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Do infants of breast-feeding mothers benefit from additional long-chain PUFA from fish oil? A 6-year follow-up

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Abstract

Fish-oil supplements are marketed as enhancing intelligence and cognitive performance. However, empirical data concerning the utility of these products in healthy term infants are mixed, particularly with respect to lasting effects into childhood. We evaluated whether fish-oil supplementation during infancy leads to better neurocognitive/behavioural development at 6 years. We conducted a double-blind randomised controlled trial of supplementation with *n*-3 long-chain PUFA in 420 healthy term infants. Infants received either fish oil (containing at least 250 mg DHA and at least 60 mg EPA) or placebo (olive oil) daily from birth to 6 months of age. Neurodevelopmental follow-up was conducted at a mean age of 6 years (SD 7 months), whereby 335 children were assessed for language, executive functioning, global intelligence quotient and behaviour. No significant differences were observed between the groups for the main neurocognitive outcomes. However in parent-report questionnaire, fish-oil supplementation was associated with negative externalising ($P = 0{\cdot}035$, $d = 0{\cdot}24$) and oppositional/defiant behaviour ($P = 0{\cdot}006$, $d = 0{\cdot}31$), particularly in boys ($P = 0{\cdot}01$, $d = 0{\cdot}45$; $P = 0{\cdot}004$, $d = 0{\cdot}40$). Our results provide evidence that fish-oil supplementation to predominantly breast-fed infants confers no significant cognitive or behavioural benefit to children at 6 years.

Key words: **Fish oil: Infants: Supplementation: Brain development**

n-3 Long-chain PUFA (*n*-3 LCPUFA), particularly DHA, are essential for the growth and maturation of the developing human brain[1]. DHA is rapidly incorporated into the frontal cortex during the 'brain growth spurt' which occurs from the last trimester to about 18 months[2]. Brain regions with the highest affinity for DHA are the frontal cortex[3] and the hippocampus[4]. These regions subserve higher-order executive functions including memory and attention[5] and interconnect with the limbic system to affect behaviour[6].

Due to the pervasive lack of *n*-3 LCPUFA in the typical Western diet[7], there is concern that infants are not receiving sufficient quantities of DHA and therefore may benefit from DHA supplementation. Based on the physicochemical effects of DHA on hippocampal- and frontal-based cognition, it is plausible that modulation of DHA availability will affect tasks that call upon executive functioning and behaviour[8].

Several observational studies have shown a positive correlation between maternal fish intake during pregnancy and child neurocognitive development[9–12]. Yet, systematic, meta-analytical and narrative reviews of intervention trials of *n*-3 LCPUFA supplementation during pregnancy and lactation[13–16] and infant formula[17–20] have revealed mixed findings. This lack of consistency has been attributed to study design variations including sample size, dosage, duration of supplementation and method of assessment. Only a small number of studies have examined the long-term effects into childhood, when neurocognitive and behavioural measurements have more predictive validity when compared with infancy.

The work presented here is part of the Infant Fish Oil Study (IFOS), which was a randomised controlled trial (RCT) of infant fish-oil supplementation from birth to 6 months of age. The trial commenced in 2005 with the goal of investigating the

Abbreviations: CELF-4, Clinical Evaluation of Language Fundamentals, 4th Edition; IFOS, Infant Fish Oil Study; IQ, intelligence quotient; LCPUFA, long-chain PUFA; RCT, randomised controlled trial; SOPT, Self-Ordered Pointing Test; WASI, Wechsler Abbreviated Scale of Intelligence – 2nd Edition.

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immunological and neurocognitive effects of fish oil during infancy[21]. We previously reported that predominantly breast-fed infants who were directly supplemented with fish oil showed improved communication at 12 and 18 months compared with placebo[22]. This study was novel in its use of direct supplementation (irrespective of mode of feeding) and the dose and length of the *n*-3 LCPUFA supplement.

The present study is a 6-year follow-up of the IFOS cohort employing a battery of sensitive neurocognitive tests to measure both global and specific aspects of cognitive and behavioural development. We predict that supplementation will have a positive neurocognitive effect, particularly on language and communication at 6 years of age – based on our earlier findings. To our knowledge, this is the first research to examine such long-term effects of fish-oil supplementation in healthy, term, primarily breast-fed infants.

Methods

The study design and methodology of the IFOS have been described previously[21]. To summarise, women in their third trimester of pregnancy were recruited from public and private antenatal services in Perth, Western Australia between June 2005 and October 2008. To be included in the study, women needed to have a history of allergy (otherwise healthy), be non-smokers, not take fish oil during pregnancy (≥1000 mg/d) and typically consume less than three fishmeals per week. Pregnant women with a history of allergy were recruited as their infants are at a higher risk of developing allergic disease (intervention had no clinical effects on allergic outcomes[23]).

This RCT was registered at Australian New Zealand Clinical Trials Registry www.anzctr.org.au (ACTRN12606000281594). All stages of the present study were approved by the University of Western Australia and the Princess Margaret Hospital Ethics Committee. Informed, written consent was obtained from the children's parents.

Study design and intervention

Four hundred and twenty healthy, term-born, singleton infants were randomised to receive daily supplementation with either DHA-enriched fish oil (*n* 218), or image-matched placebo (*n* 202), from birth to 6 months. The fish-oil capsules contained 250–280 mg of DHA and 60–110 mg of EPA, and the placebo capsules contained 67 % *n*-9 oleic acid. The capsules were designed to be punctured so that the oil could be directly administered into the infants' mouth or incorporated into milk in bottles. Participants were asked to give the oil to the infants in the morning, prior to/during feeding. Fatty acid intake during the period of intervention was accounted for via semi-quantitative FFQ along with fatty acid measurement of breast milk samples.

Blinding

The study was designed as a double-blind RCT, where both participants and staff were unaware of group allocation. The randomisation schedule was prepared by an independent biostatistician and stratified according to maternal and paternal allergic history and parity. Participants were unblinded after the 30-month immunological follow-up by a researcher independent to the present work. Participants were asked not to discuss their group allocation with the researcher conducting the 6-year neurocognitive assessments. The researcher responsible for conducting the 6-year follow-up assessments and associated results remained blinded throughout the trial. Following all data collection, groups were masked as A or B until all end points were analysed.

Fatty acids

Plasma phospholipid and erythrocyte DHA concentrations in the fish-oil group were statistically significantly increased compared with placebo at 6 months (data reported previously[22]). This increase was considered modest considering the dose and has been attributed to the high DHA status of the infants at birth, the high proportion of breast-fed infants (98 %) along with high DHA status of the breast milk, reduced capsule adherence (59 %) and the bioavailability of the ethyl ester supplement.

The 6-year follow-up

The purpose of the 6-year follow-up was to evaluate neurocognitive outcomes through a battery of neurocognitive tests and (parent and teacher) questionnaires. The tests were delivered in the same order for each participant and took approximately 2 h to perform. The tests were performed by one post-graduate student under the direction of a clinical neuropsychologist. Blood *n*-3 LCPUFA concentrations or dietary intake were not measured at the 6-year follow-up.

Language and communication

A Core Language Composite score was derived from performance on the Clinical Evaluation of Language Fundamentals, 4th Edition (CELF-4)[24]. This is an age-standardised test for the assessment of child language and communication skills. Adjunct measures of language and communication included the Renfrew Bus Story test[25], which evaluated the child's ability to retell a narrative to sentence length, complexity and vocabulary. Parents also completed the Children's Communication Checklist[26].

Behaviour

Parents and teachers completed questionnaires regarding the child's behaviour at home and at school. Parents completed the Autism Spectrum Quotient: Children's Version[27] and the Child Behaviour Checklist[28], and teachers completed the Teachers Report Form and the Gifted Rating Scale[29].

Working memory and executive function

An age-adjusted Working Memory Composite score was derived from the CELF-4. Two supplementary tests of executive function, Rapid Automatic Naming and Word Association, were also derived from the CELF-4. To further assess executive functioning and working memory, we created the Fruit Stroop Test based on the previously established protocol[30] (for further information on the Fruit Stroop Test design, see online Supplementary Information). Participants achieved a score based on how many

colours they correctly identified and a total error score. To assess non-spatial, complex working memory, we created the Self-Ordered Pointing Test (SOPT) based on previously established protocol[31] (for further information on the SOPT design, see online Supplementary Information). Participants achieved a score quantifying the number of errors made, that is, selection of any picture more than once.

Global intelligence

The Wechsler Abbreviated Scale of Intelligence – 2nd Edition (WASI) was used to evaluate general intelligence in those aged 6 to 90 years of age[32]. The test informs verbal intelligence quotient (IQ), performance IQ and full-scale IQ scores[33]. School teachers completed the Gifted Rating Scale questionnaire, which provides information on intelligence, academic ability, creativity, artistic talents, leadership ability and motivation[29].

Statistical analysis

Statistical analysis was performed using the IBM statistical software, Statistical Package for the Social Sciences version 21 for PC. Statistical significance was assessed at the two-tailed $P < 0{\cdot}05$. Outcomes were assessed on the basis of 'intention to treat'. Thus, all children were included in the analysis, irrespective of compliance with the intervention.

Power estimates for the present study (*post hoc*) were based upon a study in which scores on the Peabody Picture Vocabulary Test in 5-year-old children differed with regard to the duration of breast-feeding and the LCPUFA content of the breast milk[34]. It is appropriate to base the power estimate on this study, given that breast milk is the exclusive source of LCPUFA delivery in non-supplemented infants. The study observed an 8·2 point advantage for girls following adjustment for confounders, and a 5·8 point advantage for boys. Therefore, a sample size of approximately 135 participants per group was required to detect a 5·8 point minimum difference in the full-scale IQ scores between groups using an independent-groups *t* test, with 89 % power ($\alpha = 0{\cdot}05$)[35].

Any difference in demographic or neurocognitive characteristics between the groups was determined by independent *t* tests where data were normally distributed (normality having been checked through histograms and confirmed using Q–Q plots). Where variables were not normally distributed, logarithm and square root transformations were performed. However, untransformed data are referred to in the descriptive statistics for ease of interpretation, as transformation did not alter the final results. Pearson's χ^2 tests were used for nominal/categorical data. Where normality was not achieved and could not be improved by natural log transformation, non-parametric Mann–Whitney *U* tests were performed.

Many of the scores from the neurocognitive tests/questionnaires were age-standardised according to the test. Subsequently, age unadjusted analyses (independent *t* tests) were performed using the composite T scores derived from the CELF-4, Children's Communication Checklist, Child Behaviour Checklist, Teachers Report Form, WASI and the Gifted Rating Scale to compare the fish-oil and placebo groups. For the remaining data that were not age-standardised (Renfrew Bus Story, SOPT, Fruit Stroop, Autism Spectrum Quotient: Children's Version, Rapid Automatic Naming and Word Association test), multivariate linear regression analyses were used, controlling for age (in months) at the time of assessment. Regression models were also employed to analyse the raw scores of the WASI individual sub-tests (vocabulary, similarities, block design and matrix reasoning) while adjusting for age. This was necessary since the standardised WASI IQ scores were only accurate from age 6 years, yet not all participants had reached 6 years of age by the time of their assessment. Effect sizes were calculated using Cohen's *d*, with scores 0·2 indicating a small effect, 0·5 indicating a medium effect and ≥0·8 indicating a large effect.

Results

At the 6-year follow-up, 335 children participated (80 % of initial enrolment): 156 from the placebo group and 179 from the fish-oil group. The trial design and number of individuals at each stage are shown in Fig. 1. A higher number of participants from the fish-oil group (*n* 25) did not attend the follow-up compared with placebo (*n* 1). Reasons were not provided, but considering this was outside the supplementation period, this is unlikely to be related to the intervention. Overall, the mean age of children at the time of the assessment was 72 (SD 7) months, and the range was between 61 and 97 months.

Baseline characteristics of randomised groups

There were no differences between the fish-oil and placebo groups regarding their baseline population characteristics with the exception of the length of gestation, whereby the fish-oil group was born approximately 2·5 d earlier on average. However, the clinical significance of this is likely negligible so was not adjusted for in subsequent analyses (Table 1). There were no differences in the baseline characteristics of the participants who did not attend the 6-year follow-up compared with those presented here (data not shown).

Language and communication

As shown in Table 2, there was no difference between the fish-oil and placebo groups for any of the language scores including the Core Language Composite score. Nor were their significant differences between the groups for the sentence length, information or use of subordinate clauses according to the Renfrew Bus Story, either before or after adjusting for age. Results from the Children's Communication Checklist identified no significant differences between the two groups in terms of parents' perceptions of their child's communicative skills.

Behavioural outcomes

The fish-oil group displayed significantly more externalising behaviours, with the mean externalising *T* score of the fish-oil group 2·3 points higher than placebo (Table 3). Further analysis of the sub-tests comprising externalising behaviour revealed that the mean *T* scores of oppositional defiance were 1·6 points higher in the fish-oil group (95 % CI 0·40, 2·75; $P = 0{\cdot}006$, $d = 0{\cdot}24$). *Post hoc* analyses revealed that these behavioural effects were

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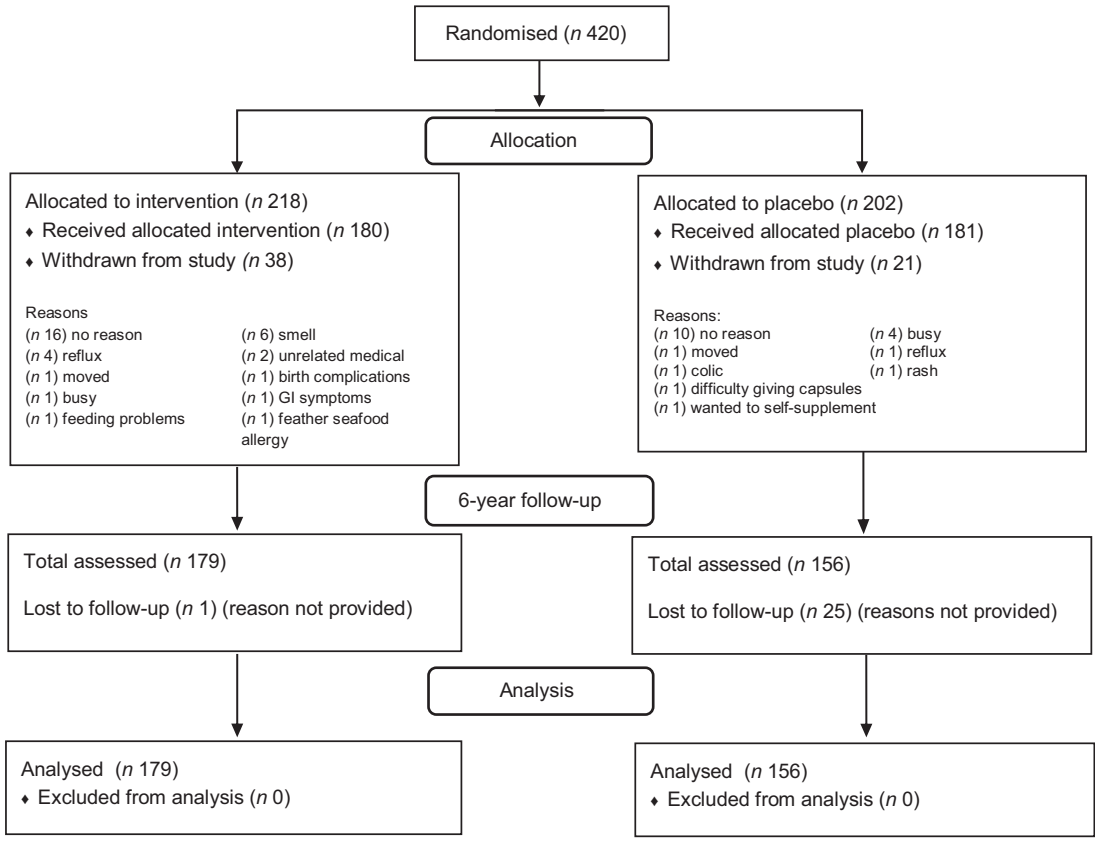

Fig. 1. Flow chart for study design, participant progress and data collection. GI, gastrointestinal.

Table 1. Baseline characteristics of the two groups seen at the 6-year follow-up (Mean values and standard deviations)

	Placebo (*n* 156)		Fish oil (*n* 179)		
	Mean	SD	Mean	SD	*P*
Neonatal anthropometrics					
Birth weight (g)	3516	486	3465	432	0·361
Gestation (weeks)	39·59	1·27	39·29	1·17	0·010*
Head circumference (cm)	34·64	1·33	34·92	1·48	0·121
Ethnicity (% Caucasian)	94·1		90·7		0·432
First/only child	47·1		48·7		0·809
Sex (% male)	46·4		52·8		0·329
Family characteristics					
Maternal age (years)	32·45	5·37	33·23	5·21	0·063
Maternal education (years)	13·50	2·83	14·90	3·59	0·110
Partner smokes (% yes)	12·9		10·5		0·560
Participant characteristics					
Capsules consumed (% total)	70	2·4	62	3	0·089
Age ceased breast-feeding (months)	6·32	3·03	6·53	3·65	0·790
Ever breast-fed (% yes)	100		97·5		0·600
Breast-fed beyond 6 months (% yes)	45·8		54·1		0·668

* $P < 0·05$, difference reaching statistical significance.

stronger in boys (*n* 165). Boys in the fish-oil group attained mean externalising behaviour *T* scores four points higher than boys in the placebo group (95 % CI 1·24, 6·58; $P = 0·004$, $d = 0·45$). Similarly, oppositional defiance was two points higher for boys in the fish-oil group compared with boys in the placebo group (95 % CI 0·49, 3·52; $P = 0·010$, $d = 0·40$). However, this was not a pre-planned analysis and thus statistical power is reduced accordingly.

No other significant differences were observed for the Child Behaviour Checklist questionnaire including Internalising or Total Behavioural scores. The Teacher Report Form identified no significant differences between the two groups, as was

Table 2. Language scores of the placebo group compared with the fish-oil group (Mean values and standard deviations)

	Placebo		Fish oil			
Test/score	Mean	SD	Mean	SD	*P**	Cohen's *d*
CELF-4 (*n*)	144		160			
Core Language Composite	104·01	13·15	102·92	11·83	0·361	0·09
The Renfrew Bus Story test (*n*)	140		153			
Information score	34·85	8·13	33·56	8·40	0·184	0·16
Sentence length	11·68	2·61	11·45	2·29	0·427	0·09
Subordinate clauses	1·85	1·60	1·80	1·58	0·778	0·03
CCC-2 (*n*)	150		150			
General Communication Composite	79·01	17·94	78·68	17·33	0·870	0·02

CELF-4, Clinical Evaluation of Language Fundamentals; CCC-2, Children's Communication Checklist – 2nd ed.
* *P* values are based on independent-group *t* tests (CELF-4, CCC-2) or multivariate linear regression controlling for age (Renfrew).

Table 3. Behaviour scores of the placebo group compared with the fish-oil group (Mean values and standard deviations)

	Placebo		Fish oil			
Test/score	Mean	SD	Mean	SD	*P**	Cohen's *d*
CBCL (*n*)	152		155			
Internalising *T* score	49·18	9·39	48·07	10·45	0·326	0·11
Externalising *T* score	48·46	9·46	50·74	9·14	0·031†	0·24
Total behaviours *T* score	48·29	9·14	48·51	10·13	0·842	0·02
Total competence *T* score	46·61	9·63	46·56	8·90	0·965	0·005
AQ-Child (*n*)	153		150			
General Communication Composite	46·06	15·44	45·22	15·59	0·637	0·05

CBCL, Child Behaviour Checklist – Parent Report Form; AQ-Child, Autism Spectrum Questionnaire – Children's Version.
* *P* values are based on independent group *t* tests (CBCL) or multivariate linear regression controlling for age (AQ-Child).
† Indicates statistically significant difference between the placebo and fish-oil groups.

Table 4. Working memory scores of the placebo group compared with the fish-oil group (Mean values and standard deviations)

	Placebo		Fish oil			
Test/score	Mean	SD	Mean	SD	*P**	Cohen's *d*
Working memory (*n*)	145		156			
Working Memory Composite	103·21	13·86	104·40	13·16	0·448	0·09
No. repetition forwards	10·85	2·76	10·19	2·75	0·040†	0·24
No. repetition backwards	10·07	2·58	10·11	2·54	0·876	0·02
Familiar sequences	10·59	2·77	11·27	2·70	0·031†	0·24
Rapid Autonomic Naming (*n*)	124		127			
	121·17	38·88	118·0	39·34	0·563	0·08
Word Association (*n*)	147		137			
	24·98	7·51	24·31	7·32	0·459	0·09
Additional tests: executive functioning, memory and inhibition (*n*)	124		121			
Fruit Stroop	2·87	15·44	45·22	15·59	0·637	0·05
Fruit Stroop errors	4·79	3·98	4·14	3·61	0·188	0·17
Self-Ordered Pointing Test (*n*)	127		129			
	2·66	2·46	2·32	2·06	0·226	0·15

* *P* values are based on multivariate linear regression controlling for age.
† Statistically significant difference between the placebo and fish-oil groups.

the case for the Autism Spectrum Quotient: Children's Version questionnaire.

Working memory and specialised executive functions

As seen in Table 4, there was no difference between the fish-oil and placebo groups for the composite score of working memory derived from the CELF-4. However, sub-test analysis found participants in the fish-oil group scored lower in number repetition (forwards) than the placebo group (95 % CI 0·03, 1·29; $P = 0·040$, $d = 0·24$). Whereas, in the familiar sequences sub-test, the fish-oil group scored higher compared with placebo (95 % CI −1·30, 0·062; $P = 0·031$, $d = 0·24$). There were no group differences for the secondary outcomes of the CELF-4, that is, the two

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Table 5. Global intelligence scores of the placebo group compared with the fish-oil group (Mean values and standard deviations)

	Placebo		Fish oil			
Test/score	Mean	SD	Mean	SD	*P**	Cohen's *d*
Wechsler Abbreviated Scale of Intelligence – 2nd ed. (*n*)	147		162			
Verbal IQ	111·17	13·41	111·49	12·96	0·833	0·02
Performance IQ	103·28	11·41	103·98	11·94	0·601	0·06
Full-scale IQ	108·03	12·03	108·67	11·18	0·629	0·05
Gifted Rating Scale (*n*)	30		36			
Intellect	49·20	9·35	48·62	7·76	0·801	0·06

IQ, intelligent quotient.
* *P* values are based on independent-group *t* tests.

supplementary tests, Word Associations and Rapid Automatic Naming after adjusting for age. Similarly, there were no differences between the fish-oil and placebo groups regarding the Fruit Stroop Test or the SOPT.

Global intelligence

There were no significant group differences between the fish-oil and placebo groups for the global measures of IQ (verbal IQ, performance IQ or full-scale IQ) derived from the WASI (Table 5). Given that the WASI IQ scores were age-standardised from 6 years of age, we also analysed the group differences for each of the WASI sub-test raw scores while adjusting for age (in months). There were no significant differences between the groups for any of the WASI raw scores before or after adjusting for exact age. Furthermore, results from the Gifted Rating Scale identified no significant differences between the two groups for intelligence or any other measures including academic, creativity, artistic, leadership or motivation.

Discussion

We predicted that fish-oil supplementation during infancy would have a positive neurocognitive effect, particularly on language and communication at 6 years of age – based on our earlier findings at 12 and 18 months[22]. Further, we expected the long-term effects to be more pronounced at 6 years due to the increased validity of neurocognitive assessments and the emergence of additional language and cognitive capacities subsequent to ongoing brain maturation. However, we found no evidence that the fish-oil group was superior to the placebo group at 6 years in terms of their communication or language development, despite the use of several neurocognitive tests specifically chosen to investigate this. This long-term follow-up indicates that any benefit of fish-oil supplementation on communication during toddlerhood was transient and did not permanently alter neurocognitive ability. It may be that current dietary intake of DHA (not measured in the present study) is more relevant than past intake, even during a period of rapid neurodevelopment.

Externalising behavioural problems were found to be significantly higher in the fish-oil group (although still within the normal range). The adverse finding of infant fish-oil supplementation on behaviour is in line with our previous findings in this sample, which reported a trend for higher anxiety/depression in the fish-oil group, although not significant ($P = 0{\cdot}081$)[22]. It is not clear why fish-oil supplementation in early life would cause negative behavioural effects in childhood. However, a small number of other prenatal DHA supplementation trials have revealed similar findings. A large Australian RCT of prenatal DHA supplementation found significantly more behavioural problems in the DHA group *v.* placebo, when followed up at 4 years[36] and again at age 7 years[37]. Similarly, Meldrum *et al.* recently found a comparable trend for externalising behavioural traits in 12-year-old children whose mothers had undergone DHA supplementation during pregnancy[38]. Furthermore, the RCT by Cheatham *et al.* reported lower pro-social scores in 7-year-old children whose mothers were supplemented with fish oil during breast-feeding, compared with controls[39]. Of these previous studies, few have offered plausible explanations for this curious result, instead citing reasons such as chance (i.e. type I statistical errors), which is possible. Negative effects on behaviour are in contrast to both animal trials and trials of mental health and behaviour in adult populations[40,41]. This finding requires replication in future large studies before conclusions can be drawn.

There was no significant difference between the fish-oil and placebo groups in terms of the composite measures of working memory, although there were some effects within the sub-tests of working memory. Since the directions of these effects were inconsistent (i.e. the fish-oil group performed significantly better in the familiar sequences sub-test yet significantly worse recalling numbers), these may well be random effects. The null effect with respect to overall working memory performance was echoed in the additional tests of executive functioning and inhibitory control (the Fruit Stoop and SOPT) which we developed to overcome some of the limitations of global tests of neurocognitive outcomes and investigate more specific cognitive domains. These tests are believed to be more specialised and sensitive, with greater potential to detect more modest effects on cognitive performance[42]. The utility and merit of these tests have been evaluated, and experts agree that there is a pressing need for psychometrically sound instruments capable of detecting subtle differences in executive functioning in children[43].

The IFOS study attempted to provide a high-dose *n*-3 LCPUFA within fish oil; however, the actual increase in DHA was modest and thought to be as a result of the form (ethyl ester) and mode of supplementation (directly to the infant via liquid squired from the capsule). Consequently, the present results

may be attributable to an insufficient dose. Yet, a high dosage has conferred no consistent additional benefit in an RCT of infant formula supplementation utilising a variable dose research design[44,45]. Future research examining high dosages of *n*-3 LCPUFA supplement during infancy with long-term follow-up into later childhood is required to assist in the interpretation of the present findings.

The IFOS sample population were well educated with high-income and high breast-feeding rates. The social characteristics of our sample population may enhance the real-world utility of this study as parents within this demographic are more likely to possess the financial means to purchase commodities such as infant fish-oil products in the belief that they may confer a neurocognitive advantage. However, this does potentially limit the generalisability of the present study, especially to families with lower rates of income and education, with high rates of infant formula feeding. Such populations are likely to have lower DHA consumption[46].

To our knowledge, this is the first published RCT to examine the long-term neurocognitive and behavioural effects of fish-oil supplementation in healthy, term, primarily breast-fed infants. The percentage of participants retained within the study was higher than other large-scale long-term longitudinal studies in this field[39,47]. While the nutritional supplement and infant formula industries market DHA as a conduit to optimise brain development and child learning, our study found infant fish-oil supplementation provided no advantage to well-nourished, Australian children.

Possible weaknesses include that our trial was designed to assess the role of fish oil in allergy prevention[21]. Therefore, infants at high risk of developing allergic disease due to maternal allergy were recruited, as they were at a high risk of developing allergy. Considering that allergic disease has been associated with neurodevelopment in a bidirectional manner[48], this population may differ when compared with a non-allergic population, although the exact nature of this potential effect remains unclear. A second weakness is the lack of blinding. At 18 months, the majority of participants in the fish-oil group correctly guessed their infant's group allocation and participants were then unblinded at 30 months of age. It is feasible that parents within the fish-oil supplementation group may have altered perceptions of their child's skills, particularly considering the group differences observed in this cohort in parent-report measures at both 18 months and within the present follow-up, compared with clinician-observed measurement where no differences were observed[22]. While the likelihood that such as bias would extend until 6 years of age is questionable, this remains a consideration in the interpretation of the results.

In addition, a large number of statistical comparisons were performed. Statistical corrections were not undertaken, as it has been validly argued that this would not be appropriate in the case of consistent, repeated and biologically plausible patterns[49]. This is supported by the current finding of increased externalising behavioural problems in the supplemented group of in line with our previous trends at 18 months. However, it remains possible that the present results were due to chance.

Conclusion

Fish-oil supplementation during infancy for predominantly breast-fed infants is not recommended for the purposes of enhancing long-term neurocognitive or behavioural outcomes in early childhood. Our results indicate no positive behavioural effects among 6-year-old children (especially in boys), running counter to the hypothesis that fish-oil supplementation during infancy incites long-term improvement in brain development.

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S. J. M., K. S. and S. L. P. conceptualised and designed the study and procured funding for the present neurodevelopmental follow-up. S. J. M. and J. K. F. selected the neurocognitive tests and supervised the psychometric assessment. A. E. H. coordinated cohort maintenance and performed the neurocognitive tests. A. E. H. and S. J. M. analysed and interpreted the results. All authors critically reviewed the results and added intellectual content. S. J. M. and A. E. H. participated in manuscript drafting, review and preparation. All authors revised and approved the submitted manuscript.

There are no potential conflicts of interest to declare for any of the listed authors.

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