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Does n-3 LCPUFA supplementation during pregnancy increase the Intelligence Quotient of children at school age? : Follow-up of a Randomised Controlled Trial

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ABSTRACT

Introduction

Despite recommendations that pregnant women increase their docosahexaenoic acid (DHA) intake to support fetal brain development, a recent systematic review found a lack of high-quality data to support the long term-effects of DHA supplementation on children's neurodevelopment.

Methods and Analysis

We will assess child neurodevelopment at 7 years of age in follow-up of a multi-centre double-blind randomised controlled trial of DHA supplementation in pregnancy.

In 2010-2012, n=2399 Australian women with a singleton pregnancy <21 weeks' gestation were randomised to receive three capsules daily containing a total dose of 800 mg DHA/day or a vegetable oil placebo until birth. N=726 children from Adelaide (all n=97 born preterm, random sample of n=630 born at term) were selected for neurodevelopmental follow-up and n= 638 (preterm n=85) are still enrolled at 7 years of age.

At the 7-year follow-up, a psychologist will assess the primary outcome, intelligence quotient, with the Wechsler Abbreviated Scale of Intelligence, 2nd edition. Specific measures of executive functioning (Fruit Stroop and the Rey Complex Figure), attention (Test of Everyday Attention for Children), memory and learning (Rey Auditory Verbal Learning Test), language (Clinical Evaluation of Language Fundamentals, 4th edition) and basic educational skills (Wide Range Achievement Test, 4th edition) will also be administered.

Caregivers will be asked to complete questionnaires measuring behaviour and executive functioning.

Families, clinicians and research personnel are blinded to group assignment with the exception of families who requested un-blinding prior to the follow-up. All analyses will be conducted according to the intention-to-treat principal.

Ethics and Dissemination

All procedures will be approved by the relevant institutional ethics committees prior to commencement of the study. The results of this study will be disseminated in peer reviewed journal publications and academic presentations.

Trial Registration

Australian New Zealand Clinical Trials Registry: www.anzctr.org.au

ACTRN1260500056906 & ACTRN12614000770662

Strengths and Limitations

- This follow-up study builds on a well powered and well-conducted randomised controlled trial
- This follow-up will be one of only 3 to explore the effects of prenatal DHA supplements on child development beyond the age of 3 years
- A comprehensive range of neurological domains are measured in this follow-up
- Given the high usage of prenatal supplements containing DHA internationally, this is likely one of the last opportunities for a large trial to compare a DHA intervention with a placebo
- No planned adjustments for multiple comparisons may be a limitation

Key words

Docosahexaenoic acid, neurodevelopment, omega-3 fatty acids, prenatal, LCPUFA, cognition, child development

INTRODUCTION

The omega-3 long-chain polyunsaturated fatty acid (n-3 LCPUFA), docosahexaenoic acid (DHA, 22:6 n-3), is a crucial nutrient for the developing brain. It is known to be involved in neurogenesis, signal transduction and neurotransmission[1]. During pregnancy DHA is preferentially transferred across the placenta to the fetus in high amounts[2], where it accumulates in developing neural tissues, particularly during the fetal brain growth spurt in the last trimester of pregnancy[3]. The frontal areas of the brain are a primary area of DHA accretion and undergo rapid growth at this time. This area of the brain, specifically the frontal cortex, is important for language, memory and higher-order cognitive functioning, including purposeful, goal-directed behaviours which are often referred to as executive functions[4]. The importance of adequate DHA during this key period of brain development is demonstrated in studies of preterm infants who are denied the full gestation period to accumulate DHA. Infants who are born preterm have lower concentrations of DHA in brain tissues[2] and are at increased risk of developmental delay[5], impaired executive functioning[6], attention problems[7] and attention deficit/hyperactivity disorder[8] compared with their term-born counterparts.

Prenatal DHA Intake and Child Development: Evidence from Cohort Studies

A supply of DHA in the diet is considered important during pregnancy, with fish being the richest source of DHA. The most compelling data linking maternal DHA intake from fish and seafood during pregnancy with childhood intelligent quotient (IQ) comes from a well-conducted cohort study of 5449 mother-child pairs from the Avon Longitudinal Study of Pregnancy and Childhood[9]. Fish and seafood intake above the level recommended for pregnancy by the US government was associated with a

decreased risk of being in the lowest quartile for verbal IQ and suboptimum pro-social behaviour, fine motor, communication and social development scores at 8 years of age[9]. These findings are supported by other smaller cohort studies reporting that seafood intake in pregnancy is associated with developmental benefits in childhood such as advanced motor development, social development[10 11] and language skills at 18 months[10], higher receptive vocabulary at 3 years[12], higher IQ, language and motor development scores at 4 years[13], and reduced hyperactivity, as well as higher verbal IQ at 9 years[14]. Similarly, cohort studies in which blood DHA concentrations were measured at the end of pregnancy reported associations between higher DHA status and improved attention, and reduced distractibility in infants from birth to 18 months[15 16], and better motor development and fewer internalising behaviour problems in children at 7 years of age[17]. Although these epidemiological studies controlled for numerous confounding factors, there is always the possibility that residual or unknown confounding influenced the results[18]. Thus randomised controlled trials (RCT) are essential to establish the extent of benefit of gestational DHA supply on cognition in childhood.

RCT's of Maternal DHA Supplementation and Child Development

There are 11 RCTs investigating the effect of prenatal DHA supplementation on childhood cognitive outcomes that are published or are awaiting publication[16 17 19-33]. However, the majority of these studies have limitations that potentially influenced their results. Most studies did not clearly report, or did not have adequate processes to independently generate the randomisation sequence, or to conceal the random allocation, increasing the risk of selection bias[34]. Furthermore, all studies suffer high attrition (between 27-86%)[15 16 19-27 33], compounding the fact that

many trials were relatively small and therefore underpowered to detect clinically meaningful differences in cognitive outcomes from the beginning[15 16 19-27 32 33]. Other potential biases include systematic post-randomisation losses such as greater attrition from the DHA-supplemented group compared with the control group[19 20], or post-randomisation exclusion criteria[19 20 24 27] and possible publication bias where results from completed trials are not published in full[25 29]. One trial modified the eligibility criteria to include participants taking prenatal supplements containing low dose DHA (up to 200mg/day) after the trial had commenced because the high use of supplements was causing recruitment problems[28].

Given the variation in trial quality, it is not surprising that the results of RCTs investigating the effect of DHA supplementation during that last half of pregnancy on measures of child neurodevelopment have been mixed and largely demonstrated no effect of supplementation[34]. For example, of the 9 trials in which development quotient (DQ) or IQ were assessed, 6 reported no effect of DHA supplementation on DQ or IQ at 10 months[22], 12 months[32], 18 months[27 30 31], 2.5 years[26], 6.5 years[24], 7 years[20] or 12 years of age[33], although positive effects of DHA supplementation are reported by 4 trials on one subtest of a DQ assessment at 18 months[31], 2.5 years[26] and 4 years of age[29], as well as the DQ score at 4 years of age[19]. Most trials assessed too few children (15 to 125 per group) and did not have the statistical power to detect the sort of differences that might realistically be expected between groups as a result of DHA supplementation[34].

The DOMInO (DHA to Optimise Mother Infant Outcome) Trial

Our DOMInO RCT (trial registration #12605000569606 at www.anzctr.org.au) was designed to evaluate the effects of a substantial dose of DHA during the second half

of pregnancy on symptoms of postnatal depression to 6 months postpartum and infant cognitive development at 18 months of age[35]. Women were eligible if they had a singleton pregnancy less than 21 weeks' gestation and were able to give informed consent. Women were excluded if there was a known fetal abnormality, a bleeding disorder, a history of drug or alcohol abuse or English was not spoken in the home (as children undergoing developmental testing were required to understand and take instructions from a psychologist in English). At study entry, women were randomly assigned to receive either a fish oil concentrate (800 mg DHA/day) or a blend of vegetable oils (no DHA) in capsules that were identical in appearance from ~20 weeks' gestation until birth. The DOMInO Trial is the largest RCT of maternal DHA supplementation in pregnancy with n=2,399 women enrolled around Australia.

Outcomes at 18 months: The primary neurodevelopmental outcome was the cognitive scale of the Bayley Scales of Infant Development, Third Edition (Bayley-III) at 18 months of age in a subset of n=726 children (powered to detect a clinically meaningful (4-point) difference in development). The neurodevelopment cohort subset consisted of all preterm children and a random sample of term born children whose mothers were recruited from Adelaide. Secondary outcomes included Bayley-III language and motor scales, as well as developmental delay. We assessed 694 of the 726 (95.6%) infants selected for the neurodevelopment cohort. We found no significant difference between groups in the mean cognitive scores of children whose mothers were assigned to receive DHA supplements compared with those assigned to receive placebo (101.8±11.1 vs 101.8±12.6), although fewer children from the DHA group had scores indicative of mildly delayed cognitive development (DQ<85,

2.7% vs 6.6%, RR 0.41, 95% CI 0.22 to 0.78, p=0.007)[35]. These data are consistent with the Avon Longitudinal Study of Pregnancy and Childhood finding [9] and provide evidence that DHA supplementation is effective at preventing developmental delay in early childhood. Mean language and motor scores did not differ between the groups, although there was a surprising treatment by sex interaction for the language outcome, which indicated that girls and boys responded differently to DHA treatment.

In addition to the 18 month follow-up, a nested side-study assessed early emergence of executive functioning in a nested side-study (n=185) of term-born DOMInO children[36]. The measures used were specialised to detect differences in the early development of the executive functioning skills attention, working memory and inhibitory control. We found no significant group differences[36].

Outcomes at 4 years: To further explore the differences found at 18 months, we assessed neurodevelopment again at 4 years with n=646 (92% of the 726 children in the neurodevelopment cohort) consenting to an assessment with a psychologist (trial registration #12611001125910 at www.anzctr.org.au)[37]. The primary outcome was DQ as assessed by the Differential Ability Scales 2nd Edition. Secondary outcomes were general language ability measured with the Clinical Evaluation of Language Fundamentals Preschool, 2nd Edition, inhibitory control measured with the efficiency score of the Day-Night Stroop, and short term memory measured with the Recognition of Pictures and Recall of Digits Forward tests from the Differential Ability Scales. Parents completed the Strengths and Difficulties Questionnaire (SDQ) and the Behaviour Rating Inventory of Executive Function (BRIEF)-Preschool and provided information regarding family demographics, the child's dietary intake of

DHA-rich foods, such as fish, eggs, DHA-enriched breads and yogurts, use of DHA supplements, parent reported medical diagnosis of Attention Deficit Hyperactivity Disorder (ADHD), autism and behavioural or learning disorders of children, as well as the home environment (Home Screening Questionnaire (HSQ))[38], life events (the Recent Life Events (RLE))[39], and family functioning (the Family Assessment Device (FAD))[40]. As at 18 months, we found no significant mean group difference in general cognitive functioning of children whose mothers were assigned to receive DHA supplements compared with those assigned to receive placebo at 4 years (DHA group DQ=99.6, 95% CI 98.4 to 100.8 vs Control group DQ=99.4, 95% CI 98.3 to 100.6)[25]. We also found that there was no longer a group difference in cognitive delay, and no sex by treatment interactions, although children from the DHA group had slightly poorer scores on the parent-completed measures of behaviour and executive functioning than control-group children, indicating increased parent-perceived problems in the DHA group[37].

Rationale for the Current Follow-Up

We propose to conduct a neurodevelopmental assessment focussed primarily on cognitive functioning at 7 years of age in the DOMInO neurodevelopment cohort. Whilst the previous assessment at 4-years for these children provided an indication of neurodevelopment in the preschool years and school readiness, new skills develop with age, and as a result, long-term effects on cognitive functioning and deficits that emerge in later years need to be examined.

Cognitive skills develop rapidly during early childhood[41] and by age 7 years most cognitive domains can be reliably assessed using valid and standardised instruments[42]. Importantly, a measure of IQ at 7 years of age is predictive of adult

IQ and adult attained education and occupation[43],occupational status, material well-being[44] and mortality risk[45]. Although an assessment after 7 years of age will also provide a predictive measure of adulthood IQ, there is a greater chance of loss to follow-up as the children get older. Assessment at age 7 provides us with the best compromise between an assessment of cognitive function that is predictive of adult functioning with maximal follow-up and the lowest risk of attrition. The suite of developmental assessments at early, middle and late (18 months, 4 and 7 years) childhood is complementary and will provide a more complete picture of the effect of DHA supplementation in pregnancy on children's developmental trajectory during early-mid childhood. Follow-up at 7 years is vital to complete the picture and provide the long-term outcome data necessary to indicate the permanency of any effects of prenatal DHA supplementation. Furthermore, given the information relating to previous trial quality and low power, the methodologically robust and well-powered DOMInO RCT is expected to provide robust data regarding the effect of DHA supplementation in pregnancy on long-term cognitive development of children[34].

Aims and Hypothesis

Our aim is to determine whether DHA supplementation during pregnancy enhances cognitive function at 7 years of age, with our primary outcome being IQ. We hypothesise that children who were exposed to a DHA-rich environment during the second half of gestation will have higher IQ scores at 7 years of age than children whose mothers consumed a regular Australian diet typically low in DHA.

METHODS AND ANALYSIS

Study Design

This is a prospective, follow-up study of children born to women who participated in the DOMInO Trial. Children will be invited to undergo a cognitive assessment with a psychologist when they are 7 years (± 3 months) of age (corrected age for preterm birth). Families could request to be unblinded after completion of analysis of the 18 month results. Families who requested unblinding were given the telephone number of an independent statistician who held the randomisation sequence, and were asked not to discuss treatment allocation with study staff. All families, clinicians and study staff are blinded to group treatment allocation, with the exception of families who requested un-blinding prior to the follow-up.

Setting

Children in the neurodevelopment cohort were born at the Women's & Children's Hospital, or the Flinders Medical Centre, Adelaide, Australia. Appointments will be conducted in study clinics at the hospital and medical centre, where possible. If necessary, appointments will be conducted at the participant's home, or at a location close to their home such as a school or community centre. Appointments will commence March 2013 and will be completed by August 2015.

Participants and Recruitment

All children included in the DOMInO Trial neurodevelopment cohort, who have not died and whose parents have not withdrawn consent, will be invited to participate in the 7 year-follow-up (n =638, 88.2% of the original n=726). Primary carers for eligible children will be initially contacted via an invitation letter sent 3 months prior to the child's 7th birthday, followed by a telephone call. Children in the DOMInO Trial are all

from singleton pregnancies. Figure 1 is a flow chart of the neurodevelopment cohort follow-up assessments according to the CONSORT statement.

Measures

See Table 1 for a summary of all outcomes assessed and measures used.

Primary Outcome: Full scale IQ at 7 years of age will be assessed using the Wechsler Abbreviated Scale of Intelligence, Second Edition (WASI-II)[46]. The WASI-II consists of four subtests (Block Design, Vocabulary, Matrix Reasoning, and Similarities) and provides a brief (average 30 minutes) and reliable estimate of the child's general intellectual functioning. Full scale IQ, Verbal Comprehension Index and Perceptual Reasoning Index scores will be calculated. Each scale is age standardised with a mean of 100 (SD 15). Mild intellectual impairment will be defined as a full-scale IQ from 70 to 84 (from -2 to < -1 SD from the mean), and major intellectual impairment defined as an IQ < 70 (i.e. < -2 SD from the mean). Corrected age will be used to standardise the scores of children who were born preterm.

Secondary Outcomes: Secondary outcomes include neurobehavioural domains that are thought to be sensitive to DHA depletion and are important indicators of child development: executive function, attention, memory and learning and behaviour.

1. *Executive function* will be assessed with the Rey Complex Figure (RCF)[47], the Fruit Stroop test (F-Stroop)[48], the Number Repetition Subtest of the Clinical Evaluation of Language Fundamentals 4th Edition (CELF-4)[49] and the BRIEF[50]. The RCF requires participants to copy a complex geometric figure and evaluates spatial organisation (the ability to perceive and interpret complex spatial stimuli) and strategic decision making (the capacity to plan

ahead and devise efficient and effective strategies to reach a specific goal). The F-Stroop assesses behavioural inhibition and mental flexibility. The Number Repetition from the CELF-4 requires participants to recall a series of digits in the order they were presented, and in the reversed order. It measures working memory, a core element of executive functioning. The BRIEF is a parent-completed questionnaire that is an important adjunct to formal assessment of executive functioning as some elements of executive dysfunction are more obvious in everyday settings such as the home and kindergarten.

2. *Attention* will be assessed using subtests from the Test of Everyday Attention for Children (TEACh)[51]. The TEACh provides a comprehensive assessment of attention skills across different modalities. The subtests to be administered will be Sky Search (selective attention), Score! (sustained attention), Creature Counting (attentional control) and Sky Search Dual Task (divided attention). The divided attention score will be calculated by multiplying the proportion of visual stimuli found by the proportion of auditory stimuli counted, multiplied by 10 (with 10 signifying a perfect score)[52].
3. *Memory & learning* will be assessed with the Rey Auditory Verbal Learning Test (RAVLT)[53].This test is used extensively to assess immediate verbal memory, learning ability and delayed recall. It requires the child to learn a list of 15 spoken words over 5 trials. Delayed recall and recognition trials will also be administered.
4. *Language* will be measured with the core subtests of the Clinical Evaluation of Language Fundamentals 4th Edition (CELF-4)[49]. This test will provide a

Core Language score with a mean of 100 and SD of 15 as a measure of general language abilities.

5. *Behaviour* will be evaluated with the parent version of the Strength and Difficulties Questionnaire (SDQ). The SDQ is a well-validated questionnaire that assesses overall behaviour problems, emotional symptoms, hyperactivity/inattention, peer relationship problems, and prosocial behaviour. As there is growing speculation that DHA plays a role in preventing and reducing ADHD, we will also administer a specific ADHD diagnostic questionnaire, namely the Conners' ADHD/DSM-IV Scales, which will be completed by parents[54].
6. *Academic Abilities/Educational Progress* will be captured with the Word Reading, Spelling and Math Computation subtests of the Wide Range Achievement Test, 4th edition (WRAT-4)[55].

Other Outcomes: Children will have their head, waist and hip circumferences measured, they will be weighed and their height will be measured at the time of the cognitive assessment as an index of the nutritional well-being of children.

Additional Data: Socio-demographic data (such as parental age, education, employment, gestational age at birth, birth weight, birth order, sex) were collected for the DOMInO Trial at trial entry or at birth. Parental education and employment were collected again at the 4 year follow-up and will also be collected at the 7-year follow up, as these details may change over time. Information regarding duration of exclusive breastfeeding, type of infant formula used, age at introduction to solid foods and the number of, and reason for, hospitalisations have all been collected as

part of the DOMInO Trial. At the 7-year follow-up we will again seek information about the child's medically diagnosed conditions (such as Autism Spectrum Disorder or ADHD), medications and hospitalisations. The recent use of DHA supplements and DHA-rich or fortified foods were collected at 18 months and 4 years of age, and will again be collected at 7 years. All hospital admissions >24 hours will be documented as possible adverse events and the frequency of events will be compared between the treatment and control groups. Admission to intensive (Level 3) care or death will be treated as possible serious adverse events.

Table 1. Assessments used at 7 years of age to capture child development

Domains	*Measure*	Respondent
General intellectual ability	Wechsler Abbreviated Scale of Intelligence-2nd Edition	Child
Executive Function	Rey Complex Figure	Child
Executive Function	Fruit Stroop Task	Child
Executive Function	Number Repetition from the Clinical Evaluation of Language Fundamentals-4th Edition	Child
Executive Function	Behaviour Rating Inventory of Executive Functioning	Parent
Attention	Test of Everyday Attention for Children	Child
Memory & learning	Rey Auditory Verbal Learning Test	Child
Language	Clinical Evaluation of Language Fundamentals-4th Edition	Child
Behaviour	Strengths and Difficulties Questionnaire	Parent
Behaviour - ADHD	Conners' ADHD/DSM-IV Scales	Parent
Educational Progress	Wide Range Achievement Test-4th Edition	Child
Growth	Anthropometry	Child
Demographics	Background questions	Parent

Sample Size and Statistical Analysis

There were originally 726 children selected for developmental follow-up. If 80% of the original 726 participate in the 7 year follow-up, we will have at least 89% power to detect a 4-point difference in Full Scale IQ (mean 100, SD 15) between the treatment and control groups (alpha = 0.05, 2-sided). If 75%, or even 70%, of the

original 726 are successfully followed-up at age 7 years, the power remains high (at least 87% and 85% respectively). To achieve a minimum of 80% power, we would need to successfully follow-up 61% of participants (222 per group). Detection of a 4-point difference in IQ is realistic and comparable to the magnitude of cognitive benefit found in 8-year-old children whose mothers consumed 2-3 servings of fish/week during pregnancy compared with those who ate <1 serving/week[9] and the findings from one RCT[19]. Similar sized differences in DQ and IQ have been observed between iron deficient anaemic and non-anaemic children[56] and also in children who were exposed to high and low levels of lead from the environment[57]. In both cases public health policy was changed as a result; infant cereals were fortified with iron and the lead is now removed from petrol and the environment as much as possible.

All analyses will be performed on an intention-to-treat basis according to the mother's allocation to the treatment or control group. No interim analyses will be conducted for this study and all analyses will be performed according to the pre-specified statistical analysis plan. Analyses will be performed using SAS Version 9.3 or later, and Stata Release 13 or later. No data transformations are planned or expected. Both adjusted and unadjusted analyses will be performed, with the adjusted results used to draw conclusion about the effect of treatment on the outcomes of interest. Results will be presented as differences in means for continuous outcomes, or relative risks for binary outcomes, with 95% confidence intervals and 2-sided p-values. Statistical significance will be assessed at the 5% level and no adjustment will be made for the number of analyses planned, as a single primary outcome has been pre-specified for the study.

Potential Confounders: Since recruiting centre and parity were used as stratification variables in the randomisation process, all analyses will be adjusted for centre and parity.

Adjustment will also be made for additional baseline variables that are potential confounders for some outcomes specified *a priori*; these include smoking during pregnancy, maternal secondary education, maternal further education and infant sex.

Primary Outcome: Mean IQ scores will be compared between the treatment groups using a linear regression model.

Secondary Outcomes: Will be analysed using linear regression models for continuous (normally distributed) outcomes and log binomial regression models for binary outcomes.

Secondary Analyses: We will test for evidence of effect modification by sex by including a treatment by sex interaction for primary and secondary neurodevelopmental outcomes, as we have previously found differential effects of DHA supplementation on aspects of the neurodevelopment of boys and girls[35 58].

Missing Data: Data collected on participants up to the point of withdrawal will be included in the analysis. Children who are missing scores on psychological assessments because they were untestable for developmental reasons will be reviewed by a psychologist (who is blinded to treatment group) to determine whether the lowest possible score should be assigned. Multiple imputation will be used to

create 100 complete datasets for analysis using the fully conditional specification method separately by treatment group.

Analyses will be performed on both the raw and imputed data, with conclusions to be drawn based on the results of the analyses performed on the imputed data. Imputed datasets will include all children whose primary carer consented to the follow-up study. Sensitivity analyses will be performed using different imputation models and for all 726 children in the originally selected sub-sample, excluding known deaths.

Accounting for Study Design: The selection procedure for the neurodevelopmental follow-up was stratified by preterm status, sex, recruiting centre and time period. Sampling weights were calculated for each infant as the inverse of the probability of selection. Infants will be weighted according to these sampling weights and the stratification variables will be specified in all analyses.

Ethics and Dissemination

Approval in writing from the Human Research Ethics Committee at each study site (the Women's and Children's Hospital, Adelaide and Flinders Medical Centre, Adelaide) shall be granted prior to the initiation of the study at that site. This study will be carried out in accordance with the Australian National Statement on Ethical Conduct in Research Involving Humans which builds upon the ethical codes of the Declaration of Helsinki and the Principles of International Conference on Harmonisation Good Clinical Practice (as adopted in Australia). Caregivers will be required to provide written informed consent prior to participation in the follow-up study, and will be given a copy of the signed Consent Form and Participant

Information Sheet. Parents will be advised that they are free to decline any aspect of the 7 year follow-up, or withdraw from the study at any time without prejudice.

This is a follow-up study with no active intervention and is considered a low-risk study. The developmental assessments described in this protocol will be conducted by a team of trained assessors supervised by a psychologist. The full suite of assessments will take each child about 2 ½ to 3 hours, including a break between neurodevelopmental assessments. The assessments do not pose any apparent physical risk to children and are enjoyable for 7-year-old children. Given the short and engaging nature of the tasks, children generally maintain interest and concentration throughout the assessment. If a child becomes upset or uncooperative during the assessment, the child will be given time to recover or parents offered the opportunity to return and complete the assessment on another occasion.

Parents will be given $50 to cover travel/parking and childcare expenses of other siblings not attending the appointment.

The results of this follow-up study will be published in peer-reviewed journals and presented at academic conferences. No individual participants will be identified or identifiable. All data will be analysed in de-identified form. Data, both paper copies and the electronic database, will be kept (locked and password protected) for 30 years after completion of the study and publication of the results.

DISCUSSION

Despite the paucity of evidence, recommendations exist internationally to increase DHA intake during pregnancy[59-61] and the nutritional supplement industry markets prenatal DHA supplements to optimise fetal brain development. This project addresses national[62] and international[63 64] calls for rigorous scientific evidence

regarding benefits of fish oil supplementation during pregnancy for child development from RCTs. Such trials are fundamental to establishing a causal link between DHA exposure during gestation and child development. Our follow-up study can provide robust data regarding the potential long-term effects of supplementing the diets of pregnant women with DHA on cognitive functioning in middle childhood. IQ at 7 years is an important outcome as it is known to predict adult IQ, academic achievement, income[43] and employment[43 44]. In fact, a one-point increase in a nation's average IQ is associated with a 0.11% annual increase in quality of life as assessed by gross domestic product per capita[65]. If the results of this study indicate beneficial effects of DHA supplementation, changes to public health policy and subsequent strengthening of human capital has the potential for enormous economic benefits for Australia and the World.

Only two other RCTs have followed children through to 7 years of age after supplementing pregnant women with DHA, however one study only assessed 143 children of the 590 participants included in the trial (75% attrition)[20] and the other study only included 50 of 98 children (49% attrition)[33] which meant the randomisation integrity may not have been maintained in either study.

Strengths of our study include building on a well-powered, well-conducted multi-centre RCT with the highest retention rates to date, and assessment of a range of neurodevelopmental domains. The DOMInO Trial has the broadest inclusion criteria of all the RCTs of DHA supplementation in pregnancy to maximise representativeness of the sample.

The DOMInO Trial is likely one of the last opportunities to compare the effect of a DHA supplement with a placebo due to the high use of prenatal supplements that

contain DHA by pregnant women today. We will provide the first robust data regarding the long-term effects of maternal DHA supplementation during pregnancy.

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Competing Interests

Professor Makrides reports serving on scientific advisory boards for Nestle, Fonterra, and Nutricia. Professor Gibson reports serving on scientific advisory board for Fonterra and Ferrero. Associated honoraria for Professors Makrides and Gibson are paid to their institutions to support conference travel and continuing education for postgraduate students and early career researchers. The remaining authors (JFG, LGS, LNY, KT, PJA, AJMP) and investigators declare that they have no financial disclosures or competing interests.

List of Abbreviations

ADHD	Attention Deficit Hyperactivity Disorder
Bayley-III	Bayley Scales of Infant Development, 3rd Edition
CELF-4	Clinical Evaluation of Language Fundamentals, 4thEdition
DHA	Docosahexaenoic acid
DOMInO	Docosohexanoic Acid to Optimise Mother Infant Outcome
DQ	Development Quotient
F-Stroop	Fruit Stroop Test
IQ	Intelligence Quotient
n-3 LCPUFA	Omega-3 Long Chain Polyunsaturated Fatty Acid
RAVLT	Rey Auditory Verbal Learning Test
RCF	Rey Complex Figure
RCT	Randomised Controlled Trial
SDQ	Strengths and Difficulties Questionnaire
TEACh	Test of Everyday Attention for Children
WASI-II	Wechsler Abbreviated Scale of Intelligence, 2ndEdition
WRAT-4	Wide Range Achievement Test, 4th Edition

Authors Contributions

Study concept and design: Makrides, Smithers, Yelland, Treyvaud, Gould, Anderson, Gibson, McPhee.

Drafting the protocol: Gould, Makrides.

Comment and approval of the final draft of the protocol: Gould, Makrides, Smithers, Yelland, Treyvaud, Anderson, Gibson, McPhee.

Statistical expertise: Yelland, Makrides, Smithers.

Obtained funding: Makrides, Smithers, Yelland, Treyvaud.

Administrative, technical, or material support: Gould, Makrides, Smithers, Yelland, Treyvaud, Anderson, Gibson, McPhee.

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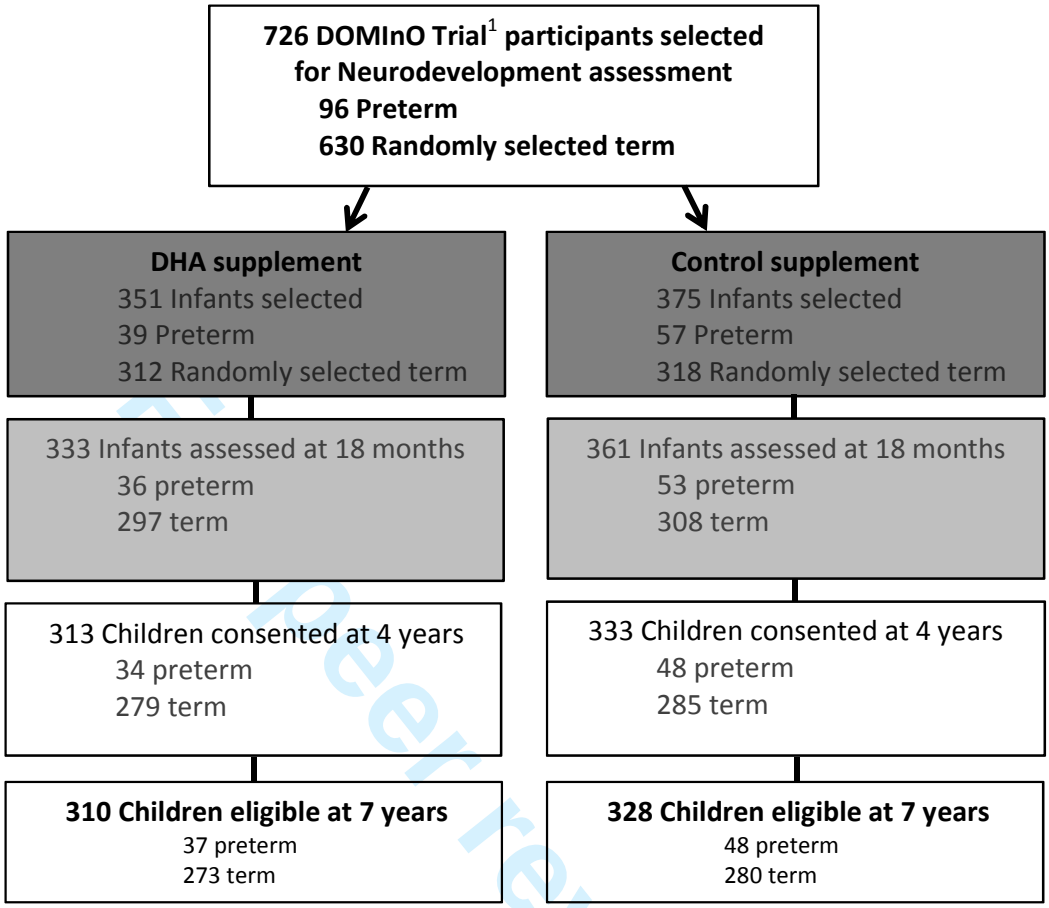
Figure 1. Flow chart of participants selected for neurodevelopment follow-up assessment in the DOMInO Trial

[1] Docosahexaenoic acid to Optimise Mother Infant Outcome Trial



SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

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

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	4
	2b	All items from the World Health Organization Trial Registration Data Set	1-25
Protocol version	3	Date and version identifier	NA
Funding	4	Sources and types of financial, material, and other support	25
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1-2, 26-27
	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)	25-27

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5-12
	6b	Explanation for choice of comparators	9
Objectives	7	Specific objectives or hypotheses	12
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	9,13
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	13
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	9, 13
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	NA
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	NA
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	NA
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	14-18
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)	Figure, 13

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	___18-19___
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	____13_____
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	___NA_____
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	____NA_____
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	_____NA____
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	____13_____
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	____13_____
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	14-16,18-21__
	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	___NA____

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	_____18-21___
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	____19-20____
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	____19-20_____
	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)	_____20-21___
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	_____NA_____
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	____NA_____
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	___17_____
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	_____NA_____
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	_____21-22__
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	____NA_____

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	_____21-22__
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	_____NA____
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	_____22____
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	_____25_____
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	_____22_____
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	___NA_____
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	____NA_____
	31b	Authorship eligibility guidelines and any intended use of professional writers	_26-27
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	_____NA____
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Attached here
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	_____NA

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

BMJ Open

Does n-3 LCPUFA supplementation during pregnancy improve the Intelligence Quotient of children at school age? Follow-up of a Randomised Controlled Trial

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Primary Subject Heading:	Obstetrics and gynaecology
Secondary Subject Heading:	Paediatrics, Nutrition and metabolism
Keywords:	Docosahexaenoic acid, neurodevelopment, omega-3 fatty acids, prenatal, LCPUFA, cognition

Does n-3 LCPUFA supplementation during pregnancy increase the Intelligence Quotient of children at school age? : Follow-up of a Randomised Controlled Trial

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ABSTRACT

Introduction

Despite recommendations that pregnant women increase their docosahexaenoic acid (DHA) intake to support fetal brain development, a recent systematic review found a lack of high-quality data to support the long term-effects of DHA supplementation on children's neurodevelopment.

Methods and Analysis

We will assess child neurodevelopment at 7 years of age in follow-up of a multi-centre double-blind randomised controlled trial of DHA supplementation in pregnancy.

In 2010-2012, n=2399 Australian women with a singleton pregnancy <21 weeks' gestation were randomised to receive three capsules daily containing a total dose of 800 mg DHA/day or a vegetable oil placebo until birth. N=726 children from Adelaide (all n=97 born preterm, random sample of n=630 born at term) were selected for neurodevelopmental follow-up and n= 638 (preterm n=85) are still enrolled at 7 years of age.

At the 7-year follow-up, a psychologist will assess the primary outcome, intelligence quotient, with the Wechsler Abbreviated Scale of Intelligence, 2nd edition. Specific measures of executive functioning (Fruit Stroop and the Rey Complex Figure), attention (Test of Everyday Attention for Children), memory and learning (Rey Auditory Verbal Learning Test), language (Clinical Evaluation of Language Fundamentals, 4th edition) and basic educational skills (Wide Range Achievement Test, 4th edition) will also be administered.

Caregivers will be asked to complete questionnaires measuring behaviour and executive functioning.

Families, clinicians and research personnel are blinded to group assignment with the exception of families who requested un-blinding prior to the follow-up. All analyses will be conducted according to the intention-to-treat principal.

Ethics and Dissemination

All procedures will be approved by the relevant institutional ethics committees prior to commencement of the study. The results of this study will be disseminated in peer reviewed journal publications and academic presentations.

Trial Registration

Australian New Zealand Clinical Trials Registry: www.anzctr.org.au

ACTRN1260500056906 & ACTRN12614000770662

Strengths and Limitations

- This follow-up study builds on a well powered and well-conducted randomised controlled trial
- This follow-up of the DOMInO trial will be one of only 4 randomised controlled trials to explore the effects of prenatal DHA supplements on child development beyond the age of 3 years
- A comprehensive range of neurological domains are measured in this follow-up
- Given the high usage of prenatal supplements containing DHA internationally, this is likely one of the last opportunities for a large trial to compare a DHA intervention with a placebo
- No planned adjustments for multiple comparisons may be a limitation

Key words

Docosahexaenoic acid, neurodevelopment, omega-3 fatty acids, prenatal, LCPUFA, cognition, child development

INTRODUCTION

The omega-3 long-chain polyunsaturated fatty acid (n-3 LCPUFA), docosahexaenoic acid (DHA, 22:6 n-3), is a crucial nutrient for the developing brain. It is known to be involved in neurogenesis, signal transduction and neurotransmission[1]. During pregnancy DHA is transferred across the placenta to the fetus in high amounts[2], where it accumulates in developing neural tissues, particularly during the fetal brain growth spurt in the last trimester of pregnancy[3]. The frontal areas of the brain are a primary area of DHA accretion and undergo rapid growth at this time. This area of the brain, specifically the frontal cortex, is important for language, memory and higher-order cognitive functioning, including purposeful, goal-directed behaviours which are often referred to as executive functions[4]. The importance of adequate DHA during this key period of brain development is indicated in studies of preterm infants who are denied the full gestation period to accumulate DHA. Infants who are born preterm have lower concentrations of DHA in brain tissues[2] and are at increased risk of developmental delay[5], impaired executive functioning[6], attention problems[7] and attention deficit/hyperactivity disorder[8] compared with their term-born counterparts.

Prenatal DHA Intake and Child Development: Evidence from Cohort Studies

A supply of DHA in the diet is considered important during pregnancy, with fish being the richest source of DHA. The most compelling data linking maternal DHA intake from fish and seafood during pregnancy with childhood intelligent quotient (IQ) comes from a well-conducted cohort study of 5449 mother-child pairs from the Avon Longitudinal Study of Pregnancy and Childhood[9]. Fish and seafood intake above

the level recommended for pregnancy by the US government was associated with a decreased risk of being in the lowest quartile for verbal IQ and suboptimum pro-social behaviour, fine motor, communication and social development scores at 8 years of age[9]. These findings are supported by other smaller cohort studies reporting that seafood intake in pregnancy is associated with developmental benefits in childhood such as advanced motor development, social development[10 11] and language skills at 18 months[10], higher receptive vocabulary at 3 years[12], higher IQ, language and motor development scores at 4 years[13], and reduced hyperactivity, as well as higher verbal IQ at 9 years[14]. Similarly, cohort studies in which blood DHA concentrations were measured at the end of pregnancy reported associations between higher DHA status and improved attention, and reduced distractibility in infants from birth to 18 months[15 16], and better motor development and fewer internalising behaviour problems in children at 7 years of age[17]. Although these epidemiological studies controlled for numerous confounding factors, there is always the possibility that residual or unknown confounding influenced the results[18]. Thus randomised controlled trials (RCT) are essential to establish the extent of benefit of gestational DHA supply on cognition in childhood.

RCT's of Maternal DHA Supplementation and Child Development

There are 11 RCTs investigating the effect of prenatal DHA supplementation on childhood cognitive outcomes that are published or are awaiting publication[16 17 19-33]. However, the majority of these studies have limitations that potentially influenced their results. Most studies did not clearly report, or did not have adequate processes to independently generate the randomisation sequence, or to conceal the random allocation, increasing the risk of selection bias[34]. Furthermore, all studies

suffer high attrition (between 27-86%)[15 16 19-27 33], compounding the fact that many trials were relatively small and therefore underpowered to detect clinically meaningful differences in cognitive outcomes from the beginning[15 16 19-27 32 33]. Other potential biases include systematic post-randomisation losses such as greater attrition from the DHA-supplemented group compared with the control group[19 20], or post-randomisation exclusion criteria[19 20 24 27] and possible publication bias where results from completed trials are not published in full[25 29]. One trial modified the eligibility criteria to include participants taking prenatal supplements containing low dose DHA (up to 200mg/day) after the trial had commenced because the high use of supplements was causing recruitment problems[28].

Given the variation in trial quality, it is not surprising that the results of RCTs investigating the effect of DHA supplementation during that last half of pregnancy on measures of child neurodevelopment have been mixed and largely demonstrated no effect of supplementation[34]. For example, of the 9 trials in which development quotient (DQ) or IQ were assessed, 6 reported no effect of DHA supplementation on DQ or IQ at 10 months[22], 12 months[32], 18 months[27 30 31], 2.5 years[26], 6.5 years[24], 7 years[20] or 12 years of age[33], although positive effects of DHA supplementation are reported by 4 trials on one subtest of a DQ assessment at 18 months[31], 2.5 years[26] and 4 years of age[29], as well as the DQ score at 4 years of age[19]. Most trials assessed too few children (15 to 125 per group) and did not have the statistical power to detect the sort of differences that might realistically be expected between groups as a result of DHA supplementation[34].

The DOMInO (DHA to Optimise Mother Infant Outcome) Trial

Our DOMInO RCT (trial registration #12605000569606 at www.anzctr.org.au) was designed to evaluate the effects of a substantial dose of DHA during the second half of pregnancy on symptoms of postnatal depression to 6 months postpartum and infant cognitive development at 18 months of age[35]. Women were eligible if they had a singleton pregnancy less than 21 weeks' gestation and were able to give informed consent. Women were excluded if there was a known fetal abnormality, a bleeding disorder, a history of drug or alcohol abuse or English was not spoken in the home (as children undergoing developmental testing were required to understand and take instructions from a psychologist in English). At study entry, women were randomly assigned to receive either a fish oil concentrate (800 mg DHA/day) or a blend of vegetable oils (no DHA) in capsules that were identical in appearance from ~20 weeks' gestation until birth. The DOMInO Trial is the largest RCT of maternal DHA supplementation in pregnancy with n=2,399 women enrolled around Australia.

Outcomes at 18 months: The primary neurodevelopmental outcome was the cognitive scale of the Bayley Scales of Infant Development, Third Edition (Bayley-III) at 18 months of age in a subset of n=726 children (powered to detect a clinically meaningful (4-point) difference in development). The neurodevelopment cohort subset consisted of all preterm children and a random sample of term born children whose mothers were recruited from Adelaide. Secondary outcomes included Bayley-III language and motor scales, as well as developmental delay. We assessed 694 of the 726 (95.6%) infants selected for the neurodevelopment cohort. We found no significant difference between groups in the mean cognitive scores of children whose mothers were assigned to receive DHA supplements compared with those assigned

to receive placebo (101.8±11.1 vs 101.8±12.6), although fewer children from the DHA group had scores indicative of mildly delayed cognitive development (DQ<85, 2.7% vs 6.6%, RR 0.41, 95% CI 0.22 to 0.78, p=0.007)[35]. These data are consistent with the Avon Longitudinal Study of Pregnancy and Childhood finding [9] and provide evidence that DHA supplementation is effective at preventing developmental delay in early childhood. Mean language and motor scores did not differ between the groups, although there was a surprising treatment by sex interaction for the language and adaptive behaviour outcomes, which indicated that girls and boys responded differently to DHA treatment [35].

In addition to the 18 month follow-up, a side-study assessed early emergence of executive functioning in a nested side-study (n=185) of term-born DOMInO children[36]. The measures used were specialised to detect differences in the early development of the executive functioning skills attention, working memory and inhibitory control. We found no significant group differences[36].

Outcomes at 4 years: To further explore the differences found at 18 months, we assessed neurodevelopment again at 4 years with n=646 (92% of the 726 children in the neurodevelopment cohort) consenting to an assessment with a psychologist (trial registration #12611001125910 at www.anzctr.org.au)[37]. The primary outcome was DQ as assessed by the Differential Ability Scales 2nd Edition. Secondary outcomes were general language ability measured with the Clinical Evaluation of Language Fundamentals Preschool, 2nd Edition, inhibitory control measured with the efficiency score of the Day-Night Stroop, and short term memory measured with the Recognition of Pictures and Recall of Digits Forward tests from the Differential Ability Scales. Parents completed the Strengths and Difficulties Questionnaire (SDQ) and

the Behaviour Rating Inventory of Executive Function (BRIEF)-Preschool and provided information regarding family demographics, the child's dietary intake of DHA-rich foods, such as fish, eggs, DHA-enriched breads and yogurts, use of DHA supplements, parent reported medical diagnosis of Attention Deficit Hyperactivity Disorder (ADHD), autism and behavioural or learning disorders of children, as well as the home environment (Home Screening Questionnaire (HSQ))[38], life events (the Recent Life Events (RLE))[39], and family functioning (the Family Assessment Device (FAD))[40]. As at 18 months, we found no significant mean group difference in general cognitive functioning of children whose mothers were assigned to receive DHA supplements compared with those assigned to receive placebo at 4 years (DHA group DQ=99.6, 95% CI 98.4 to 100.8 vs Control group DQ=99.4, 95% CI 98.3 to 100.6)[25]. We also found that there was no longer a group difference in cognitive delay, and no sex by treatment interactions, although children from the DHA group had slightly poorer scores on the parent-completed measures of behaviour and executive functioning than control-group children, indicating increased parent-perceived problems in the DHA group[37].

Rationale for the Current Follow-Up

We propose to conduct a neurodevelopmental assessment focussed primarily on cognitive functioning at 7 years of age in the DOMInO neurodevelopment cohort. Whilst the previous assessment at 4-years for these children provided an indication of neurodevelopment in the preschool years and school readiness, new skills develop with age, and as a result, long-term effects on cognitive functioning and deficits that emerge in later years need to be examined.

Cognitive skills develop rapidly during early childhood[41] and by age 7 years most cognitive domains can be reliably assessed using valid and standardised instruments[42]. Importantly, a measure of IQ at 7 years of age is predictive of adult IQ and adult attained education and occupation[43],occupational status, material well-being[44] and mortality risk[45]. Although an assessment after 7 years of age will also provide a predictive measure of adulthood IQ, there is a greater chance of loss to follow-up as the children get older. Assessment at age 7 provides us with the best compromise between an assessment of cognitive function that is predictive of adult functioning with maximal follow-up and the lowest risk of attrition. The suite of developmental assessments at early, middle and late (18 months, 4 and 7 years) childhood is complementary and will provide a more complete picture of the effect of DHA supplementation in pregnancy on children's developmental trajectory during early-mid childhood. Follow-up at 7 years is vital to complete the picture and provide the long-term outcome data necessary to indicate the permanency of any effects of prenatal DHA supplementation. Furthermore, given the information relating to previous trial quality and low power, the methodologically robust and well-powered DOMInO RCT is expected to provide robust data regarding the effect of DHA supplementation in pregnancy on long-term cognitive development of children[34].

Aims and Hypothesis

Our aim is to determine whether DHA supplementation during pregnancy enhances cognitive function at 7 years of age, with our primary outcome being IQ. The areas of the brain thought to be most susceptible to DHA exposure in the last trimester of pregnancy when our intervention took place are the frontal lobes. These lobes undergo two critical periods of development during childhood. We expect that the

hypothesised benefits of prenatal DHA supplementation on the performance of these lobes will be detectable following these key development periods[4]. We hypothesise that children who were exposed to a DHA-rich environment during the second half of gestation will have higher IQ scores at 7 years of age than children whose mothers consumed a regular Australian diet typically low in DHA.

METHODS AND ANALYSIS

Study Design

This is a prospective, follow-up study of children born to women who participated in the DOMInO Trial. Children will be invited to undergo a cognitive assessment with a psychologist when they are 7 years (± 3 months) of age (corrected age for preterm birth). Families could request to be unblinded after completion of analysis of the 18 month results. Families who requested unblinding were given the telephone number of an independent statistician who held the randomisation sequence, and were asked not to discuss treatment allocation with study staff. All families, clinicians and study staff are blinded to group treatment allocation, with the exception of families who requested un-blinding prior to the follow-up. At the time of the four-year follow-up, 2% of treatment group families and 5% of control group families had requested to be unblinded, and this knowledge did not appear to influence child DHA intake from food or supplements.

Setting

Children in the neurodevelopment cohort were born at the Women's & Children's Hospital, or the Flinders Medical Centre, Adelaide, Australia. Appointments will be conducted in study clinics at the hospital and medical centre, where possible. If

necessary, appointments will be conducted at the participant's home, or at a location close to their home such as a school or community centre. Appointments will commence March 2013 and will be completed by August 2015.

Participants and Recruitment

All children included in the DOMInO Trial neurodevelopment cohort, who have not died and whose parents have not withdrawn consent, will be invited to participate in the 7 year-follow-up (n =638, 88.2% of the original n=726). Primary carers for eligible children will be initially contacted via an invitation letter sent 3 months prior to the child's 7th birthday, followed by a telephone call. Figure 1 is a flow chart of the neurodevelopment cohort follow-up assessments according to the CONSORT statement.

Measures

See Table 1 for a summary of all outcomes assessed and measures used. The majority of the measures used will be psychologist administered, with the addition of some parent-completed questionnaires. The assessors for the follow-up study will be specifically trained and supervised by a supervising psychologist to ensure standardisation between assessors. All assessors will need to correctly administer an assessment on a non-study participant for approval by the supervising psychologist prior to conducting study appointments. The supervising psychologist will audit the first 20 assessments completed by each assessor, and if standardisation is assured, every fourth assessment by each assessor will be audited.

Primary Outcome: Full scale IQ at 7 years of age will be assessed using the Wechsler Abbreviated Scale of Intelligence, Second Edition (WASI-II)[46]. The WASI-II consists of four subtests (Block Design, Vocabulary, Matrix Reasoning, and Similarities) and provides a brief (average 30 minutes) and reliable estimate of the child's general intellectual functioning. Full scale IQ, Verbal Comprehension Index and Perceptual Reasoning Index scores will be calculated. Each scale is age standardised with a mean of 100 (SD 15). Mild intellectual impairment will be defined as a full-scale IQ from 70 to 84 (from -2 to < -1 SD from the mean), and major intellectual impairment defined as an IQ < 70 (i.e. < -2 SD from the mean). Corrected age will be used to standardise the scores of children who were born preterm.

Secondary Outcomes: Secondary outcomes include neurobehavioural domains that are thought to be sensitive to DHA depletion and are important indicators of child development: executive function, attention, memory and learning and behaviour.

1. *Executive function* will be assessed with the Rey Complex Figure (RCF)[47], the Fruit Stroop test (F-Stroop)[48], the Number Repetition Subtest of the Clinical Evaluation of Language Fundamentals 4th Edition (CELF-4)[49] and the BRIEF[50]. The RCF requires participants to copy a complex geometric figure and evaluates spatial organisation (the ability to perceive and interpret complex spatial stimuli) and strategic decision making (the capacity to plan ahead and devise efficient and effective strategies to reach a specific goal). The F-Stroop assesses behavioural inhibition and mental flexibility. The Number Repetition from the CELF-4 requires participants to recall a series of digits in the order they were presented, and in the reversed order. It measures working memory, a core element of executive functioning. The BRIEF is a

parent-completed questionnaire that is an important adjunct to formal assessment of executive functioning as some elements of executive dysfunction are more obvious in everyday settings such as the home and kindergarten.

2. *Attention* will be assessed using subtests from the Test of Everyday Attention for Children (TEACh)[51]. The TEACh provides a comprehensive assessment of attention skills across different modalities. The subtests to be administered will be Sky Search (selective attention), Score! (sustained attention), Creature Counting (attentional control) and Sky Search Dual Task (divided attention). The divided attention score will be calculated by multiplying the proportion of visual stimuli found by the proportion of auditory stimuli counted, multiplied by 10 (with 10 signifying a perfect score)[52].
3. *Memory & learning* will be assessed with the Rey Auditory Verbal Learning Test (RAVLT)[53].This test is used extensively to assess immediate verbal memory, learning ability and delayed recall. It requires the child to learn a list of 15 spoken words over 5 trials. Delayed recall and recognition trials will also be administered.
4. *Language* will be measured with the core subtests of the Clinical Evaluation of Language Fundamentals 4th Edition (CELF-4)[49]. This test will provide a Core Language score with a mean of 100 and SD of 15 as a measure of general language abilities.
5. *Behaviour* will be evaluated with the parent version of the Strength and Difficulties Questionnaire (SDQ). The SDQ is a well-validated questionnaire that assesses overall behaviour problems, emotional symptoms, hyperactivity/inattention, peer relationship problems, and prosocial behaviour.

As there is growing speculation that DHA plays a role in preventing and reducing ADHD, we will also administer a specific ADHD diagnostic questionnaire, namely the Conners' ADHD/DSM-IV Scales, which will be completed by parents[54].

6. *Academic Abilities/Educational Progress* will be captured with the Word Reading, Spelling and Math Computation subtests of the Wide Range Achievement Test, 4th edition (WRAT-4)[55].

Other Outcomes: Children will have their head, waist and hip circumferences measured, they will be weighed and their height will be measured at the time of the cognitive assessment as an index of the nutritional well-being of children.

Additional Data: Socio-demographic data (such as parental age, education, employment, gestational age at birth, birth weight, birth order, sex) were collected for the DOMInO Trial at trial entry or at birth. Parental education and employment were collected again at the 4 year follow-up and will also be collected at the 7-year follow up, as these details may change over time. Information regarding duration of exclusive breastfeeding, type of infant formula used, age at introduction to solid foods and the number of, and reason for, hospitalisations have all been collected as part of the DOMInO Trial. At the 7-year follow-up we will again seek information about the child's medically diagnosed conditions (such as Autism Spectrum Disorder or ADHD), medications and hospitalisations. All hospital admissions >24 hours will be documented as possible adverse events and the frequency of events will be compared between the treatment and control groups. Admission to intensive (Level 3) care or death will be treated as possible serious adverse events. The recent use

of DHA supplements and DHA-rich or fortified foods were collected at 18 months and 4 years of age, and will again be collected at 7 years.

Table 1. Assessments used at 7 years of age to capture child development

Domains	*Measure*	Respondent
General intellectual ability	Wechsler Abbreviated Scale of Intelligence-2nd Edition	Child
Executive Function	Rey Complex Figure	Child
Executive Function	Fruit Stroop Task	Child
Executive Function	Number Repetition from the Clinical Evaluation of Language Fundamentals-4th Edition	Child
Executive Function	Behaviour Rating Inventory of Executive Functioning	Parent
Attention	Test of Everyday Attention for Children	Child
Memory & learning	Rey Auditory Verbal Learning Test	Child
Language	Clinical Evaluation of Language Fundamentals-4th Edition	Child
Behaviour	Strengths and Difficulties Questionnaire	Parent
Behaviour - ADHD	Conners' ADHD/DSM-IV Scales	Parent
Educational Progress	Wide Range Achievement Test-4th Edition	Child
Growth	Anthropometry	Child
Demographics	Background questions	Parent

Sample Size and Statistical Analysis

There were originally 726 children selected for developmental follow-up. If 80% of the original 726 participate in the 7 year follow-up, we will have at least 89% power to detect a 4-point difference in Full Scale IQ (mean 100, SD 15) between the treatment and control groups (alpha = 0.05, 2-sided). If 75%, or even 70%, of the

original 726 are successfully followed-up at age 7 years, the power remains high (at least 87% and 85% respectively). To achieve a minimum of 80% power, we would need to successfully follow-up 61% of participants (222 per group). Detection of a 4-point difference in IQ is realistic and comparable to the magnitude of cognitive benefit found in 8-year-old children whose mothers consumed 2-3 servings of fish/week during pregnancy compared with those who ate <1 serving/week[9] and the findings from one RCT[19]. Similar sized differences in DQ and IQ have been observed between iron deficient anaemic and non-anaemic children[56] and also in children who were exposed to high and low levels of lead from the environment[57]. In both cases public health policy was changed as a result; infant cereals were fortified with iron and the lead is now removed from petrol and the environment as much as possible.

All analyses will be performed on an intention-to-treat basis according to the mother's allocation to the treatment or control group. No interim analyses will be conducted for this study and all analyses will be performed according to the pre-specified statistical analysis plan. Analyses will be performed using SAS Version 9.3 or later, and Stata Release 13 or later. No data transformations are planned or expected. Both adjusted and unadjusted analyses will be performed, with the adjusted results used to draw conclusion about the effect of treatment on the outcomes of interest. Results will be presented as differences in means for continuous outcomes, or relative risks for binary outcomes, with 95% confidence intervals and 2-sided p-values. Statistical significance will be assessed at the 5% level and no adjustment will be made for the number of analyses planned, as a single primary outcome has been pre-specified for the study.

Potential Confounders: Since recruiting centre and parity were used as stratification variables in the randomisation process, all analyses will be adjusted for centre and parity.

Adjustment will also be made for additional baseline variables that are potential confounders for some outcomes specified *a priori*; these include smoking during pregnancy, maternal secondary education, maternal further education and infant sex.

Primary Outcome: Mean IQ scores will be compared between the treatment groups using a linear regression model.

Secondary Outcomes: Will be analysed using linear regression models for continuous (normally distributed) outcomes and log binomial regression models for binary outcomes.

Secondary Analyses: We will test for evidence of effect modification by sex by including a treatment by sex interaction for primary and secondary neurodevelopmental outcomes, as we have previously found differential effects of DHA supplementation on aspects of the neurodevelopment of boys and girls[35 58].

Missing Data: Data collected on participants up to the point of withdrawal will be included in the analysis. Children who are missing scores on psychological assessments because they were untestable for developmental reasons will be reviewed by a psychologist (who is blinded to treatment group) to determine whether the lowest possible score should be assigned. Multiple imputation will be used to

create 100 complete datasets for analysis using the fully conditional specification method separately by treatment group.

Analyses will be performed on both the raw and imputed data, with conclusions to be drawn based on the results of the analyses performed on the imputed data. Imputed datasets will include all children whose primary carer consented to the follow-up study. Sensitivity analyses will be performed using different imputation models and for all 726 children in the originally selected sub-sample, excluding known deaths.

Accounting for Study Design: The selection procedure for the neurodevelopmental follow-up was stratified by preterm status, sex, recruiting centre and time period. Sampling weights were calculated for each infant as the inverse of the probability of selection. Infants will be weighted according to these sampling weights and the stratification variables will be specified in all analyses.

Ethics and Dissemination

Approval in writing from the Human Research Ethics Committee at each study site (the Women's and Children's Hospital, Adelaide and Flinders Medical Centre, Adelaide) shall be granted prior to the initiation of the study at that site. This study will be carried out in accordance with the Australian National Statement on Ethical Conduct in Research Involving Humans which builds upon the ethical codes of the Declaration of Helsinki and the Principles of International Conference on Harmonisation Good Clinical Practice (as adopted in Australia). Caregivers will be required to provide written informed consent prior to participation in the follow-up study, and will be given a copy of the signed Consent Form and Participant

Information Sheet. Parents will be advised that they are free to decline any aspect of the 7 year follow-up, or withdraw from the study at any time without prejudice.

This is a follow-up study with no active intervention and is considered a low-risk study. The developmental assessments described in this protocol will be conducted by a team of trained assessors supervised by a psychologist. The full suite of assessments will take each child about 2 ½ to 3 hours, including a break between neurodevelopmental assessments. The assessments do not pose any apparent physical risk to children and are enjoyable for 7-year-old children. Given the short and engaging nature of the tasks, children generally maintain interest and concentration throughout the assessment. If a child becomes upset or uncooperative during the assessment, the child will be given time to recover or parents offered the opportunity to return and complete the assessment on another occasion.

Parents will be given $50 to cover travel/parking and childcare expenses of other siblings not attending the appointment.

The results of this follow-up study will be published in peer-reviewed journals and presented at academic conferences. No individual participants will be identified or identifiable. All data will be analysed in de-identified form. Data, both paper copies and the electronic database, will be kept (locked and password protected) for 30 years after completion of the study and publication of the results.

DISCUSSION

Despite the paucity of evidence, recommendations exist internationally to increase DHA intake during pregnancy[59-61] and the nutritional supplement industry markets prenatal DHA supplements to optimise fetal brain development. This project addresses national[61] and international[62 63] calls for rigorous scientific evidence

regarding benefits of fish oil supplementation during pregnancy for child development from RCTs. Such trials are fundamental to establishing a causal link between DHA exposure during gestation and child development. Our follow-up study can provide robust data regarding the potential long-term effects of supplementing the diets of pregnant women with DHA on cognitive functioning in middle childhood. IQ at 7 years is an important outcome as it is known to predict adult IQ, academic achievement, income[43] and employment[43 44]. In fact, a one-point increase in a nation's average IQ is associated with a 0.11% annual increase in quality of life as assessed by gross domestic product per capita[64]. If the results of this study indicate beneficial effects of DHA supplementation, changes to public health policy and subsequent strengthening of human capital has the potential for enormous economic benefits for Australia and the World.

Only two other RCTs have followed children through to 7 years of age after supplementing pregnant women with DHA, however one study only assessed 143 children of the 590 participants included in the trial (75% attrition)[20] and the other study only included 50 of 98 children (49% attrition)[33] which meant the randomisation integrity may not have been maintained in either study.

Strengths of our study include building on a well-powered, well-conducted multi-centre RCT with the highest retention rates to date, and assessment of a range of neurodevelopmental domains. The DOMInO Trial has the broadest inclusion criteria of all the RCTs of DHA supplementation in pregnancy to maximise representativeness of the sample.

The DOMInO Trial is likely one of the last opportunities to compare the effect of a DHA supplement with a placebo due to the high use of prenatal supplements that

contain DHA by pregnant women today. We will provide the first robust data regarding the long-term effects of maternal DHA supplementation during pregnancy.

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Competing Interests

Professor Makrides reports serving on scientific advisory boards for Nestle, Fonterra, and Nutricia. Professor Gibson reports serving on scientific advisory board for Fonterra and Ferrero. Associated honoraria for Professors Makrides and Gibson are paid to their institutions to support conference travel and continuing education for postgraduate students and early career researchers. The remaining authors (JFG, LGS, LNY, KT, PJA, AJMP) and investigators declare that they have no financial disclosures or competing interests.

List of Abbreviations

ADHD	Attention Deficit Hyperactivity Disorder
Bayley-III	Bayley Scales of Infant Development, 3rd Edition
CELF-4	Clinical Evaluation of Language Fundamentals, 4thEdition
DHA	Docosahexaenoic acid
DOMInO	Docosohexanoic Acid to Optimise Mother Infant Outcome
DQ	Development Quotient
F-Stroop	Fruit Stroop Test
IQ	Intelligence Quotient
n-3 LCPUFA	Omega-3 Long Chain Polyunsaturated Fatty Acid
RAVLT	Rey Auditory Verbal Learning Test
RCF	Rey Complex Figure
RCT	Randomised Controlled Trial
SDQ	Strengths and Difficulties Questionnaire
TEACh	Test of Everyday Attention for Children
WASI-II	Wechsler Abbreviated Scale of Intelligence, 2ndEdition
WRAT-4	Wide Range Achievement Test, 4th Edition

Authors Contributions

Study concept and design: Makrides, Smithers, Yelland, Treyvaud, Gould, Anderson, Gibson, McPhee.

Drafting the protocol: Gould, Makrides.

Comment and approval of the final draft of the protocol: Gould, Makrides, Smithers, Yelland, Treyvaud, Anderson, Gibson, McPhee.

Statistical expertise: Yelland, Makrides, Smithers.

Obtained funding: Makrides, Smithers, Yelland, Treyvaud.

Administrative, technical, or material support: Gould, Makrides, Smithers, Yelland, Treyvaud, Anderson, Gibson, McPhee.

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Figure 1. Flow chart of participants selected for neurodevelopment follow-up assessment in the DOMInO Trial

[1] Docosahexaenoic acid to Optimise Mother Infant Outcome Trial

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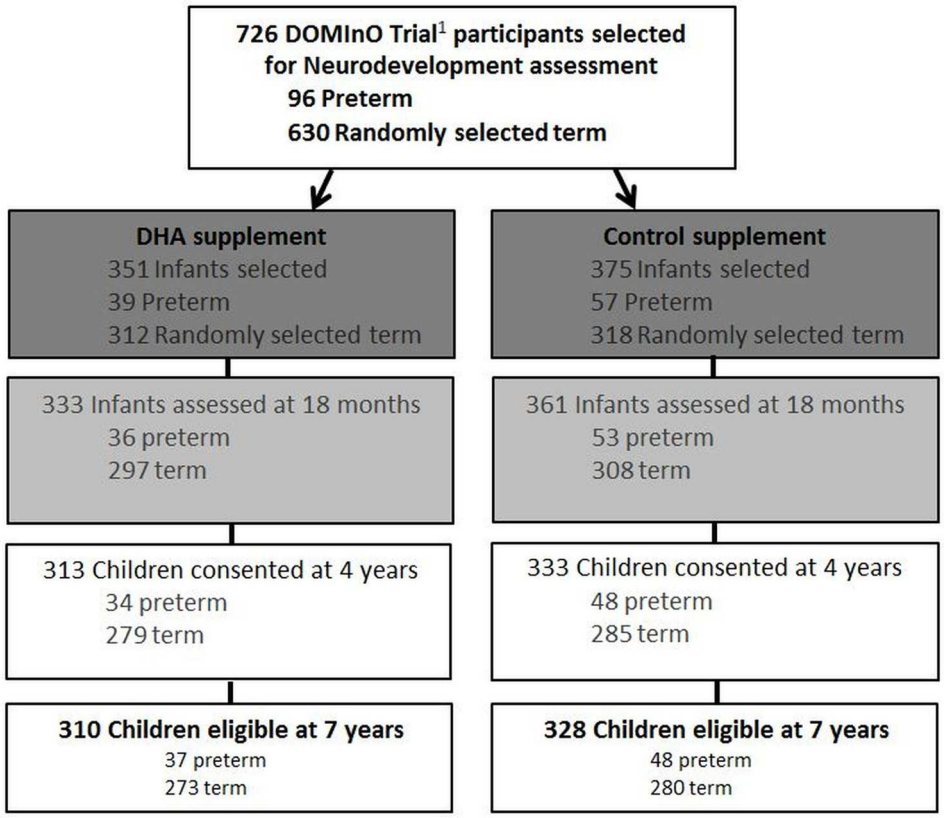

Figure 1. Flow chart of participants selected for neurodevelopment follow-up assessment in the DOMInO Trial
1 Docosahexaenoic acid to Optimise Mother Infant Outcome Trial

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SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

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

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	_____1_____
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	_____4_____
	2b	All items from the World Health Organization Trial Registration Data Set	_____1-25
Protocol version	3	Date and version identifier	_____NA____
Funding	4	Sources and types of financial, material, and other support	____25_____
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	__1-2, 26-27_____
	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)	_____25-27_____

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5-12
	6b	Explanation for choice of comparators	9
Objectives	7	Specific objectives or hypotheses	12
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	9,13
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	13
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	9, 13
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	NA
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	NA
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	NA
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	14-18
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)	Figure, 13

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	___18-19___
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	____13_____
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	___NA_____
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	____NA_____
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	_____NA____
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	____13_____
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	____13_____
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	14-16,18-21__
	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	___NA____

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	_____18-21___
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	____19-20____
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	____19-20_____
	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)	_____20-21___
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	_____NA_____
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	____NA_____
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	___17_____
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	_____NA_____
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	_____21-22__
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	____NA_____

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	_____21-22__
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	_____NA____
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	_____22____
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	_____25_____
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	_____22_____
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	___NA_____
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	____NA_____
	31b	Authorship eligibility guidelines and any intended use of professional writers	_26-27
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	_____NA____
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Attached here
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	_____NA

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