 oil capsule which found significant improvements in reading, spelling and behaviour in a group size of 55 school children in each treatment arm [27]. To account for possible drop-out, we increased the required number by 20% or 66 in each group.

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Outcome measures

The main outcome measures for this study were behavioural (ADHD symptoms) and cognitive functioning. All tests were carried out before and at the end of intervention at school by trained researchers, undergraduate psychologists trained to dispense the outcome measures uniformly under the supervision of a board certified neuropsychologist. *Vanderbilt Assessment Scales-Teacher Assessment Scale* also known as the *National Initiative for Children's Health Quality Vanderbilt Assessment Scales-Teacher Assessment Scale* (NICHQ Vanderbilt Assessment Scales) were performed by teachers in order to quantify the presence of ADHD.

Three tests were used to assess various domains of cognitive functioning including verbal ability, learning and remembering and executive functioning. In order to avoid 'learning effect', different versions of the outcome (*Verbal Fluency Test*; *Buschke Selective Reminding Test*) were therefore employed.

The *Verbal Fluency Test* or also known as Controlled Oral Word Association Test was used to examine lexical ability and initiation speed of verbal responses. The respondents were given 60 seconds to produce as many unique words as possible within a semantic category such as FAS. In this study, the letters are derived from the Arabic alphabet, *taa, raa, waaw* as described elsewhere [18].

The Buschke Selective Reminding Test was used to tap into immediate recall which represents working memory and attentional capacity [19]. The participants were read a list of 12 words and asked to recall as many as possible in a total of 3 trials. The scoring represents the mean total items recalled out of the three trials.

Part B of the Trail Making Test was used to assess executive functioning: attention, visual search and scanning, sequencing and shifting, flexibility, ability to execute and modify a plan

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of action, and ability to maintain two trains of thought simultaneously. The participants are requested to draw lines to connect the circles in an ascending order by alternating between the numbers and letters (i.e., 1-A-2-B-3-C, etc.). These domains have generally been attributed to constitute executive functions [20, 21].

A standardised Arabic version of the *Vanderbilt Assessment Scales-Teacher Assessment Scale* was used to examine behavioural and emotional functioning [22, 23]. This tool has 55 questions divided into two sections soliciting 'symptoms' and 'performance' scales based on teachers' observations. The SYMPTOMS section contains 47 items that are divided into various sub-sections: items 1-18 tap into the symptoms of ADHD. The present analysis focused only on the ADHD symptoms. The formula used for scoring *Vanderbilt Assessment Scales-Teacher Assessment Scale* has been detailed elsewhere [24]. The research team taught teachers how to complete *Vanderbilt Assessment Scales-Teacher Assessment Scale* by circling only one of the numbers on the scale for each item and basing their answers in reference to the child's behaviour over a six-month period.

In addition, students were tested for their intellectual ability using the *Coloured Progressive Matrices* (CPM), which has been designed for children aged 5 through 11 years-of-age. CPM is accorded as 'culture free' test [25]. It consists of 36 items grouped into three sets. Each item contains a pattern problem with one part removed and between six and eight pictured inserts of which one contains the correct pattern. The subject points to the pattern s/he selects as correct. Possible scores, therefore, can range from 0 – 36. For the present study, the raw scores were reported. This test was carried out once during recruitment.

Red cell fatty acid analysis

Fasting blood samples were collected in ethylenediaminetetraacetic acid treated tube (BD Vacutainer® EDTA tubes) and separated into red cells and plasma by cold (4°C) centrifugation at 1500g for 10 min. After removing the plasma, the remaining red cells were

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washed with saline (0.9%) twice and transferred to another tube. This initial stage of sample preparation was carried out at the Royal Hospital, Oman and subsequently transported to Lipidomics and Nutrition Research Centre, London and kept in a -70°C freezer until analysis. The total red cell lipid was extracted using a mixture of chloroform and methanol, and fatty acid methyl esters were prepared in 15% acetyl chloride in dry methanol solution and separated using a gas-liquid chromatography (HRGC MEGA 2 Series; Fisons Instruments, Milan, Italy) fitted with a BP20 capillary column (25m x 0.32 mm i.d., 0.25µm film). Hydrogen was used as a carrier gas and the injector, oven, and detector temperatures were maintained at 250, 200, and 280°C, respectively. The fatty acid methyl esters were identified by comparing retention time with authentic standards. Peak areas were quantified by a computer chromatography data system (EZChrom Chromatography Data System; Scientific Software, Inc., San Ramon, CA).

Statistical analyses

Group comparisons of outcome measures, mean post-intervention score minus baseline score), were carried out. An intention to treat (ITT) method was the main statistical analysis. Comparison of mean change from the baseline to end line, was carried out by calculating the differences in mood scores (mean, standard deviation (SD) or median, interquartile range (IQR)) using the student test if normal, otherwise using Mann Whitney U test. The denominator would be the number randomised. The differences in red cell omega-3 fatty acid levels between two groups were examined using the independent t-test. The paired t - test was used to compare the changes in the red cell omega-3 fatty acids within each group. Data was entered in EPIDATA software and analysed using SPSS software.

Results

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One hundred thirty two children, 66 in each group, were randomised to receive either fish oil or fish meal. (Figure 1 provides the CONSORT flow chart). Demographic data showed no significant group differences (Table 1), demonstrating appropriate sampling selection. Boys comprised slightly less than half of the sample; the average age of participants was 9.5 years. The reasoning capacity of both groups were within normal range. The children were fairly similar in terms of the birth history and socio-economic background. Compliance rate was high (97%) with no known side effects (i.e. nausea, loose stools, fish aftertaste) of fish oil supplement reported.

Red cell omega-3 fatty acids

Both groups of children had similar levels of long-chain polyunsaturated omega-3 fatty acids, namely EPA, docosapentaenoic acid (DPA) and DHA in red cells before intervention (Table 2). As expected, fish oil supplement increased the DHA level by 72% compared to pre-intervention (3.6 ± 1.4 to 6.2 ± 1.5, p=0.000). Similarly, the DHA level was 64% higher in the fish meal group (3.4 ± 1.4 to 5.6 ± 1.2, p=0.000). However, the proportional increase was significantly higher in the fish oil group (p=0.014) compared to the fish meal group. Interestingly, the sum of long-chain omega-3 fatty acids between the two groups did not differ after intervention as the slightly lower level of DHA in children from the fish meal group was compensated by the higher proportion of DPA. Although arachidonic acid (ARA) was positively associated with the omega-3 fatty acid index post-intervention in both groups (fish oil r = 0.394, p<0.0001; fish meal r = 0.213, p<0.05, data not shown); the association of total omega-6 fatty acid with oemga-3 fatty acid was observed only in the fish oil group (fish oil r = 0.414, p<0.0001; fish meal r = - 0.032, p>0.05).

Cognitive functioning

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Of the three tests for cognitive function presented in Table 3, the *Trail Making Test* for executive functioning was the only one that demonstrated marked differences between the groups. The median IQR difference between pre and post intervention in the *Trail Making* Part B score was 61.5 (19.3, 103.2) in the fish oil group and was markedly lower in the fish meal group, 24.5 (-15.2, 74.7). Thus, the fish oil group had significantly higher change as compared to fish meal group (p=0.005).

Emotional and behavioural functioning

The Vanderbilt Assessment Scales measuring symptom of ADHD demonstrated marked differences between groups. The mean (SE) score for the fish oil group at the pre-test was 22.1 (2.0), while this was 23.0 (2.4) at the post test. Similarly, the mean (SE) *Vanderbilt Assessment Scales-Teacher Assessment Scale* was 25.7 (2.2) and 18.0 (2.1) in the fish oil and fish meal groups respectively. Although the fish oil group saw an improvement (Mean: 1.4, SE 1.6), a marked decrease was observed in the fish meal group (Mean: -7.2, SE 0.7).

Discussion

This study is one of the first studies in the Arab world examining the benefits of long chain polyunsaturated omega-3 fatty acid consumption, DHA in particular, on cognitive and behavioural functioning among healthy school children. The findings, particularly the significantly greater benefit of fish oil consumption compared to fish, adds to the available evidence largely from North America and Western Europe [3, 7, 8, 10, 14]. Studies have generally shown global cognitive improvement (attention and concentration, learning and remembering, executive functioning) among children taking omega-3 fatty acid supplements [12, 28, 29]. Our 12-week intervention study suggests improvement in verbal fluency and executive functioning in all children; however, significantly greater improvement was seen

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only with executive functioning among those who consumed omega-3 oil supplement five-days a week compared to those who consumed fish. Previous studies have linked executive functioning to perceptual motor speed, visual searching and sequencing, and the ability to make alternating conceptual shifts, planning and goal-directed behaviour [30]. They also have suggested that omega-3 fatty acid supplement has the potential to impact executive functioning [7, 31, 32]. A related study found that omega-3 fatty acid supplementation mitigates vitamin D deficiency prevalence in Omani school children [33]. Thus, consideration should be made for child-focused program of food fortification.

In a systematic review and meta-analysis of randomised placebo-controlled trials examining omega-3 fatty acid supplementation among children with ADHD, Bloch and Qawasmi [34] concluded that omega-3 fatty acid supplementation attenuates the ADHD symptoms. Other systematic reviews and meta-analyses had similar findings [35, 36]. It is interesting to note that ADHD has been suggested to manifest de-executive disorder [37]. Some of the spectrum of deficits in children with ADHD include impaired self-regulation or poor impulse control, lack of planning or goal directed behaviour [38-40]. Pending further scrutiny, this study suggests that ADHD-related symptoms are amenable with omega-3 fatty acid supplementation. The retrospective Cape Cod Health Study found that an increased fish consumption was associated with increased odds of children having ADHD-related symptoms but showed no other meaningful associations to childhood learning and behaviour disorders [41]. They observed that it may be because of insufficient fish consumption or the type of fish consumed. It is possible that our finding of increased teacher observed ADHD-related symptoms with fish consumption may also be due to insufficient fish consumption; in our study, the fish provided around 150 – 200 mg DHA, less than half the amount provided by the supplements.

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The positive correlation seen between ARA and omega-3 fatty acids corresponds with observations made in other studies [42, 43]. For example, Luxwolda et al. have found a bell curve relationship between erythrocyte omega--3 fatty acid and ARA[42]. It appears that the incorporation of omega--3 and omega--6 long chain polyunsaturated fatty acids into cell membrane is highly regulated and the balance between the two fatty acid families is critical for orderly structural organisation and function of cellular and subcellular membranes. At the same time, studies have found that ARA supplementation improves infant neurodevelopment including cognition and language development [43-45]. Further studies are required to better understand the role of ARA in normal growth and development.

The findings from this study have several limitations. First, the amount of omega-3 fatty acids was not the same across the two groups; the fish oil capsules were likely to have uniform nutritional contents while the omega-3 fatty acid levels of the variety of fish served were not examined. These discrepancies could act as a confounder for the present comparison. According to meta-analysis of 21 cohort studies reported by Zhang et al [6,], both capsule supplementation and fish appears to be associated with lower risk of cognitive impairment, there is no established linear dose-response relation in the existing literature. In another meta-analysis, it was suggested that omega-3 fatty acid capsules have a greater potential for enhancing cognitive performance, especially in growing children [47]. Future studies should aim to establish the optimal intake of omega-3 fatty acids for optimum effect of cognitive and behavioural functioning in children; preferably using a double blind study methodology rather than an open label one as used in this study and with varying amounts of omega-3 fatty acids. Secondly, ADHD symptoms were solicited by the *Vanderbilt*

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Assessment Scales-Teacher Assessment Scale which has not been standardised for use with the Omani population. These symptoms were solicited using a checklist rather than a clinician-based semi-structured interview. The development of a reliable and valid Arabic version of these Scales would greatly improve our confidence in the measure, particularly if this process of standardisation is carried out with an eye toward the cultural specificities relevant to the Omani school population. Finally, students were only recruited from Muscat, the capital area, thus, results cannot be generalized to the Omani population.

This small intervention study contributes to available evidence on the cognitive and behavioural benefits in healthy school children of omega-3 fatty acids consumption, particularly as a supplement. However, further research is required to determine optimal intake. Given these benefits, public health authorities should not only continue to promote fish consumption but may wish to consider enhancing the food fortification programme as part of broader policy to improve child health and their performance in school.

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Figure 1: CONSORT flow diagram of study participants

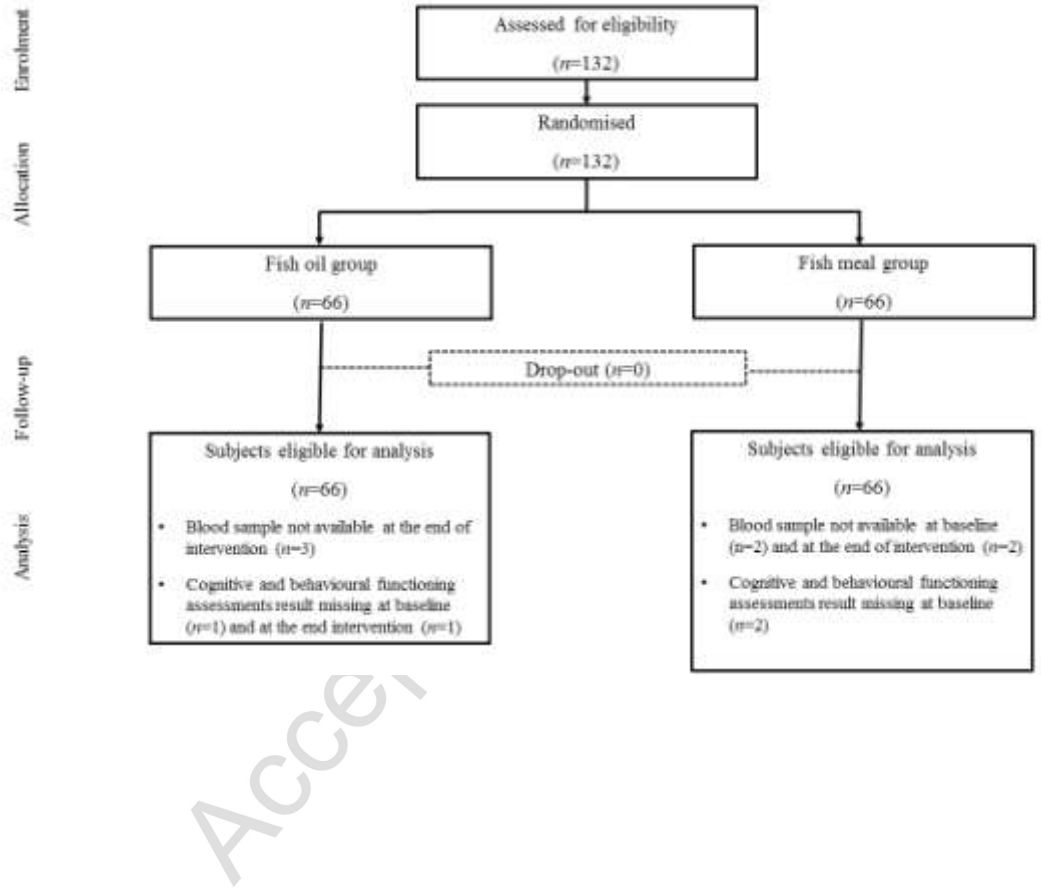

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Table 1: Description of study participants

	Fish Oil Group (*n*66)	**Fish Meal Group (*n*66)**
Boys (%)	40.9	45.5
Mean Age (in years, mean ± SD)	9.58 ± 0.4	9.56 ± 0.5
Intellectual Functioning (Coloured Progressive Matrices, mean ± SD)	27.6 ± 6.2	29.4 ± 5.5
Birth History		
Anthropometric measures at birth		
Mean weight at birth (kg, mean ± SD)	3.09 ± 0.4	3.11 ± 0.5
Mean length at birth (cm, mean ± SD)	50.6 ± 3.8	50.5 ± 5.7
Problems during pregnancy (%)		
None	75.4	61.1
Gestational Diabetes	11.5	19.4
Hypertension	1.6	8.3
Iron Deficiency Anaemia	3.3	2.8
Other	8.2	8.3
Birth-related issues (%)		
None	87.5	87.7
Caesarean section	7.8	7.7
Low birth weight	1.6	4.6
Pre-term baby	1.6	0.0
Other	1.6	0.0
Parental Consanguinity		
First degree	28.1	23.1
Second degree	10.9	16.9
Distantly	15.6	9.2
No relationship	45.3	50.8
Parental Socioeconomic Background		
Mother's education (%)		
Preparatory or less	6.3	4.5
Secondary	25	37.9

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	Fish Oil Group (*n*66)	**Fish Meal Group (*n*66)**
More than secondary	18.8	21.2
Mother's employment status (%)		
Employed	7.9	8.1
Not employed	92.1	91.9
Father's education (%)		
Preparatory or less	9.4	6.2
Secondary	25	30.8
More than secondary	20.3	15.4
Father's employment status (%)	87.5	87.7
Employed	12.5	12.3
Not employed		
Mean Household Monthly Income (Omani Rial)	1376.0 ± 788.0	1406.7 ± 875.4

SD, standard deviation

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Table 2. Red cell total lipid eicosahexaenoic acid (EPA, 20:5ω3), docosapentaenoic acid (DPA, 22:5ω3), docosahexaenoic acid (DHA, 22:6ω3), and sum of long chain polyunsaturated omega-3 fatty acid levels before and after intervention[1]

	Fish oil group (*n* 66)[2]				Fish meal group (*n* 64)[3]			
	EPA	DHA	DPA	Sum of long-chain omega-3 fatty acids	EPA	DHA	DPA	Sum of long-chain omega-3 fatty acids
Before intervention	0.3 ± 0.1	3.6 ± 1.4	0.9 ± 0.3	4.8 ± 1.7	0.3 ± 0.1	3.4 ± 1.4	1.0 ± 0.3	4.7 ± 1.8
After intervention	0.4 ± 0.1[a]	6.2 ± 1.5[c]	1.0 ± 0.2	7.6 ± 1.8[c]	0.4 ± 0.2[*, b]	5.6 ± 1.2[*, c]	1.4 ± 0.3[**, c]	7.4 ± 1.5[c]

[*, **] Significantly different from those of fish oil group at $p<0.05$ and $p<0.0001$ (independent sample t-test, 2-tailed).

[a, b, c] Significantly different from the values measured before intervention at $p<0.05$, $p<0.01$, and $p<0.0001$, respectively (paired sample t-test, 2 tailed).

[1] Values are expressed as mean (% of total fatty acids identified) ± standard deviation.

[2] The mean value for the "After intervention" was based on 63 subjects as blood samples were not available from three subjects.

[3] Blood samples were not available from two subjects at baseline and two at the end of intervention.

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Table 3. Cognitive and behavioural functioning assessments

	Fish Oil Group		Fish Meal Group		Mean difference of change between groups (95% CI)	P Value
	Mean/ Median	SE/IQR	Mean/ Median	SE/IQR		
Verbal Fluency Test						
ITT						
Pre	9.0	6.0, 12.0	7.0	5.0, 10.0		
Post	12.0	9.0, 15.5	11.5	7.0, 16.0		
Difference	-4.0	-6.5, 0.5	-3.5	-6.2, -2.0	NA	0.235*
Buschke Selective Reminding Test						
ITT						
Pre	24.9	0.4	23.6	0.5		
Post	25.3	0.4	23.2	0.6		
Difference	-0.4	0.5	0.1	0.6	-0.5 (-2.2, 1.2)	0.548
Trail Making - Part B						
ITT						
Pre	184.0	139.5, 232.5	183.5	135.5, 219.2		
Post	111.0	85.0, 145.0	142.0	98.5, 194.2		
Difference	61.5	19.3, 103.2	24.5	-15.2, 74.7	NA	0.005*
Vanderbilt Assessment Scales-Teacher Assessment Scale						
ITT						
Pre	22.1	2.0	25.7	2.2		
Post	23.0	2.4	18.0	2.1		
Difference	1.4	1.6	-7.2	0.7	8.6 (5.0, 12.2)	<0.001

*P value based on Mann-Whitney test
NA,Not Applicable

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IQR, Inter Quartile Range

ITT, Intention to Treat

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