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Protocol for assessing if behavioural functioning of infants born <29 weeks' gestation is improved by omega-3 long-chain polyunsaturated fatty acids: follow-up of a randomised controlled trial

Jacqueline F Gould ,[1,2] Rachel M Roberts,[3] Peter J Anderson,[4] Maria Makrides,[1,5] Thomas R Sullivan,[1,6] Robert A Gibson,[1,7] Andrew J McPhee,[1,8] Lex William Doyle ,[9] Gillian Opie,[10] Javeed Travadi,[11,12] Jeanie L Y Cheong ,[13] Peter G Davis ,[13] Mary Sharp,[14] Karen Simmer,[15] Kenneth Tan,[16,17] Scott Morris,[18] Kei Lui,[19] Srinivas Bolisetty,[19] Helen Liley,[20] Jacqueline Stack,[21] Karen P Best,[1,5] Carmel T Collins[5,22]

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For numbered affiliations see end of article.

Correspondence to
Dr Jacqueline F Gould;
jacqueline.gould@sahmri.com

ABSTRACT

Introduction During the last trimester of pregnancy, the fetal brain undergoes a rapid growth spurt and accumulates essential nutrients including docosahexaenoic acid (DHA). This takes place ex-utero for infants born <29 weeks' gestation, without the in-utero provisions of DHA. Infants born <29 weeks' are more likely to experience behavioural and emotional difficulties than their term-born counterparts. It has been hypothesised that supplementing preterm infants with dietary DHA may alleviate insufficiency and subsequently prevent or minimise behavioural problems. This protocol describes a follow-up of infants born <29 weeks gestation who were enrolled in a randomised controlled trial (RCT) of DHA supplementation. We aim to determine whether DHA supplementation improves the behaviour, and general health of these infants.

Methods and analysis Infants born <29 weeks' gestation were enrolled in a multicentre blinded RCT of enteral DHA supplementation. Infants were randomised to receive an enteral emulsion that provided 60 mg/kg/day of DHA or a control emulsion commenced within the first 3 days of enteral feeding, until 36 weeks' postmenstrual age or discharge home, whichever occurred first. Families of surviving children (excluding those who withdrew from the study) from the Australian sites (up to 955) will be invited to complete a survey. The survey will include questions regarding child behavioural and emotional functioning, executive functioning, respiratory health and general health. We hypothesise that the DHA intervention will have a benefit on the primary outcome, parent-rated behaviour and emotional status as measured using the Total Difficulties score of the Strengths and Difficulties Questionnaire. Detecting a 2-point difference between groups (small effect size of 0.25 SD) with 90% power will require follow-up of 676 participants.

Ethics and dissemination The Women's and Children Health Network Human Research Ethics Committee reviewed and approved the study (HREC/16/WCHN/184). Results will be disseminated in peer-reviewed publications and conference presentations.

Trial registration number ACTRN12612000503820.

Strengths and limitations of this study

- This study will be the largest follow-up of a randomised controlled trial of enteral docosahexaenoic acid (DHA) supplementation for infants born preterm.
- This follow-up will determine the effect of enteral DHA supplementation for infants born <29 weeks' gestation on child behaviour and emotional state.
- This follow-up study will assess a range of general health outcomes for infants born <29 weeks' gestation.
- Loss to follow-up 5 years after enrolment into the trial may contribute to risk of bias.

INTRODUCTION

Docosahexaenoic acid (DHA, 22:6n-3) is an omega-3 long-chain polyunsaturated fatty acid (n-3 LCPUFA) that is crucial for the developing brain. DHA is present in all cell membranes as a structural component of the phospholipid bilayer with an integral role in membrane fluidity.[1] It is concentrated in the brain[2 3] where it is known to be involved in neurogenesis, signal transduction and neurotransmission.[4] In the last trimester of a normal pregnancy, it is estimated that the

fetus acquires ~70 mg of n-3 LCPUFA per day, largely as DHA[5] and this peak period of neural tissue DHA accumulation coincides with the fetal brain growth spurt.[6]

Preterm birth can slow the fetal brain growth spurt as well as interrupts the usual placental supply of nutrients such as DHA. Infants born preterm typically have lower neural tissue DHA levels than term-born infants.[6 7] They are more likely to experience poor neurobehavioural outcomes compared with infants born at term.[8–19] The risk of suboptimal development increases as gestational age at birth decreases,[9 13 20–22] and among those born <28 weeks' gestational age, 52% of survivors will have some neurobehavioural disability.[23] Behavioural problems,[9 11 13–17 19 24–26] such as attention difficulties,[9 18 27 28] and anxiety,[26 27 29 30] as well as executive functioning difficulties (struggling with skills essential for undertaking goal-oriented behaviours including inhibitory control)[10 30 31] are common issues for children born preterm when compared with their term-born counterparts.

Insufficient dietary DHA in the neonatal period for preterm infants may be a modifiable contributing factor to the increased rate of poor neurobehavioural outcomes. Randomised controlled trials (RCTs) of infants born preterm have compared treatment with supplemental DHA with control to assess the impact on child development. These have produced inconclusive results.[32–34] The most promising evidence comes from two trials that supplemented preterm infants with a dose equivalent to in-utero accretion of DHA (60 mg/kg/day) compared with the standard dose of DHA (20 mg/kg/day present in breastmilk and in preterm infant formula).[35 36] Study authors reported benefits of higher doses of DHA supplementation in infants born <1500 g on problem solving[35] and attention[37] and, in infants born <1250 g a reduction in the prevalence of cognitive delay.[36] Follow-up assessments at 7 years in one of these trials revealed an unexpected potentially adverse interaction effect with DHA where a few instances of parent-rated behaviour and executive functioning were worse in girls who received extra DHA compared with girls who received the standard dose of DHA.[38 39] However, these findings were based on secondary analyses and hence were insufficient to change clinical practice.

In N–3 Fatty Acids for Improvement in Respiratory Outcomes (N3RO), the largest trial to date, 1273 infants born <29 weeks' gestation were randomised to receive oral supplementation of DHA (60 mg/kg/day) or control from within the 3 days of commencing enteral feeds until 36 weeks' postmenstrual age or discharge home, whichever occurred first.[40 41] DHA provided no benefit and may have increased the risk of bronchopulmonary dysplasia (the primary outcome).[41] A similar recent study also showed an increased risk of bronchopulmonary dysplasia in infants born <29 weeks', although the trial was ceased early after interim analyses suggested poorer outcomes in the DHA group.[42] Given these recent findings we can no longer assume safety. Equally the potential neurobehavioral benefit or harm has never been adequately demonstrated in extremely preterm infants.

The N3RO trial recruited infants at high risk of adverse neurobehavioural outcomes[23] and offers a unique opportunity to determine whether DHA supplementation improves neurobehavioural outcomes.

We aim to follow-up the N3RO trial children to evaluate the effect of DHA supplementation in infants born <29 weeks' gestation on behavioural, emotional and executive functioning at early school age. We hypothesise that infants <29 weeks' gestation provided with oral DHA supplements in the first months of life in amounts resembling transplacental acquisition will have fewer behavioural, emotional and executive function problems at 5 years' corrected age compared with infants who received the control intervention.

METHODS

The N3RO trial

Infants were eligible for N3RO if they were born <29 weeks' gestation, were able to be recruited within 3 days of their first enteral feed and had a parent or guardian capable of providing written informed consent. Infants were ineligible if they were participating in another fatty acid intervention trial, if they had a major congenital or chromosomal abnormality, if a breastfeeding mother was taking DHA containing supplements of more than 250 mg/day, or if they were receiving intravenous lipids containing fish oil.[41] A total of 1273 infants were enrolled into the N3RO trial from 13 centres in Australia, New Zealand and Singapore between 2012 and 2015.

On enrolment, infants were randomised to receive an enteral emulsion that provided either 60 mg of DHA per kg of body weight per day (intervention group), or a control emulsion without DHA (control group).[41] Infants received the study emulsion from enrolment to either discharge home or 36 weeks' postmenstrual age, whichever occurred first. Intervention and control emulsions were identical in viscosity, colour and packaging to ensure blinding of families, clinical staff and study personnel.[41] Infants were randomised through a secure web-based server using an independently generated randomisation schedule stratified for gestational age at birth <27 weeks or 27–28 weeks, sex and centre; infants from multiple births were randomised individually.

Follow-up study procedure

This is a follow-up study of infants enrolled in the N3RO trial from the Australian centres who had not died or withdrawn from the trial. This follow-up was not specified in the original N3RO trial protocol, however at enrolment into the N3RO trial families gave consent to be contacted in the future for follow-up studies. Hospital records from the enrolment centres will be checked to confirm children are not deceased prior to contacting families. Caregivers of surviving N3RO trial infants will be invited to complete an online survey when their child reaches 5 years' corrected age. Study personnel are blinded to randomisation.

Families had the opportunity to request knowledge of their group allocation after completion of the N3RO trial primary outcome analyses. The number of families who requested to be unblinded overall will be reported, along with the number of families who were unblinded and participated in the follow-up.

The survey comprises multiple parent-rated measures of child behaviour, behavioural manifestations of executive functioning, health-related quality of life, symptoms of asthma and allergy, child general health, family functioning, parenting style, current dietary intake of food and supplement sources of DHA and the quality of the social and emotional support available in the home environment. The entire survey takes on average 40 min (between 20 min to 60 min) to complete. The survey can be completed online (via a personalised link sent through email or text message), or if caregivers prefer, a posted hard-copy or via interview over the telephone with study staff.

Caregivers of surviving, eligible N3RO trial children will be emailed a letter of invitation to the follow-up study 2 months before their child reaches 5 years' corrected age, followed by a telephone call to answer any questions, clarify willingness to participate and ascertain mode of preferred survey completion. Contact details provided at enrolment in the N3RO trial, and updated details provided during yearly mail-outs will be used to contact eligible families. Age is corrected for prematurity to avoid a known bias in standardised test scores for children born preterm.[43] Families will be reimbursed with an $A40 gift voucher for completing the survey. Assessments for this follow-up study commenced 29 August 2018 and are expected to be completed by June 2021.

Participants

Children are eligible and will be invited to participate in this follow-up if they were enrolled at any of the Australian sites; the Flinders Medical Centre (South Australia), John Hunter Hospital (New South Wales), King Edward Memorial Hospital (Western Australia), Liverpool Hospital (New South Wales), Mater Mothers' Hospital (Queensland), Mercy Hospital for Women (Victoria), Monash Children's Hospital (Victoria), Royal Hospital for Women (New South Wales), The Royal Women's Hospital (Victoria) and the Women's and Children's Hospital (South Australia). Families will not be approached if their child was withdrawn from the N3RO trial or if N3RO study staff are notified that the child has died.

Outcomes and measures

For this study, all outcome measures are derived from questionnaires (see table 1) to be completed by caregivers using the Research Electronic Data Capture (REDCap) software platform.[44 45] Surveys completed in hard copy or via interview over the phone will be entered into REDCap.

Primary outcome

The primary outcome is behavioural functioning as assessed by the Total Difficulties Score of the Strengths and Difficulties Questionnaire (SDQ). The SDQ is a parent-completed rating of symptoms of behavioural problems.[46] The parent-completed version of the SDQ for children age 4–10 years will be used. Caregivers are asked to rate their child's behaviour and emotional state in comparison to other children of the same age. The SDQ has good test–retest stability (r=0.62, after 4–6 months) and internal consistency for the Total Difficulties scale (α=0.82).[47] Despite being a screening test, the parent-rated version of the SDQ Total Difficulties scale has demonstrated good sensitivity and positive predictive validity for psychiatric diagnoses in children.[47]

Secondary Outcomes

Other SDQ scales

The SDQ contains 25 items, split evenly into five scale scores; Emotional Symptoms, Conduct Problems, Hyperactivity/Inattention, Peer Relationship Problems and Prosocial Behaviour.[46] Higher scores for all scales and composites (excepting Prosocial Behaviour), indicate more perceived symptoms of a behavioural problem. Although scores on the SDQ are not standardised, they will be categorised as normal (<14) or abnormal/indicative of dysfunction (14 or higher) according to Australian norms[48 49] and compared between the groups, in addition to comparing mean scores.

Behaviour Rating Inventory of Executive Functioning-Preschool edition

The parent version of the Behaviour Rating Inventory of Executive Functioning-Preschool edition (BRIEF-P) will be administered to assess behavioural manifestations of young children's (two to 2–5 years) executive functions in everyday settings.[50] The BRIEF-P is an important addition to formal, performance-based assessment of executive functioning as some elements of executive dysfunction are only obvious in everyday situations, such as the home. The BRIEF-P has five scales (Inhibit Scale, Emotional Control Scale, Shift Scale, Working Memory Scale and Plan/Organise Scale) that make up three indices (Inhibitory Self Control Index, Flexibility Index and Emergent Metacognition Index) and an overall Global Executive Composite score.

Scores are age-standardised to a mean of 50, SD=10, and higher scores indicate more symptoms of executive dysfunction.[50] Scaled scores at or above 65 are categorised as dysfunctional and scores below 65 are categorised as normal and will be compared between groups, as will mean scores for each domain. Scores will be age-standardised according to corrected age (corrected for preterm birth) at the time of survey completion. For children who are older than 6 years' corrected age at the time of the survey completion, caregivers will be asked to complete the BRIEF2 (BRIEF, Second Edition, for children aged 6–18 years).[51] The BRIEF2 has slightly different

Table 1 Description of sample characteristics, and primary and secondary outcomes assessments

Characteristics (descriptive presentation)		
To be described for each group	Baseline characteristics	Pregnancy information (birth hospital, maternal smoking during pregnancy, maternal omega-3 long-chain polyunsaturated fatty-acid supplements during pregnancy, antenatal corticosteroids, singleton pregnancy)
		Socio-demographic characteristics (parental age, education, maternal ethnicity, employment)
		Infant characteristics (gestational age, weight, sex, age at enrolment, docosahexaenoic level in whole blood at randomisation)
To be described for each group	Post-randomisation characteristics	Infant characteristics (age at first intervention dose, sepsis, necrotising enterocolitis, bronchopulmonary dysplasia, retinopathy of prematurity, cerebral cystic formation, intraventricular haemorrhage, length of hospital stay)
		Home environment and child characteristics at follow-up (family structure, English the primary language spoken at home, whether the child attends or attended pre-school or full-time primary school, age at time of participation, family unblinded to intervention)
		Docosahexaenoic acid (DHA) in the child's diet (DHA containing supplements, fish meals)
	Short Family Assessment Device	General Family Functioning score
	StimQ	Parental Involvement in Developmental Advance score
	Parenting Scale	Total score
		Laxness score
		Over-reactivity score
		Hostility score
Outcomes (compared between groups)		
Primary outcome	Strengths and Difficulties Questionnaire	Total difficulties score
Secondary outcomes	Strengths and Difficulties Questionnaire	Emotional symptoms score
		Conduct problems score
		Hyperactivity/Inattention score
		Peer relationship problems score
		Prosocial Behaviour score
		Impact score
	Behaviour Rating Inventory of Executive Function-Preschool	Global executive composite score
		Emergent Metacognition index score
		Inhibitory Self-Control index score
		Flexibility index score
		Inhibit scale score

Continued

Table 1 Continued

Characteristics (descriptive presentation)		
		Shift scale score
		Emotional control scale score
		Working memory scale score
		Plan/organise scale score
	General health	Medically diagnosed neurological condition (cognitive, behavioural disorder, autism spectrum disorder)
		Medically diagnosed physical condition (blindness, hearing-loss or deafness, physical disability or other medical conditions)
		Allied health service use (whether the child has received services of a physiotherapist, speech therapist, occupational therapist, psychologist, behavioural therapist, psychiatrist or other allied health professional)
		Hospitalisations (any hospitalisations for respiratory conditions since discharge from hospital)
		Any surgeries (since discharge from hospital)
	International Study of Asthma and Allergies in Childhood	Wheeze ever
		Current wheeze
		Parent reported 'Asthma' diagnosis ever
	Paediatric Quality of Life Inventory	Total score
		Physical Functioning score
		Emotional Functioning score
	Social Functioning score	
		School Functioning score
		Psychosocial Functioning score

(age-appropriate) subscales and indices to the BRIEF-P but has the same overall standardised Global Executive Composite score.

General health

Child's health status will be compared between the groups in terms of whether they have been diagnosed (yes/no) with a cognitive, behavioural or emotional disorder, blindness, hearing-loss or deafness, physical disability, autism spectrum disorder or other medical conditions by a health professional, and whether the child has received services of a physiotherapist, speech therapist, occupational therapist, psychologist, behavioural therapist, psychiatrist or other allied health professional. Caregivers will be asked about any surgical procedures that the child has undergone since discharge from hospital, as well as any respiratory-related hospital admissions.

International Study of Asthma and Allergies in Childhood

The International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire is a brief parent-completed measure of the frequency and severity of allergic disease, including respiratory symptoms in the previous 12 months.[52] As the primary outcome of the original N3RO trial was bronchopulmonary dysplasia,[41] the ISAAC questionnaire will be important for providing an indication of any effect of DHA on respiratory health.

Paediatric Quality of Life Inventory

The Paediatric Quality of Life Inventory (PedsQL) is a parent-rated measure of general well-being and the health-related quality of life in healthy children, and in children with acute or chronic health conditions. A total of 23 items assess Physical Functioning, Emotional Functioning, Social Functioning and School Functioning and provide mean scores for each domain as well as an overall score to be compared between groups.[53]

Background information and sample characteristics

There will be a descriptive comparison of background characteristics and the following post-randomisation characteristics. Socio-demographic data were collected at enrolment into the N3RO trial (such as parental age, education, employment) through interview with the caregiver. Clinical characteristics of infants from baseline to discharge home, or 40 weeks' postmenstrual age (whichever occurred first) were collected from medical records as part of the N3RO trial.

As part of this protocol, current details about the child's home and medical background will be recorded. Caregivers will be asked about the child's family structure, whether English is the primary language spoken at home, whether the child attends or attended pre-school or full-time primary school. Caregivers will also be asked about whether the child has consumed dietary DHA from DHA containing supplements (on at least 3 days per week) and the number of fish meals (one meal=60–80 g of fish) in the last month.

The home environment

Indicators of the quality of the home environment will be captured and descriptively compared with to check for balance between groups as stimulation within the home, parenting and family functioning all have an influence on child development and behaviour.[54–57] The family home environment will be assessed through three parent-completed questionnaires. Families with more than one child in the follow-up study will only need to complete these questionnaires once. The 12-item General Family Functioning scale (considered the short Family Assessment Device) of the Family Assessment Device will be administered to measure problem solving by the family as a whole.[58] The Parental Involvement in Developmental Advance subscale of the StimQ, will be used to assess parental involvement in child learning activities.[59]

The Parenting Scale captures parenting style in different scenarios to measure dysfunctional parenting when discipline is needed.[60] The 30-item questionnaire assesses the probability of using different disciplinary strategies. The scale generates an overall score as well as scales for Laxness (permissive or inconsistent style), Over-reactivity (emotional, irritable, harsh or authoritarian style) and Hostility (use of verbal or physical force).[60]

Sample size

A total of 1028 children were randomised in the 10 Australian sites in the N3RO trial. Excluding 66 deaths and 7 withdrawals from the original N3RO trial, 955 children are potentially available for follow-up. A sample size of 338 children per group (676 total, approximately 70% of those potentially available) will provide 90% power, two-tailed alpha 0.05, to detect a 0.25 SD mean difference (small effect size) between groups in the Total Difficulties score of the SDQ. Based on our earlier follow-up of 7-year-old children born <33 weeks' gestation enrolled in a DHA RCT,[38] we expect an effect size of 0.25 SD to correspond to a mean difference in the Total Difficulties score of approximately 2 points.

No adjustment to the sample size is needed for clustering due to multiple births, since children were randomised individually in N3RO and the design effect for continuous outcomes is one in this case.[61] The sample size calculation was performed with Stata V.15 software (StataCorp LP) assuming a linear regression model for analysis (equivalently a two-sample t-test with equal variances).

Statistical analysis and data management

All analyses will be undertaken on an intention-to-treat basis for all surviving children from eligible centres according to a prespecified statistical analysis plan approved by the N3RO trial Steering Committee. All data will be identified through the study randomisation identification numbers assigned at enrolment into the N3RO trial. Identification numbers are associated with a code to indicate group allocation (available to the trial statistician

only) so that data can be analysed blinded to treatment group.

The following data will be descriptively compared between the groups to determine comparability of sample characteristics; baseline characteristics (such as infant sex and maternal education), characteristics and 5 years' corrected age (such as family structure and attendance at school) and the home environment (such as the Family Assessment Device, the StimQ and the Parenting Scale scores). Outcomes to be compared between randomisation groups are all outcomes from the SDQ, BRIEF-P, ISAAC and the PedsQL, as well as the general health of the children at the time of the follow-up (such as presence of neurological diagnoses).

Outcomes of intervention and control group children will be compared using generalised linear models, with generalised estimated equations used to account for clustering due to multiple births within the same family. Continuous and binary outcomes will be analysed using linear and log binomial models, respectively, with adjustment for variables used to stratify the randomisation: sex, centre enrolled and gestational age (<27 completed weeks or 27–28 weeks). For all outcomes, preplanned subgroup analyses will be performed to test for evidence of effect modification by sex and gestational age (less than 27 weeks, 27 to less than 29 weeks). Effect modification will be assessed by including an interaction term between the subgroup variable and treatment group into the regression model for each outcome. Estimates of the treatment effect within each subgroup will be reported, independent of the degree of evidence for effect modification, since these treatment effects are a priori of interest. No adjustment will be made for multiple preplanned comparisons, as the single primary outcome of interest is the SDQ Total Difficulties score and all other analyses will be hypothesis-generating secondary outcomes.

Missing outcome data will be addressed using multiple imputation, with imputation performed separately by treatment group using fully conditional specification.[62] Imputed data sets will include all surviving children from the 10 included centres.

Data will be entered into a REDCap database, which uses a MySQL database via a secure web interface with data checks used during data entry to ensure data quality. Data from the survey will be stored on secure servers within the South Australian Health and Medical Research Institute for a minimum of 30 years. Data will be accessible only by study staff and investigators.

Ethical considerations and dissemination of results

This study will be carried out in accordance with the Australian National Statement on Ethical Conduct in Research Involving Humans,[63] which builds on the ethical codes of the Declaration of Helsinki and the Principles of International Conference on Harmonisation[64] Good Clinical Practice (as adopted in Australia).[65] All procedures and study materials have been reviewed and approved by the Women's and Children's Health Network Human Research Ethics Committee (HREC/16/WCHN/184), as well as the Research Governance Offices at each site. The N3RO Trial and this follow-up are registered on the Australia and New Zealand Clinical Trial Registry.

Caregivers will be provided with detailed written information about the study and will provide informed consent for their child's involvement in the form of either e-consent built into the survey, or a hard-copy written form. Data collected will be treated with confidence and caregivers will be free to withdraw their children from the study at any time without prejudice.

The results of this follow-up study will be presented at academic conferences and published in peer-reviewed journals. No participants will be identified in the dissemination of study results.

Access to data

Individual participant data, including data dictionaries, may be shared after de-identification on reasonable request. Proposals to access the data must be scientifically and methodologically sound and must be reviewed and approved by the N3RO trial Steering Committee and the Women's and Children's Human Research Ethics Committee. To gain access, data requestors will need to sign a data access agreement. Proposals should be directed to Jacqueline Gould through email (Jacqueline.gould@sahmri.com).

Patient and public involvement

Neither caregivers or families of patients nor the public were directly involved in the development of the research question or design of this follow-up study. However, our primary outcome of behaviour is based on reported concerns over long-term developmental concerns from parents of preterm infants.[66]

A Community Board, comprising parents (including parents of a child born preterm) as well as clinicians and researchers specialising in paediatrics will be consulted for the dissemination of the study findings to participants, including reviewing the study results and format of dissemination.

DISCUSSION

Approximately 15 million infants are born preterm worldwide each year,[67] with increasing survival into childhood of infants born as early as 23 weeks' gestation. However, the risk of long-term behavioural and emotional difficulties in survivors remains high[68 69] and there is evidence that the prevalence of behavioural problems is increasing.[16 70–74]

The N3RO trial, along with another trial in breastfed infants, showed no benefit of the DHA intervention on bronchopulmonary dysplasia, but the effect of DHA on childhood neurobehavioural outcomes in this specific population–infants born <29 weeks' gestation–is unknown.[41 42] A preliminary follow-up of a small subgroup (n<100) of the N3RO children in infancy[75] detected no effect of the DHA intervention on their

executive function, visual attention or on cognition, motor or language abilities[76] although the sample was under-powered to detect an effect on cognition, motor or language development.[75 76] Further follow-up of a larger sample of the N3RO trial children is needed to determine the effectiveness of the DHA intervention on behavioural outcomes.[77]

This study is the largest follow-up study after a DHA intervention in preterm infants, and is the first early DHA intervention study with behaviour as a main outcome.[32 77–82] In addition to behavioural and emotional status, we will assess the effect of the DHA intervention on the manifestations of executive functioning skills in everyday behaviour as well as child respiratory health and general health and well-being.

Among all preterm infants, the <29 week infants such as those recruited to the N3RO trial were at risk of prolonged exposure to low DHA exposure and are at particularly high-risk for problematic neurobehavioural development. Hence, they represent an ideal sample for evaluating whether enteral DHA supplementation improves neurobehavioural development.[77 83] A possible limitation of the study design is that a subset of the 1273 children from the original trial from the 10 Australian sites, of the 13 international enrolling centres, will be invited to participate in this survey, possibly introducing bias due to differences in populations and clinical care between enrolling centres, although there is no evidence of systematic differences in the intervention according to site or country. Loss to follow-up 5 years after the trial enrolment may introduce attrition bias. Parents were able to request their group allocation after analysis of the primary outcome, and although few families were unblinded, knowledge of the intervention received may introduce bias.

The result of this follow-up will contribute to the evaluation of the risks and benefits of providing preterm infants with high-dose DHA during the neonatal period. Any potential benefit detected in the course of this 5-year follow-up study would need to be balanced against the possible short-term adverse effect on the risk of bronchopulmonary dysplasia.

Author affiliations
[1]SAHMRI Women and Kids, South Australian Health and Medical Research Institute, Adelaide, South Australia, Australia
[2]School of Psychology and Discipline of Paediatrics, Faculty of Health and Medical Sciences, The University of Adelaide, Adelaide, South Australia, Australia
[3]School of Psychology, Faculty of Health and Medical Sciences, University of Adelaide, Adelaide, South Australia, Australia
[4]Turner Institute for Brain and Mental Health, School of Psychological Sciences, Monash University, Melbourne, Victoria, Australia
[5]Discipline of Paediatrics, Faculty of Health and Medical Sciences, The University of Adelaide, Adelaide, South Australia, Australia
[6]School of Public Health, Faculty of Health and Medical Sciences, The University of Adelaide, Adelaide, South Australia, Australia
[7]School of Agriculture, Food and Wine, University of Adelaide, Waite Campus, Glen Osmond, South Australia, Australia
[8]Neonatal Medicine, Women's and Children's Hospital Adelaide, North Adelaide, South Australia, Australia
[9]Department Obstetrics and Gynaecology, Royal Women's Hospital, Parkville, Victoria, Australia
[10]Neonatal Services, Mercy Hospital for Women, Heidelberg, Victoria, Australia
[11]Newborn Services, John Hunter Children's Hospital, New Lambton Heights, New South Wales, Australia
[12]School of Medicine and Public Health, The University of Newcastle, Newcastle, New South Wales, Australia
[13]Neonatal Medicine, Royal Women's Hospital, Parkville, Melbourne, Australia
[14]Neonatal Follow up, King Edward Memorial Hospital for Women Perth, Perth, Western Australia, Australia
[15]Neonatal Research, The University of Western Australia, Perth, Western Australia, Australia
[16]Department of Paediatrics, Monash University, Melbourne, Victoria, Australia
[17]Monash Children's Hospital, Clayton, New South Wales, Australia
[18]Paediatric Neonatal Clinic, Flinders Medical Centre, Bedford Park, South Australia, Australia
[19]Newborn Care Centre, Royal Hospital for Women, Randwick, New South Wales, Australia
[20]Mater Research – The Faculty of Medicine, The University of Queensland, South Brisbane, Queensland, Australia
[21]Neonatal Intensive Care Unit, Liverpool Hospital, Liverpool, New South Wales, Australia
[22]Women and Kids, South Australian Health and Medical Research Institute, North Adelaide, South Australia, Australia

Twitter Karen P Best @kpb2901

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Contributors Study concept and design: JFG, RMR, CTC, PJA, MM, TS. Drafting the protocol: JFG, CTC, TS. Comment and approval of the final draft of the protocol: JFG, CTC, RMR, TS, MM, RAG, PJA, AJM, PGD, LWD, GO, JT, JC, MS, KS, KT, SM, KL, SB, HL, JS, KPB. Statistical expertise: TS. Obtained funding: JFG, CTC, RMR. Administrative, technical or material support: JFG, CTC, RMR, PJA, MM, RAG, TS, AJM, PGD, LWD, GO, JT, JC, MS, KS, KT, SM, KL, SB, HL, JS, KPB.

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Competing interests Study product for the original trial was donated by Clover Corporation Limited (Melbourne, Australia). MM and RAG report holding a patent relating to methods and compositions for promoting the neurological development for preterm infants (2009201540), owned by the South Australian Health and Medical Research Institute and licensed to Clover Corporation Limited. MM is supported by an Australian NHMRC Senior Research Fellowship ID: 1061704 and CTC is supported by a NHMRC Translating Research into Practice (TRIP) Fellowship ID 1132596. TS is supported by a NHMRC Emerging Leadership Investigator Grant ID: 1173576. KPB is supported by a Women's and Children's Hospital MS McLeod Postdoctoral Fellowship. PGD is supported by an Australian NHMRC Practitioner Fellowship ID: 1157782. JC is supported by a MRFF Career Development Fellowship ID: 1354. Honoraria have been paid to JFG's institution to support conference travel by Fonterra. MM and RAG report serving on the board for Trajan Nutrition. No other authors reported any financial disclosures. The contents of the published material are solely the responsibility of the authors and do not reflect the views of the NHMRC.

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ORCID iDs
Jacqueline F Gould http://orcid.org/0000-0003-2810-6870
Lex William Doyle http://orcid.org/0000-0002-7667-7312
Jeanie L Y Cheong http://orcid.org/0000-0001-5901-0455
Peter G Davis http://orcid.org/0000-0001-6742-7314

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