## PEER REVIEW HISTORY



## ARTICLE DETAILS

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| **TITLE (PROVISIONAL)** | 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 |
| **AUTHORS** | Gould, Jacqueline; Makrides, Maria; Sullivan, Thomas; Anderson, Peter; Gibson, Robert; Best, Karen; McPhee, Andrew; Doyle, Lex; Opie, Gillian; Travadi, Javeed; Cheong, Jeanie; Davis, Peter; Sharp, Mary; Simmer, Karen; Collins, Carmel |

## VERSION 1 – REVIEW

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| **REVIEWER** | Jane Alsweiler<br>University of Auckland,<br>New Zealand |
| **REVIEW RETURNED** | 24-Aug-2020 |

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| **GENERAL COMMENTS** | This paper is the protocol for the 5 year cognitive follow up of the N3RO trial of DHA supplementation in preterm babies. The protocol addresses an important research question and is well written. I have a few comments:<br>1. The instructions from the editors were that dates of the study should be included in the manuscript, please add these. Has the follow-up already started?<br>2. This follow-up is only to assess cognition, executive function and anthropometry. It seems a bit of a wasted opportunity to not also assess the motor, sensory and respiratory function of these children. Indeed the protocol discusses what will be done if the child is blind or has cerebral palsy but does not mention how these conditions will be assessed. I realise it will be too late to change the protocol now, but wondered what the rationale was for not including the other assessments.<br>3. The Strengths and Limitations section - only includes two strengths, which are pretty much the same, and no limitations. Please re-word. There is also no mention of limitations in the Discussion, please add (could be point 2 above).<br>4. Are the authors concerned that schooling will have influenced the results? Consider adding the age at which children start school in Australia, and how much schooling the children will have had at the time of assessment.<br><br>Minor<br>Line 183 "with only 200" and "less than 70" needs to have "children" or "participants" added i.e. with only 200 children.<br>5. Ref 17 and 18 are the same Cochrane review, one is the newer version - I can't see a reason to also include the old version |

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| **REVIEWER** | Betty Vohr, MD<br>Women & Infants Hospital of Rhode Island<br>Department of Pediatrics<br>101 Dudley Street<br>Providence, RI 02905 |
| **REVIEW RETURNED** | 31-Aug-2020 |

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| **GENERAL COMMENTS** | A protocol for assessing whether cognition of preterm infants< 29 weeks' gestation can be improved by an intervention with omega-3 long-chain polyunsaturated fatty acid docosahexaenoic acid (DHA): a follow-up of randomized controlled trial.<br><br>The background and rationale for studying the effects of supplemental DHA on 5 year corrected age outcomes of infants < 29 weeks' gestation (at increased risk of neurocognitive deficits) are well described.<br>Original established Cohort: Children all participated in a randomized multicenter controlled trial of enteral DHA supplementation of 60mg/kg/day or control emulsion without DHA for preterm infants < 29 weeks to determine effects on BPD. Children enrolled in this subsample will be from 5 of the largest Australian recruiting centers. 655/702 are eligible. They plan to enroll 296/group, for a total of 592. This indicates an impressive 90% five year enrollment rate, 592/655.<br>As the authors state, nutrition is a modifiable influence on outcomes. Because cognitive and executive function skill deficits occur frequently in preterm survivors < 29 weeks the experienced investigators who have collaborated together in the past have selected the Wechsler Preschool and Primary Scale of Intelligence IV (WPPSI IV) intelligence quotient as their primary outcome. Research staff will administer the WPPSI IV and measure inhibition control and mental flexibility with the Fruit Stroop to assess executive function as a secondary outcome, in addition to child anthropometrics. Short term effects of DHA have been somewhat mixed in prior reports and this cohort provides a unique opportunity to study Kindergarten age effects of supplemental DHA on a well described cohort of children from the neonatal trial.<br><br>Statistical Analysis and Power: With a cohort of 592 children they have determined a 90% power to detect a 4-point difference in IQ, which is the primary outcome. Statistical analyses are well described and include generalized linear models, separate analyses by sex and by gestational age groups and imputation for missing values. The cohort has appropriate exclusion criteria and randomization.<br>The trial has been registered with the Australian and New Zealand Clinical Trial Registry and informed consent will be obtained for the 5-year assessment.<br>Current 5-year Study: Primary Hypothesis: The authors hypothesize that providing the estimated in-utero provision of 60 mg of DHA/kg/day to infants born< 29 weeks 'gestation from enrollment to 36 weeks or discharge will result in higher cognitive scores at 5 years corrected age compared with infants who receive the control intervention.<br>The outcome assessments are appropriate.<br>Primary Outcome:<br>1.WPPSI IV full scale IQ<br>Secondary Outcomes |

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| | 1. WPPSI IV verbal comprehension, fluid reasoning, working memory, processing speed, general ability and cognitive proficiency.<br>2. The Stroop will assess 2executive functions including inhibition and mental flexibility.<br>3. Anthropometric Z scores<br>On page 27 the protocol checklist states the investigators will obtain systolic blood pressure and that it is discussed on pages 11-13. Blood pressure however or related methods for obtaining or analyzing blood pressure are not described in the application.<br>Key characteristics of child and family were collected prospectively in the original study and are in a password protected database.<br>The authors have included a Community Board comprised of clinicians, parents and researchers to be consulted as needed for study results and dissemination of study findings.<br><br>The authors provide a strong rationale and well-developed feasible protocol for this follow-up study. The importance of this 5-year follow-up is based on the availability of a known high risk population cohort that participated in a well described randomized control neonatal trial of a DHA intervention that holds promise for demonstrating early school age beneficial effects with an easy to implement intervention in the NICU. I am assuming that blood pressure was dropped as a secondary outcome by the authors from the application. |

**VERSION 1 – AUTHOR RESPONSE**

Reviewer(s)' Comments to Author:

Reviewer: 1
Reviewer Name
Jane Alsweiler

This paper is the protocol for the 5 year cognitive follow up of the N3RO trial of DHA supplementation in preterm babies. The protocol addresses an important research question and is well written. I have a few comments:

1. The instructions from the editors were that dates of the study should be included in the manuscript, please add these. Has the follow-up already started?

Response: We have added dates to the methods, lines 241-242.
Note-do we put our original end date dec 2020, OR say march next year? (I think will be understandable to say in results manuscript assessments took longer than anticipated due to COVID…)

2. This follow-up is only to assess cognition, executive function and anthropometry. It seems a bit of a wasted opportunity to not also assess the motor, sensory and respiratory function of these children. Indeed the protocol discusses what will be done if the child is blind or has cerebral palsy but does not mention how these conditions will be assessed. I realise it will be too late to change the protocol now, but wondered what the rationale was for not including the other assessments.

Response: Prior to obtaining funding for this follow-up, we received funding to conduct a survey-based follow-up of all Australian-based N3RO trial children. As part of this survey we are asking parents about blindness, cerebral palsy or other medical or neurological diagnoses, and whether there has been respiratory-related hospital admissions, as well as symptoms of asthma.
Evidence does not suggest that motor and sensory functioning are affected by DHA intervention, although they are adversely impacted by preterm birth. Respiratory functioning cannot be reliably assessed until 7 years of age. The investigative team hope to obtain funding to conduct respiratory functioning assessments when children are at least 7 years of age.

3. The Strengths and Limitations section - only includes two strengths, which are pretty much the same, and no limitations. Please re-word. There is also no mention of limitations in the Discussion, please add (could be point 2 above).

Response: We have added 3 points to the Strengths and Limitations section, see pages 5-6.
We have added limitations to the discussion, lines 397-414

4. Are the authors concerned that schooling will have influenced the results? Consider adding the age at which children start school in Australia, and how much schooling the children will have had at the time of assessment.

Response: The age at which children start school in Australia differs between states. As per the comment above, there is additional information collected in a separate but related follow-up of the N3RO trial. As part of this separate follow-up we are collecting details about schooling including when they commenced, whether the child receives special education and whether they attend/attended preschool.

Minor
Line 183 "with only 200" and "less than 70" needs to have "children" or "participants" added i.e. with only 200 children.

Response: This has been amended (line 183).

5. Ref 17 and 18 are the same Cochrane review, one is the newer version - I can't see a reason to also include the old version

Response: The older Cochrane review (Schulzke 2011) has been removed.

Reviewer: 2
Reviewer Name
Betty Vohr, MD

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

The background and rationale for studying the effects of supplemental DHA on 5 year corrected age outcomes of infants < 29 weeks' gestation (at increased risk of neurocognitive deficits) are well described.
Original established Cohort: Children all participated in a randomized multicenter controlled trial of enteral DHA supplementation of 60mg/kg/day or control emulsion without DHA for preterm infants < 29 weeks to determine effects on BPD. Children enrolled in this subsample will be from 5 of the largest Australian recruiting centers. 655/702 are eligible. They plan to enroll 296/group, for a total of 592. This indicates an impressive 90% five year enrollment rate, 592/655.
As the authors state, nutrition is a modifiable influence on outcomes. Because cognitive and executive function skill deficits occur frequently in preterm survivors < 29 weeks the experienced investigators who have collaborated together in the past have selected the Wechsler Preschool and Primary Scale of Intelligence IV (WPPSI IV) intelligence quotient as their primary outcome. Research staff will administer the WPPSI IV and measure inhibition control and mental flexibility with the Fruit Stroop to assess executive function as a secondary outcome, in addition to child anthropometrics. Short term effects of DHA have been somewhat mixed in prior reports and this cohort provides a unique opportunity to study Kindergarten age effects of supplemental DHA on a well described cohort of children from the neonatal trial.

Statistical Analysis and Power: With a cohort of 592 children they have determined a 90% power to detect a 4-point difference in IQ, which is the primary outcome. Statistical analyses are well described and include generalized linear models, separate analyses by sex and by gestational age groups and imputation for missing values. The cohort has appropriate exclusion criteria and randomization.
The trial has been registered with the Australian and New Zealand Clinical Trial Registry and informed consent will be obtained for the 5-year assessment.
Current 5-year Study: Primary Hypothesis: The authors hypothesize that providing the estimated in-utero provision of 60 mg of DHA/kg/day to infants born< 29 weeks 'gestation from enrollment to 36 weeks or discharge will result in higher cognitive scores at 5 years corrected age compared with infants who receive the control intervention.
The outcome assessments are appropriate.
Primary Outcome:
1.WPPSI IV full scale IQ
Secondary Outcomes
1. WPPSI IV verbal comprehension, fluid reasoning, working memory, processing speed, general ability and cognitive proficiency.
2. The Stroop will assess 2executive functions including inhibition and mental flexibility.
3. Anthropometric Z scores
On page 27 the protocol checklist states the investigators will obtain systolic blood pressure and that it is discussed on pages 11-13. Blood pressure however or related methods for obtaining or analyzing blood pressure are not described in the application.

Response: Blood pressure is not being collected in this follow-up. The protocol checklist text is the standard template text, study authors are expected to add page numbers/relevant details to the column "Addressed on page number" only. Item 12 refers to the outcome assessment of primary, secondary and other outcomes. Blood pressure is listed as an example outcome as part of the standard text and was not added by the study authors.

Key characteristics of child and family were collected prospectively in the original study and are in a password protected database.
The authors have included a Community Board comprised of clinicians, parents and researchers to be consulted as needed for study results and dissemination of study findings.

The authors provide a strong rationale and well-developed feasible protocol for this follow-up study. The importance of this 5-year follow-up is based on the availability of a known high risk population cohort that participated in a well described randomized control neonatal trial of a DHA intervention that holds promise for demonstrating early school age beneficial effects with an easy to implement intervention in the NICU. I am assuming that blood pressure was dropped as a secondary outcome by the authors from the application.

Response: Blood pressure was not a planned outcome of this follow-up, please see response above.

**VERSION 2 – REVIEW**

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| **REVIEWER** | Jane Alsweiler<br>University of Auckland,<br>New Zealand |
| **REVIEW RETURNED** | 17-Nov-2020 |

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| **GENERAL COMMENTS** | Thank you for addressing my queries.<br>I agree with inserting the original dates and talking about the effect of COVID in the results paper - it could all change again between now and then!<br>I didn't understand this line - "No adjustment to the sample size is needed for<br>clustering due to multiple births, since children were randomised individually in N3RO and the design effect for continuous outcomes is one in this case". I agree with the message of not adjusting the sample size for multiples for a follow-up, but didn't understand the rationale - or why you would reference a paper on sample size calculation for randomized trials, since this is a follow-up rather than a trial. Surely the sample size is predetermined from the original trial? |

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| **REVIEWER** | Betty Vohr, MD<br>Women & Infants Hospital of Rhode Island<br>United States |
| **REVIEW RETURNED** | 03-Nov-2020 |

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| **GENERAL COMMENTS** | Rationale is clear and of significant clinical importance:<br>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. Infants born <29 weeks' gestation are deprived the normal in-utero supply of DHA during this period of rapid brain development. Evidence for the efficacy of DHA supplementation effects on cognition is provided in the introduction along with limitations of prior randomized studies with inadequate sample size and or design issues.<br>Methods:<br>Sample: Subjects are from the N3RO which was a randomized 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). No significant effects were identified. In this follow-up at five years' corrected age, a predefined subset (n=655) of children |

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| | from five Australian sites will be invited to participate.<br>Assessments include validated tests including the Wechsler Preschool and Primary Scale of Intelligence (4th edition), the Fruit Stroop, a measure of inhibitory control, and growth parameters.<br><br>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 t groups. 80% power also provided should there be lower participation.<br>Statistical Analysis plan expands methods: 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 randomized individually in N3RO and the design effect for continuous outcomes is one in this case.<br><br>This statement on page 14 in protocol seems contradictory regarding multiples : Outcomes of intervention and control group children will be compared using generalized linear models, with generalized estimated equations used to account for clustering due to multiple births within the same family.<br>Strengths and Limitations are clearly stated:<br>Strengths:<br>• First adequately powered randomised controlled trial to assess cognitive development following well described neonatal study of docosahexaenoic acid supplementation in preterms born <29 weeks' gestation.<br>• 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.<br>• Will include a comprehensive assessment of well-described cohort at school age, 5 years corrected age.<br>• WPPSI IV and Fruit Stroop are both well validated assessments<br>• Secondary cognitive outcomes are examined in addition to executive function & growth<br>• Human Research Ethics Committee approval and informed consent obtained<br>• Data in Redcap database<br>• Global significance; > 1 million preterm infants< 29 weeks born each year<br>Limitations<br>• Loss to follow-up five years after enrolment into the trial may contribute to risk of bias.<br>• Partial unblinding of study group allocation permitted under the primary protocol may contribute to risk of bias<br>• Although bronchopulmonary dysplasia was the primary outcome of the original N3RO trial, childhood respiratory functioning is not assessed in this follow-up cohort. |

**VERSION 2 – AUTHOR RESPONSE**

**Reviewer: 1**
**Reviewer Name: Jane Alsweiler**
**Institution and Country: University of Auckland,**

**New Zealand**

**Comments to the Author**

Thank you for addressing my queries.
I agree with inserting the original dates and talking about the effect of COVID in the results paper - it could all change again between now and then!
I didn't understand this line - "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". I agree with the message of not adjusting the sample size for multiples for a follow-up, but didn't understand the rationale - or why you would reference a paper on sample size calculation for randomized trials, since this is a follow-up rather than a trial. Surely the sample size is predetermined from the original trial?

*Response: We have amended this sentence for clarity (page 13, lines 309-311). There were 1,273 children enrolled in the N3RO trial between Australia, New Zealand and Singapore. Rather than attempting to assess the IQ of all children in N3RO, we calculated the sample size that would be necessary to detect a realistic, meaningful difference in IQ. In any setting with multiple births, it is important to consider the effects of clustering due to multiple births on sample size estimates. The referenced article provides guidance on calculating design effects for studies involving clustered data and randomised comparisons, which is applicable given our interest in randomised comparisons in this follow-up study.*

**Reviewer: 2**
**Reviewer Name: Betty Vohr, MD**
**Institution and Country: Women & Infants Hospital of Rhode Island**
**United States**

**Comments to the Author**

Rationale is clear and of significant clinical importance: 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. Infants born <29 weeks' gestation are deprived the normal in-utero supply of DHA during this period of rapid brain development. Evidence for the efficacy of DHA supplementation effects on cognition is provided in the introduction along with limitations of prior randomized studies with inadequate sample size and or design issues.
Methods:
Sample: Subjects are from the N3RO which was a randomized 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). No significant effects were identified. In this follow-up at five years' corrected age, a predefined subset (n=655) of children from five Australian sites will be invited to participate. Assessments include validated tests including the Wechsler Preschool and Primary Scale of Intelligence (4th edition), the Fruit Stroop, a measure of inhibitory control, and growth parameters.

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 t groups. 80% power also

provided should there be lower participation.
Statistical Analysis plan expands methods: 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 randomized individually in N3RO and the design effect for continuous outcomes is one in this case.

This statement on page 14 in protocol seems contradictory regarding multiples : Outcomes of intervention and control group children will be compared using generalized linear models, with generalized estimated equations used to account for clustering due to multiple births within the same family.

*Response: We have reworded our sample size calculation so it doesn't appear contradictory to the analysis methods. Clustering due to multiple births should always be considered in sample size calculations and during analysis, however in this study the inclusion of multiple births had no impact on sample size estimates (since children were randomised individually in the original trial*

Strengths and Limitations are clearly stated:
Strengths:
- First adequately powered randomised controlled trial to assess cognitive development following well described neonatal study of docosahexaenoic acid supplementation in preterms 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.
- Will include a comprehensive assessment of well-described cohort at school age, 5 years corrected age.
- WPPSI IV and Fruit Stroop are both well validated assessments
- Secondary cognitive outcomes are examined in addition to executive function & growth
- Human Research Ethics Committee approval and informed consent obtained
- Data in Redcap database
- Global significance; > 1 million preterm infants< 29 weeks born each year

Limitations
- 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 cohort.