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

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3 110 **ABSTRACT**  
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6 111 **Introduction**  
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9 112 Docosahexaenoic acid (DHA) is an omega-3 (n-3) fatty acid that accumulates into neural  
10  
11 113 tissue during the last trimester of pregnancy, as the fetal brain is undergoing a growth spurt.  
12  
13 114 Infants born <29 weeks' gestation are deprived the normal in-utero supply of DHA during  
14  
15 115 this period of rapid brain development. Insufficient dietary DHA postnatally may contribute  
16  
17 116 to the cognitive impairments common among this population. This follow-up of the N-3 fatty  
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19 117 acids for improvement in Respiratory Outcomes (N3RO) randomised controlled trial aims to  
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21 118 determine if enteral DHA supplementation in infants born <29 weeks' gestation during the  
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23 119 first months of life improves cognitive development at five-years of age corrected for  
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25 120 prematurity.  
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33 122 **Methods and Analysis**  
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36 123 N3RO was a randomised controlled trial of enteral DHA supplementation (60 mg/kg/day) or  
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38 124 a control emulsion (without DHA) in 1,273 infants born <29 weeks' gestation to determine  
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40 125 the effect on bronchopulmonary dysplasia (BPD). We showed that DHA supplementation did  
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42 126 not reduce the risk of BPD and may have increased the risk.  
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44 127 In this follow-up at five years' corrected age, a predefined subset (n=655) of children from  
45  
46 128 five Australian sites will be invited to attend a cognitive assessment with a psychologist.  
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48 129 Children will be administered the Wechsler Preschool and Primary Scale of Intelligence (4<sup>th</sup>  
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50 130 edition) and a measure of inhibitory control (Fruit Stroop), while height, weight and head  
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52 131 circumference will be measured.  
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3 132 The primary outcome is Full-Scale intelligence quotient (IQ). To ensure 90% power, a  
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5 133 minimum of 592 children are needed to detect a four-point difference in IQ between the  
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8 134 groups.

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10 135 Research personnel and families remain blinded to group assignment.  
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### 16 137 **Ethics and Dissemination**

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19 138 The Women's and Children Health Network Human Research Ethics Committee reviewed  
20  
21 139 and approved the study (HREC/17/WCHN/187). Caregivers will give informed consent prior  
22  
23 140 to taking part in this follow-up study. Findings of this study will be disseminated through  
24  
25  
26 141 peer reviewed publications and conference presentations.  
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28  
29 142

### 32 143 **Trial Registration**

33  
34  
35 144 Australian and New Zealand Clinical Trial Registry: [anzctr.org.au](http://anzctr.org.au): [ACTRN12612000503820](https://www.anzctr.org.au/Trial/Registration/Trial.jsp?ACTRN12612000503820).  
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### 41 146 **Strengths and Limitations**

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44 147 • This will be the first adequately powered randomised controlled trial to assess  
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46 148 cognitive development following docosahexaenoic acid supplementation in preterm  
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48 149 infants born <29 weeks' gestation.
- 50  
51 150 • This follow-up of the N3RO trial will provide sound evidence for the effect of enteral  
52  
53 151 DHA supplementation on the cognitive development of infants born <29 weeks'  
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55 152 gestation.  
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3 154 **Key words:** intelligence quotient, cognition, preterm infant, docosahexaenoic acid,  
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5 155 randomised control trial  
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## 157 INTRODUCTION

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

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

172 Several randomised controlled trials (RCT) have attempted to evaluate this  
173 hypothesis, with mixed results.[17-19] Two RCTs compared the standard dose of DHA in  
174 breastmilk and preterm infant formula (20 mg/kg/day) to the estimated in-utero accretion rate  
175 (60 mg/kg/day).[20 21] In one trial the DHA group showed greater problem solving skills at  
176 6 months[21] and improved sustained attention at 20 months,[22] although attrition was high.  
177 In the larger trial, assessment at 18 months revealed no difference in overall mean cognitive  
178 scores but fewer infants had developmental delay in the DHA group.[20] No overall  
179 differences in intelligence quotient (IQ) were detected in follow-up of these trials at  
180 seven[23] or eight years of age.[24] Interestingly, both trials suggested a benefit of extra  
181 DHA in infants born at the earliest gestations (<29 weeks or <1250 g) who are most

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3 182 vulnerable to experiencing neurodevelopmental deficit.[20 21] While this is promising, both  
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5 183 trials were significantly underpowered (with only 200 in one trial[20] and under 70 in the  
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8 184 other[21]) to detect an effect in this subgroup.  
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10  
11 185 It is clear that current neonatal feeding practices are unable to replace the placental  
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13 186 transfer of DHA[16] and despite decades of research, we still do not know whether meeting  
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15 187 the estimated requirement of DHA during the neonatal period improves cognitive outcomes  
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17 188 in the most vulnerable sub-population of preterm infants.[17 20 21 23 24]  
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21 189 The N-3 Fatty Acids for Improvement in Respiratory Outcomes (N3RO) RCT was  
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23 190 designed to determine the effect of an enteral DHA emulsion (providing 60 mg/kg/day) on  
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25 191 the incidence of bronchopulmonary dysplasia (BPD).[25] The DHA intervention did not  
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27 192 lower the incidence of BPD in infants born <29 weeks' gestation and may have resulted in a  
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29  
30 193 greater risk of BPD.[25] However, the N3RO trial offers an ideal opportunity to resolve  
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32 194 whether DHA supplementation is beneficial for the cognitive development of these most  
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34 195 vulnerable preterm infants.  
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37 196 The N3RO trial infants are now reaching five years of age. Cognition develops  
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39 197 rapidly across early childhood[26] and by five years most cognitive domains can be reliably  
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42 198 assessed using standardised psychometric tests.[27] IQ tests are considered a robust method  
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44 199 of estimating an individual's overall cognitive ability. Executive function is an umbrella term  
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46 200 referring to those skills essential for undertaking goal-oriented behaviours and includes  
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48 201 inhibitory control which has been reported to be an area of concern for children born  
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50 202 preterm.[6]  
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54 203 By assessing the cognition of the N3RO infants as they turn five years of age we can  
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56 204 determine whether providing infants born <29 weeks' gestation with DHA emulsion  
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58 205 improves cognitive development. We hypothesise that providing the estimated in-utero  
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206 provisions of DHA to infants born <29 weeks' gestation will result in higher cognitive scores  
207 at five years' corrected age compared with infants who received the control intervention.

208

## 209 **METHODS**

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

### 213 **The N3RO trial**

214 1,273 infants born <29 weeks' gestation were enrolled into the N3RO trial within 3  
215 days of their first enteral feed. Infants were recruited between June 2012 and September 2015  
216 from 13 centres in Australia, New Zealand and Singapore.[25] Infants were excluded if they  
217 had a major congenital or chromosomal abnormality, were participating in another fatty acid  
218 intervention trial, were receiving intravenous lipids containing fish oil, or if a breast feeding  
219 mother was taking greater than 250 mg/day DHA through supplements.[25]

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

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3 230 gestation. Infants from multiple births were randomised individually. A statistician not  
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5 231 otherwise involved in the N3RO trial generated the randomisation schedule.  
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### 10 11 233 **Five-year follow-up study procedure**

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14 234 This is a follow-up of a predefined sub-sample of the N3RO trial infants from five of  
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16 235 the Australian recruiting centres. No additional interventions will be administered. Eligible  
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18 236 N3RO infants will be invited to attend an appointment with a psychologist when they are 5-  
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20 237 years' corrected age to measure child abilities on selected cognitive domains; age is corrected  
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22 238 for prematurity to avoid a known bias in cognitive test scores.[28] Appointments will take  
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24 239 between 45 minutes to 1.5 hours, depending on the child's abilities and speed whilst working  
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26 240 through the IQ test tasks, and assessments will be conducted by personnel blinded to group  
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28 241 allocation.  
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33 242 Families of eligible children will be emailed a letter of invitation two months before  
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35 243 their child reaches 5 years' corrected age, followed by a telephone call to answer any  
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37 244 questions and book appointments with families that wish to participate. Where necessary,  
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39 245 families will be offered appointments at the family's home or at a location close to their home  
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41 246 such as a school or community centre.  
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### 47 48 248 **Participants**

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51 249 Children who participated in the N3RO Trial and were recruited from the five largest  
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53 250 recruiting centres, John Hunter Hospital (New South Wales), King Edward Memorial  
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55 251 Hospital (Western Australia), Mercy Hospital for Women (Victoria), Royal Women's  
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57 252 Hospital (Victoria), and the Women's and Children's Hospital (South Australia) in Australia  
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3 253 will be invited to participate in this follow-up study. Children will not be invited if they have  
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5 254 previously been withdrawn from the N3RO trial or have died. Of the n=702 children enrolled  
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8 255 between the five centres, n=655 will be eligible to be approached for the five-year follow-up  
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10 256 once deaths (n=4) and withdrawals (n=43) are excluded.  
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## 16 258 **Outcomes and Measures**

### 17 18 19 259 *Primary outcome*

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22 260 The primary outcome is Full-Scale IQ, as assessed by the Wechsler Preschool and  
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24 261 Primary Scale of Intelligence - Fourth Edition, Australian and New Zealand (WPPSI-IV).  
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26 262 The WPPSI-IV is a battery of subtests that provides an assessment of general cognitive  
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29 263 ability for pre-schoolers and young children (2:6 to 7:7 years). The WPPSI-IV has strong  
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31 264 internal consistency and test–retest stability and sound psychometric properties.[29] The  
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33 265 average reliability coefficient for the Full-Scale IQ is 0.95.[29]  
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### 39 267 *Secondary outcomes*

#### 40 41 42 268 *WPPSI-IV*

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45 269 Other outcomes from the WPPSI-IV will be included as secondary outcomes. These  
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47 270 include Verbal Comprehension, Fluid Reasoning, Working Memory and the Processing  
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50 271 Speed, General Ability and Cognitive Proficiency Primary Index Scales.  
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53 272 The WPPSI-IV has Australian/New Zealand norms that are age-standardised with a  
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55 273 mean of 100 and SD 15. Intellectual impairment will be defined as Full-Scale IQ <85 (i.e. <-1  
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57 274 SD), and moderate-severe intellectual impairment as Full-Scale IQ <70 (i.e. <-2 SD). Any  
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3 275 impairment on any of the WPPSI-IV Primary Index Scales will be defined as an Index Scale  
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5 276 score <85 (i.e. <-1 SD).  
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11 278 *Fruit Stroop*  
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14 279 The Fruit Stroop was administered to assess two executive functions, inhibition and  
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16 280 mental flexibility.[30] The child is required to identify a the correct, natural colour of a series  
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18 281 of fruits and vegetables in four 45 s trials under a series of conditions that increase in  
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20 282 complexity. The outcome is an interference score calculated as the difference between the  
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22 283 number of correct responses on the final (inhibition) trial, and predicted scores on the first  
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24 284 and third trials, where lower or negative values indicate more interference.  
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32 286 *Growth*  
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35 287 Anthropometrics including child height, weight and head circumference will be  
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37 288 measured at the appointment as measures of the nutritional well-being of the children.  
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39 289 Measurements will be converted to Z (SD) scores appropriate for corrected age and sex.[31]  
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45 291 *Background information and characteristics*  
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48 292 At enrolment into the N3RO trial a range of socio-demographic data were collected  
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50 293 through interview with the caregiver (including parental age, education, and employment). As  
51  
52 294 part of the N3RO trial infant medical records were used to determine a range of baseline and  
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54 295 outcome clinical characteristics up to 40 weeks' postmenstrual age or first discharge home,  
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56 296 whichever occurred first, including for e.g., gestational age, birth weight, sex, and instances  
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59 297 of intraventricular haemorrhage.  
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56 299 **Sample size**  
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9 300 A sample size of 296 children per group (total 592) will provide 90% power (two-  
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11 301 tailed alpha 0.05) to detect a 4-point (0.27 standard deviation) mean difference in the primary  
12  
13 302 outcome of Full-Scale IQ between groups. No adjustment to the sample size is needed for  
14  
15 303 clustering due to multiple births, since children were randomised individually in N3RO and  
16  
17 304 the design effect for continuous outcomes is one in this case.[32] Should enrolment be lower  
18  
19 305 than planned, the study will have 80% power to detect a 4-point difference between groups  
20  
21 306 provided at least 222 children per group (total 444) provide follow-up data.  
22  
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26 307  
2728  
29 308 **Statistical analysis and data management**  
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32 309 All participants were assigned a study identification number at enrolment into the  
33  
34 310 N3RO trial. Throughout the follow-up and analyses, the identification number will be used to  
35  
36 311 identify data. Data will be entered into a REDCap database, which uses a MySQL database  
37  
38 312 via a secure web interface with data checks used during data entry to ensure data quality.  
39  
40 313 REDCap includes a complete suite of features to support the Health Insurance Portability and  
41  
42 314 Accountability Act of 1996 compliance, including a full audit trail, user-based privileges, and  
43  
44 315 integration with the institutional LDAP server.  
45  
46  
47

48 316 All analyses will be conducted according to a pre-specified statistical analysis plan.  
49  
50 317 Analyses will not commence until the N3RO trial Steering Committee has approved the  
51  
52 318 statistical analysis plan. Intervention groups will be dummy coded to allow analyses to be  
53  
54 319 performed blinded to treatment group.  
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2  
3 320 Outcomes of intervention and control group children will be compared using  
4  
5 321 generalised linear models, with generalised estimated equations used to account for clustering  
6  
7 322 due to multiple births within the same family. Continuous and binary outcomes will be  
8  
9  
10 323 analysed using linear and log binomial models, respectively, with adjustment for variables  
11  
12 324 used to stratify the randomisation: sex, centre enrolled, and gestational age (<27 completed  
13  
14 325 weeks' or 27 to <29 weeks' at birth). Pre-planned subgroup analyses will examine the effects  
15  
16 326 of DHA separately for girls or boys (all outcomes), and for infants born at <27 weeks'  
17  
18 327 gestation or 27 to <29 weeks' gestation (primary outcome only). No adjustment will be made  
19  
20 328 for multiple pre-planned comparisons, as the single overall comparison of Full-Scale IQ  
21  
22 329 between groups is of primary interest.

23  
24  
25  
26  
27 330 Missing outcome data will be addressed using multiple imputation, with imputation  
28  
29 331 performed separately by treatment group using fully conditional specification.[33] Imputed  
30  
31 332 datasets will include all surviving children from the five included centres. Children who are  
32  
33 333 missing scores on psychological assessments because they were unable to complete the  
34  
35 334 assessment for cognitive or physical reasons (such as blindness or cerebral palsy) will be  
36  
37 335 reviewed by a psychologist to determine whether assigning the lowest possible score is  
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39 336 appropriate.

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### 45 46 338 **Ethical considerations and dissemination of results**

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48  
49 339 This follow-up study will be carried out in accordance with the Australian National  
50  
51 340 Statement on Ethical Conduct in Research Involving Humans, which builds upon the ethical  
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53 341 codes of the Declaration of Helsinki and the Principles of International Conference on  
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55 342 Harmonisation Good Clinical Practice (as adopted in Australia). All procedures and study  
56  
57 343 materials have been reviewed and approved by the Women's and Children's Health Network  
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3 344 Human Research Ethics Committee (HREC/17/WCHN/187), as well as the Research  
4  
5 345 Governance officers at each site. The N3RO Trial and this follow-up are registered on the  
6  
7 346 Australia and New Zealand Clinical Trial Registry (ANZCTR: ACTRN12612000503820).  
8  
9

10 347 Caregivers will be provided with a detailed information sheet about the study and will  
11  
12 348 provide informed consent for their child's involvement in the study. Caregivers will be free to  
13  
14 349 re-negotiate consent for each procedure in the follow-up study and are able to decline any  
15  
16 350 part of the follow-up. Caregivers will be free to withdraw their children from the study at any  
17  
18 351 time.  
19  
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23 352 The results of this follow-up study will be presented at academic conferences and  
24  
25 353 published in peer-reviewed journals. Participating families will receive a lay-report of the  
26  
27 354 study findings. No participants will be identified in the dissemination of study results and  
28  
29 355 data collected will be treated with confidence.  
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### 32 356

### 33 357 **Access to Data**

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35  
36 358 Individual participant data, including data dictionaries, may be shared after de-  
37  
38 359 identification upon reasonable request. Proposals to access the data must be scientifically and  
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40 360 methodologically sound and must be reviewed and approved by the N3RO trial Steering  
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42 361 Committee and the Women's and Children's Human Research Ethics Committee. To gain  
43  
44 362 access, data requestors will need to sign a data access agreement. Proposals should be  
45  
46 363 directed to Jacqueline Gould through email ([Jacqueline.gould@sahmri.com](mailto:Jacqueline.gould@sahmri.com)).  
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50 364

### 51 365 **Patient and public involvement**

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54 366 Neither patients nor the public were directly involved in the development of the  
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56 367 research question or design of this follow-up study. However, our primary outcome of IQ is  
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3 368 based on reported concerns over long-term developmental concerns from parents of preterm  
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5 369 infants.[34]  
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8 370 A Community Board, comprising parents (including parents of a child born preterm)  
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10 371 as well as clinicians and researchers specialising in paediatrics will be consulted for the  
11  
12 372 dissemination of the study findings to participants, including reviewing the study results and  
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14 373 format of dissemination.  
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## 20 375 **DISCUSSION**

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23 376 This protocol details a follow-up of a RCT of a DHA enteral emulsion (60 mg/kg/day)  
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25 377 compared with a control emulsion (no DHA), for preterm infants born <29 weeks' gestation  
26  
27 378 in the first months of life, to evaluate the effect on child cognitive ability at 5 years of age.

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29  
30 379 Unlike previous DHA RCTs in preterm populations,[17-19] our follow-up has the benefits of  
31  
32 380 a population likely to be insufficient in DHA,[35] and a robust method of intervention.[25]  
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35  
36 381 We previously conducted a follow-up of a small sub-group of the N3RO trial infants  
37  
38 382 when they were aged 18 months' corrected age. Children underwent an experimental  
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40 383 assessment of visual attention (considered to be a basic, early emergence of higher order  
41  
42 384 cognitive skills known as the executive functions).[36] Where available, Bayley Scales of  
43  
44 385 Infant and Toddler Development-3<sup>rd</sup> edition Cognition, Motor and Language assessment  
45  
46 386 results were collected from hospital records.[36] No statistically significant differences were  
47  
48 387 found for attention, cognition, motor or language abilities (manuscript currently under  
49  
50  
51 388 review). However, assessments of cognition during infancy are considered poor predictors of  
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53 389 later performance,[37-41] and the sample was small and under-powered to detect a clinically  
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55 390 important effect on cognition.[36]  
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3 391 For this follow-up we have carefully selected a robust assessment of general cognitive  
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5 392 abilities, including executive functioning (both of which domains are likely to be adversely  
6  
7 393 affected by very preterm birth)[42-44] to be administered at an age when cognitive domains  
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9  
10 394 can be reliably assessed[27 45], as well as ensuring a large, adequately powered sample. As  
11  
12 395 per the recommendations of a consortium of parents and clinicians caring for high-risk  
13  
14 396 preterm infants, we are assessing general cognitive ability using a Wechsler scale, which is  
15  
16  
17 397 considered the gold standard, and have included an assessment of growth.[46]  
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19

20 398 This project has global significance, with over one million infants born <29 weeks'  
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22 399 gestation each year, and the number rising.[47] The potential benefit of DHA on cognitive  
23  
24 400 performance has never been adequately demonstrated in this population. However, because  
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26  
27 401 of the N3RO primary results it is extremely unlikely that such a trial will be repeated. The  
28  
29 402 N3RO cohort may represent the only children in which the longer-term cognitive and  
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31 403 behavioural effects of DHA supplementation in these infants can be assessed.  
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4

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6  
7 406 will participate in the follow-up study, and the N3RO Steering Committee, Investigative  
8  
9 407 Team and research staff.  
10  
11

12 408

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14  
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16

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18  
19 411 Council (NHMRC) Australia (ID: 1022112 - N3RO trial, 1146806 – 5-year follow-up) and  
20  
21 412 Clover Corporation Limited (Melbourne, Australia).  
22  
23

24 413

25  
26 414 **Competing Interests**  
27

28 415 Study product for the original trial was donated by Clover Corporation Limited (Melbourne,  
29  
30 416 Australia). MM and RAG report holding a patent relating to methods and compositions for  
31  
32 417 promoting the neurological development for preterm infants (2009201540), owned by the  
33  
34 418 South Australian Health and Medical Research Institute and licensed to Clover Corporation  
35  
36 419 Limited.  
37  
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41  
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43

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45

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49

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431 responsibility of the authors and do not reflect the views of the NHMRC.

432

### 433 **List of Abbreviations**

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

441

### 442 **Authors Contributions**

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

444 *Drafting the protocol:* Gould, Collins, Sullivan.

445 *Comment and approval of the final draft of the protocol:* Gould, Collins, Makrides, Sullivan,

446 Anderson, Gibson, McPhee, Best, Davis, Doyle, Opie, Travadi, Cheong, Sharp, Simmer.

447 *Statistical expertise:* Sullivan.

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

449 *Administrative, technical, or material support:* Gould, Collins, Makrides, Gibson, Sullivan,

450 McPhee, Anderson, Best, Davis, Doyle, Opie, Travadi, Cheong, Sharp, Simmer.

451



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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
<b>Administrative information</b>			
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 ___

## 1 Introduction

2			
3	Background and	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
4	rationale		_____ 7-8 _____
5			
6		6b	Explanation for choice of comparators
7			_____ 7-8 _____
8	Objectives	7	Specific objectives or hypotheses
9			_____ 8-9 _____
10	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)
11			_____ 9-14 _____
12			
13			
14	<b>Methods: Participants, interventions, and outcomes</b>		
15			
16	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
17			_____ 10 _____
18			
19	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)
20			_____ 9-10 _____
21			
22	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered
23			_____ 9 _____
24			
25		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)
26			_____ NA _____
27			
28		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
29			_____ NA _____
30			
31		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial
32			_____ NA _____
33			
34	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
35			_____ 11-13 _____
36			
37	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)
38			_____ 9 _____
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1	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___
2				
3				
4	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	___10-11___
5				

## 6 **Methods: Assignment of interventions (for controlled trials)**

### 7 Allocation:

8				
9				
10	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___
11				
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14				
15				
16	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___
17				
18				
19				
20	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	___NA___
21				
22				
23				
24	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	___9-10___
25				
26				
27		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	___NA___
28				
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## 31 **Methods: Data collection, management, and analysis**

32				
33	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___
34				
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39		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___
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1	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 _____
2				
3				
4				
5	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 _____
6				
7				
8		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	_____ 13-14 _____
9				
10		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 _____
11				
12				
13				
14	<b>Methods: Monitoring</b>			
15				
16	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 _____
17				
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22		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 _____
23				
24				
25	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 _____
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27				
28	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	_____ NA _____
29				
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32	<b>Ethics and dissemination</b>			
33				
34	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	_____ 14-15 _____
35				
36				
37	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 _____
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1	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
2				
3				
4		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
5				
6				
7	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
8				
9				
10	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	18
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13	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
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16	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
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20	Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, health care 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
21				
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23				
24		31b	Authorship eligibility guidelines and any intended use of professional writers	NA
25				
26		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA
27				
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29	<b>Appendices</b>			
30				
31	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Available upon request
32				
33				
34	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
35				
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\*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](https://creativecommons.org/licenses/by/4.0/)" 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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2 **A protocol for assessing whether cognition of preterm infants <29 weeks' gestation can**  
3 **be improved by an intervention with the omega-3 long-chain polyunsaturated fatty acid**  
4 **docosahexaenoic acid (DHA): a follow-up of a randomised controlled trial**

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For peer review only

1  
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3 110 **ABSTRACT**  
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6 111 **Introduction**  
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9 112 Docosahexaenoic acid (DHA) is an omega-3 (n-3) fatty acid that accumulates into neural  
10  
11 113 tissue during the last trimester of pregnancy, as the fetal brain is undergoing a growth spurt.  
12  
13 114 Infants born <29 weeks' gestation are deprived the normal in-utero supply of DHA during  
14  
15 115 this period of rapid brain development. Insufficient dietary DHA postnatally may contribute  
16  
17 116 to the cognitive impairments common among this population. This follow-up of the N-3 fatty  
18  
19 117 acids for improvement in Respiratory Outcomes (N3RO) randomised controlled trial aims to  
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21 118 determine if enteral DHA supplementation in infants born <29 weeks' gestation during the  
22  
23 119 first months of life improves cognitive development at five-years of age corrected for  
24  
25 120 prematurity.  
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32  
33 122 **Methods and Analysis**  
34  
35

36 123 N3RO was a randomised controlled trial of enteral DHA supplementation (60 mg/kg/day) or  
37  
38 124 a control emulsion (without DHA) in 1,273 infants born <29 weeks' gestation to determine  
39  
40 125 the effect on bronchopulmonary dysplasia (BPD). We showed that DHA supplementation did  
41  
42 126 not reduce the risk of BPD and may have increased the risk.  
43  
44 127 In this follow-up at five years' corrected age, a predefined subset (n=655) of children from  
45  
46 128 five Australian sites will be invited to attend a cognitive assessment with a psychologist.  
47  
48 129 Children will be administered the Wechsler Preschool and Primary Scale of Intelligence (4<sup>th</sup>  
49  
50 130 edition) and a measure of inhibitory control (Fruit Stroop), while height, weight and head  
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52 131 circumference will be measured.  
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3 132 The primary outcome is Full-Scale intelligence quotient (IQ). To ensure 90% power, a  
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5 133 minimum of 592 children are needed to detect a four-point difference in IQ between the  
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8 134 groups.

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10 135 Research personnel and families remain blinded to group assignment.  
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### 16 137 **Ethics and Dissemination**

17  
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19 138 The Women's and Children Health Network Human Research Ethics Committee reviewed  
20  
21 139 and approved the study (HREC/17/WCHN/187). Caregivers will give informed consent prior  
22  
23 140 to taking part in this follow-up study. Findings of this study will be disseminated through  
24  
25  
26 141 peer reviewed publications and conference presentations.  
27  
28  
29 142

### 32 143 **Trial Registration**

33  
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35 144 Australian and New Zealand Clinical Trial Registry: [anzctr.org.au](http://anzctr.org.au): [ACTRN12612000503820](https://www.anzctr.org.au/Trial/Registration/Trial.jsp?ACTRN12612000503820).  
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### 41 146 **Strengths and Limitations**

- 42  
43  
44 147 • This will be the first adequately powered randomised controlled trial to assess  
45  
46 148 cognitive development following docosahexaenoic acid supplementation in preterm  
47  
48 149 infants born <29 weeks' gestation.
- 50  
51 150 • This follow-up of the N3RO trial will provide sound evidence for the effect of enteral  
52  
53 151 DHA supplementation on the cognitive development of infants born <29 weeks'  
54  
55 152 gestation.
- 56  
57 153 • Loss to follow-up five years after enrolment into the trial may contribute to risk of  
58  
59  
60 154 bias.

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3 155 • Partial unblinding of study group allocation permitted under the primary protocol may  
4  
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6 156 contribute to risk of bias  
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8 157 • Although bronchopulmonary dysplasia was the primary outcome of the original  
9  
10 158 N3RO trial, childhood respiratory functioning is not assessed in this follow-up  
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14  
15 160 **Key words:** intelligence quotient, cognition, preterm infant, docosahexaenoic acid,  
16  
17 161 randomised control trial  
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## 163 INTRODUCTION

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

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

178 Several randomised controlled trials (RCT) have attempted to evaluate this  
179 hypothesis, with mixed results.[17 18] Two RCTs compared the standard dose of DHA in  
180 breastmilk and preterm infant formula (20 mg/kg/day) to the estimated in-utero accretion rate  
181 (60 mg/kg/day).[19 20] In one trial the DHA group showed greater problem solving skills at  
182 6 months[20] and improved sustained attention at 20 months,[21] although attrition was high.  
183 In the larger trial, assessment at 18 months revealed no difference in overall mean cognitive  
184 scores but fewer infants had developmental delay in the DHA group.[19] No overall  
185 differences in intelligence quotient (IQ) were detected in follow-up of these trials at  
186 seven[22] or eight years of age.[23] Interestingly, both trials suggested a benefit of extra  
187 DHA in infants born at the earliest gestations (<29 weeks or <1250 g) who are most

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3 188 vulnerable to experiencing neurodevelopmental deficit.[19 20] While this is promising, both  
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5 189 trials were significantly underpowered (with only 200 children in one trial[19] and under 70  
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8 190 in the other[20]) to detect an effect in this subgroup.  
9

10  
11 191 It is clear that current neonatal feeding practices are unable to replace the placental  
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13 192 transfer of DHA[16] and despite decades of research, we still do not know whether meeting  
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15 193 the estimated requirement of DHA during the neonatal period improves cognitive outcomes  
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17 194 in the most vulnerable sub-population of preterm infants.[17 19 20 22 23]

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21 195 The N-3 Fatty Acids for Improvement in Respiratory Outcomes (N3RO) RCT was  
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23 196 designed to determine the effect of an enteral DHA emulsion (providing 60 mg/kg/day) on  
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25 197 the incidence of bronchopulmonary dysplasia (BPD).[24] The DHA intervention did not  
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27 198 lower the incidence of BPD in infants born <29 weeks' gestation and may have resulted in a  
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30 199 greater risk of BPD.[24] However, the N3RO trial offers an ideal opportunity to resolve  
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32 200 whether DHA supplementation is beneficial for the cognitive development of these most  
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34 201 vulnerable preterm infants.  
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37 202 The N3RO trial infants are now reaching five years of age. Cognition develops  
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39 203 rapidly across early childhood[25] and by five years most cognitive domains can be reliably  
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41 204 assessed using standardised psychometric tests.[26] IQ tests are considered a robust method  
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43 205 of estimating an individual's overall cognitive ability. Executive function is an umbrella term  
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45 206 referring to those skills essential for undertaking goal-oriented behaviours and includes  
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47 207 inhibitory control which has been reported to be an area of concern for children born  
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49 208 preterm.[6]  
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54 209 By assessing the cognition of the N3RO infants as they turn five years of age we can  
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56 210 determine whether providing infants born <29 weeks' gestation with DHA emulsion  
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58 211 improves cognitive development. We hypothesise that providing the estimated in-utero  
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212 provisions of DHA to infants born <29 weeks' gestation will result in higher cognitive scores  
213 at five years' corrected age compared with infants who received the control intervention.

214

## 215 **METHODS**

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

### 219 **The N3RO trial**

220 1,273 infants born <29 weeks' gestation were enrolled into the N3RO trial within 3  
221 days of their first enteral feed. Infants were recruited between June 2012 and September 2015  
222 from 13 centres in Australia, New Zealand and Singapore.[24] Infants were excluded if they  
223 had a major congenital or chromosomal abnormality, were participating in another fatty acid  
224 intervention trial, were receiving intravenous lipids containing fish oil, or if a breast feeding  
225 mother was taking greater than 250 mg/day DHA through supplements.[24]

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

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3 236 gestation. Infants from multiple births were randomised individually. A statistician not  
4  
5 237 otherwise involved in the N3RO trial generated the randomisation schedule.  
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### 10 11 239 **Five-year follow-up study procedure**

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13  
14 240 This is a follow-up of a predefined sub-sample of the N3RO trial infants from five of  
15  
16 241 the Australian recruiting centres. No additional interventions will be administered. Eligible  
17  
18 242 N3RO infants will be invited to attend an appointment with a psychologist when they are 5-  
19  
20 243 years' corrected age to measure child abilities on selected cognitive domains; age is corrected  
21  
22 244 for prematurity to avoid a known bias in cognitive test scores.[27] Appointments will take  
23  
24 245 between 45 minutes to 1.5 hours, depending on the child's abilities and speed whilst working  
25  
26 246 through the IQ test tasks, and assessments will be conducted by personnel blinded to group  
27  
28 247 allocation. Assessments for this follow-up study commenced 29<sup>th</sup> August 2018 and are  
29  
30 248 expected to be completed on the 31<sup>st</sup> December 2020.  
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35 249 Families of eligible children will be emailed a letter of invitation two months before  
36  
37 250 their child reaches 5 years' corrected age, followed by a telephone call to answer any  
38  
39 251 questions and book appointments with families that wish to participate. Where necessary,  
40  
41 252 families will be offered appointments at the family's home or at a location close to their home  
42  
43 253 such as a school or community centre.  
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### 49 50 51 255 **Participants**

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54 256 Children who participated in the N3RO Trial and were recruited from the five largest  
55  
56 257 recruiting centres, John Hunter Hospital (New South Wales), King Edward Memorial  
57  
58 258 Hospital (Western Australia), Mercy Hospital for Women (Victoria), Royal Women's  
59  
60

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

264

## 265 **Outcomes and Measures**

### 266 *Primary outcome*

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

273

### 274 *Secondary outcomes*

#### 275 *WPPSI-IV*

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

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

1  
2  
3 282 impairment on any of the WPPSI-IV Primary Index Scales will be defined as an Index Scale  
4  
5 283 score <85 (i.e. <-1 SD).  
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### 10 11 285 *Fruit Stroop* 12

13  
14 286 The Fruit Stroop was administered to assess two executive functions, inhibition and  
15  
16 287 mental flexibility.[29] The child is required to identify a the correct, natural colour of a series  
17  
18 288 of fruits and vegetables in four 45 s trials under a series of conditions that increase in  
19  
20 289 complexity. The outcome is an interference score calculated as the difference between the  
21  
22 290 number of correct responses on the final (inhibition) trial, and predicted scores on the first  
23  
24 291 and third trials, where lower or negative values indicate more interference.  
25  
26  
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### 31 293 *Growth* 32

33  
34 294 Anthropometrics including child height, weight and head circumference will be  
35  
36 295 measured at the appointment as measures of the nutritional well-being of the children.  
37  
38 296 Measurements will be converted to Z (SD) scores appropriate for corrected age and sex.[30]  
39  
40

41 297  
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### 45 298 *Background information and characteristics* 46

47  
48 299 At enrolment into the N3RO trial a range of socio-demographic data were collected  
49  
50 300 through interview with the caregiver (including parental age, education, and employment). As  
51  
52 301 part of the N3RO trial infant medical records were used to determine a range of baseline and  
53  
54 302 outcome clinical characteristics up to 40 weeks' postmenstrual age or first discharge home,  
55  
56 303 whichever occurred first, including for e.g., gestational age, birth weight, sex, and instances  
57  
58 304 of intraventricular haemorrhage.  
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56 306 **Sample size**  
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9 307 A sample size of 296 children per group (total 592) will provide 90% power (two-  
10  
11 308 tailed alpha 0.05) to detect a 4-point (0.27 standard deviation) mean difference in the primary  
12  
13 309 outcome of Full-Scale IQ between groups. No adjustment to the sample size is needed for  
14  
15 310 clustering due to multiple births, since children were randomised individually in N3RO and  
16  
17 311 the design effect for continuous outcomes is one in this case.[31] Should enrolment be lower  
18  
19 312 than planned, the study will have 80% power to detect a 4-point difference between groups  
20  
21 313 provided at least 222 children per group (total 444) provide follow-up data.  
22  
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29 315 **Statistical analysis and data management**  
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32 316 All participants were assigned a study identification number at enrolment into the  
33  
34 317 N3RO trial. Throughout the follow-up and analyses, the identification number will be used to  
35  
36 318 identify data. Data will be entered into a REDCap database, which uses a MySQL database  
37  
38 319 via a secure web interface with data checks used during data entry to ensure data quality.  
39  
40 320 REDCap includes a complete suite of features to support the Health Insurance Portability and  
41  
42 321 Accountability Act of 1996 compliance, including a full audit trail, user-based privileges, and  
43  
44 322 integration with the institutional LDAP server.  
45  
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47

48 323 All analyses will be conducted according to a pre-specified statistical analysis plan.  
49  
50 324 Analyses will not commence until the N3RO trial Steering Committee has approved the  
51  
52 325 statistical analysis plan. Intervention groups will be dummy coded to allow analyses to be  
53  
54 326 performed blinded to treatment group.  
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3 327 Outcomes of intervention and control group children will be compared using  
4  
5 328 generalised linear models, with generalised estimated equations used to account for clustering  
6  
7  
8 329 due to multiple births within the same family. Continuous and binary outcomes will be  
9  
10 330 analysed using linear and log binomial models, respectively, with adjustment for variables  
11  
12 331 used to stratify the randomisation: sex, centre enrolled, and gestational age (<27 completed  
13  
14 332 weeks' or 27 to <29 weeks' at birth). Pre-planned subgroup analyses will examine the effects  
15  
16 333 of DHA separately for girls or boys (all outcomes), and for infants born at <27 weeks'  
17  
18 334 gestation or 27 to <29 weeks' gestation (primary outcome only). No adjustment will be made  
19  
20 335 for multiple pre-planned comparisons, as the single overall comparison of Full-Scale IQ  
21  
22 336 between groups is of primary interest.  
23  
24  
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27 337 Missing outcome data will be addressed using multiple imputation, with imputation  
28  
29 338 performed separately by treatment group using fully conditional specification.[32] Imputed  
30  
31 339 datasets will include all surviving children from the five included centres. Children who are  
32  
33 340 missing scores on psychological assessments because they were unable to complete the  
34  
35 341 assessment for cognitive or physical reasons (such as blindness or cerebral palsy) will be  
36  
37 342 reviewed by a psychologist to determine whether assigning the lowest possible score is  
38  
39 343 appropriate.  
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#### 46 345 **Ethical considerations and dissemination of results**

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49 346 This follow-up study will be carried out in accordance with the Australian National  
50  
51 347 Statement on Ethical Conduct in Research Involving Humans, which builds upon the ethical  
52  
53 348 codes of the Declaration of Helsinki and the Principles of International Conference on  
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55 349 Harmonisation Good Clinical Practice (as adopted in Australia). All procedures and study  
56  
57 350 materials have been reviewed and approved by the Women's and Children's Health Network  
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3 351 Human Research Ethics Committee (HREC/17/WCHN/187), as well as the Research  
4  
5 352 Governance officers at each site. The N3RO Trial and this follow-up are registered on the  
6  
7 353 Australia and New Zealand Clinical Trial Registry (ANZCTR: ACTRN12612000503820).  
8  
9

10 354 Caregivers will be provided with a detailed information sheet about the study and will  
11  
12 355 provide informed consent for their child's involvement in the study. Caregivers will be free to  
13  
14 356 re-negotiate consent for each procedure in the follow-up study and are able to decline any  
15  
16 357 part of the follow-up. Caregivers will be free to withdraw their children from the study at any  
17  
18 358 time.  
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23 359 The results of this follow-up study will be presented at academic conferences and  
24  
25 360 published in peer-reviewed journals. Participating families will receive a lay-report of the  
26  
27 361 study findings. No participants will be identified in the dissemination of study results and  
28  
29 362 data collected will be treated with confidence.  
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### 32 363

### 33 364 **Access to Data**

34  
35 365 Individual participant data, including data dictionaries, may be shared after de-  
36  
37 366 identification upon reasonable request. Proposals to access the data must be scientifically and  
38  
39 367 methodologically sound and must be reviewed and approved by the N3RO trial Steering  
40  
41 368 Committee and the Women's and Children's Human Research Ethics Committee. To gain  
42  
43 369 access, data requestors will need to sign a data access agreement. Proposals should be  
44  
45 370 directed to Jacqueline Gould through email ([Jacqueline.gould@sahmri.com](mailto:Jacqueline.gould@sahmri.com)).  
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51 371

### 52 372 **Patient and public involvement**

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55 373 Neither patients nor the public were directly involved in the development of the  
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57 374 research question or design of this follow-up study. However, our primary outcome of IQ is  
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3 375 based on reported concerns over long-term developmental concerns from parents of preterm  
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5 376 infants.[33]  
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8 377 A Community Board, comprising parents (including parents of a child born preterm)  
9  
10 378 as well as clinicians and researchers specialising in paediatrics will be consulted for the  
11  
12 379 dissemination of the study findings to participants, including reviewing the study results and  
13  
14 380 format of dissemination.  
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18 381

## 20 382 **DISCUSSION**

23 383 This protocol details a follow-up of a RCT of a DHA enteral emulsion (60 mg/kg/day)  
24  
25 384 compared with a control emulsion (no DHA), for preterm infants born <29 weeks' gestation  
26  
27 385 in the first months of life, to evaluate the effect on child cognitive ability at 5 years of age.

28  
29 386 Unlike previous DHA RCTs in preterm populations,[17 18] our follow-up has the benefits of  
30  
31 387 a population likely to be insufficient in DHA,[34] and a robust method of intervention.[24]  
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34

35 388 We previously conducted a follow-up of a small sub-group of the N3RO trial infants  
36  
37 389 when they were aged 18 months' corrected age. Children underwent an experimental  
38  
39 390 assessment of visual attention (considered to be a basic, early emergence of higher order  
40  
41 391 cognitive skills known as the executive functions).[35] Where available, Bayley Scales of  
42  
43 392 Infant and Toddler Development-3<sup>rd</sup> edition Cognition, Motor and Language assessment  
44  
45 393 results were collected from hospital records.[35] No statistically significant differences were  
46  
47 394 found for attention, cognition, motor or language abilities.[36] However, assessments of  
48  
49 395 cognition during infancy are considered poor predictors of later performance,[37-41] and the  
50  
51 396 sample was small and under-powered to detect a clinically important effect on cognition.[35]  
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55  
56 397 Our sample size calculation for the primary outcome requires a 90% follow-up rate of  
57  
58 398 the N3RO trial children, five years after enrolment. More than 10% loss to follow-up may  
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1  
2  
3 399 introduce attrition bias. After completion of the N3RO trial primary outcome analyses,  
4  
5 400 families had the opportunity to request knowledge of their group allocation. Although few  
6  
7 401 families requested this, knowledge of their randomisation group prior to the five-year follow-  
8  
9 402 up assessment may introduce additional bias to the results.  
10  
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12

13 403 For this follow-up we have carefully selected a robust assessment of general cognitive  
14  
15 404 abilities, including executive functioning (both of which domains are likely to be adversely  
16  
17 405 affected by very preterm birth)[42-44] to be administered at an age when cognitive domains  
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19 406 can be reliably assessed[26 45], as well as ensuring a large, adequately powered sample. As  
20  
21 407 per the recommendations of a consortium of parents and clinicians caring for high-risk  
22  
23 408 preterm infants, we are assessing general cognitive ability using a Wechsler scale, which is  
24  
25 409 considered the gold standard, and have included an assessment of growth.[46] Assessments  
26  
27 410 of respiratory functioning are unreliable in early childhood and hence were not included in  
28  
29 411 this follow-up. It is important that the long-term respiratory effects of DHA supplementation  
30  
31 412 in infants born <29 weeks' gestation is addressed when the N3RO trial children reach an  
32  
33 413 appropriate age.  
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39 414 This project has global significance, with over one million infants born <29 weeks'  
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41 415 gestation each year, and the number rising.[47] The potential benefit of DHA on cognitive  
42  
43 416 performance has never been adequately demonstrated in this population. However, because  
44  
45 417 of the N3RO primary results it is extremely unlikely that such a trial will be repeated. The  
46  
47 418 N3RO cohort may represent the only children in which the longer-term cognitive and  
48  
49 419 behavioural effects of DHA supplementation in these infants can be assessed.  
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1  
2  
3 420 **Acknowledgements**  
4

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6  
7 422 will participate in the follow-up study, and the N3RO Steering Committee, Investigative  
8  
9 423 Team and research staff.  
10  
11

12 424

13  
14  
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16

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18  
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20  
21 428 Clover Corporation Limited (Melbourne, Australia).  
22  
23

24 429

25  
26 430 **Competing Interests**  
27

28 431 Study product for the original trial was donated by Clover Corporation Limited (Melbourne,  
29  
30 432 Australia). MM and RAG report holding a patent relating to methods and compositions for  
31  
32 433 promoting the neurological development for preterm infants (2009201540), owned by the  
33  
34 434 South Australian Health and Medical Research Institute and licensed to Clover Corporation  
35  
36 435 Limited.  
37  
38

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41  
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49

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51

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4  
5 445 Drs Makrides and Gibson report serving on the board for Trajan Nutrition. No other authors  
6  
7 reported any financial disclosures. The contents of the published material are solely the  
8 446  
9  
10 447 responsibility of the authors and do not reflect the views of the NHMRC.  
11  
12 448

### 14 449 **List of Abbreviations**

16 450	BPD	Bronchopulmonary dysplasia
17		
18 451	DHA	Docosahexaenoic acid
19		
20 452	IQ	Intelligence Quotient
21		
22 453	n-3	Omega-3
23		
24 454	N3RO	N-3 (omega-3) Fatty Acids for Improvement in Respiratory Outcomes
25		
26 455	RCT	Randomised controlled trial
27		
28 456	WPPSI-IV	Wechsler Preschool and Primary Scale of Intelligence - Fourth Edition
29		
30 457		

### 32 458 **Authors Contributions**

33  
34  
35 459 *Study concept and design:* Collins, Gould, Makrides, McPhee, Anderson, Gibson, Sullivan.  
36  
37 460 *Drafting the protocol:* Gould, Collins, Sullivan.  
38  
39 461 *Comment and approval of the final draft of the protocol:* Gould, Collins, Makrides, Sullivan,  
40  
41 Anderson, Gibson, McPhee, Best, Davis, Doyle, Opie, Travadi, Cheong, Sharp, Simmer.  
42 462  
43  
44 463 *Statistical expertise:* Sullivan.  
45  
46  
47 464 *Obtained funding:* Collins, Gould, Makrides, McPhee, Gibson, Sullivan, Best.  
48  
49 465 *Administrative, technical, or material support:* Gould, Collins, Makrides, Gibson, Sullivan,  
50  
51  
52 466 McPhee, Anderson, Best, Davis, Doyle, Opie, Travadi, Cheong, Sharp, Simmer.  
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SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents\*

Section/item	Item No	Description	Addressed on page number
<b>Administrative information</b>			
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 ___

## 1 Introduction

2			
3	Background and	6a	Description of research question and justification for undertaking the trial, including summary of relevant
4	rationale		studies (published and unpublished) examining benefits and harms for each intervention
5			
6		6b	Explanation for choice of comparators
7			
8	Objectives	7	Specific objectives or hypotheses
9			
10	Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group),
11			allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)
12			
13			
14	<b>Methods: Participants, interventions, and outcomes</b>		
15			
16	Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will
17			be collected. Reference to where list of study sites can be obtained
18			
19	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and
20			individuals who will perform the interventions (eg, surgeons, psychotherapists)
21			
22	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be
23			administered
24			
25		11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose
26			change in response to harms, participant request, or improving/worsening disease)
27			
28		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence
29			(eg, drug tablet return, laboratory tests)
30			
31		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial
32			
33	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood
34			pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg,
35			median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen
36			efficacy and harm outcomes is strongly recommended
37			
38	Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for
39			participants. A schematic diagram is highly recommended (see Figure)
40			
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1	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___
2				
3				
4	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	___10-11___
5				

## 6 **Methods: Assignment of interventions (for controlled trials)**

### 7 Allocation:

10	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___
11				
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16	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___
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20	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	___NA___
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23				
24	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	___9-10___
25				
26				
27		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	___NA___
28				
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## 31 **Methods: Data collection, management, and analysis**

33	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___
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39		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___
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1	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 _____
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5	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 _____
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8		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	_____ 13-14 _____
9				
10		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 _____
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14	<b>Methods: Monitoring</b>			
15				
16	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 _____
17				
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22		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 _____
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25	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 _____
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28	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	_____ NA _____
29				
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32	<b>Ethics and dissemination</b>			
33				
34	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	_____ 14-15 _____
35				
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37	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 _____
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1	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
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4		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
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7	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
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10	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	18
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13	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
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16	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
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20	Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, health care 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
21				
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24		31b	Authorship eligibility guidelines and any intended use of professional writers	NA
25				
26		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA
27				
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29	<b>Appendices</b>			
30				
31	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Available upon request
32				
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34	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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\*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](https://creativecommons.org/licenses/by/4.0/)" 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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<b>Primary Subject Heading</b>:	Paediatrics
Secondary Subject Heading:	Nutrition and metabolism
Keywords:	NEONATOLOGY, NUTRITION & DIETETICS, Developmental neurology & neurodisability < PAEDIATRICS

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

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107 **Word Count: 2861**

For peer review only

1  
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3 110 **ABSTRACT**  
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6 111 **Introduction**  
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9 112 Docosahexaenoic acid (DHA) is an omega-3 (n-3) fatty acid that accumulates into neural  
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11 113 tissue during the last trimester of pregnancy, as the fetal brain is undergoing a growth spurt.  
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13 114 Infants born <29 weeks' gestation are deprived the normal in-utero supply of DHA during  
14  
15 115 this period of rapid brain development. Insufficient dietary DHA postnatally may contribute  
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17 116 to the cognitive impairments common among this population. This follow-up of the N-3 fatty  
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19 117 acids for improvement in Respiratory Outcomes (N3RO) randomised controlled trial aims to  
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21 118 determine if enteral DHA supplementation in infants born <29 weeks' gestation during the  
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23 119 first months of life improves cognitive development at five-years of age corrected for  
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25 120 prematurity.  
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33 122 **Methods and Analysis**  
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36 123 N3RO was a randomised controlled trial of enteral DHA supplementation (60 mg/kg/day) or  
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38 124 a control emulsion (without DHA) in 1,273 infants born <29 weeks' gestation to determine  
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40 125 the effect on bronchopulmonary dysplasia (BPD). We showed that DHA supplementation did  
41  
42 126 not reduce the risk of BPD and may have increased the risk.  
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44 127 In this follow-up at five years' corrected age, a predefined subset (n=655) of children from  
45  
46 128 five Australian sites will be invited to attend a cognitive assessment with a psychologist.  
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48 129 Children will be administered the Wechsler Preschool and Primary Scale of Intelligence (4<sup>th</sup>  
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50 130 edition) and a measure of inhibitory control (Fruit Stroop), while height, weight and head  
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52 131 circumference will be measured.  
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3 132 The primary outcome is Full-Scale intelligence quotient (IQ). To ensure 90% power, a  
4  
5 133 minimum of 592 children are needed to detect a four-point difference in IQ between the  
6  
7  
8 134 groups.

9  
10 135 Research personnel and families remain blinded to group assignment.  
11  
12  
13 136

### 16 137 **Ethics and Dissemination**

17  
18  
19 138 The Women's and Children Health Network Human Research Ethics Committee reviewed  
20  
21 139 and approved the study (HREC/17/WCHN/187). Caregivers will give informed consent prior  
22  
23 140 to taking part in this follow-up study. Findings of this study will be disseminated through  
24  
25  
26 141 peer reviewed publications and conference presentations.  
27  
28  
29 142

### 32 143 **Trial Registration**

33  
34  
35 144 Australian and New Zealand Clinical Trial Registry: anzctr.org.au: [ACTRN12612000503820](https://www.anzctr.org.au/Trial/Registration/Trial.jsp?ACTRN12612000503820).  
36  
37  
38 145

### 41 146 **Strengths and Limitations**

- 42  
43  
44 147 • This will be the first adequately powered randomised controlled trial to assess  
45  
46 148 cognitive development following docosahexaenoic acid supplementation in preterm  
47  
48 149 infants born <29 weeks' gestation.
- 49  
50  
51 150 • This follow-up of the N3RO trial will provide sound evidence for the effect of enteral  
52  
53 151 DHA supplementation on the cognitive development of infants born <29 weeks'  
54  
55 152 gestation.
- 56  
57  
58 153 • Loss to follow-up five years after enrolment into the trial may contribute to risk of  
59  
60 154 bias.

- 1  
2  
3 155 • Partial unblinding of study group allocation permitted under the primary protocol may  
4  
5  
6 156 contribute to risk of bias  
7  
8 157 • Although bronchopulmonary dysplasia was the primary outcome of the original  
9  
10 158 N3RO trial, childhood respiratory functioning is not assessed in this follow-up  
11  
12  
13 159

14  
15 160 **Key words:** intelligence quotient, cognition, preterm infant, docosahexaenoic acid,  
16  
17 161 randomised control trial  
18  
19  
20 162

## 163 INTRODUCTION

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

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

178 Several randomised controlled trials (RCT) have attempted to evaluate this  
179 hypothesis, with mixed results.[17 18] Two RCTs compared the standard dose of DHA in  
180 breastmilk and preterm infant formula (20 mg/kg/day) to the estimated in-utero accretion rate  
181 (60 mg/kg/day).[19 20] In one trial the DHA group showed greater problem solving skills at  
182 6 months[20] and improved sustained attention at 20 months,[21] although attrition was high.  
183 In the larger trial, assessment at 18 months revealed no difference in overall mean cognitive  
184 scores but fewer infants had developmental delay in the DHA group.[19] No overall  
185 differences in intelligence quotient (IQ) were detected in follow-up of these trials at  
186 seven[22] or eight years of age.[23] Interestingly, both trials suggested a benefit of extra  
187 DHA in infants born at the earliest gestations (<29 weeks or <1250 g) who are most

1  
2  
3 188 vulnerable to experiencing neurodevelopmental deficit.[19 20] While this is promising, both  
4  
5 189 trials were significantly underpowered (with only 200 children in one trial[19] and under 70  
6  
7  
8 190 in the other[20]) to detect an effect in this subgroup.  
9

10  
11 191 It is clear that current neonatal feeding practices are unable to replace the placental  
12  
13 192 transfer of DHA[16] and despite decades of research, we still do not know whether meeting  
14  
15 193 the estimated requirement of DHA during the neonatal period improves cognitive outcomes  
16  
17 194 in the most vulnerable sub-population of preterm infants.[17 19 20 22 23]  
18  
19

20  
21 195 The N-3 Fatty Acids for Improvement in Respiratory Outcomes (N3RO) RCT was  
22  
23 196 designed to determine the effect of an enteral DHA emulsion (providing 60 mg/kg/day) on  
24  
25 197 the incidence of bronchopulmonary dysplasia (BPD).[24] The DHA intervention did not  
26  
27 198 lower the incidence of BPD in infants born <29 weeks' gestation and may have resulted in a  
28  
29 199 greater risk of BPD.[24] However, the N3RO trial offers an ideal opportunity to resolve  
30  
31 200 whether DHA supplementation is beneficial for the cognitive development of these most  
32  
33 201 vulnerable preterm infants.  
34  
35  
36

37 202 The N3RO trial infants are now reaching five years of age. Cognition develops  
38  
39 203 rapidly across early childhood[25] and by five years most cognitive domains can be reliably  
40  
41 204 assessed using standardised psychometric tests.[26] IQ tests are considered a robust method  
42  
43 205 of estimating an individual's overall cognitive ability. Executive function is an umbrella term  
44  
45 206 referring to those skills essential for undertaking goal-oriented behaviours and includes  
46  
47 207 inhibitory control which has been reported to be an area of concern for children born  
48  
49 208 preterm.[6]  
50  
51  
52  
53

54 209 By assessing the cognition of the N3RO infants as they turn five years of age we can  
55  
56 210 determine whether providing infants born <29 weeks' gestation with DHA emulsion  
57  
58 211 improves cognitive development. We hypothesise that providing the estimated in-utero  
59  
60



1  
2  
3 212 provisions of DHA to infants born <29 weeks' gestation will result in higher cognitive scores  
4  
5 213 at five years' corrected age compared with infants who received the control intervention.  
6  
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9 214

## 10 11 215 **METHODS AND ANALYSIS** 12 13

14 216 This protocol details the methods for a follow-up at five years of age of infants  
15  
16 217 enrolled in the N3RO trial. Detailed methods of the N3RO trial have been published  
17  
18 218 previously[24] and are summarised here.  
19  
20  
21

### 22 219 **The N3RO trial** 23 24

25 220 1,273 infants born <29 weeks' gestation were enrolled into the N3RO trial within 3  
26  
27 221 days of their first enteral feed. Infants were recruited between June 2012 and September 2015  
28  
29 222 from 13 centres in Australia, New Zealand and Singapore.[24] Infants were excluded if they  
30  
31 223 had a major congenital or chromosomal abnormality, were participating in another fatty acid  
32  
33 224 intervention trial, were receiving intravenous lipids containing fish oil, or if a breast feeding  
34  
35 225 mother was taking greater than 250 mg/day DHA through supplements.[24] Infants were  
36  
37 226 randomised to the intervention or control group through a secure web-based computer-  
38  
39 227 generated schedule stratified for the 13 centres, sex and gestational age at birth <27 weeks' or  
40  
41 228 27 to <29 weeks' gestation. Infants from multiple births were randomised individually. A  
42  
43 229 statistician not otherwise involved in the N3RO trial generated the randomisation schedule.  
44  
45  
46  
47

### 48 230 **The N3RO trial intervention** 49 50

51 231 Infants were randomised to receive a DHA emulsion that provided 60 mg of DHA per  
52  
53 232 kg of body weight per day (intervention group, n=631), or a control emulsion without DHA  
54  
55 233 (control group, n=642).[24] Infants received the study intervention from enrolment to 36  
56  
57 234 weeks' postmenstrual age or discharge home, whichever occurred first. The emulsion was  
58  
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60

1  
2  
3 235 administered three times per day, immediately before an enteral feed through a nasogastric or  
4  
5 236 orogastric tube for the duration of the intervention period. The DHA and control emulsions  
6  
7 237 were iso-caloric and identical in viscosity, colour, and packaging and families, clinical staff  
8  
9  
10 238 and study personnel were blinded to group allocation.[24]  
11  
12

13 239  
14  
15

### 16 240 **Five-year follow-up study procedure**

17  
18  
19 241 This is a follow-up of a predefined sub-sample of the N3RO trial infants from five of  
20  
21 242 the Australian recruiting centres. No additional interventions will be administered. Eligible  
22  
23 243 N3RO infants will be invited to attend an appointment with a psychologist when they are 5-  
24  
25 244 years' corrected age to measure child abilities on selected cognitive domains; age is corrected  
26  
27 245 for prematurity to avoid a known bias in cognitive test scores.[27] Appointments will take  
28  
29 246 between 45 minutes to 1.5 hours, depending on the child's abilities and speed whilst working  
30  
31 247 through the IQ test tasks, and assessments will be conducted by personnel blinded to group  
32  
33 248 allocation. Assessments for this follow-up study commenced 29<sup>th</sup> August 2018 and are  
34  
35 249 expected to be completed on the 31<sup>st</sup> December 2020.  
36  
37  
38  
39

40 250 Families of eligible children will be emailed a letter of invitation two months before  
41  
42 251 their child reaches 5 years' corrected age, followed by a telephone call to answer any  
43  
44 252 questions and book appointments with families that wish to participate. Where necessary,  
45  
46 253 families will be offered appointments at the family's home or at a location close to their home  
47  
48 254 such as a school or community centre.  
49  
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51  
52 255  
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54

### 55 256 **Participants and sample selection**

1  
2  
3 257 Children who participated in the N3RO Trial and were recruited from the five largest  
4  
5 258 recruiting centres, John Hunter Hospital (New South Wales), King Edward Memorial  
6  
7  
8 259 Hospital (Western Australia), Mercy Hospital for Women (Victoria), Royal Women's  
9  
10 260 Hospital (Victoria), and the Women's and Children's Hospital (South Australia) in Australia  
11  
12 261 will be invited to participate in this follow-up study. Children will not be invited if they have  
13  
14 262 previously been withdrawn from the N3RO trial or have died. Of the n=702 children enrolled  
15  
16 263 between the five centres, n=655 will be eligible to be approached for the five-year follow-up  
17  
18 264 once deaths (n=4) and withdrawals (n=43) are excluded.  
19  
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21  
22  
23  
24

265

## 266 **Outcomes and Measures**

### 267 *Primary outcome*

268 The primary outcome is Full-Scale IQ, as assessed by the Wechsler Preschool and  
269 Primary Scale of Intelligence - Fourth Edition, Australian and New Zealand (WPPSI-IV).  
270 The WPPSI-IV is a battery of subtests that provides an assessment of general cognitive  
271 ability for pre-schoolers and young children (2:6 to 7:7 years). The WPPSI-IV has strong  
272 internal consistency and test-retest stability and sound psychometric properties.[28] The  
273 average reliability coefficient for the Full-Scale IQ is 0.95.[28]  
274

274

### 275 *Secondary outcomes*

#### 276 *WPPSI-IV*

277 Other outcomes from the WPPSI-IV will be included as secondary outcomes. These  
278 include Verbal Comprehension, Fluid Reasoning, Working Memory and the Processing  
279 Speed, General Ability and Cognitive Proficiency Primary Index Scales.  
280

1  
2  
3 280 The WPPSI-IV has Australian/New Zealand norms that are age-standardised with a  
4  
5 281 mean of 100 and SD 15. Intellectual impairment will be defined as Full-Scale IQ <85 (i.e. <-1  
6  
7 282 SD), and moderate-severe intellectual impairment as Full-Scale IQ<70 (i.e. <-2 SD). Any  
8  
9 283 impairment on any of the WPPSI-IV Primary Index Scales will be defined as an Index Scale  
10  
11 284 score <85 (i.e. <-1 SD).  
12  
13  
14  
15  
16  
17

285

### 286 *Fruit Stroop*

21 287 The Fruit Stroop was administered to assess two executive functions, inhibition and  
22  
23 288 mental flexibility.[29] The child is required to identify a the correct, natural colour of a series  
24  
25 289 of fruits and vegetables in four 45 s trials under a series of conditions that increase in  
26  
27 290 complexity. The outcome is an interference score calculated as the difference between the  
28  
29 291 number of correct responses on the final (inhibition) trial, and predicted scores on the first  
30  
31 292 and third trials, where lower or negative values indicate more interference.  
32  
33  
34  
35  
36  
37

293

### 294 *Growth*

41 295 Anthropometrics including child height, weight and head circumference will be  
42  
43 296 measured at the appointment as measures of the nutritional well-being of the children.  
44  
45 297 Measurements will be converted to Z (SD) scores appropriate for corrected age and sex.[30]  
46  
47  
48  
49  
50  
51

298

### 299 *Background information and characteristics*

55 300 At enrolment into the N3RO trial a range of socio-demographic data were collected  
56  
57 301 through interview with the caregiver (including parental age, education, and employment). As  
58  
59 302 part of the N3RO trial infant medical records were used to determine a range of baseline and  
60

1  
2  
3 303 outcome clinical characteristics up to 40 weeks' postmenstrual age or first discharge home,  
4  
5 304 whichever occurred first, including for e.g., gestational age, birth weight, sex, and instances  
6  
7  
8 305 of intraventricular haemorrhage.  
9

10  
11 306

### 12 13 14 307 **Sample size calculation**

15  
16 308 A sample size of 296 children per group (total 592) will provide 90% power (two-  
17  
18 309 tailed alpha 0.05) to detect a 4-point (0.27 standard deviation) mean difference in the primary  
19  
20 310 outcome of Full-Scale IQ between groups. The power calculation assumes a design effect due  
21  
22 311 to the inclusion of multiple births of one, since children from a multiple birth were  
23  
24 312 randomized individually in N3RO.[31] Should enrolment be lower than planned, the study  
25  
26 313 will have 80% power to detect a 4-point difference between groups provided at least 222  
27  
28 314 children per group (total 444) provide follow-up data.  
29  
30

31  
32  
33 315

### 34 35 36 316 **Data management and analysis plan**

37  
38  
39 317 All participants were assigned a study identification number at enrolment into the  
40  
41 318 N3RO trial. Throughout the follow-up and analyses, the identification number will be used to  
42  
43 319 identify data. Data will be entered into a REDCap database, which uses a MySQL database  
44  
45 320 via a secure web interface with data checks used during data entry to ensure data quality.  
46  
47 321 REDCap includes a complete suite of features to support the Health Insurance Portability and  
48  
49 322 Accountability Act of 1996 compliance, including a full audit trail, user-based privileges, and  
50  
51 323 integration with the institutional LDAP server.  
52  
53

54  
55  
56 324 All analyses will be conducted according to a pre-specified statistical analysis plan.

57  
58 325 Analyses will not commence until the N3RO trial Steering Committee has approved the  
59  
60

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3 326 statistical analysis plan. Intervention groups will be dummy coded to allow analyses to be  
4  
5 327 performed blinded to treatment group.  
6  
7

8 328 Outcomes of intervention and control group children will be compared using  
9  
10 329 generalised linear models, with generalised estimated equations used to account for clustering  
11  
12 330 due to multiple births within the same family. Continuous and binary outcomes will be  
13  
14 331 analysed using linear and log binomial models, respectively, with adjustment for variables  
15  
16 332 used to stratify the randomisation: sex, centre enrolled, and gestational age (<27 completed  
17  
18 333 weeks' or 27 to <29 weeks' at birth). Pre-planned subgroup analyses will examine the effects  
19  
20 334 of DHA separately for girls or boys (all outcomes), and for infants born at <27 weeks'  
21  
22 335 gestation or 27 to <29 weeks' gestation (primary outcome only). No adjustment will be made  
23  
24 336 for multiple pre-planned comparisons, as the single overall comparison of Full-Scale IQ  
25  
26 337 between groups is of primary interest.  
27  
28  
29  
30  
31

32 338 Missing outcome data will be addressed using multiple imputation, with imputation  
33  
34 339 performed separately by treatment group using fully conditional specification.[32] Imputed  
35  
36 340 datasets will include all surviving children from the five included centres. Children who are  
37  
38 341 missing scores on psychological assessments because they were unable to complete the  
39  
40 342 assessment for cognitive or physical reasons (such as blindness or cerebral palsy) will be  
41  
42 343 reviewed by a psychologist to determine whether assigning the lowest possible score is  
43  
44 344 appropriate.  
45  
46  
47  
48  
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51

## 52 346 **Ethics and dissemination**

53  
54

55 347 This follow-up study will be carried out in accordance with the Australian National  
56  
57 348 Statement on Ethical Conduct in Research Involving Humans, which builds upon the ethical  
58  
59 349 codes of the Declaration of Helsinki and the Principles of International Conference on  
60

1  
2  
3 350 Harmonisation Good Clinical Practice (as adopted in Australia). All procedures and study  
4  
5 351 materials have been reviewed and approved by the Women's and Children's Health Network  
6  
7 352 Human Research Ethics Committee (HREC/17/WCHN/187), as well as the Research  
8  
9  
10 353 Governance officers at each site. The N3RO Trial and this follow-up are registered on the  
11  
12 354 Australia and New Zealand Clinical Trial Registry (ANZCTR: ACTRN12612000503820).

13  
14  
15 355 Caregivers will be provided with a detailed information sheet about the study and will  
16  
17 356 provide informed consent for their child's involvement in the study. Caregivers will be free to  
18  
19 357 re-negotiate consent for each procedure in the follow-up study and are able to decline any  
20  
21 358 part of the follow-up. Caregivers will be free to withdraw their children from the study at any  
22  
23 359 time.

24  
25  
26  
27 360 The results of this follow-up study will be presented at academic conferences and  
28  
29 361 published in peer-reviewed journals. Participating families will receive a lay-report of the  
30  
31 362 study findings. No participants will be identified in the dissemination of study results and  
32  
33 363 data collected will be treated with confidence.

34  
35  
36  
37 364

### 38 39 365 **Access to Data**

40  
41 366 Individual participant data, including data dictionaries, may be shared after de-  
42  
43 367 identification upon reasonable request. Proposals to access the data must be scientifically and  
44  
45 368 methodologically sound and must be reviewed and approved by the N3RO trial Steering  
46  
47 369 Committee and the Women's and Children's Human Research Ethics Committee. To gain  
48  
49 370 access, data requestors will need to sign a data access agreement. Proposals should be  
50  
51 371 directed to Jacqueline Gould through email ([Jacqueline.gould@sahmri.com](mailto:Jacqueline.gould@sahmri.com)).

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55 372

### 56 57 373 **Patient and public involvement**

1  
2  
3 374 Neither patients nor the public were directly involved in the development of the  
4  
5 375 research question or design of this follow-up study. However, our primary outcome of IQ is  
6  
7  
8 376 based on reported concerns over long-term developmental concerns from parents of preterm  
9  
10 377 infants.[33]

11  
12  
13 378 A Community Board, comprising parents (including parents of a child born preterm)  
14  
15 379 as well as clinicians and researchers specialising in paediatrics will be consulted for the  
16  
17  
18 380 dissemination of the study findings to participants, including reviewing the study results and  
19  
20 381 format of dissemination.

21  
22  
23 382

## 24 25 383 **DISCUSSION**

26  
27  
28 384 This protocol details a follow-up of a RCT of a DHA enteral emulsion (60 mg/kg/day)  
29  
30 385 compared with a control emulsion (no DHA), for preterm infants born <29 weeks' gestation  
31  
32 386 in the first months of life, to evaluate the effect on child cognitive ability at 5 years of age.  
33  
34  
35 387 Unlike previous DHA RCTs in preterm populations,[17 18] our follow-up has the benefits of  
36  
37 388 a population likely to be insufficient in DHA,[34] and a robust method of intervention.[24]

38  
39  
40 389 We previously conducted a follow-up of a small sub-group of the N3RO trial infants  
41  
42 390 when they were aged 18 months' corrected age. Children underwent an experimental  
43  
44 391 assessment of visual attention (considered to be a basic, early emergence of higher order  
45  
46 392 cognitive skills known as the executive functions).[35] Where available, Bayley Scales of  
47  
48 393 Infant and Toddler Development-3<sup>rd</sup> edition Cognition, Motor and Language assessment  
49  
50 394 results were collected from hospital records.[35] No statistically significant differences were  
51  
52 395 found for attention, cognition, motor or language abilities.[36] However, assessments of  
53  
54 396 cognition during infancy are considered poor predictors of later performance,[37-41] and the  
55  
56  
57  
58 397 sample was small and under-powered to detect a clinically important effect on cognition.[35]  
59  
60



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2  
3 398 Our sample size calculation for the primary outcome requires a 90% follow-up rate of  
4  
5 399 the N3RO trial children, five years after enrolment. More than 10% loss to follow-up may  
6  
7  
8 400 introduce attrition bias. After completion of the N3RO trial primary outcome analyses,  
9  
10 401 families had the opportunity to request knowledge of their group allocation. Although few  
11  
12 402 families requested this, knowledge of their randomisation group prior to the five-year follow-  
13  
14  
15 403 up assessment may introduce additional bias to the results.

16  
17  
18 404 For this follow-up we have carefully selected a robust assessment of general cognitive  
19  
20 405 abilities, including executive functioning (both of which domains are likely to be adversely  
21  
22 406 affected by very preterm birth)[42-44] to be administered at an age when cognitive domains  
23  
24 407 can be reliably assessed[26 45], as well as ensuring a large, adequately powered sample. As  
25  
26  
27 408 per the recommendations of a consortium of parents and clinicians caring for high-risk  
28  
29 409 preterm infants, we are assessing general cognitive ability using a Wechsler scale, which is  
30  
31 410 considered the gold standard, and have included an assessment of growth.[46] Assessments  
32  
33 411 of respiratory functioning are unreliable in early childhood and hence were not included in  
34  
35  
36 412 this follow-up. It is important that the long-term respiratory effects of DHA supplementation  
37  
38 413 in infants born <29 weeks' gestation is addressed when the N3RO trial children reach an  
39  
40  
41 414 appropriate age.

42  
43  
44 415 This project has global significance, with over one million infants born <29 weeks'  
45  
46 416 gestation each year, and the number rising.[47] The potential benefit of DHA on cognitive  
47  
48 417 performance has never been adequately demonstrated in this population. However, because  
49  
50 418 of the N3RO primary results it is extremely unlikely that such a trial will be repeated. The  
51  
52  
53 419 N3RO cohort may represent the only children in which the longer-term cognitive and  
54  
55 420 behavioural effects of DHA supplementation in these infants can be assessed.  
56  
57  
58  
59  
60

1  
2  
3 421 **Acknowledgements**  
4

5 422 We would like to thank the families who generously participated in the N3RO trial and who  
6  
7 423 will participate in the follow-up study, and the N3RO Steering Committee, Investigative  
8  
9 424 Team and research staff.  
10  
11

12 425

13  
14  
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16  
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18  
19 428 Council (NHMRC) Australia (ID: 1022112 - N3RO trial, 1146806 – 5-year follow-up) and  
20  
21 429 Clover Corporation Limited (Melbourne, Australia).  
22

23  
24 430

25  
26 431 **Competing Interests**

27  
28 432 Study product for the original trial was donated by Clover Corporation Limited (Melbourne,  
29  
30 433 Australia). MM and RAG report holding a patent relating to methods and compositions for  
31  
32 434 promoting the neurological development for preterm infants (2009201540), owned by the  
33  
34 435 South Australian Health and Medical Research Institute and licensed to Clover Corporation  
35  
36 436 Limited.  
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41  
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49  
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 448 responsibility of the authors and do not reflect the views of the NHMRC.

449

#### 450 **List of Abbreviations**

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

458

#### 459 **Authors Contributions**

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

461 *Drafting the protocol:* Gould, Collins, Sullivan.

462 *Comment and approval of the final draft of the protocol:* Gould, Collins, Makrides, Sullivan,

463 Anderson, Gibson, McPhee, Best, Davis, Doyle, Opie, Travadi, Cheong, Sharp, Simmer.

464 *Statistical expertise:* Sullivan.

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

466 *Administrative, technical, or material support:* Gould, Collins, Makrides, Gibson, Sullivan,

467 McPhee, Anderson, Best, Davis, Doyle, Opie, Travadi, Cheong, Sharp, Simmer.

468

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

Section/item	Item No	Description	Addressed on page number
<b>Administrative information</b>			
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 ___

## 1 Introduction

2			
3	Background and	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
4	rationale		_____ 7-8 _____
5			
6		6b	Explanation for choice of comparators
7			_____ 7-8 _____
8	Objectives	7	Specific objectives or hypotheses
9			_____ 8-9 _____
10	Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, or single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)
11			_____ 9-14 _____
12			
13			
14	<b>Methods: Participants, interventions, and outcomes</b>		
15			
16	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
17			_____ 10 _____
18			
19	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)
20			_____ 9-10 _____
21			
22	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered
23			_____ 9 _____
24			
25		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)
26			_____ NA _____
27			
28		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
29			_____ NA _____
30			
31		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial
32			_____ NA _____
33			
34	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
35			_____ 11-13 _____
36			
37	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)
38			_____ 9 _____
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1	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___
2				
3				
4	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	___10-11___
5				

## 6 **Methods: Assignment of interventions (for controlled trials)**

### 7 Allocation:

10	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___
11				
12				
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16	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___
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19				
20	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	___NA___
21				
22				
23				
24	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	___9-10___
25				
26				
27		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	___NA___
28				
29				
30				

## 31 **Methods: Data collection, management, and analysis**

33	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___
34				
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39		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___
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1	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 _____
2				
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5	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 _____
6				
7				
8		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	_____ 13-14 _____
9				
10		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 _____
11				
12				
13				
14	<b>Methods: Monitoring</b>			
15				
16	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 _____
17				
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22		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 _____
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25	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 _____
26				
27				
28	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	_____ NA _____
29				
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32	<b>Ethics and dissemination</b>			
33				
34	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	_____ 14-15 _____
35				
36				
37	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 _____
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1	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
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3				
4		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
5				
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7	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
8				
9				
10	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	18
11				
12				
13	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
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16	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
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20	Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, health care 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
21				
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24		31b	Authorship eligibility guidelines and any intended use of professional writers	NA
25				
26		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA
27				
28				
29	<b>Appendices</b>			
30				
31	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Available upon request
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33				
34	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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\*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](https://creativecommons.org/licenses/by/4.0/)" license.