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A protocol for assessing whether cognition of preterm infants <29 weeks' gestation can be improved by an intervention with the omega-3 long-chain polyunsaturated fatty acid docosahexaenoic acid (DHA): a follow-up of a randomised controlled trial

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ABSTRACT

Introduction

Docosahexaenoic acid (DHA) is an omega-3 (n-3) fatty acid that accumulates into neural tissue during the last trimester of pregnancy, as the fetal brain is undergoing a growth spurt. Infants born <29 weeks' gestation are deprived the normal in-utero supply of DHA during this period of rapid brain development. Insufficient dietary DHA postnatally may contribute to the cognitive impairments common among this population. This follow-up of the N−3 fatty acids for improvement in Respiratory Outcomes (N3RO) randomised controlled trial aims to determine if enteral DHA supplementation in infants born <29 weeks' gestation during the first months of life improves cognitive development at five-years of age corrected for prematurity.

Methods and Analysis

N3RO was a randomised controlled trial of enteral DHA supplementation (60 mg/kg/day) or a control emulsion (without DHA) in 1,273 infants born <29 weeks' gestation to determine the effect on bronchopulmonary dysplasia (BPD). We showed that DHA supplementation did not reduce the risk of BPD and may have increased the risk.

In this follow-up at five years' corrected age, a predefined subset (n=655) of children from five Australian sites will be invited to attend a cognitive assessment with a psychologist. Children will be administered the Wechsler Preschool and Primary Scale of Intelligence (4th edition) and a measure of inhibitory control (Fruit Stroop), while height, weight and head circumference will be measured.

The primary outcome is Full-Scale intelligence quotient (IQ). To ensure 90% power, a minimum of 592 children are needed to detect a four-point difference in IQ between the groups.

Research personnel and families remain blinded to group assignment.

Ethics and Dissemination

The Women's and Children Health Network Human Research Ethics Committee reviewed and approved the study (HREC/17/WCHN/187). Caregivers will give informed consent prior to taking part in this follow-up study. Findings of this study will be disseminated through peer reviewed publications and conference presentations.

Trial Registration

Australian and New Zealand Clinical Trial Registry: anzctr.org.au: ACTRN12612000503820.

Strengths and Limitations

- This will be the first adequately powered randomised controlled trial to assess cognitive development following docosahexaenoic acid supplementation in preterm infants born <29 weeks' gestation.
- This follow-up of the N3RO trial will provide sound evidence for the effect of enteral DHA supplementation on the cognitive development of infants born <29 weeks' gestation.

Key words: intelligence quotient, cognition, preterm infant, docosahexaenoic acid, randomised control trial

INTRODUCTION

Medical and technological advances in the care of infants born preterm have increased their survival rates. However, there is a high risk of long-term health complications and neurological deficits with preterm birth[1-4], including higher risks of cognitive deficits[5 6] and behavioural problems[3 6-11] compared with term-born counterparts. The risk and severity of poor outcome increases as gestational age decreases.[4 8 12 13]

Nutrition is thought to be one modifiable influence on neurodevelopment in preterm infants, in particular the omega-3 (n-3) long-chain polyunsaturated fatty acid (LCPUFA), docosahexaenoic acid (DHA). During the last trimester of pregnancy, the fetus is estimated to acquire ~70 mg/day of n-3 LCPUFA, largely as DHA.[14] Infants born preterm are deprived of the placental transfer of DHA and hence have lower neural tissue levels of DHA compared with infants born at term.[15] It has been hypothesised that providing infants born preterm with DHA may enhance normal neurodevelopment and the most recent recommendations are that the preterm infant needs approximately 60 mg/kg/day DHA (about 1% of total dietary fatty acids) to approximate the fetal accumulation rate.[16]

Several randomised controlled trials (RCT) have attempted to evaluate this hypothesis, with mixed results.[17-19] Two RCTs compared the standard dose of DHA in breastmilk and preterm infant formula (20 mg/kg/day) to the estimated in-utero accretion rate (60 mg/kg/day).[20 21] In one trial the DHA group showed greater problem solving skills at 6 months[21] and improved sustained attention at 20 months,[22] although attrition was high. In the larger trial, assessment at 18 months revealed no difference in overall mean cognitive scores but fewer infants had developmental delay in the DHA group.[20] No overall differences in intelligence quotient (IQ) were detected in follow-up of these trials at seven[23] or eight years of age.[24] Interestingly, both trials suggested a benefit of extra DHA in infants born at the earliest gestations (<29 weeks or <1250 g) who are most

vulnerable to experiencing neurodevelopmental deficit.[20 21] While this is promising, both trials were significantly underpowered (with only 200 in one trial[20] and under 70 in the other[21]) to detect an effect in this subgroup.

It is clear that current neonatal feeding practices are unable to replace the placental transfer of DHA[16] and despite decades of research, we still do not know whether meeting the estimated requirement of DHA during the neonatal period improves cognitive outcomes in the most vulnerable sub-population of preterm infants.[17 20 21 23 24]

The N−3 Fatty Acids for Improvement in Respiratory Outcomes (N3RO) RCT was designed to determine the effect of an enteral DHA emulsion (providing 60 mg/kg/day) on the incidence of bronchopulmonary dysplasia (BPD).[25] The DHA intervention did not lower the incidence of BPD in infants born <29 weeks' gestation and may have resulted in a greater risk of BPD.[25] However, the N3RO trial offers an ideal opportunity to resolve whether DHA supplementation is beneficial for the cognitive development of these most vulnerable preterm infants.

The N3RO trial infants are now reaching five years of age. Cognition develops rapidly across early childhood[26] and by five years most cognitive domains can be reliably assessed using standardised psychometric tests.[27] IQ tests are considered a robust method of estimating an individual's overall cognitive ability. Executive function is an umbrella term referring to those skills essential for undertaking goal-oriented behaviours and includes inhibitory control which has been reported to be an area of concern for children born preterm.[6]

By assessing the cognition of the N3RO infants as they turn five years of age we can determine whether providing infants born <29 weeks' gestation with DHA emulsion improves cognitive development. We hypothesise that providing the estimated in-utero

provisions of DHA to infants born <29 weeks' gestation will result in higher cognitive scores at five years' corrected age compared with infants who received the control intervention.

METHODS

This protocol details the methods for a follow-up at five years of age of infants enrolled in the N3RO trial. Detailed methods of the N3RO trial have been published previously[25] and are summarised here.

The N3RO trial

1,273 infants born <29 weeks' gestation were enrolled into the N3RO trial within 3 days of their first enteral feed. Infants were recruited between June 2012 and September 2015 from 13 centres in Australia, New Zealand and Singapore.[25] Infants were excluded if they had a major congenital or chromosomal abnormality, were participating in another fatty acid intervention trial, were receiving intravenous lipids containing fish oil, or if a breast feeding mother was taking greater than 250 mg/day DHA through supplements.[25]

Infants were randomised to receive a DHA emulsion that provided 60 mg of DHA per kg of body weight per day (intervention group, n=631), or a control emulsion without DHA (control group, n=642).[25] Infants received the study intervention from enrolment to 36 weeks' postmenstrual age or discharge home, whichever occurred first. The emulsion was administered three times per day, immediately before an enteral feed through a nasogastric or orogastric tube for the duration of the intervention period. The DHA and control emulsions were iso-caloric and identical in viscosity, colour, and packaging and families, clinical staff and study personnel were blinded to group allocation.[25] Infants were randomised to the intervention or control group through a secure web-based computer-generated schedule stratified for the 13 centres, sex and gestational age at birth <27 weeks' or 27 to <29 weeks'

gestation. Infants from multiple births were randomised individually. A statistician not otherwise involved in the N3RO trial generated the randomisation schedule.

Five-year follow-up study procedure

This is a follow-up of a predefined sub-sample of the N3RO trial infants from five of the Australian recruiting centres. No additional interventions will be administered. Eligible N3RO infants will be invited to attend an appointment with a psychologist when they are 5-years' corrected age to measure child abilities on selected cognitive domains; age is corrected for prematurity to avoid a known bias in cognitive test scores.[28] Appointments will take between 45 minutes to 1.5 hours, depending on the child's abilities and speed whilst working through the IQ test tasks, and assessments will be conducted by personnel blinded to group allocation.

Families of eligible children will be emailed a letter of invitation two months before their child reaches 5 years' corrected age, followed by a telephone call to answer any questions and book appointments with families that wish to participate. Where necessary, families will be offered appointments at the family's home or at a location close to their home such as a school or community centre.

Participants

Children who participated in the N3RO Trial and were recruited from the five largest recruiting centres, John Hunter Hospital (New South Wales), King Edward Memorial Hospital (Western Australia), Mercy Hospital for Women (Victoria), Royal Women's Hospital (Victoria), and the Women's and Children's Hospital (South Australia) in Australia

will be invited to participate in this follow-up study. Children will not be invited if they have previously been withdrawn from the N3RO trial or have died. Of the n=702 children enrolled between the five centres, n=655 will be eligible to be approached for the five-year follow-up once deaths (n=4) and withdrawals (n=43) are excluded.

Outcomes and Measures

Primary outcome

The primary outcome is Full-Scale IQ, as assessed by the Wechsler Preschool and Primary Scale of Intelligence - Fourth Edition, Australian and New Zealand (WPPSI-IV). The WPPSI-IV is a battery of subtests that provides an assessment of general cognitive ability for pre-schoolers and young children (2:6 to 7:7 years). The WPPSI-IV has strong internal consistency and test–retest stability and sound psychometric properties.[29] The average reliability coefficient for the Full-Scale IQ is 0.95.[29]

Secondary outcomes

WPPSI-IV

Other outcomes from the WPPSI-IV will be included as secondary outcomes. These include Verbal Comprehension, Fluid Reasoning, Working Memory and the Processing Speed, General Ability and Cognitive Proficiency Primary Index Scales.

The WPPSI-IV has Australian/New Zealand norms that are age-standardised with a mean of 100 and SD 15. Intellectual impairment will be defined as Full-Scale IQ <85 (i.e. <-1 SD), and moderate-severe intellectual impairment as Full-Scale IQ<70 (i.e. <-2 SD). Any

impairment on any of the WPPSI-IV Primary Index Scales will be defined as an Index Scale score <85 (i.e. <-1 SD).

Fruit Stroop

The Fruit Stroop was administered to assess two executive functions, inhibition and mental flexibility.[30] The child is required to identify a the correct, natural colour of a series of fruits and vegetables in four 45 s trials under a series of conditions that increase in complexity. The outcome is an interference score calculated as the difference between the number of correct responses on the final (inhibition) trial, and predicted scores on the first and third trials, where lower or negative values indicate more interference.

Growth

Anthropometrics including child height, weight and head circumference will be measured at the appointment as measures of the nutritional well-being of the children. Measurements will be converted to Z (SD) scores appropriate for corrected age and sex.[31]

Background information and characteristics

At enrolment into the N3RO trial a range of socio-demographic data were collected through interview with the caregiver (including parental age, education, and employment). As part of the N3RO trial infant medical records were used to determine a range of baseline and outcome clinical characteristics up to 40 weeks' postmenstrual age or first discharge home, whichever occurred first, including for e.g., gestational age, birth weight, sex, and instances of intraventricular haemorrhage.

Sample size

A sample size of 296 children per group (total 592) will provide 90% power (two-tailed alpha 0.05) to detect a 4-point (0.27 standard deviation) mean difference in the primary outcome of Full-Scale IQ between groups. 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.[32] Should enrolment be lower than planned, the study will have 80% power to detect a 4-point difference between groups provided at least 222 children per group (total 444) provide follow-up data.

Statistical analysis and data management

All participants were assigned a study identification number at enrolment into the N3RO trial. Throughout the follow-up and analyses, the identification number will be used to identify data. 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. REDCap includes a complete suite of features to support the Health Insurance Portability and Accountability Act of 1996 compliance, including a full audit trail, user-based privileges, and integration with the institutional LDAP server.

All analyses will be conducted according to a pre-specified statistical analysis plan. Analyses will not commence until the N3RO trial Steering Committee has approved the statistical analysis plan. Intervention groups will be dummy coded to allow analyses to be performed blinded to treatment group.

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 to <29 weeks' at birth). Pre-planned subgroup analyses will examine the effects of DHA separately for girls or boys (all outcomes), and for infants born at <27 weeks' gestation or 27 to <29 weeks' gestation (primary outcome only). No adjustment will be made for multiple pre-planned comparisons, as the single overall comparison of Full-Scale IQ between groups is of primary interest.

Missing outcome data will be addressed using multiple imputation, with imputation performed separately by treatment group using fully conditional specification.[33] Imputed datasets will include all surviving children from the five included centres. Children who are missing scores on psychological assessments because they were unable to complete the assessment for cognitive or physical reasons (such as blindness or cerebral palsy) will be reviewed by a psychologist to determine whether assigning the lowest possible score is appropriate.

Ethical considerations and dissemination of results

This follow-up study will be carried out in accordance with the Australian National Statement on Ethical Conduct in Research Involving Humans, which builds upon the ethical codes of the Declaration of Helsinki and the Principles of International Conference on Harmonisation Good Clinical Practice (as adopted in Australia). All procedures and study materials have been reviewed and approved by the Women's and Children's Health Network

Human Research Ethics Committee (HREC/17/WCHN/187), as well as the Research Governance officers at each site. The N3RO Trial and this follow-up are registered on the Australia and New Zealand Clinical Trial Registry (ANZCTR: ACTRN12612000503820).

Caregivers will be provided with a detailed information sheet about the study and will provide informed consent for their child's involvement in the study. Caregivers will be free to re-negotiate consent for each procedure in the follow-up study and are able to decline any part of the follow-up. Caregivers will be free to withdraw their children from the study at any time.

The results of this follow-up study will be presented at academic conferences and published in peer-reviewed journals. Participating families will receive a lay-report of the study findings. No participants will be identified in the dissemination of study results and data collected will be treated with confidence.

Access to Data

Individual participant data, including data dictionaries, may be shared after de-identification upon 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 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 IQ is

based on reported concerns over long-term developmental concerns from parents of preterm infants.[34]

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

This protocol details a follow-up of a RCT of a DHA enteral emulsion (60 mg/kg/day) compared with a control emulsion (no DHA), for preterm infants born <29 weeks' gestation in the first months of life, to evaluate the effect on child cognitive ability at 5 years of age. Unlike previous DHA RCTs in preterm populations,[17-19] our follow-up has the benefits of a population likely to be insufficient in DHA,[35] and a robust method of intervention.[25]

We previously conducted a follow-up of a small sub-group of the N3RO trial infants when they were aged 18 months' corrected age. Children underwent an experimental assessment of visual attention (considered to be a basic, early emergence of higher order cognitive skills known as the executive functions).[36] Where available, Bayley Scales of Infant and Toddler Development-3rd edition Cognition, Motor and Language assessment results were collected from hospital records.[36] No statistically significant differences were found for attention, cognition, motor or language abilities (manuscript currently under review). However, assessments of cognition during infancy are considered poor predictors of later performance,[37-41] and the sample was small and under-powered to detect a clinically important effect on cognition.[36]

For this follow-up we have carefully selected a robust assessment of general cognitive abilities, including executive functioning (both of which domains are likely to be adversely affected by very preterm birth)[42-44] to be administered at an age when cognitive domains can be reliably assessed[27 45], as well as ensuring a large, adequately powered sample. As per the recommendations of a consortium of parents and clinicians caring for high-risk preterm infants, we are assessing general cognitive ability using a Wechsler scale, which is considered the gold standard, and have included an assessment of growth.[46]

This project has global significance, with over one million infants born <29 weeks' gestation each year, and the number rising.[47] The potential benefit of DHA on cognitive performance has never been adequately demonstrated in this population. However, because of the N3RO primary results it is extremely unlikely that such a trial will be repeated. The N3RO cohort may represent the only children in which the longer-term cognitive and behavioural effects of DHA supplementation in these infants can be assessed.

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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.

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List of Abbreviations

BPD	Bronchopulmonary dysplasia
DHA	Docosahexaenoic acid
IQ	Intelligence Quotient
n-3	Omega-3
N3RO	N−3 (omega-3) Fatty Acids for Improvement in Respiratory Outcomes
RCT	Randomised controlled trial
WPPSI-IV	Wechsler Preschool and Primary Scale of Intelligence - Fourth Edition

Authors Contributions

Study concept and design: Collins, Gould, Makrides, McPhee, Anderson, Gibson, Sullivan.

Drafting the protocol: Gould, Collins, Sullivan.

Comment and approval of the final draft of the protocol: Gould, Collins, Makrides, Sullivan, Anderson, Gibson, McPhee, Best, Davis, Doyle, Opie, Travadi, Cheong, Sharp, Simmer.

Statistical expertise: Sullivan.

Obtained funding: Collins, Gould, Makrides, McPhee, Gibson, Sullivan, Best.

Administrative, technical, or material support: Gould, Collins, Makrides, Gibson, Sullivan, McPhee, Anderson, Best, Davis, Doyle, Opie, Travadi, Cheong, Sharp, Simmer.

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SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	5
	2b	All items from the World Health Organization Trial Registration Data Set	1-21
Protocol version	3	Date and version identifier	NA
Funding	4	Sources and types of financial, material, and other support	17-18
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 18
	5b	Name and contact information for the trial sponsor	NA
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	NA
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	17

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	7-8
	6b	Explanation for choice of comparators	7-8
Objectives	7	Specific objectives or hypotheses	8-9
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	9-14
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	10
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	9-10
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	NA
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	NA
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	NA
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	11-13
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	9

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	___12_____
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	___10-11_____
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	___9-10_____
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	____9-10_____
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	_____NA____
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	____9-10_____
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	____NA_____
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	____9-14___
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	___10____

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	_____13-15____
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	____13-14____
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	____13-14___
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	_____14____
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	_____NA_____
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	____NA_____
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	___NA_____
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	_____NA_____
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	_____14-15__
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	____NA_____

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9-10, 14-15
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	13, 15
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	18
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	15
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	NA
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	15-16
	31b	Authorship eligibility guidelines and any intended use of professional writers	*NA*
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Available upon request
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

A protocol for assessing whether cognition of preterm infants <29 weeks' gestation can be improved by an intervention with the omega-3 long-chain polyunsaturated fatty acid docosahexaenoic acid (DHA): a follow-up of a randomised controlled trial

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A protocol for assessing whether cognition of preterm infants <29 weeks' gestation can be improved by an intervention with the omega-3 long-chain polyunsaturated fatty acid docosahexaenoic acid (DHA): a follow-up of a randomised controlled trial

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ABSTRACT

Introduction

Docosahexaenoic acid (DHA) is an omega-3 (n-3) fatty acid that accumulates into neural tissue during the last trimester of pregnancy, as the fetal brain is undergoing a growth spurt. Infants born <29 weeks' gestation are deprived the normal in-utero supply of DHA during this period of rapid brain development. Insufficient dietary DHA postnatally may contribute to the cognitive impairments common among this population. This follow-up of the N−3 fatty acids for improvement in Respiratory Outcomes (N3RO) randomised controlled trial aims to determine if enteral DHA supplementation in infants born <29 weeks' gestation during the first months of life improves cognitive development at five-years of age corrected for prematurity.

Methods and Analysis

N3RO was a randomised controlled trial of enteral DHA supplementation (60 mg/kg/day) or a control emulsion (without DHA) in 1,273 infants born <29 weeks' gestation to determine the effect on bronchopulmonary dysplasia (BPD). We showed that DHA supplementation did not reduce the risk of BPD and may have increased the risk.

In this follow-up at five years' corrected age, a predefined subset (n=655) of children from five Australian sites will be invited to attend a cognitive assessment with a psychologist. Children will be administered the Wechsler Preschool and Primary Scale of Intelligence (4th edition) and a measure of inhibitory control (Fruit Stroop), while height, weight and head circumference will be measured.

The primary outcome is Full-Scale intelligence quotient (IQ). To ensure 90% power, a minimum of 592 children are needed to detect a four-point difference in IQ between the groups.

Research personnel and families remain blinded to group assignment.

Ethics and Dissemination

The Women's and Children Health Network Human Research Ethics Committee reviewed and approved the study (HREC/17/WCHN/187). Caregivers will give informed consent prior to taking part in this follow-up study. Findings of this study will be disseminated through peer reviewed publications and conference presentations.

Trial Registration

Australian and New Zealand Clinical Trial Registry: anzctr.org.au: ACTRN12612000503820.

Strengths and Limitations

- This will be the first adequately powered randomised controlled trial to assess cognitive development following docosahexaenoic acid supplementation in preterm infants born <29 weeks' gestation.
- This follow-up of the N3RO trial will provide sound evidence for the effect of enteral DHA supplementation on the cognitive development of infants born <29 weeks' gestation.
- Loss to follow-up five years after enrolment into the trial may contribute to risk of bias.

- Partial unblinding of study group allocation permitted under the primary protocol may contribute to risk of bias
- Although bronchopulmonary dysplasia was the primary outcome of the original N3RO trial, childhood respiratory functioning is not assessed in this follow-up

Key words: intelligence quotient, cognition, preterm infant, docosahexaenoic acid, randomised control trial

INTRODUCTION

Medical and technological advances in the care of infants born preterm have increased their survival rates. However, there is a high risk of long-term health complications and neurological deficits with preterm birth[1-4], including higher risks of cognitive deficits[5 6] and behavioural problems[3 6-11] compared with term-born counterparts. The risk and severity of poor outcome increases as gestational age decreases.[4 8 12 13]

Nutrition is thought to be one modifiable influence on neurodevelopment in preterm infants, in particular the omega-3 (n-3) long-chain polyunsaturated fatty acid (LCPUFA), docosahexaenoic acid (DHA). During the last trimester of pregnancy, the fetus is estimated to acquire ~70 mg/day of n-3 LCPUFA, largely as DHA.[14] Infants born preterm are deprived of the placental transfer of DHA and hence have lower neural tissue levels of DHA compared with infants born at term.[15] It has been hypothesised that providing infants born preterm with DHA may enhance normal neurodevelopment and the most recent recommendations are that the preterm infant needs approximately 60 mg/kg/day DHA (about 1% of total dietary fatty acids) to approximate the fetal accumulation rate.[16]

Several randomised controlled trials (RCT) have attempted to evaluate this hypothesis, with mixed results.[17 18] Two RCTs compared the standard dose of DHA in breastmilk and preterm infant formula (20 mg/kg/day) to the estimated in-utero accretion rate (60 mg/kg/day).[19 20] In one trial the DHA group showed greater problem solving skills at 6 months[20] and improved sustained attention at 20 months,[21] although attrition was high. In the larger trial, assessment at 18 months revealed no difference in overall mean cognitive scores but fewer infants had developmental delay in the DHA group.[19] No overall differences in intelligence quotient (IQ) were detected in follow-up of these trials at seven[22] or eight years of age.[23] Interestingly, both trials suggested a benefit of extra DHA in infants born at the earliest gestations (<29 weeks or <1250 g) who are most

vulnerable to experiencing neurodevelopmental deficit.[19 20] While this is promising, both trials were significantly underpowered (with only 200 children in one trial[19] and under 70 in the other[20]) to detect an effect in this subgroup.

It is clear that current neonatal feeding practices are unable to replace the placental transfer of DHA[16] and despite decades of research, we still do not know whether meeting the estimated requirement of DHA during the neonatal period improves cognitive outcomes in the most vulnerable sub-population of preterm infants.[17 19 20 22 23]

The N−3 Fatty Acids for Improvement in Respiratory Outcomes (N3RO) RCT was designed to determine the effect of an enteral DHA emulsion (providing 60 mg/kg/day) on the incidence of bronchopulmonary dysplasia (BPD).[24] The DHA intervention did not lower the incidence of BPD in infants born <29 weeks' gestation and may have resulted in a greater risk of BPD.[24] However, the N3RO trial offers an ideal opportunity to resolve whether DHA supplementation is beneficial for the cognitive development of these most vulnerable preterm infants.

The N3RO trial infants are now reaching five years of age. Cognition develops rapidly across early childhood[25] and by five years most cognitive domains can be reliably assessed using standardised psychometric tests.[26] IQ tests are considered a robust method of estimating an individual's overall cognitive ability. Executive function is an umbrella term referring to those skills essential for undertaking goal-oriented behaviours and includes inhibitory control which has been reported to be an area of concern for children born preterm.[6]

By assessing the cognition of the N3RO infants as they turn five years of age we can determine whether providing infants born <29 weeks' gestation with DHA emulsion improves cognitive development. We hypothesise that providing the estimated in-utero

provisions of DHA to infants born <29 weeks' gestation will result in higher cognitive scores at five years' corrected age compared with infants who received the control intervention.

METHODS

This protocol details the methods for a follow-up at five years of age of infants enrolled in the N3RO trial. Detailed methods of the N3RO trial have been published previously[24] and are summarised here.

The N3RO trial

1,273 infants born <29 weeks' gestation were enrolled into the N3RO trial within 3 days of their first enteral feed. Infants were recruited between June 2012 and September 2015 from 13 centres in Australia, New Zealand and Singapore.[24] Infants were excluded if they had a major congenital or chromosomal abnormality, were participating in another fatty acid intervention trial, were receiving intravenous lipids containing fish oil, or if a breast feeding mother was taking greater than 250 mg/day DHA through supplements.[24]

Infants were randomised to receive a DHA emulsion that provided 60 mg of DHA per kg of body weight per day (intervention group, n=631), or a control emulsion without DHA (control group, n=642).[24] Infants received the study intervention from enrolment to 36 weeks' postmenstrual age or discharge home, whichever occurred first. The emulsion was administered three times per day, immediately before an enteral feed through a nasogastric or orogastric tube for the duration of the intervention period. The DHA and control emulsions were iso-caloric and identical in viscosity, colour, and packaging and families, clinical staff and study personnel were blinded to group allocation.[24] Infants were randomised to the intervention or control group through a secure web-based computer-generated schedule stratified for the 13 centres, sex and gestational age at birth <27 weeks' or 27 to <29 weeks'

gestation. Infants from multiple births were randomised individually. A statistician not otherwise involved in the N3RO trial generated the randomisation schedule.

Five-year follow-up study procedure

This is a follow-up of a predefined sub-sample of the N3RO trial infants from five of the Australian recruiting centres. No additional interventions will be administered. Eligible N3RO infants will be invited to attend an appointment with a psychologist when they are 5-years' corrected age to measure child abilities on selected cognitive domains; age is corrected for prematurity to avoid a known bias in cognitive test scores.[27] Appointments will take between 45 minutes to 1.5 hours, depending on the child's abilities and speed whilst working through the IQ test tasks, and assessments will be conducted by personnel blinded to group allocation. Assessments for this follow-up study commenced 29th August 2018 and are expected to be completed on the 31st December 2020.

Families of eligible children will be emailed a letter of invitation two months before their child reaches 5 years' corrected age, followed by a telephone call to answer any questions and book appointments with families that wish to participate. Where necessary, families will be offered appointments at the family's home or at a location close to their home such as a school or community centre.

Participants

Children who participated in the N3RO Trial and were recruited from the five largest recruiting centres, John Hunter Hospital (New South Wales), King Edward Memorial Hospital (Western Australia), Mercy Hospital for Women (Victoria), Royal Women's

Hospital (Victoria), and the Women's and Children's Hospital (South Australia) in Australia will be invited to participate in this follow-up study. Children will not be invited if they have previously been withdrawn from the N3RO trial or have died. Of the n=702 children enrolled between the five centres, n=655 will be eligible to be approached for the five-year follow-up once deaths (n=4) and withdrawals (n=43) are excluded.

Outcomes and Measures

Primary outcome

The primary outcome is Full-Scale IQ, as assessed by the Wechsler Preschool and Primary Scale of Intelligence - Fourth Edition, Australian and New Zealand (WPPSI-IV). The WPPSI-IV is a battery of subtests that provides an assessment of general cognitive ability for pre-schoolers and young children (2:6 to 7:7 years). The WPPSI-IV has strong internal consistency and test–retest stability and sound psychometric properties.[28] The average reliability coefficient for the Full-Scale IQ is 0.95.[28]

Secondary outcomes

WPPSI-IV

Other outcomes from the WPPSI-IV will be included as secondary outcomes. These include Verbal Comprehension, Fluid Reasoning, Working Memory and the Processing Speed, General Ability and Cognitive Proficiency Primary Index Scales.

The WPPSI-IV has Australian/New Zealand norms that are age-standardised with a mean of 100 and SD 15. Intellectual impairment will be defined as Full-Scale IQ <85 (i.e. <-1 SD), and moderate-severe intellectual impairment as Full-Scale IQ<70 (i.e. <-2 SD). Any

impairment on any of the WPPSI-IV Primary Index Scales will be defined as an Index Scale score <85 (i.e. <-1 SD).

Fruit Stroop

The Fruit Stroop was administered to assess two executive functions, inhibition and mental flexibility.[29] The child is required to identify a the correct, natural colour of a series of fruits and vegetables in four 45 s trials under a series of conditions that increase in complexity. The outcome is an interference score calculated as the difference between the number of correct responses on the final (inhibition) trial, and predicted scores on the first and third trials, where lower or negative values indicate more interference.

Growth

Anthropometrics including child height, weight and head circumference will be measured at the appointment as measures of the nutritional well-being of the children. Measurements will be converted to Z (SD) scores appropriate for corrected age and sex.[30]

Background information and characteristics

At enrolment into the N3RO trial a range of socio-demographic data were collected through interview with the caregiver (including parental age, education, and employment). As part of the N3RO trial infant medical records were used to determine a range of baseline and outcome clinical characteristics up to 40 weeks' postmenstrual age or first discharge home, whichever occurred first, including for e.g., gestational age, birth weight, sex, and instances of intraventricular haemorrhage.

Sample size

A sample size of 296 children per group (total 592) will provide 90% power (two-tailed alpha 0.05) to detect a 4-point (0.27 standard deviation) mean difference in the primary outcome of Full-Scale IQ between groups. 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.[31] Should enrolment be lower than planned, the study will have 80% power to detect a 4-point difference between groups provided at least 222 children per group (total 444) provide follow-up data.

Statistical analysis and data management

All participants were assigned a study identification number at enrolment into the N3RO trial. Throughout the follow-up and analyses, the identification number will be used to identify data. 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. REDCap includes a complete suite of features to support the Health Insurance Portability and Accountability Act of 1996 compliance, including a full audit trail, user-based privileges, and integration with the institutional LDAP server.

All analyses will be conducted according to a pre-specified statistical analysis plan. Analyses will not commence until the N3RO trial Steering Committee has approved the statistical analysis plan. Intervention groups will be dummy coded to allow analyses to be performed blinded to treatment group.

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 to <29 weeks' at birth). Pre-planned subgroup analyses will examine the effects of DHA separately for girls or boys (all outcomes), and for infants born at <27 weeks' gestation or 27 to <29 weeks' gestation (primary outcome only). No adjustment will be made for multiple pre-planned comparisons, as the single overall comparison of Full-Scale IQ between groups is of primary interest.

Missing outcome data will be addressed using multiple imputation, with imputation performed separately by treatment group using fully conditional specification.[32] Imputed datasets will include all surviving children from the five included centres. Children who are missing scores on psychological assessments because they were unable to complete the assessment for cognitive or physical reasons (such as blindness or cerebral palsy) will be reviewed by a psychologist to determine whether assigning the lowest possible score is appropriate.

Ethical considerations and dissemination of results

This follow-up study will be carried out in accordance with the Australian National Statement on Ethical Conduct in Research Involving Humans, which builds upon the ethical codes of the Declaration of Helsinki and the Principles of International Conference on Harmonisation Good Clinical Practice (as adopted in Australia). All procedures and study materials have been reviewed and approved by the Women's and Children's Health Network

Human Research Ethics Committee (HREC/17/WCHN/187), as well as the Research Governance officers at each site. The N3RO Trial and this follow-up are registered on the Australia and New Zealand Clinical Trial Registry (ANZCTR: ACTRN12612000503820).

Caregivers will be provided with a detailed information sheet about the study and will provide informed consent for their child's involvement in the study. Caregivers will be free to re-negotiate consent for each procedure in the follow-up study and are able to decline any part of the follow-up. Caregivers will be free to withdraw their children from the study at any time.

The results of this follow-up study will be presented at academic conferences and published in peer-reviewed journals. Participating families will receive a lay-report of the study findings. No participants will be identified in the dissemination of study results and data collected will be treated with confidence.

Access to Data

Individual participant data, including data dictionaries, may be shared after de-identification upon 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 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 IQ is

based on reported concerns over long-term developmental concerns from parents of preterm infants.[33]

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

This protocol details a follow-up of a RCT of a DHA enteral emulsion (60 mg/kg/day) compared with a control emulsion (no DHA), for preterm infants born <29 weeks' gestation in the first months of life, to evaluate the effect on child cognitive ability at 5 years of age. Unlike previous DHA RCTs in preterm populations,[17 18] our follow-up has the benefits of a population likely to be insufficient in DHA,[34] and a robust method of intervention.[24]

We previously conducted a follow-up of a small sub-group of the N3RO trial infants when they were aged 18 months' corrected age. Children underwent an experimental assessment of visual attention (considered to be a basic, early emergence of higher order cognitive skills known as the executive functions).[35] Where available, Bayley Scales of Infant and Toddler Development-3rd edition Cognition, Motor and Language assessment results were collected from hospital records.[35] No statistically significant differences were found for attention, cognition, motor or language abilities.[36] However, assessments of cognition during infancy are considered poor predictors of later performance,[37-41] and the sample was small and under-powered to detect a clinically important effect on cognition.[35]

Our sample size calculation for the primary outcome requires a 90% follow-up rate of the N3RO trial children, five years after enrolment. More than 10% loss to follow-up may

introduce attrition bias. After completion of the N3RO trial primary outcome analyses, families had the opportunity to request knowledge of their group allocation. Although few families requested this, knowledge of their randomisation group prior to the five-year follow-up assessment may introduce additional bias to the results.

For this follow-up we have carefully selected a robust assessment of general cognitive abilities, including executive functioning (both of which domains are likely to be adversely affected by very preterm birth)[42-44] to be administered at an age when cognitive domains can be reliably assessed[26 45], as well as ensuring a large, adequately powered sample. As per the recommendations of a consortium of parents and clinicians caring for high-risk preterm infants, we are assessing general cognitive ability using a Wechsler scale, which is considered the gold standard, and have included an assessment of growth.[46] Assessments of respiratory functioning are unreliable in early childhood and hence were not included in this follow-up. It is important that the long-term respiratory effects of DHA supplementation in infants born <29 weeks' gestation is addressed when the N3RO trial children reach an appropriate age.

This project has global significance, with over one million infants born <29 weeks' gestation each year, and the number rising.[47] The potential benefit of DHA on cognitive performance has never been adequately demonstrated in this population. However, because of the N3RO primary results it is extremely unlikely that such a trial will be repeated. The N3RO cohort may represent the only children in which the longer-term cognitive and behavioural effects of DHA supplementation in these infants can be assessed.

Acknowledgements

We would like to thank the families who generously participated in the N3RO trial and who will participate in the follow-up study, and the N3RO Steering Committee, Investigative Team and research staff.

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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.

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List of Abbreviations

BPD	Bronchopulmonary dysplasia
DHA	Docosahexaenoic acid
IQ	Intelligence Quotient
n-3	Omega-3
N3RO	N−3 (omega-3) Fatty Acids for Improvement in Respiratory Outcomes
RCT	Randomised controlled trial
WPPSI-IV	Wechsler Preschool and Primary Scale of Intelligence - Fourth Edition

Authors Contributions

Study concept and design: Collins, Gould, Makrides, McPhee, Anderson, Gibson, Sullivan.

Drafting the protocol: Gould, Collins, Sullivan.

Comment and approval of the final draft of the protocol: Gould, Collins, Makrides, Sullivan, Anderson, Gibson, McPhee, Best, Davis, Doyle, Opie, Travadi, Cheong, Sharp, Simmer.

Statistical expertise: Sullivan.

Obtained funding: Collins, Gould, Makrides, McPhee, Gibson, Sullivan, Best.

Administrative, technical, or material support: Gould, Collins, Makrides, Gibson, Sullivan, McPhee, Anderson, Best, Davis, Doyle, Opie, Travadi, Cheong, Sharp, Simmer.

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SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	5
	2b	All items from the World Health Organization Trial Registration Data Set	1-21
Protocol version	3	Date and version identifier	NA
Funding	4	Sources and types of financial, material, and other support	17-18
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 18
	5b	Name and contact information for the trial sponsor	NA
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	NA
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	17

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	7-8
	6b	Explanation for choice of comparators	7-8
Objectives	7	Specific objectives or hypotheses	8-9
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	9-14
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	10
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	9-10
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	NA
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	NA
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	NA
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	11-13
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	9

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	___12_____
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	___10-11_____
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	___9-10_____
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	____9-10_____
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	_____NA____
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	____9-10_____
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	____NA_____
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	____9-14___
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	___10____

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	_____13-15____
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	____13-14____
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	____13-14___
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	_____14____
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	_____NA_____
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	____NA_____
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	___NA_____
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	_____NA_____
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	_____14-15__
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	____NA_____

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9-10, 14-15
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	13, 15
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	18
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	15
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	NA
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	15-16
	31b	Authorship eligibility guidelines and any intended use of professional writers	*NA*
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Available upon request
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

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A protocol for assessing whether cognition of preterm infants <29 weeks' gestation can be improved by an intervention with the omega-3 long-chain polyunsaturated fatty acid docosahexaenoic acid (DHA): a follow-up of a randomised controlled trial

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A protocol for assessing whether cognition of preterm infants <29 weeks' gestation can be improved by an intervention with the omega-3 long-chain polyunsaturated fatty acid docosahexaenoic acid (DHA): a follow-up of a randomised controlled trial

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ABSTRACT

Introduction

Docosahexaenoic acid (DHA) is an omega-3 (n-3) fatty acid that accumulates into neural tissue during the last trimester of pregnancy, as the fetal brain is undergoing a growth spurt. Infants born <29 weeks' gestation are deprived the normal in-utero supply of DHA during this period of rapid brain development. Insufficient dietary DHA postnatally may contribute to the cognitive impairments common among this population. This follow-up of the N−3 fatty acids for improvement in Respiratory Outcomes (N3RO) randomised controlled trial aims to determine if enteral DHA supplementation in infants born <29 weeks' gestation during the first months of life improves cognitive development at five-years of age corrected for prematurity.

Methods and Analysis

N3RO was a randomised controlled trial of enteral DHA supplementation (60 mg/kg/day) or a control emulsion (without DHA) in 1,273 infants born <29 weeks' gestation to determine the effect on bronchopulmonary dysplasia (BPD). We showed that DHA supplementation did not reduce the risk of BPD and may have increased the risk.

In this follow-up at five years' corrected age, a predefined subset (n=655) of children from five Australian sites will be invited to attend a cognitive assessment with a psychologist. Children will be administered the Wechsler Preschool and Primary Scale of Intelligence (4th edition) and a measure of inhibitory control (Fruit Stroop), while height, weight and head circumference will be measured.

The primary outcome is Full-Scale intelligence quotient (IQ). To ensure 90% power, a minimum of 592 children are needed to detect a four-point difference in IQ between the groups.

Research personnel and families remain blinded to group assignment.

Ethics and Dissemination

The Women's and Children Health Network Human Research Ethics Committee reviewed and approved the study (HREC/17/WCHN/187). Caregivers will give informed consent prior to taking part in this follow-up study. Findings of this study will be disseminated through peer reviewed publications and conference presentations.

Trial Registration

Australian and New Zealand Clinical Trial Registry: anzctr.org.au: ACTRN12612000503820.

Strengths and Limitations

- This will be the first adequately powered randomised controlled trial to assess cognitive development following docosahexaenoic acid supplementation in preterm infants born <29 weeks' gestation.
- This follow-up of the N3RO trial will provide sound evidence for the effect of enteral DHA supplementation on the cognitive development of infants born <29 weeks' gestation.
- Loss to follow-up five years after enrolment into the trial may contribute to risk of bias.

- Partial unblinding of study group allocation permitted under the primary protocol may contribute to risk of bias
- Although bronchopulmonary dysplasia was the primary outcome of the original N3RO trial, childhood respiratory functioning is not assessed in this follow-up

Key words: intelligence quotient, cognition, preterm infant, docosahexaenoic acid, randomised control trial

INTRODUCTION

Medical and technological advances in the care of infants born preterm have increased their survival rates. However, there is a high risk of long-term health complications and neurological deficits with preterm birth[1-4], including higher risks of cognitive deficits[5 6] and behavioural problems[3 6-11] compared with term-born counterparts. The risk and severity of poor outcome increases as gestational age decreases.[4 8 12 13]

Nutrition is thought to be one modifiable influence on neurodevelopment in preterm infants, in particular the omega-3 (n-3) long-chain polyunsaturated fatty acid (LCPUFA), docosahexaenoic acid (DHA). During the last trimester of pregnancy, the fetus is estimated to acquire ~70 mg/day of n-3 LCPUFA, largely as DHA.[14] Infants born preterm are deprived of the placental transfer of DHA and hence have lower neural tissue levels of DHA compared with infants born at term.[15] It has been hypothesised that providing infants born preterm with DHA may enhance normal neurodevelopment and the most recent recommendations are that the preterm infant needs approximately 60 mg/kg/day DHA (about 1% of total dietary fatty acids) to approximate the fetal accumulation rate.[16]

Several randomised controlled trials (RCT) have attempted to evaluate this hypothesis, with mixed results.[17 18] Two RCTs compared the standard dose of DHA in breastmilk and preterm infant formula (20 mg/kg/day) to the estimated in-utero accretion rate (60 mg/kg/day).[19 20] In one trial the DHA group showed greater problem solving skills at 6 months[20] and improved sustained attention at 20 months,[21] although attrition was high. In the larger trial, assessment at 18 months revealed no difference in overall mean cognitive scores but fewer infants had developmental delay in the DHA group.[19] No overall differences in intelligence quotient (IQ) were detected in follow-up of these trials at seven[22] or eight years of age.[23] Interestingly, both trials suggested a benefit of extra DHA in infants born at the earliest gestations (<29 weeks or <1250 g) who are most

vulnerable to experiencing neurodevelopmental deficit.[19 20] While this is promising, both trials were significantly underpowered (with only 200 children in one trial[19] and under 70 in the other[20]) to detect an effect in this subgroup.

It is clear that current neonatal feeding practices are unable to replace the placental transfer of DHA[16] and despite decades of research, we still do not know whether meeting the estimated requirement of DHA during the neonatal period improves cognitive outcomes in the most vulnerable sub-population of preterm infants.[17 19 20 22 23]

The N−3 Fatty Acids for Improvement in Respiratory Outcomes (N3RO) RCT was designed to determine the effect of an enteral DHA emulsion (providing 60 mg/kg/day) on the incidence of bronchopulmonary dysplasia (BPD).[24] The DHA intervention did not lower the incidence of BPD in infants born <29 weeks' gestation and may have resulted in a greater risk of BPD.[24] However, the N3RO trial offers an ideal opportunity to resolve whether DHA supplementation is beneficial for the cognitive development of these most vulnerable preterm infants.

The N3RO trial infants are now reaching five years of age. Cognition develops rapidly across early childhood[25] and by five years most cognitive domains can be reliably assessed using standardised psychometric tests.[26] IQ tests are considered a robust method of estimating an individual's overall cognitive ability. Executive function is an umbrella term referring to those skills essential for undertaking goal-oriented behaviours and includes inhibitory control which has been reported to be an area of concern for children born preterm.[6]

By assessing the cognition of the N3RO infants as they turn five years of age we can determine whether providing infants born <29 weeks' gestation with DHA emulsion improves cognitive development. We hypothesise that providing the estimated in-utero

provisions of DHA to infants born <29 weeks' gestation will result in higher cognitive scores at five years' corrected age compared with infants who received the control intervention.

METHODS AND ANALYSIS

This protocol details the methods for a follow-up at five years of age of infants enrolled in the N3RO trial. Detailed methods of the N3RO trial have been published previously[24] and are summarised here.

The N3RO trial

1,273 infants born <29 weeks' gestation were enrolled into the N3RO trial within 3 days of their first enteral feed. Infants were recruited between June 2012 and September 2015 from 13 centres in Australia, New Zealand and Singapore.[24] Infants were excluded if they had a major congenital or chromosomal abnormality, were participating in another fatty acid intervention trial, were receiving intravenous lipids containing fish oil, or if a breast feeding mother was taking greater than 250 mg/day DHA through supplements.[24] Infants were randomised to the intervention or control group through a secure web-based computer-generated schedule stratified for the 13 centres, sex and gestational age at birth <27 weeks' or 27 to <29 weeks' gestation. Infants from multiple births were randomised individually. A statistician not otherwise involved in the N3RO trial generated the randomisation schedule.

The N3RO trial intervention

Infants were randomised to receive a DHA emulsion that provided 60 mg of DHA per kg of body weight per day (intervention group, n=631), or a control emulsion without DHA (control group, n=642).[24] Infants received the study intervention from enrolment to 36 weeks' postmenstrual age or discharge home, whichever occurred first. The emulsion was

administered three times per day, immediately before an enteral feed through a nasogastric or orogastric tube for the duration of the intervention period. The DHA and control emulsions were iso-caloric and identical in viscosity, colour, and packaging and families, clinical staff and study personnel were blinded to group allocation.[24]

Five-year follow-up study procedure

This is a follow-up of a predefined sub-sample of the N3RO trial infants from five of the Australian recruiting centres. No additional interventions will be administered. Eligible N3RO infants will be invited to attend an appointment with a psychologist when they are 5-years' corrected age to measure child abilities on selected cognitive domains; age is corrected for prematurity to avoid a known bias in cognitive test scores.[27] Appointments will take between 45 minutes to 1.5 hours, depending on the child's abilities and speed whilst working through the IQ test tasks, and assessments will be conducted by personnel blinded to group allocation. Assessments for this follow-up study commenced 29th August 2018 and are expected to be completed on the 31st December 2020.

Families of eligible children will be emailed a letter of invitation two months before their child reaches 5 years' corrected age, followed by a telephone call to answer any questions and book appointments with families that wish to participate. Where necessary, families will be offered appointments at the family's home or at a location close to their home such as a school or community centre.

Participants and sample selection

Children who participated in the N3RO Trial and were recruited from the five largest recruiting centres, John Hunter Hospital (New South Wales), King Edward Memorial Hospital (Western Australia), Mercy Hospital for Women (Victoria), Royal Women's Hospital (Victoria), and the Women's and Children's Hospital (South Australia) in Australia will be invited to participate in this follow-up study. Children will not be invited if they have previously been withdrawn from the N3RO trial or have died. Of the n=702 children enrolled between the five centres, n=655 will be eligible to be approached for the five-year follow-up once deaths (n=4) and withdrawals (n=43) are excluded.

Outcomes and Measures

Primary outcome

The primary outcome is Full-Scale IQ, as assessed by the Wechsler Preschool and Primary Scale of Intelligence - Fourth Edition, Australian and New Zealand (WPPSI-IV). The WPPSI-IV is a battery of subtests that provides an assessment of general cognitive ability for pre-schoolers and young children (2:6 to 7:7 years). The WPPSI-IV has strong internal consistency and test–retest stability and sound psychometric properties.[28] The average reliability coefficient for the Full-Scale IQ is 0.95.[28]

Secondary outcomes

WPPSI-IV

Other outcomes from the WPPSI-IV will be included as secondary outcomes. These include Verbal Comprehension, Fluid Reasoning, Working Memory and the Processing Speed, General Ability and Cognitive Proficiency Primary Index Scales.

The WPPSI-IV has Australian/New Zealand norms that are age-standardised with a mean of 100 and SD 15. Intellectual impairment will be defined as Full-Scale IQ <85 (i.e. <-1 SD), and moderate-severe intellectual impairment as Full-Scale IQ<70 (i.e. <-2 SD). Any impairment on any of the WPPSI-IV Primary Index Scales will be defined as an Index Scale score <85 (i.e. <-1 SD).

Fruit Stroop

The Fruit Stroop was administered to assess two executive functions, inhibition and mental flexibility.[29] The child is required to identify a the correct, natural colour of a series of fruits and vegetables in four 45 s trials under a series of conditions that increase in complexity. The outcome is an interference score calculated as the difference between the number of correct responses on the final (inhibition) trial, and predicted scores on the first and third trials, where lower or negative values indicate more interference.

Growth

Anthropometrics including child height, weight and head circumference will be measured at the appointment as measures of the nutritional well-being of the children. Measurements will be converted to Z (SD) scores appropriate for corrected age and sex.[30]

Background information and characteristics

At enrolment into the N3RO trial a range of socio-demographic data were collected through interview with the caregiver (including parental age, education, and employment). As part of the N3RO trial infant medical records were used to determine a range of baseline and

outcome clinical characteristics up to 40 weeks' postmenstrual age or first discharge home, whichever occurred first, including for e.g., gestational age, birth weight, sex, and instances of intraventricular haemorrhage.

Sample size calculation

A sample size of 296 children per group (total 592) will provide 90% power (two-tailed alpha 0.05) to detect a 4-point (0.27 standard deviation) mean difference in the primary outcome of Full-Scale IQ between groups. The power calculation assumes a design effect due to the inclusion of multiple births of one, since children from a multiple birth were randomized individually in N3RO.[31] Should enrolment be lower than planned, the study will have 80% power to detect a 4-point difference between groups provided at least 222 children per group (total 444) provide follow-up data.

Data management and analysis plan

All participants were assigned a study identification number at enrolment into the N3RO trial. Throughout the follow-up and analyses, the identification number will be used to identify data. 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. REDCap includes a complete suite of features to support the Health Insurance Portability and Accountability Act of 1996 compliance, including a full audit trail, user-based privileges, and integration with the institutional LDAP server.

All analyses will be conducted according to a pre-specified statistical analysis plan. Analyses will not commence until the N3RO trial Steering Committee has approved the

statistical analysis plan. Intervention groups will be dummy coded to allow analyses to be performed blinded to treatment group.

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 to <29 weeks' at birth). Pre-planned subgroup analyses will examine the effects of DHA separately for girls or boys (all outcomes), and for infants born at <27 weeks' gestation or 27 to <29 weeks' gestation (primary outcome only). No adjustment will be made for multiple pre-planned comparisons, as the single overall comparison of Full-Scale IQ between groups is of primary interest.

Missing outcome data will be addressed using multiple imputation, with imputation performed separately by treatment group using fully conditional specification.[32] Imputed datasets will include all surviving children from the five included centres. Children who are missing scores on psychological assessments because they were unable to complete the assessment for cognitive or physical reasons (such as blindness or cerebral palsy) will be reviewed by a psychologist to determine whether assigning the lowest possible score is appropriate.

Ethics and dissemination

This follow-up study will be carried out in accordance with the Australian National Statement on Ethical Conduct in Research Involving Humans, which builds upon the ethical codes of the Declaration of Helsinki and the Principles of International Conference on

Harmonisation Good Clinical Practice (as adopted in Australia). All procedures and study materials have been reviewed and approved by the Women's and Children's Health Network Human Research Ethics Committee (HREC/17/WCHN/187), as well as the Research Governance officers at each site. The N3RO Trial and this follow-up are registered on the Australia and New Zealand Clinical Trial Registry (ANZCTR: ACTRN12612000503820).

Caregivers will be provided with a detailed information sheet about the study and will provide informed consent for their child's involvement in the study. Caregivers will be free to re-negotiate consent for each procedure in the follow-up study and are able to decline any part of the follow-up. Caregivers will be free to withdraw their children from the study at any time.

The results of this follow-up study will be presented at academic conferences and published in peer-reviewed journals. Participating families will receive a lay-report of the study findings. No participants will be identified in the dissemination of study results and data collected will be treated with confidence.

Access to Data

Individual participant data, including data dictionaries, may be shared after de-identification upon 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 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 IQ is based on reported concerns over long-term developmental concerns from parents of preterm infants.[33]

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

This protocol details a follow-up of a RCT of a DHA enteral emulsion (60 mg/kg/day) compared with a control emulsion (no DHA), for preterm infants born <29 weeks' gestation in the first months of life, to evaluate the effect on child cognitive ability at 5 years of age. Unlike previous DHA RCTs in preterm populations,[17 18] our follow-up has the benefits of a population likely to be insufficient in DHA,[34] and a robust method of intervention.[24]

We previously conducted a follow-up of a small sub-group of the N3RO trial infants when they were aged 18 months' corrected age. Children underwent an experimental assessment of visual attention (considered to be a basic, early emergence of higher order cognitive skills known as the executive functions).[35] Where available, Bayley Scales of Infant and Toddler Development-3rd edition Cognition, Motor and Language assessment results were collected from hospital records.[35] No statistically significant differences were found for attention, cognition, motor or language abilities.[36] However, assessments of cognition during infancy are considered poor predictors of later performance,[37-41] and the sample was small and under-powered to detect a clinically important effect on cognition.[35]

Our sample size calculation for the primary outcome requires a 90% follow-up rate of the N3RO trial children, five years after enrolment. More than 10% loss to follow-up may introduce attrition bias. After completion of the N3RO trial primary outcome analyses, families had the opportunity to request knowledge of their group allocation. Although few families requested this, knowledge of their randomisation group prior to the five-year follow-up assessment may introduce additional bias to the results.

For this follow-up we have carefully selected a robust assessment of general cognitive abilities, including executive functioning (both of which domains are likely to be adversely affected by very preterm birth)[42-44] to be administered at an age when cognitive domains can be reliably assessed[26 45], as well as ensuring a large, adequately powered sample. As per the recommendations of a consortium of parents and clinicians caring for high-risk preterm infants, we are assessing general cognitive ability using a Wechsler scale, which is considered the gold standard, and have included an assessment of growth.[46] Assessments of respiratory functioning are unreliable in early childhood and hence were not included in this follow-up. It is important that the long-term respiratory effects of DHA supplementation in infants born <29 weeks' gestation is addressed when the N3RO trial children reach an appropriate age.

This project has global significance, with over one million infants born <29 weeks' gestation each year, and the number rising.[47] The potential benefit of DHA on cognitive performance has never been adequately demonstrated in this population. However, because of the N3RO primary results it is extremely unlikely that such a trial will be repeated. The N3RO cohort may represent the only children in which the longer-term cognitive and behavioural effects of DHA supplementation in these infants can be assessed.

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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.

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List of Abbreviations

BPD	Bronchopulmonary dysplasia
DHA	Docosahexaenoic acid
IQ	Intelligence Quotient
n-3	Omega-3
N3RO	N−3 (omega-3) Fatty Acids for Improvement in Respiratory Outcomes
RCT	Randomised controlled trial
WPPSI-IV	Wechsler Preschool and Primary Scale of Intelligence - Fourth Edition

Authors Contributions

Study concept and design: Collins, Gould, Makrides, McPhee, Anderson, Gibson, Sullivan.

Drafting the protocol: Gould, Collins, Sullivan.

Comment and approval of the final draft of the protocol: Gould, Collins, Makrides, Sullivan, Anderson, Gibson, McPhee, Best, Davis, Doyle, Opie, Travadi, Cheong, Sharp, Simmer.

Statistical expertise: Sullivan.

Obtained funding: Collins, Gould, Makrides, McPhee, Gibson, Sullivan, Best.

Administrative, technical, or material support: Gould, Collins, Makrides, Gibson, Sullivan, McPhee, Anderson, Best, Davis, Doyle, Opie, Travadi, Cheong, Sharp, Simmer.

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SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	5
	2b	All items from the World Health Organization Trial Registration Data Set	1-21
Protocol version	3	Date and version identifier	NA
Funding	4	Sources and types of financial, material, and other support	17-18
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 18
	5b	Name and contact information for the trial sponsor	NA
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	NA
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	17

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	7-8
	6b	Explanation for choice of comparators	7-8
Objectives	7	Specific objectives or hypotheses	8-9
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	9-14
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	10
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	9-10
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	NA
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	NA
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	NA
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	11-13
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	9

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	___12_____
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	___10-11_____
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	___9-10_____
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	____9-10_____
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	_____NA____
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	____9-10_____
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	____NA_____
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	____9-14___
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	___10____

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	_____13-15____
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	____13-14____
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	____13-14___
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	_____14____
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	_____NA_____
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	____NA_____
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	___NA_____
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	_____NA_____
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	_____14-15__
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	____NA_____

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9-10, 14-15
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	13, 15
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	18
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	15
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	NA
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	15-16
	31b	Authorship eligibility guidelines and any intended use of professional writers	*NA*
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Available upon request
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.