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Study protocol for a randomized controlled trial evaluating the effect of prenatal omega-3 LCPUFA supplementation to reduce the incidence of preterm birth: The ORIP trial

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Abbreviations

AA, arachidonic acid; ACTRN, Australia and New Zealand Clinical Trial Registry; CRF, case report form; DOMInO, DHA to Optimise Mother and Infant Outcome; DHA, docosahexaenoic acid; DMAC, data management and analysis centre; EPTB, Early preterm birth; EPA, eicosapentaenoic acid; EDD, estimated date of delivery; GA, gestational age; Good Clinical Practice; GMP, code of good manufacturing practice; HREC, Human Research Ethics Committee; ID, Identifier; LA, linoleic acid; LCPUFA, long-chain polyunsaturated fatty acids; mg, milligram; ω-3, omega-3; ω-6, omega-6; ORIP, the acronym is derived from 'Omega-3 fatty acids to reduce the incidence of prematurity'; NHMRC, National Health and Medical Research Council of Australia; RR, Relative Risk; SAE, Serious Adverse Event.

ABSTRACT

Introduction: Preterm birth accounts for more than 85% of all perinatal complications and deaths. Seventy-five percent of early preterm births occur spontaneously and without identifiable risk factors. The need for a broadly applicable, effective strategy for primary prevention is paramount. Secondary outcomes from the Docosahexaenoic Acid (DHA) to Optimize Mother Infant Outcome (DOMInO) trial showed that maternal supplementation until delivery with omega-3 long chain polyunsaturated fatty acid (LCPUFA), predominantly as DHA, resulted in a 50% reduction in the incidence of early preterm birth, but also an increase in the incidence of post-term induction or post-term pre-labour caesarean section due to extended gestation. We aim to determine the effectiveness of supplementing the maternal diet with omega-3 LCPUFA until 34 weeks' gestation on the incidence of early preterm birth.

Methods and analysis: This is a multicentre, parallel group, randomised, blinded and controlled trial. Women less than 20 weeks' gestation with a singleton or multiple pregnancy and able to give informed consent are eligible to participate. Women will be randomised to receive high DHA fish oil capsules or control capsules without DHA. Capsules will be taken from enrolment until 34 weeks' gestation. The primary outcome is the incidence of early preterm birth, defined as delivery before 34 completed weeks' gestation. Key secondary outcomes include length of gestation, incidence of post-term induction or pre-labour caesarean section and spontaneous early preterm birth. The target sample size is 5540 women (2770 per group), which will provide 85 % power to detect an absolute reduction in the incidence of preterm birth of l.16% (from 2.45% to 1.29%) between the DHA and control group (two sided α =0.05). The primary analysis will be based on the intention-to-treat principle.

Ethics and dissemination: This study has been approved by the Human Research Ethics Committee of each study site. Study findings will be disseminated via peer-reviewed publication, conference presentations, informing participants with a lay summary and the wider community through social media.

Trial Registration: Australia and New Zealand Clinical Trial Registry Number: ACTRN12613001142729

Strengths and limitations of this study

- This study is the largest randomised controlled trial with adequate power that is designed to assess the effect of ω-3 LCPUFA supplementation in pregnancy on the incidence of early preterm birth as the primary outcome.
- The study uses an innovative intervention strategy, which is unique compared with other trials in this field. The intervention targets the period before and during the time when early preterm birth is likely to occur and it is ceased at 34 weeks' gestation, at which time early preterm birth has been prevented. This strategy may reduce the risk of post-term birth.
- The study assesses maternal LCPUFA status at baseline and at the end of the intervention period, providing an independent biomarker of compliance, in addition to maternal self-report and capsule back count.
- The study is conducted in an industrialised setting where intake of ω-3 LCPUFA is low. Findings from this study may not be applicable in populations with high intake of ω-3 LCPUFA.

INTRODUCTION

Background

Preterm birth, or birth before 37 completed weeks of pregnancy, is the leading cause of death in newborns, accounting for more than 85% of all perinatal complications and death.[1] It is estimated that 10% of births (13 million babies each year worldwide) are preterm and 20% of these preterm births occur before 34 weeks, these being referred to as early preterm birth (EPTB). It is EPTB that is the major cause of perinatal mortality, serious neonatal morbidity and moderate to severe childhood disability in developed countries.[2-4] Effective, broadly applicable primary prevention strategies to reduce the risk of EPTB are lacking.

Prostaglandins and other lipid mediators derived from omega-6 (ω-6) and omega-3 (ω-3) fatty acids play essential roles in normal and pathologic initiation of labour.[5 6] The feto-placental unit is supplied with long chain polyunsaturated fatty acid (LCPUFA) from the maternal circulation, which is influenced by maternal LCPUFA intake and endogenous synthesis. Prostaglandins derived from ω-6 arachidonic acid (AA) are countered by those derived from ω-3 LCPUFA within the same tissues. The balance between the metabolites of ω-3 and ω-6 fatty acids play an important role in the maintenance of normal gestation length and are a critical element in cervical ripening and the initiation of labour.[7] If local production of ω-6 derived prostaglandins within the feto-placental unit is too high, or local accumulation of ω-3 LCPUFA is too low, the cervix may prematurely ripen and uterine contractions increase, which may in turn lead to preterm birth or EPTB.

Current Western diets are low in ω-3 LCPUFA.[8] The World Health Organization recommends an intake of 300 mg/d of ω-3 LCPUFA for pregnant women, however, the median intake in Australian women of child bearing age is only 29 mg/day[9] compared with 1000mg/d in women from nations with high-fish consumption such as Japan, Korea and Norway. The intake of ω-3 LCPUFA is further reduced among pregnant women due to a reduced intake of all fish

following advisories for pregnant women to avoid specific long-lived predatory fish due to the concern about mercury contamination.[10] The level of ω-3 LCPUFA intake estimated to achieve normal rises in the feto-placental unit for the maintenance of a full term pregnancy is well above the intake of many pregnant women, highlighting a potential insufficiency for the uterus and feto-placental unit. Optimising ω-3 LCPUFA intake in pregnancy may restore the balance between ω-3 and ω-6 fatty acids and reduce the risk of preterm birth.

At the time the trial commenced, only 2 of the 6 included trials in the Cochrane review[11] of marine oil supplementation in pregnancy reported the effect on EPTB. Both trials[12 13] were conducted in high risk pregnancies designed to assess the effect of ω-3 LCPUFA supplementation to prevent reoccurrence of intrauterine growth restriction, preterm birth or pregnancy induced hypertension. From these two relatively small trials (total of 860 women), it was seen that women allocated to receive fish oil had a lower risk of EPTB compared with control (relative risk (RR) 0.69, 95% CI 0.49 to 0.99). Systematic reviews[11 14 15] have shown no difference in the incidence of antepartum hospitalisation, caesarean section, eclampsia or other serious maternal morbidity between treatment (ω-3 LCPUFA supplements) and control groups. The relative risk of miscarriage, stillbirth and neonatal death also did not differ between the groups[11 14 15]. None of the included trials reported the effect of ω-3 LCPUFA supplementation on EPTB in the general population of pregnant women.

The strongest evidence to support the efficacy of ω-3 LCPUFA supplementation to reduce EPTB comes from our Docosahexaenoic Acid [DHA] to Optimize Mother Infant Outcome [DOMInO] trial[16], the largest trial to date of ω-3 LCPUFA supplementation in pregnancy. DOMInO was designed to assess the effect of ω-3 LCPUFA supplementation during the last half of pregnancy on the prevalence of postnatal depression in women and on early childhood neuro-developmental outcomes. We enrolled 2399 women from 5 perinatal centres around Australia who were randomly assigned to receive identical looking capsules containing either

fish oil concentrate (900 mg ω-3 LCPUFA/day) or a blend of vegetable oils (no ω-3 LCPUFA) from 20 weeks' gestation until birth. In a pre-specified secondary analysis, there were fewer EPTB in the ω-3 LCPUFA supplemented group compared with control (1.09% vs. 2.25%, adjusted RR 0.49, 95% CI 0.25 to 0.94, p=0.03) and fewer admissions to level three intensive care (1.75% vs. 3.08%, RR 0.57, 95% CI 0.34 to 0.97, p=0.04). Similar findings were reported in a smaller US trial (n=301), the Kansas University DHA Outcomes (KUDOS) Study[17], which showed that supplementation with 600 mg of DHA daily from mid-pregnancy until birth resulted in a reduction in the incidence of EPTB (0.6% vs. 4.8%, p=0.025). The effect of DHA supplementation in pregnancy in lowering the risk of EPTB observed in both the DOMInO and KUDOS trials is consistent with the Cochrane review[18] published before these two trials. Despite these encouraging findings, results have been viewed with caution by researchers and clinicians because EPTB was a secondary outcome of the DOMInO and KUDOS trials. A recent update to this Cochrane review (publication pending) includes 54 randomised trials with a total of >15,000 participants. Results show a clear reduction in EPTB (RR 0.61, 95% CI 0.46 to 0.81; 9 RCTs; n = 4351) for supplementation compared with placebo[19]. Our DOMInO trial supports the safety data from previous systematic reviews, however there were more post-term births requiring obstetric intervention (induction or caesarean section) in the ω-3 LCPUFA supplemented group compared with control (17.59% vs. 13.72%, adjusted RR 1.28, 95% CI 1.06 to 1.54, p=0.01). To determine both efficacy and safety of this intervention, we therefore planned to undertake a further trial with adequate power to assess the effect of ω-3 LCPUFA supplementation in pregnancy on EPTB as the primary outcome, with a unique design ceasing the intervention at 34 weeks' gestation to reduce the risk of post-term birth.

Hypotheses

Optimising ω-3 LCPUFA intake in pregnancy will lead to lower risk of EPTB.

METHODS

Trial design

The ORIP trial is designed as a randomised, controlled, clinician, researcher and participant/family blinded, multicentre trial with two parallel groups and a primary outcome of incidence of EPTB, defined as delivery before 34 completed weeks' gestation.

Participating Centres

The sponsoring institution and Trial Coordinating Centre is the South Australian Health and Medical Research Institute based at the Women's and Children's Hospital, Adelaide, South Australia. The trial is being conducted in the following six centres in Australia: Women's & Children's Hospital, Flinders Medical Centre and Lyell McEwin Hospital in South Australia; Joondalup Health Campus, Western Australia; Werribee Mercy Hospital, Werribee, Victoria; and Mater Mothers Hospital, Brisbane, Queensland.

Study Population

Participants are pregnant women less than 20 weeks' gestation.

Eligibility Criteria

Pregnant women are approached by trained clinical trial personnel in the antenatal clinic of participating centres.

Inclusion Criteria

Women must meet all the following criteria to be enrolled in this study:

- Singleton or multiple pregnancy and less than 20 weeks' gestation.
- Able to give informed consent.

Exclusion criteria

Women must not have any of the following criteria to be enrolled in this study:

- Fetus with a known fetal abnormality.
- Taking dietary supplements containing LCPUFA > 150mg/day.
- Taking dietary supplements containing LCPUFA ≤150mg/day and are not willing to stop.
- Bleeding disorders where fish oil is contraindicated or on anticoagulant therapy.
- History of drug or alcohol abuse.

Study treatments

Participating women will be randomised to high-DHA fish oil capsules or vegetable oil capsules. The study capsules are identical in colour, size, shape and packaging and are iso-caloric. Women will be asked to consume three capsules per day.

DHA Capsules

Each 500mg DHA enriched fish oil capsule contains approximately 300mg of ω-3 LCPUFA. Women will be asked to take three capsules per day orally, which will provide approximately 1g of ω-3 LCPUFA (800mg of DHA and 100 mg of eicosapentaenoic acid [EPA]) per day. The remaining portion of oil consists of monounsaturated and saturated fatty acids.

Control Capsules

The control capsules (also 500mg in weight) contain a blend of vegetable oils (canola, sunflower and palm oils) with a trace of tuna oil (12.6mg ω-3 LCPUFA) to assist with blinding. This blend of oil matches the fatty acid composition of the typical Australian diet.

Manufacture of study capsules

The active and control capsules are manufactured in a licensed facility (licensed according to the Code of Good Manufacturing Practice (GMP)) and have been donated to the trial by Efamol Ltd/Wassen International Ltd. The capsules are packaged and labelled in accordance with GMP including an individual product identifier (ID), batch number, expiry date and the statement “for clinical trial use only”. The pharmacist or the investigator’s designee maintain

accurate records of the receipt of all study capsules, including dates of receipt. Unused study capsules will be destroyed in compliance with applicable regulations.

Monitoring adherence to study treatment

Research personnel in each centre will maintain regular contact with participating women to monitor and encourage capsule adherence and study compliance and answer any questions as they arise. Women are asked to return unused capsules at the final study visit (34 weeks' gestation) and the proportion of capsules returned serves as an additional measure of compliance. A finger prick blood specimen collected from the woman at this final visit will be used in fatty acid analysis as an independent biomarker of adherence. All clinical care for women involved in this trial will be undertaken by her designated obstetric/midwifery care provider.

Outcome Measures

The primary outcome of this trial is the incidence of EPTB, defined as delivery before 34 completed weeks' gestation. Gestational age (GA) in days at delivery for primary and secondary outcomes will be determined from the estimated date of delivery (EDD) using the equation [280 - (EDD - date of birth)]. EDD will be determined using a combination of maternal menstrual dating and ultrasound dating in accordance with established obstetric practice, as outlined in Figure 1.

Key Secondary outcomes:

- Length of gestation
- Post-term induction or post-term pre-labour caesarean section
- Preterm birth (<37 completed weeks)
- Spontaneous EPTB,
- Birth weight
- Low birth weight (< 2500g).

- Admission to Neonatal Intensive Care Unit
- Perinatal Death (stillbirth and death within first 28 days of birth)

Other Outcomes

- Spontaneous preterm birth, pre-labour rupture of membranes, pre-labour, preterm rupture of membranes.
- Birth length, birth head circumference, small for GA, very small for GA, large for GA, very large for GA.
- The length of hospital stay of infants and mothers, length of admission in Neonatal Intensive Care Unit.
- Pregnancy and labour outcomes including; gestational diabetes, pregnancy induced hypertension, pre-eclampsia, eclampsia, maternal use of antibiotics, caesarean section, estimated blood loss at birth and post-partum haemorrhage.
- Incidence of Serious Adverse Event (SAE) defined as; maternal, neonatal or fetal deaths (still birth); fetal loss (miscarriage); maternal admissions to intensive care; admissions to intensive care for infants born after 34 weeks' gestation; and major congenital anomalies.
- Neonatal complications including jaundice (requiring phototherapy), hypoglycaemia (requiring intravenous dextrose infusion), neonatal convulsion, brain injury (including intra-ventricular haemorrhage), retinopathy of prematurity, sepsis, necrotising enterocolitis and duration of supplemental oxygen. The definitions and classifications of these neonatal complications are in accordance with the Australian and New Zealand Neonatal Network Data Dictionary.[20]
- Other side effects and tolerability of DHA supplementation assessed through data collected in the case report form.

Participant Timeline

Women will be randomised and commence study treatment capsules from enrolment (<20 weeks' gestation) until 34 weeks' gestation or birth, whichever occurs first. At enrolment, following informed consent and prior to commencement of study treatment, research personnel will collect a finger prick blood sample for baseline fatty acid status. The woman's height and weight will be measured and baseline clinical and demographic data collected. Research personnel will contact the participant two weeks following the enrolment visit and again when the woman is 28 weeks' gestation to ascertain capsule adherence and record adverse events. At 34 weeks' gestation participating women will attend a clinic appointment for collection of a finger prick blood sample for fatty acid analysis and unused capsules are returned and counted. Six weeks following delivery, research personnel will extract details of pregnancy, labour and birth from the woman and her baby's medical records for primary and secondary outcome data; see Figure 2 for a summary of the ORIP trial schema.

Sample Size

Our previous trial of DHA supplementation of pregnant women, the DOMInO trial[16], included women with singleton pregnancies and treatment resulted in a 1.16% absolute reduction (from 2.25% to 1.09%) in the incidence of EPTB. This reduction occurred in the presence of non-adherence resulting from women assigned to the intervention failing to take the trial supplements as directed, and control women taking prenatal supplements containing low dose ω-3 LCPUFA. Similar levels of non-adherence are expected in the ORIP trial. Inclusion of women with multiple pregnancies in the ORIP trial is expected to result in a small increase in the incidence of EPTB in both treatment groups, since EPTB is more common in multiple pregnancies. To demonstrate an absolute reduction in the incidence of EPTB of 1.16% (from 2.45% to 1.29%) with 85% power and overall two-sided alpha=0.05 (alpha=0.049 at the final analysis), a sample size of 2631 per group (i.e. 5262 total) is required. Allowing for a loss to follow up of 5%, a total of 5540 women is required to ensure adequate power for the primary

outcome. Since EPTB is a mother level outcome, the sample size calculations define the number of mothers that should be recruited and no adjustment for clustering due to multiple pregnancies is required for this outcome.

Recruitment

Women will be approached to enter the trial by research staff at the time of attending their routine antenatal visit at one of the centres involved. Pregnant women will also be informed about the trial by display of approved advertising material which will be circulated in hard copy and via social media avenues. A participant information sheet describing the purpose of the study, the procedures to be followed, and the risks and benefits of participation will be explained to interested women. Following a screening process to ensure inclusion and exclusion criteria are met, the investigator, or nominee will conduct the informed consent discussion and will check that information provided is understood and answer any questions about the study. A record of all women screened and their enrolment status will be maintained to adhere to the consolidated standards for the reporting of randomised controlled trials.[21] Women may withdraw their involvement in the trial at any time and wherever possible the reason for withdrawal will be recorded.

Randomisation procedures

Once the consent process has been documented by signing of the written consent form, the participant will be randomised using a web-based randomisation service. Stratification will be by centre and current supplement use (no LCPUFA vs ≤150mg LCPUFA/day prior to randomisation). Allocation follows a computer-generated randomisation schedule with balanced variable blocks, prepared using ralloc.ado version 3.7.5 in Stata version 12.1 by an independent statistician who is not involved with trial participants or data analysis. A unique six-digit study ID and a study pack with a unique product ID are assigned. The study ID

identifies the randomised woman and the product ID identifies a pack of five bottles of either active or control capsules, pre-packaged corresponding to the randomisation schedule.

Blinding

Participants and their family, care providers, data collectors, outcome assessors, research personnel and data analysts will all be blinded to randomisation group. The intervention and control capsules are identical in shape, size, colour, packaging and labelling and uniquely identified only by the product ID. In the DOMInO trial, we identified that more women in the intervention group correctly guessed their group allocation. We therefore added a small amount of tuna oil (5%) to the control capsules to enhance blinding of study participants. The randomisation code for an individual participant may be un-blinded in an emergency if the Site Investigator decides a participant cannot be adequately treated without knowing their study treatment allocation. The Principal Investigator will be notified and must contact the Data Management and Analysis Centre, where the randomisation list is securely stored. The time, date, study ID of the participant and reason for un-blinding must be documented and events leading to the emergency un-blinding will be recorded.

Data collection and trial management

A purpose-built web-based clinical trial management information system (CTMS) with password protection and defined user-level access is used to facilitate trial management. A record of all women approached, screened for eligibility, and consented is recorded in real time in the CTMS. Once consented and randomised, the CTMS automatically calculates study milestones for each participant. This information is readily available for clinical trial staff to enable scheduling of appointments and sample collection. Data entered by individual study centres is routinely scrutinized by the Coordinating Centre to monitor protocol adherence and study progress. Summary reports including screening data, enrolment, appointment attendance, sample collection, serious adverse events and study completion are generated from the CTMS

and reviewed at monthly trial steering committee meetings. Site monitoring visits to ensure compliance with good clinical practice and the study protocol are conducted at site start-up and then six monthly or as required to ensure the integrity of the trial.

Data are collected by trained clinical trial staff at each participating centre onto carbon copy paper case report forms (CRF). Carbon copies of the completed CRF remain on site at participating centres with original copies being mailed to the Data Management and Analysis Centre at the University of Adelaide for data management including data entry and data cleaning. Data validity checks are completed by data entry personnel and data queries posted to the CTMS for resolution by participating centres. Data queries generated by statisticians during regular blinded reviews of data quality and batch checks are also managed through the CTMS. Electronic data are stored on secure servers at the Data Management and Analysis Centre and released only to persons authorised to receive those data. Following study completion, copies of study documents are retained at each study site or in archives. Documents held at the Coordinating Centre will be retained for at least 30 years after study completion in line with the data retention schedules for research involving minors[22]. At the completion of this time, documentation will be destroyed using confidential document disposal. Electronic data will be stored indefinitely on secure servers with access only granted to authorised study personnel.

Statistical methods

All participants will be analysed according to the group to which they were randomised (intention-to-treat principle) for the primary analysis. The primary outcome of EPTB will be compared between intervention and control groups using a log binomial regression model. Adjustment will be made for the stratification variables centre and supplements, and results will be presented as a relative risk with confidence interval and 2-sided p-value. Statistical significance will be assessed at an alpha of 0.049 to account for the pre-specified interim analysis (see below) using the O'Brien-Fleming approach. Key secondary and other outcomes

will be compared between treatment groups using log binomial regression models for binary outcomes and linear regression models for continuous outcomes with no adjustment for multiple comparisons. Clustering due to multiple pregnancies and repeat participation in the trial will be taken into account where applicable using generalised estimating equations. Secondary analyses will be performed for the primary outcome and related key secondary and other outcomes to test for evidence of effect modification by pregnancy status (single vs multiple pregnancy), history of preterm birth and baseline DHA. Missing data will be addressed using multiple imputation under a missing at random assumption. Sensitivity analyses will be performed using the original un-imputed data. A secondary per-protocol analysis will be performed for the primary outcome including only women who were compliant with the protocol. Two separate definitions of compliance will be used. First, women will be considered compliant if they returned their remaining capsules at the end of the trial and consumed at least 75% of the number of capsules expected to be consumed. Second, women will be considered compliant if they had the 28-week telephone contact and reported they had missed less than 7 capsules in the last week out of the recommended 21 capsules.

All analyses will follow a pre-specified statistical analysis plan. There is a single planned interim analysis of the primary outcome to be performed after data collection is complete for the first 50% of the target number of randomized participants.

Data monitoring

An independent Data Monitoring Committee (DMC) has been set up to review the progress of the trial and provide feedback to the Trial Steering Committee. The DMC reviews general study progress (recruitment, compliance, loss to follow-up), the EPTB event rate and key secondary/safety outcomes. The committee meets annually or as required and consists of a neonatologist, obstetrician and statistician with clinical trials expertise. The DMC will be responsible for reviewing the results of the interim analysis.

An independent (blinded) SAE Committee has been established to review SAEs. There are no SAEs which would be anticipated as a unique consequence of participation in the trial, however all deaths, admissions to an intensive care unit of mothers or of infants born after 34 completed weeks' gestation, and major congenital abnormalities are to be reported. The SAE Committee includes experts in obstetrics, neonatology, pathology and midwifery who are not involved in the trial. The primary role of the SAE Committee is to review all SAEs and determine whether there is any likelihood that involvement in the trial could have contributed to the event. Determinations of causality will be made from medical records retrieved for this purpose. This committee meets quarterly, or as required.

ETHICS AND DISSEMINATION

Research ethics approval

This protocol and the informed consent and participant information document have been approved by the Human Research Ethics Committee (HREC) of each study site. Women's and Children's Health Network HREC (Coordinating HREC & Women's and Children's Hospital, Adelaide; Southern Adelaide Clinical HREC, Adelaide, South Australia); Mercy Health HREC (Werribee Mercy Hospital, Werribee, Victoria); Mater Mothers' Hospital, Brisbane, Queensland), University of Notradame, Joondalup Health Campus, Joondalup, Western Australia. Any subsequent modifications will be reviewed and approved by the HREC and governance of each study site. The study will be conducted in compliance with the current approved version of the protocol. Any change to the protocol document or informed consent form that affects the scientific intent, study design, patient safety, or may affect a participant's willingness to continue participation in the study will be considered a major amendment. All such amendments will be submitted to the HREC for approval. Any other changes to the protocol (such as administrative changes to dates and study personnel) will be considered minor amendments and will be notified to the HREC as appropriate.

Confidentiality

Participant confidentiality is strictly held in trust by the participating investigators, research staff and their agents. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participants. The study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorised third party, without prior written approval of the Coordinating Centre. The Coordinating Centre and regulatory authorities may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records and pharmacy records for the infants in this study subject to individuals having obtained approval/clearance through State/National Governments and HREC as required by local laws. The study site will permit access to such records. Clinical information will not be released without written permission of the parent/guardian, except as necessary for monitoring by HREC or regulatory agencies.

Dissemination plan

Study findings will be submitted for peer-reviewed publication and for presentation at appropriate local and international conferences. In addition, study findings will be disseminated to participants through a one-page lay summary. Results will be made available to the wider community through social media avenues and the South Australian Health & Medical Research Institute website.

Trial status

This enrolment phase of this trial completed on 27th April 2017 with successful attainment of sample size (n=5544). Completion of study follow up appointments and collection of labour and birth data from participant medical records will be completed by December 2017.

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Authors' contributions

MM, JQ, RAG, AJM and SJZ conceived and designed the study with KPB contributing to ongoing study implementation and management. LY provided statistical expertise. SJZ and KPB wrote the first draft of the manuscript. All authors contributed to refinement of the study protocol and approved the final manuscript.

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Competing Interests

The authors declare: financial support for the submitted work from the National Health and Medical Research Council (NHMRC) Australia (Project Grant 1050468). RAG serves on a scientific advisory board for Fonterra; MM serves on scientific advisory boards for Nestle and Fonterra. Associated Honoraria for RAG and MM are paid to their institutions to support conference travel and continuing education for postgraduate students and early career researchers. The authors declare that they have no competing interests.

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The following are members of the ORIP (Omega-3 fatty acids to Reduce the Incidence of Preterm birth) Investigative team: Gus Dekker (Northern Adelaide Health Service [Lyell McEwin Hospital and Modbury Hospital], Adelaide, South Australia. Elinor Atkinson (Flinders Medical Centre, Adelaide, South Australia), Jodie Dodd (Women's and Children's Hospital, Adelaide, South Australia), Jacqueline van Dam & Nisha Khot (Mercy Hospital, Werribee, Melbourne, Victoria), Huda Safa (Mater Mothers' Hospital, Brisbane, Queensland).

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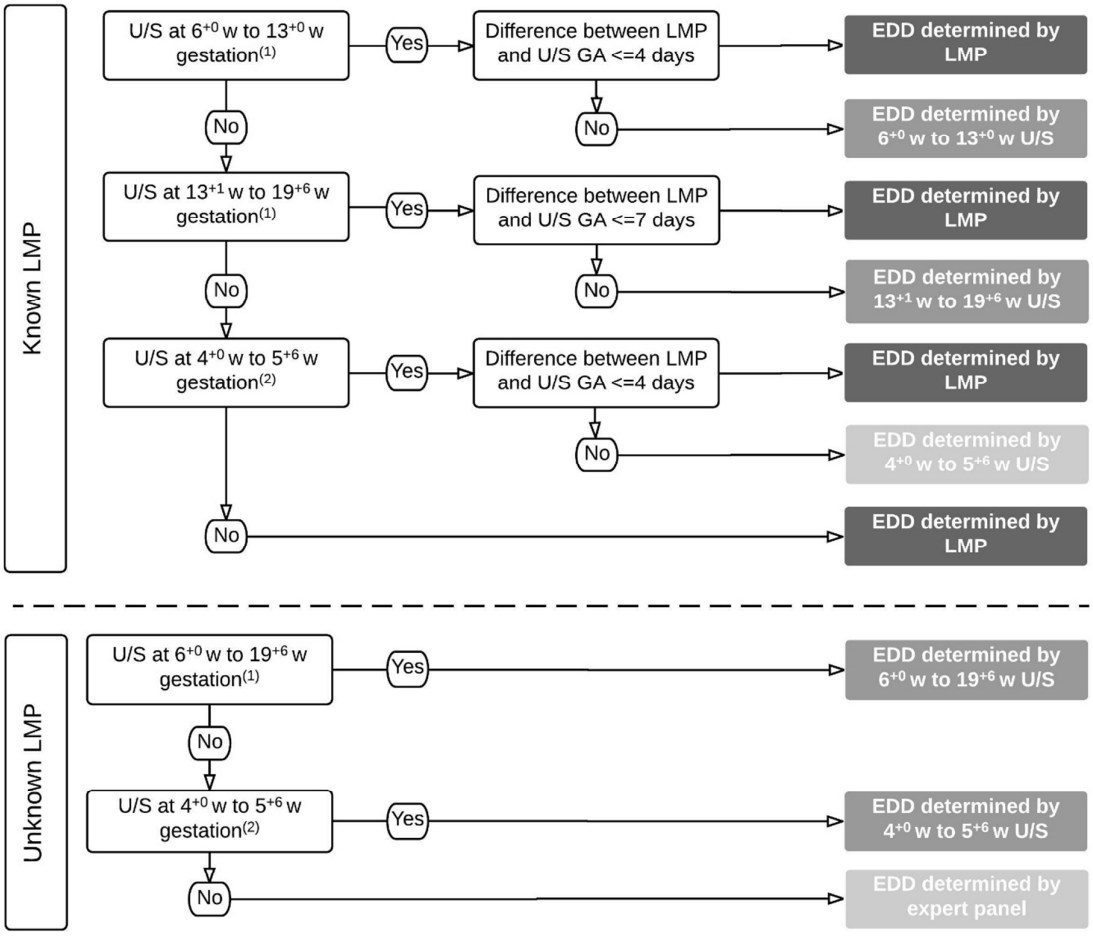
Figure 1. Flow chart for determining estimated date of delivery (EDD)

LMP, last menstrual period; GA, gestational age; U/S ultrasound; w, weeks



(1) Where multiple ultrasounds are performed within a given GA range, the earliest ultrasound will be used to determine gestational age.

(2) Where multiple ultrasounds are performed within a given GA range, the latest ultrasound will be used to determine gestational age.

Figure 2. ORIP Trial Schedule

***-t1 = <20 weeks' gestation; t1 = 2 weeks' post randomization and allocation; t2 = 28 weeks' gestation; t3 = 34 weeks' gestation or birth, whichever occurs first; t x= 6 weeks post estimated due date.*

	Enrolment	Allocation	Post-allocation			Completion
TIMEPOINT**	$-t_1$	0	t_1	t_2	t_3	t_x
ENROLMENT:						
Eligibility screening	X					
Informed consent	X					
Randomisation	X					
Allocation		X				
INTERVENTION:						
Treatment: 3x500mg capsules ω-3 LCPUFA per day						
OR						
Control: 3x500mg capsules blended vegetable oils						
ASSESSMENTS:						
Fatty Acid Analysis (finger prick)	X				X	
Maternal clinical & demographic details	X					
Compliance & side effects			X	X	X	
Labour & birth data extracted from medical records						X

ORIP STATISTICAL ANALYSIS PLAN
CONFIDENTIAL

Study Name: ORIP
NHMRC ID: APP 1050468
SAP Authors: Kara Cashman (Trial Statistician)
Lan Kelly (Trial Statistician)
Lisa Yelland (Statistical Investigator)

SAP Version: 11
SAP Date: 29/05/2017

Approved by ORIP Trial Steering Committee:

Maria Makrides	M. Makrides	Version: 11	Date: 7/6/2017
Jo Zhou		Version: 11	Date: 1.6.2017
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Robert Gibson		Version: 11	Date: 8/6/17
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Contents

ABBREVIATIONS

Abbreviation	Definition
BP	Blood Pressure
CRF	Case Report Form
DHA	Docosahexaenoic Acid
EDD	Established Date of Delivery
EPA	Eicosapentaenoic Acid
EPTB	Early Preterm Birth
GA	Gestational Age
GEE	Generalised Estimating Equation
LCPUFA	Long Chain Polyunsaturated Fatty Acid
LMP	Last Menstrual Period
MIS	Management Information System
NICU	Neonatal Intensive Care Unit
ORIP	Omega-3 fats to Reduce the Incidence of Prematurity
SAP	Statistical Analysis Plan
U/S	Ultrasound

1 PREFACE

This Statistical Analysis Plan (SAP) describes the planned analyses and reporting for the Omega-3 fats to Reduce the Incidence of Prematurity (ORIP) trial.
The following documents were reviewed in preparation of this SAP:

- ORIP NHMRC Grant Application (ID APP1050468_Zhou)
- ORIP Trial Protocol (Version 4, 14 September 2015)
- ORIP Case Report Form (CRF) (Version 1, 10 December 2013)
- ORIP ANZCTR Registration Page (www.anzctr.org.au, ACTRN 12613001142729)

2 PURPOSE OF SAP

The purpose of this SAP is to outline the planned analyses to be conducted to support the completion of the final report for the ORIP trial. The planned analyses and reporting for any side studies or follow up studies of the trial are not covered by this SAP.

3 STUDY OBJECTIVES AND OUTCOMES

3.1 Study Objectives

The primary objective of the trial is to determine the effect of supplementing the diets of pregnant women with omega-3 long chain polyunsaturated fatty acids (LCPUFA) on the incidence of early preterm birth (EPTB), defined as gestation at birth <34 completed weeks.

3.2 Outcome Variables

The primary outcome variable is the incidence of EPTB, defined as delivery before 34 completed weeks gestation. Gestational age is determined using a combination of maternal menstrual dating and ultrasound dating. The primary and secondary outcome variables are described in detail in Section 8.

4 STUDY METHODS

4.1 Overall Study Design and Plan

Randomised controlled double-blind multicentre trial.

4.2 Selection of Study Population

4.2.1 Inclusion Criteria

- Women with a singleton or multiple pregnancy and less than 20 weeks gestation.
- Women who are able to give informed consent.

4.2.2 Exclusion Criteria

- Women with a known fetal abnormality.
- Women who are taking dietary supplements containing LCPUFA > 150mg/day.
- Women who are taking dietary supplements containing LCPUFA ≤ 150mg/day and are not willing to stop.
- Women with bleeding disorders where fish oil is contraindicated or are on anticoagulant therapy.
- Women with a history of drug or alcohol abuse.

4.3 Method of Treatment Assignment and Randomisation

Women are randomly assigned to receive DHA capsules (treatment) or vegetable oil capsules (control) in the ratio 1:1 using a web-based randomisation service. The randomisation is stratified by centre and dietary supplement use (as defined in the table below) and uses randomly permuted blocks of size four and six in the ratio 1:1. The randomisation schedule was produced by an independent statistician using ralloc.ado version 3.7.5 in Stata version 12.1.

Stratification Variable	Categories
Centre	Women's & Children's Hospital, South Australia Flinders Medical Centre, South Australia Joondalup Health Service, Western Australia Werribee Mercy Hospital, Victoria Brisbane Mater Mothers Hospital, Queensland Lyell McEwin Health Service, South Australia
Use of dietary supplements containing n-3 LCPUFA in the 3 months prior to randomisation	Yes No

4.4 Treatment Masking (Blinding)

Participants, clinicians and research staff are blinded to treatment group allocation. Active and placebo capsules are identical in size, shape and colour.

4.5 Sample Size

In our DOMInO trial (Makrides et al 2010) of women with singleton pregnancies, treatment resulted in a 1.16% absolute reduction (from 2.25% to 1.09%) in the incidence of EPTB. This reduction occurred in the presence of non-adherence resulting from women assigned to the intervention failing to take the trial supplements as directed and control women taking prenatal supplements containing low dose omega-3 LCPUFA. Similar levels of non-adherence are expected in the proposed ORIP trial. Inclusion of women with multiple pregnancies in the ORIP trial is expected to result in a small increase in the incidence of EPTB in both treatment groups, since EPTB is more common in multiple pregnancies.
To demonstrate an absolute reduction in the incidence of EPTB of 1.16% (from 2.45% to 1.29%) with 85% power and two-sided alpha=0.049 at the final analysis, a sample size of

2631 per group (i.e. 5262 total) is required. Allowing for a loss to follow up of 5%, we need to enrol a total of 5540 women into the trial to ensure adequate power for the primary outcome. Since EPTB is a mother level outcome, the sample size calculations define the number of mothers that need to be recruited and no adjustment for clustering due to multiple pregnancies is required for this outcome. The sample size has not been inflated to account for mothers who take part in the trial multiple times for different pregnancies, since independent randomisations will be performed for each pregnancy and hence a design effect of approximately 1 is expected (Yelland et al 2017).

5 SEQUENCE OF PLANNED ANALYSES

5.1 Interim Analysis

An interim analysis of the primary outcome will be conducted by an independent statistician once data collection has been completed for the first 2770 (50%) randomised participants, based on the target sample size for the trial of 5540. The analysis will be performed using the method described in Section 8.2.

5.2 Final Analyses and Reporting

Once data collection and cleaning is complete, and the final version of this SAP has been approved, blinded treatment codes will be included in the database and analysis of the listed outcomes will be performed blinded to treatment group. The blinding will be broken following analysis of these outcomes. Results of the statistical analyses will be made available to the Chief Investigators.

Any post-hoc, exploratory analyses which were not identified in this SAP but are completed to support the planned study analyses listed in this SAP will be clearly identified. Any deviations from the planned analyses detailed in this SAP will be clearly documented with reasons in a post-analysis version of the SAP.

6 GENERAL ISSUES FOR STATISTICAL ANALYSIS

6.1 Analysis Software

All analyses will be performed using SAS® Software version 9.3 or later (SAS Institute Inc., Cary, NC, USA).

6.2 Analysis Approach

The planned analyses will be performed using an intention-to-treat approach; randomised participants will be analysed according to the treatment they were randomised to receive. A secondary per protocol analysis will be performed for the primary outcome only.

6.3 Methods for Withdrawals, Missing Data and Outliers

Data collected on participants up until the point of withdrawal will be included in the analysis. No data will be collected after the point of withdrawal. Participants who cease treatment and do not wish to be contacted for follow up, but who give consent to access their medical records, will be considered non compliant. Data collected up until the point they became non compliant and additional data obtained from their medical records will be included in the analysis.

Multiple imputation will be used to create 100 complete datasets for analysis. Use of 100 imputations ensures that the loss of power compared to full information maximum likelihood methods is <1%, even when the fraction of missing information is high (Graham et al., 2007). Imputation will be performed separately by treatment group using the fully conditional specification method (also known as chained equations). Imputation models will include a range of variables, including baseline characteristics and auxiliary variables measured after randomisation. For each outcome, covariates pre-specified for adjustment in the analysis model or for conducting subgroup analyses (see Sections 6.6, 6.7) will be included in the imputation model. 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.

The imputed analysis will be performed under a missing at random assumption. This assumption will be made plausible by identifying variables that predict missingness and including these in the imputation model. For the primary outcome, the sensitivity of results to the missing at random assumption will be investigated by assuming that data are missing not at random in the imputation model. Using pattern mixture models, the odds of EPTB will be assumed to be between half and twice as high in women with missing data compared to women with observed data. These differences will be applied to control group women only, treatment group women only and women in both treatment groups.

Participants who have a miscarriage or termination prior to 20 weeks gestation will be excluded from all analyses, except where this is a specified outcome, including descriptive statistics, outcomes relevant to prior to 20 weeks and safety data. Participants who have a stillbirth or termination at or after 20 weeks gestation will be excluded from analyses of neonatal outcomes, except birth anthropometrics and birth weight outcomes. These participants will not have data imputed, since outcomes are undefined rather than missing in this case. Further, the goal of the analysis is to assess the effect of treatment in survivors, rather than a hypothetical immortal setting. All other randomised participants will be included in the imputed analysis. If there is any evidence to suggest that miscarriage, termination and/or stillbirth is influenced by treatment group ($p<0.2$), the principal strata effect (i.e. the effect of treatment in the subgroup of women who would not have had a miscarriage, termination or stillbirth under either treatment) will be explored using the sensitivity analysis approach of Chiba and VanderWeele (2011).

Outliers will be queried during data collection and the statistical analysis. Unless confirmed as a data entry error through verification of the CRF or other sources of data as appropriate, outliers will not be excluded from the primary analysis.

6.4 Protocol Violations and Deviations

No participants will be excluded from the intention-to-treat analyses due to protocol

deviations. Only women who were compliant with the protocol will be included in the per protocol analysis for the primary outcome. Two separate definitions of compliance will be used. First, women will be considered compliant if they returned their remaining capsules at the end of the trial and consumed at least 75% of the number of capsules expected to be consumed. We will assume women consumed the number of capsules issued [total number of bottles dispensed (CRF reference A.6) x 90 (number of capsules per bottle)] minus the number returned (F.6), and calculate the number of capsules expected to be consumed as [3 x (min(date at 34 weeks based on GA as defined in Section 8.1, CRF reference J.5 date of birth) – CRF reference A1.1 consent date)]. Second, women will be considered compliant if they had the 28 week telephone contact and reported they had missed none, 1-3 or 4-6 capsules in the last week out of the recommended 21 capsules (CRF reference E.3).

6.5 Data Transformations

No data transformations are planned. The statistical analyses detailed in Section 8 are based on assumptions about the distribution of the outcomes. Should these assumptions turn out to be invalid, appropriate data transformations may be required. Data transformations are not planned to correct for departures from normality, since the sample size is sufficient for the central limit theorem to apply (Lumley et al., 2002).

6.6 Covariates for Adjustment

For each outcome, both unadjusted and adjusted analyses will be performed. The adjusted results will be used to draw conclusions about the effect of treatment on the outcomes of interest, with unadjusted analyses performed for completeness and to potentially confirm the results of the adjusted analyses.

The Committee for Proprietary Medicinal Products (CPMP, 2004) state that stratification variables should generally be adjusted for in the primary analysis, regardless of their effect on the outcome. It has been shown that stratification leads to positive correlation between treatment groups and failure to adjust for stratification variables in the analysis biases standard error estimates for the treatment effect upwards (Kahan and Morris, 2012). We will therefore adjust for stratification variables (as defined in Section 4.3) and draw conclusions about the effect of treatment based on the adjusted analyses. No adjustment is planned for any other baseline variables, apart from the stratification variables. If convergence is an issue, some of the stratification variables may need to be collapsed or excluded from the adjusted analysis. Any deviation from the planned analyses will be clearly identified.

6.7 Planned Treatment by Covariate Interactions

Statistical analysis of the primary outcome of EPTB and all secondary outcome variables listed in Section 8 will be performed to determine the effect of treatment on the outcome (see Section 8 for details).

For the primary outcome of EPTB and the related secondary outcomes listed below, secondary analyses will also be performed.

- Post-term induction
- Clinical diagnosis of post-term induction
- Post-term prelabour caesarean delivery
- Clinical diagnosis of post-term prelabour caesarean delivery

- Post-term induction or post-term prelabour caesarean delivery
- Clinical diagnosis of post-term induction or post-term prelabour caesarean delivery
- Preterm birth
- Early preterm spontaneous labour
- Preterm spontaneous labour
- Gestational age at birth
- Gestational age at birth (censored)
- Low birth weight
- Very low birth weight
- Small for gestational age
- Very small for gestational age
- High birth weight
- Large for gestational age
- Very large for gestational age
- Birth weight, raw and z score
- Birth length, raw and z score
- Birth head circumference, raw and z score
- Admission to neonatal intensive care unit
- Perinatal death

The secondary analyses will test for evidence of effect modification by pregnancy status (singleton vs multiple), history of preterm birth (yes vs no) and baseline DHA (split into quartiles). This will be achieved by including a main effect term for the effect modifier of interest and a treatment by effect modifier interaction term in the model. Separate estimates of treatment effect will be obtained for singleton and multiple pregnancies, history of preterm birth or not, and DHA quartiles, independent of whether the interaction term is statistically significant, since these subgroups are of interest a priori. Any additional unplanned treatment by covariate interactions must be considered exploratory and will be clearly identified in the final report.

6.8 Multiple Comparisons and Multiplicity

Multiple hypothesis tests will need to be performed to assess the effectiveness of omega-3 dietary supplementation due to multiple secondary outcomes (including key and other secondary outcomes), unadjusted and adjusted analyses, and analyses based on raw and imputed data. No adjustment will be made for the number of secondary analyses performed as these analyses are of less importance and less emphasis will be placed on the results. Since conclusions will be drawn based on the adjusted results using the imputed data, no adjustment will be made for the fact that both unadjusted and adjusted analyses are being performed on both raw and imputed data sets for each outcome.

6.9 Clustering

Some women in the trial will have a multiple pregnancy and some women will take part in the trial multiple times for different pregnancies. For all outcomes, clustering due to mother will be taken into account in the analysis using generalised estimating equations (GEEs) with an independence working correlation structure, unless otherwise specified.

7 DESCRIPTIVE STATISTICS

7.1 Flow Chart of Participants

Information will be presented by treatment group (where appropriate) on:

- The number of women screened for ORIP.
- The number of women eligible for participation in ORIP.
- The number of women not eligible for participation in ORIP by reason.
- The number of women who gave informed consent.
- The number of women who did not give informed consent by reason.
- The number of women randomised into the ORIP trial.
- The number of women who withdrew and did not give consent to access their medical records.
- The number of women who were non compliant (stopped taking treatment and requested no further contact but gave consent to access their medical records).
- The number of women who had a miscarriage or termination prior to 20 weeks gestation.
- The number of women who had a still birth or termination at or after 20 weeks gestation.
- The number of women with primary outcome data available.

7.2 Randomisation Errors

Information will be presented by treatment group on:

- The number of women randomised in the wrong stratum.
- The number of women given the wrong treatment.
- The number of women discovered to be ineligible after randomisation.
- The number of women randomised multiple times in error.

7.3 Baseline Characteristics

A descriptive comparison of the randomised groups will be conducted on the baseline characteristics collected in sections A, B and C of the CRF. Means and standard deviations, or medians and interquartile ranges will be reported for continuous variables. Frequencies and percentages will be reported for categorical variables.

7.4 Missing Data

Missing data will be assessed descriptively by treatment group for each outcome variable specified in Section 8, each potential confounder specified in Section 6.6, and each potential effect modifier specified in Section 6.7.

8 STATISTICAL ANALYSES

In this section, the following details are provided for all primary and secondary outcomes for the trial:

- Outcome - A detailed description of the outcome variable, including the type of variable, whether it is a key secondary outcome, whether it is measured on the mother or infant, the relevant variables(s) in the CRF and how it will be calculated (if applicable).
- Analysis - The type of statistical analysis to be performed and the measure of treatment effect to be reported.

For each outcome variable, 95% confidence intervals will be reported to express uncertainty about the effect of treatment and statistical significance will be assessed at the 0.05 level using a two-sided comparative test of treatment effect, unless otherwise specified.

For binary outcomes, if the number of participants experiencing the outcome is considered too small for the planned analysis to be sensible, a Fisher's exact test will be performed instead with no adjustment made for baseline covariates. For analyses performed using a log binomial model, a log Poisson model with robust variance estimation will be used if the model fails to converge.

For count outcomes, if the fit of the log Poisson model is poor, the analysis will instead be performed using a negative binomial model and/or a zero-inflated model, as appropriate.

8.1 Gestational Age Definition

Gestational age at delivery will be determined using a combination of maternal menstrual dating and ultrasound dating as follows:

If performed, the first ultrasound scan performed between 6+0 to 13+0 weeks GA will be used in determining GA. If this ultrasound scan and menstrual dating agree within an error of four days, the estimated date of delivery shall be determined using menstrual dates.

Where menstrual dating and this ultrasound disagree by more than four days, then the estimated date of delivery shall be determined by the ultrasound dating.

If no ultrasound scan was performed between 6+0 and 13+0 weeks GA, then the first scan performed after 13+0 weeks but before 20+0 weeks GA will be used in determining GA. Where this ultrasound scan and menstrual dating agree within an error of seven days, the estimated date of delivery shall be determined using menstrual dates in accordance with established obstetric practice.

Where menstrual dating and this ultrasound disagree by more than seven days, then the estimated date of delivery shall be determined by the ultrasound dating in accordance with established obstetric practice.

For women with an ultrasound performed between 4+0 and 5+6 weeks GA, but no ultrasound between 6+0 and 20+0 weeks GA, this early scan will be used in determining GA. If the latest ultrasound scan performed between 4+0 and 5+6 weeks GA and menstrual

dating agree within an error of four days, the estimated date of delivery shall be determined using menstrual dates.

Where menstrual dating and this ultrasound disagree by more than four days, then the estimated date of delivery shall be determined by the ultrasound dating.

For women with no ultrasound information before 20+0 weeks GA, the estimated date of delivery will be determined using menstrual dating.

For women with unknown LMP, the estimated date of delivery will be determined using the first ultrasound performed between 6+0 and before 20+0 weeks GA where available, or using the latest ultrasound performed between 4+0 and 5+6 weeks GA otherwise.

The GA at ultrasound (in weeks) will be determined as follows, in order of priority, depending on data recorded in CRF Section G and C.18 & C.19:

- GA from ultrasound, if recorded in CRF Section G
- 40 - (EDD from CRF Section G - date of scan)/7
- GA from medical records at date of scan in CRF section G
- (Date of scan – LMP from section C.18)/7
- 40 - (EDD from Section C.19 - date of scan)/7

Where the estimated date of delivery cannot be defined using this process, the relevant data will be provided to a team of three obstetricians who will review the data and determine the estimated date of delivery blinded to treatment group. If the three obstetricians disagree, estimated date of delivery will be determined by the majority in agreement.

The gestational age in days at birth (or other event of interest) will be determined from the estimated date of delivery using the equation [280 - (estimated date of delivery - date of birth)].

8.2 Primary Outcome Variable (EPTB)

Outcome: Mother level binary outcome. Early preterm birth defined as delivery before 34 weeks completed gestation. Gestational age is defined as in Section 8.1.

Analysis: Fit a log binomial GEE model to estimate the relative risk of early preterm birth (omega-3 LCPUFA relative to placebo). Statistical significance will be assessed using the O'Brien-Fleming type alpha spending function with symmetric stopping boundaries. The stopping boundary will be Z=±2.96259 (corresponding to alpha=0.00305) at the interim analysis and Z=±1.96857 (corresponding to alpha=0.0490) at the final analysis to maintain an overall type I error rate of alpha=0.05. Secondary analyses will be performed to test for effect modification (see Section 6.7) and in the subgroup of women who were compliant with the protocol as defined in Section 6.4.

8.3 Secondary Pregnancy Outcome Variables

8.3.1 Preterm birth

Outcome: Mother level binary outcome (key secondary outcome). Preterm birth is considered to have occurred if the gestational age at delivery (as defined in Section 8.1) was <37 weeks and 0 days.

Analysis: Fit a log binomial GEE model to estimate the relative risk of preterm birth (omega-3 LCPUFA relative to placebo) and test for evidence of effect modification (see Section 6.7).

8.3.2 Early Preterm Spontaneous Labour

Outcome: Mother level binary outcome (key secondary outcome). Early preterm spontaneous labour defined as delivery before 34 weeks completed gestation (gestational age as defined in Section 8.1) with spontaneous onset of labour (CRF reference I.2).

Analysis: Fit a log binomial GEE model to estimate the relative risk of early preterm spontaneous labour (omega-3 LCPUFA relative to placebo) and test for evidence of effect modification (see Section 6.7).

8.3.3 Preterm Spontaneous Labour

Outcome: Mother level binary outcome (key secondary outcome). Preterm spontaneous labour defined as delivery before 37 weeks completed gestation (gestational age as defined in Section 8.1) with spontaneous onset of labour (CRF reference I.2).

Analysis: Fit a log binomial GEE model to estimate the relative risk of preterm spontaneous labour (omega-3 LCPUFA relative to placebo) and test for evidence of effect modification (see Section 6.7).

8.3.4 Gestational Age at Birth

Outcome: Mother level continuous outcome (key secondary outcome). Gestational age in days as defined in Section 8.1.

Analysis: Fit a linear GEE model to estimate the difference in mean gestational age at birth (omega-3 LCPUFA minus placebo) and test for evidence of effect modification (see Section 6.7).

8.3.5 Gestational Age at Birth (Censored)

Outcome: Mother level time to event outcome, where the event is spontaneous labour (key secondary outcome). Gestational age is defined as in Section 8.1 and will be measured in days. Induction (CRF reference I.3) and pre labour caesarean section will be treated as

competing risks, since they prevent spontaneous labour from occurring. Pre labour caesarean section is considered to have occurred if the onset of labour was not spontaneous (I.2), the labour was not induced (I.3) and the mother had a caesarean section (I.4). Participants who withdraw and do not give consent to access their medical records will have their follow up time censored at the GA at which they withdraw (as defined in Section 8.1), as the event of spontaneous labour is not observed. As the planned analysis includes a censoring mechanism for missing data on the outcome variable, this analysis will be completed using raw data only, not imputed data.

Analysis: Fit a Fine and Gray competing risks regression model to estimate the sub-distribution hazard ratio of spontaneous labour (omega-3 LCPUFA relative to placebo) and test for evidence of effect modification (see Section 6.7). A cluster adjusted variance estimator will be used to account for clustering due to mothers participating in the trial during more than one pregnancy. If the proportionality assumption of the Fine and Gray model is not met, a time-varying model will be used where time to event will be split into two or more periods in which the proportionality assumption is met.

8.3.6 Pre Labour Premature Rupture of Membranes

Outcome: Mother level binary outcome. Pre labour premature rupture of membranes is considered to have occurred if premature rupture of membranes is documented (CRF reference I.5).

Analysis: Fit a log binomial GEE model to estimate the relative risk of pre labour premature rupture of membranes (omega-3 LCPUFA relative to placebo).

8.3.7 Pre Labour Preterm Premature Rupture of Membranes

Outcome: Mother level binary outcome. Pre labour preterm premature rupture of membranes is considered to have occurred if preterm premature rupture of membranes is documented (CRF reference I.6).

Analysis: Fit a log binomial GEE model to estimate the relative risk of pre labour preterm premature rupture of membranes (omega-3 LCPUFA relative to placebo).

8.3.8 Post-term Induction

Outcome: Mother level binary outcome (key secondary outcome). Post-term induction is considered to have occurred if the labour was induced (CRF reference I.3) and the gestational age at delivery (as defined in Section 8.1) was >41 weeks and 0 days.

Analysis: Fit a log binomial GEE model to estimate the relative risk of post-term induction (omega-3 LCPUFA relative to placebo) and test for evidence of effect modification (see Section 6.7).

8.3.9 Clinical diagnosis of Post-term Induction

Outcome: Mother level binary outcome (key secondary outcome). Post-term induction is considered to have occurred if the labour was induced, with the reason for induction recorded as Post term, either >41 GA, or recorded as post term by the clinician recorded under the 'other' option (CRF reference I.3).

Analysis: Fit a log binomial GEE model to estimate the relative risk of clinical diagnosis of post-term induction (omega-3 LCPUFA relative to placebo) and test for evidence of effect modification (see Section 6.7).

8.3.10 Post-term Pre Labour Caesarean Section

Outcome: Mother level binary outcome (key secondary outcome). Post-term pre labour caesarean section is considered to have occurred if the onset of labour was not spontaneous (I.2), the labour was not induced (I.3), the mother had a caesarean (I.4) and the gestational age at delivery (as defined in Section 8.1) was >41 weeks and 0 days.

Analysis: Fit a log binomial GEE model to estimate the relative risk of post-term pre labour caesarean section (omega-3 LCPUFA relative to placebo) and test for evidence of effect modification (see Section 6.7).

8.3.11 Clinical diagnosis of Post-term Pre Labour Caesarean Section

Outcome: Mother level binary outcome (key secondary outcome). Post-term pre labour caesarean section is considered to have occurred if the onset of labour was not spontaneous (I.2), the labour was not induced (I.3), the mother had a caesarean (I.4) and the reason for caesarean is recorded as post term, either >41 GA, or recorded as post term by the clinician recorded under the 'other' option (CRF reference I.4.2) .

Analysis: Fit a log binomial GEE model to estimate the relative risk of clinical diagnosis of post-term pre labour caesarean section (omega-3 LCPUFA relative to placebo) and test for evidence of effect modification (see Section 6.7).

8.3.12 Post-term Induction or Post-term Pre Labour Caesarean Section

Outcome: Mother level binary outcome (key secondary outcome). Post-term induction is considered to have occurred if the labour was induced (CRF reference I.3) and the gestational age at delivery (as defined in Section 8.1) was >41 weeks and 0 days. Post-term pre labour caesarean section is considered to have occurred if the onset of labour was not

spontaneous (I.2), the labour was not induced (I.3), the mother had a caesarean (I.4) and the gestational age at delivery (as defined in Section 8.1) was >41 weeks and 0 days.

Analysis: Fit a log binomial GEE model to estimate the relative risk of post-term induction or post-term pre labour caesarean section (omega-3 LCPUFA relative to placebo) and test for evidence of effect modification (see Section 6.7).

8.3.13 Clinical diagnosis of Post-term Induction or Post-term Pre Labour Caesarean Section

Outcome: Mother level binary outcome (key secondary outcome). Post-term induction is considered to have occurred if the labour was induced with the reason for induction recorded as Post term, either >41 GA, or recorded as post term by the clinician recorded under the 'other' option (CRF reference I.3) . Post-term pre labour caesarean section is considered to have occurred if the onset of labour was not spontaneous (I.2), the labour was not induced (I.3), the mother had a caesarean (I.4) and the reason for caesarean is recorded as post term, either >41 GA, or recorded as post term by the clinician recorded under the 'other' option (CRF reference I.4.2) .

Analysis: Fit a log binomial GEE model to estimate the relative risk of clinical diagnosis of post-term induction or post-term pre labour caesarean section (omega-3 LCPUFA relative to placebo) and test for evidence of effect modification (see Section 6.7).

8.3.14 Prolonged Gestation

Outcome: Mother level binary outcome. Prolonged gestation is considered to have occurred if the gestational age at delivery (as defined in Section 8.1) was >42 weeks and 0 days.

Analysis: Fit a log binomial GEE model to estimate the relative risk of prolonged gestation (omega-3 LCPUFA relative to placebo).

8.3.15 Caesarean Section

Outcome: Mother level binary outcome. Caesarean section as recorded on the CRF (reference I.4).

Analysis: Fit a log binomial GEE model to estimate the relative risk of caesarean section (omega-3 LCPUFA relative to placebo).

8.3.16 Gestational Diabetes Definition 1

Outcome: Mother level binary outcome. The presence of gestational diabetes will be indicated by either:

1) baseline blood glucose level >5.5mmol/L (CRF reference H.2.2)

OR

2) 2 hour blood glucose level >8mmol/L (CRF reference H.2.4).

Women who have a glucose challenge test (CRF reference H.1) but not a glucose tolerance test (CRF reference H.2) will be defined as not having gestational diabetes.

Analysis: Fit a log binomial GEE model to estimate the relative risk of gestational diabetes (omega-3 LCPUFA relative to placebo).

8.3.17 Gestational Diabetes Definition 2

Outcome: Mother level binary outcome. The presence of gestational diabetes will be indicated by at least one of:

1) baseline blood glucose level ≥5.1 mmol/L (CRF reference H.2.2)

OR

2) 1 hour blood glucose level ≥10.0 mmol/L (CRF reference H.2.3)

OR

3) 2 hour blood glucose level ≥8.5 mmol/L (CRF reference H.2.4).

Women who have a glucose challenge test (CRF reference H.1) but not a glucose tolerance test (CRF reference H.2) will be defined as not having gestational diabetes.

Analysis: Fit a log binomial GEE model to estimate the relative risk of gestational diabetes (omega-3 LCPUFA relative to placebo).

8.3.18 Clinical Diagnosis of Gestational Diabetes

Outcome: Mother level binary outcome. Clinical diagnosis of gestational diabetes, as recorded on the CRF (reference H.3).

Analysis: Fit a log binomial GEE model to estimate the relative risk of a clinical diagnosis of gestational diabetes (omega-3 LCPUFA relative to placebo).

8.3.19 Pregnancy Induced Hypertension

Outcome: Mother level binary outcome. Pregnancy induced hypertension is considered to have occurred if there has been one systolic BP ≥160mmHg and/or one diastolic BP ≥110mmHg recorded after 20 weeks gestation (CRF reference H.4.1), or two consecutive systolic BP ≥140mmHg and/or diastolic BP ≥90mmHg, four or more hours apart, have been recorded after 20 weeks gestation (CRF reference H.4.2).

Analysis: Fit a log binomial GEE model to estimate the relative risk of pregnancy induced hypertension (omega-3 LCPUFA relative to placebo).

8.3.20 Pre-Eclampsia

Outcome: Mother level binary outcome. The presence of pre-eclampsia during pregnancy is indicated by the presence of both pregnancy induced hypertension (as defined in 8.3.19) and proteinuria, indicated by either

1) spot urine protein:creatinine ratio ≥ 30mg/mmol (CRF reference H.5.1)
OR
2) one 24 hour urine collection with total protein ≥ 0.3g/L (CRF reference H.5.2)
OR
3) two random urine collections ≥ 1g/L (2+) (CRF reference H.5.3).

Analysis: Fit a log binomial GEE model to estimate the relative risk of pre-eclampsia (omega-3 LCPUFA relative to placebo).

8.3.21 Clinical Diagnosis of Pre-Eclampsia

Outcome: Mother level binary outcome. Clinical diagnosis of pre-eclampsia, as recorded on the CRF (reference H.6).

Analysis: Fit a log binomial GEE model to estimate the relative risk of a clinical diagnosis of pre-eclampsia (omega-3 LCPUFA relative to placebo).

8.3.22 Eclampsia

Outcome: Mother level binary outcome. Hospital admission for eclampsia, as recorded on the CRF (reference H.12).

Analysis: Fit a log binomial GEE model to estimate the relative risk of a hospital admission for eclampsia (omega-3 LCPUFA relative to placebo).

8.3.23 Postpartum Haemorrhage

Outcome: Mother level binary outcome. Clinical diagnosis of postpartum haemorrhage, as recorded on the CRF (reference I.9).

Analysis: Fit a log binomial GEE model to estimate the relative risk of a clinical diagnosis of postpartum haemorrhage (omega-3 LCPUFA relative to placebo).

8.3.24 Estimated Blood Loss at Birth

Outcome: Mother level continuous outcome. Estimated blood loss at birth (CRF reference I.10).

Analysis: Fit a linear GEE model to estimate the difference in mean estimated blood loss at birth (omega-3 LCPUFA minus placebo).

8.3.25 Maternal Serious Adverse Events

Outcome: Mother level binary outcomes. Any recorded maternal serious adverse event, where 'mother' is indicated on the CRF (Section L):

- Death
- Level 3 hospitalisation: Maternal admission to ICU
- Any of the above

Analysis: Fit separate log binomial GEE models to estimate the relative risk of each serious adverse event (omega-3 LCPUFA relative to placebo).

8.3.26 Length of Hospital Stay (Mother)

Outcome: Mother level count outcome. Number of days in hospital from birth, calculated as mother discharge date minus baby date of birth (CRF references I.12, J.5).

Analysis: Fit a log Poisson GEE model to estimate the ratio of mean number of days in hospital (omega-3 LCPUFA relative to placebo).

8.3.27 Maternal Use of Antibiotics

Outcome: Mother level binary outcome. Maternal use of antibiotics during pregnancy (CRF reference H.10), excluding prophylactic use for Caesarean sections.

Analysis: Fit a log binomial GEE model to estimate the relative risk of use of antibiotics (omega-3 LCPUFA relative to placebo).

8.4 Safety and Tolerability of DHA Supplementation

Outcome: Mother level binary outcomes. Reported occurrence of the following symptoms two weeks post randomisation (CRF reference D.4), at 28 weeks gestation (CRF reference E.4) and 34 weeks gestation (as CRF reference F.4):

- Diarrhoea
- Nausea
- Vomiting
- Burping
- Constipation
- Vaginal blood loss
- Nose bleeds
- Any gastro intestinal related symptom (diarrhoea, nausea, vomiting, burping or constipation)
- Any blood loss (vaginal blood loss or nose bleeds)
- Any of the above

Analysis: Fit separate repeated measures log binomial GEE models to estimate the relative risk of each safety and tolerability symptom (omega-3 LCPUFA relative to placebo). An interaction term between treatment group and time (2 weeks post randomisation, 28 weeks and 34 weeks) will be included in the initial model. If the interaction term is significant ($p<0.05$), relative risks for treatment will be reported for each time point. If the interaction term is not significant ($p\geq0.05$), the model will be re-fit without the interaction and one overall relative risk for treatment will be reported.

8.5 Secondary Neonatal Outcome Variables

8.5.1 Low Birth Weight

Outcome: Infant level binary outcome (key secondary outcome). Defined as birth weight less than 2500g (CRF reference J.9).

Analysis: Fit a log binomial GEE model to estimate the relative risk of low birth weight (omega-3 LCPUFA relative to placebo) and test for evidence of effect modification (see Section 6.7).

8.5.2 Very Low Birth Weight

Outcome: Infant level binary outcome. Defined as birth weight less than 1500g (CRF reference J.9).

Analysis: Fit a log binomial GEE model to estimate the relative risk of very low birth weight (omega-3 LCPUFA relative to placebo) and test for evidence of effect modification (see Section 6.7).

8.5.3 Very Small for Gestational Age

Outcome: Infant level binary outcome. Defined as birth weight (CRF reference J.9) less than 3rd percentile for corresponding GA (as defined in Section 8.1) and gender (CRF reference J.7), using percentiles as defined by Beeby PJ et al (1996).

Analysis: Fit a log binomial GEE model to estimate the relative risk of small for gestational age (omega-3 LCPUFA relative to placebo) and test for evidence of effect modification (see Section 6.7).

8.5.4 Small for Gestational Age

Outcome: Infant level binary outcome. Defined as birth weight (CRF reference J.9) less than 10th percentile for corresponding GA (as defined in Section 8.1) and gender (CRF reference J.7), using percentiles as defined by Beeby PJ et al (1996).

Analysis: Fit a log binomial GEE model to estimate the relative risk of small for gestational age (omega-3 LCPUFA relative to placebo) and test for evidence of effect modification (see Section 6.7).

8.5.5 High Birth Weight

Outcome: Infant level binary outcome. Defined as birth weight greater than 4000g (CRF reference J.9).

Analysis: Fit a log binomial GEE model to estimate the relative risk of high birth weight (omega-3 LCPUFA relative to placebo) and test for evidence of effect modification (see Section 6.7).

8.5.6 Large for Gestational Age

Outcome: Infant level binary outcome. Defined as birth weight (CRF reference J.9) greater than 90th percentile for corresponding GA (as defined in Section 8.1) and gender (CRF reference J.7), using percentiles as defined by Beeby PJ et al (1996).

Analysis: Fit a log binomial GEE model to estimate the relative risk of large for gestational age (omega-3 LCPUFA relative to placebo) and test for evidence of effect modification (see Section 6.7).

8.5.7 Very Large for Gestational Age

Outcome: Infant level binary outcome. Defined as birth weight (CRF reference J.9) greater than 97th percentile for corresponding GA (as defined in Section 8.1) and gender (CRF reference J.7), using percentiles as defined by Beeby PJ et al (1996).

Analysis: Fit a log binomial GEE model to estimate the relative risk of large for gestational age (omega-3 LCPUFA relative to placebo) and test for evidence of effect modification (see Section 6.7).

8.5.8 Birth Anthropometrics

Outcomes: Infant level continuous outcomes. The following birth anthropometrics will be analysed, using both the raw measurements (CRF reference J.9) and Z-scores for corresponding GA (as defined in Section 8.1) and gender (CRF reference J.7), using means and standard deviations as defined by Beeby PJ et al (1996):

- Birth weight (key secondary outcome)
- Birth length
- Birth head circumference

Analysis: Fit separate linear GEE models to estimate the difference in means for each birth anthropometric (omega-3 LCPUFA minus placebo) and test for evidence of effect modification (see Section 6.7).

8.5.9 Neonatal Complications

Outcome: Infant level binary outcomes. The following complications will be analysed:

- Intermittent positive pressure ventilation at birth, with or without cardiac compressions (CRF reference J.12)
- Use of supplemental oxygen (CRF reference J.15.1, number of days >0)
- Jaundice requiring phototherapy (CRF reference J.16.1)
- Hypoglycaemia requiring intravenous dextrose infusion (CRF reference J.16.2)
- Neonatal convulsion (CRF reference J.16.3)
- Any Brain Injury (CRF reference J.16.4)
- Surgery (CRF reference J.16.5)
- Necrotising enterocolitis (CRF reference J.16.6)
- Sepsis (CRF reference J.16.7)
- Retinopathy of prematurity (CRF reference J.16.8)
- Other neonatal complication (CRF reference J.16.9)
- Intraventricular Haemorrhage, grade 2 or higher (CRF J.16.4 and associated brain injury form)

Analysis: Fit separate log binomial GEE models to estimate the relative risk of each neonatal complication (omega-3 LCPUFA relative to placebo).

8.5.10 Duration of Supplemental Oxygen

Outcome: Infant level count outcome. Number of days of supplemental oxygen for those infants who required oxygen (CRF reference J.15.1, number of days >0).

Analysis: Fit a log Poisson GEE model to estimate the ratio of mean number of days of supplemental oxygen (omega-3 LCPUFA relative to placebo).

8.5.11 Infant Serious Adverse Event

Outcome: Infant level binary outcomes. Any recorded infant serious adverse event:

- Perinatal Death, defined as still birth, termination greater than 20 weeks gestation or death within 28 days of birth (CRF references J.3, J.17, Section L) (key secondary outcome)
- Still Birth (CRF reference J.3)
- Termination greater than 20 weeks gestation (CRF reference Section L)
- Death within 28 days of birth (CRF reference J.17)
- Major congenital abnormalities (CRF reference J.10)

- Admissions to intensive care up to 28 days post birth (CRF reference J.14), for infants born >34 weeks gestation (as defined in Section 8.1)
- Miscarriage before 20 weeks GA
- Pregnancy termination before 20 weeks GA
- Any of the above

Analysis: Fit separate log binomial GEE models to estimate the relative risk of each infant serious adverse event (omega-3 LCPUFA relative to placebo).

8.5.12 Admission to Neonatal Intensive Care

Outcome: Infant level binary outcome (key secondary outcome). Any admission to neonatal intensive care up to 28 days post birth (CRF reference J.14).

Analysis: Fit separate log binomial GEE models to estimate the relative risk of admission to neonatal intensive care (omega-3 LCPUFA relative to placebo) and test for evidence of effect modification (see Section 6.7).

8.5.13 Length of Stay in Neonatal Intensive Care Unit

Outcome: Infant level count outcome. Number of days in neonatal intensive care for those infants who were admitted, calculated as baby discharge date minus baby admission date (CRF references J.14.2 and J.14.1). If infants have multiple NICU admissions, the total number of days spent in NICU will be summed across all admissions.

Analysis: Fit a log Poisson GEE model to estimate the ratio of mean number of days in neonatal intensive care (omega-3 LCPUFA relative to placebo).

8.5.14 Length of Hospital Stay (Infant)

Outcome: Infant level time to event outcome, where the event is hospital discharge. Time until hospital discharge will be calculated as baby discharge date minus baby date of birth (CRF references J.5, J.18). Death prior to discharge will be treated as a competing risk because it prevents hospital discharge from occurring.

Analysis: Fit a Fine and Gray competing risks regression model to estimate the sub-distribution hazard ratio of hospital discharge (omega-3 LCPUFA relative to placebo). A cluster adjusted variance estimator will be used to account for clustering due to siblings. If the proportionality assumption of the Fine and Gray model is not met, a time-varying model will be used where time to event will be split into two or more periods in which the proportionality assumption is met.

8.6 Secondary Outcome Variables Related to Trial Quality

8.6.1 Correctly Guessed Treatment Assignment

Outcome: Mother level binary outcome. Correctly guessed treatment assignment (CRF reference F.7). Responses recorded as 'Don't know' will be classified as incorrect guesses.

Analysis: Fit a log binomial GEE model to estimate the relative risk of correctly guessing treatment assignment (omega-3 LCPUFA relative to placebo). Calculate blindness index (Bang et al., 2004), which estimates the proportion of correct guesses beyond the level expected by chance separately for each treatment group.

8.6.2 Number of Capsules Missed

Outcome: Mother level categorical outcome. Number of capsules missed in the past week, recorded at 2 weeks post randomisation, 28 weeks and 34 weeks gestation (CRF references D.3, E.3, F.3). Number of capsules missed are categorised as None, 1-3, 4-6, 7-10, >10, Stopped completely.

Analysis: Fit a repeated measures ordinal logistic GEE model to estimate the odds of missing a greater number of capsules (omega-3 LCPUFA relative to placebo). An interaction term between treatment and time (2 weeks post randomisation, 28 weeks and 34 weeks) will be included in the initial model. If the interaction term is significant ($p<0.05$), odds ratios for treatment will be reported for each time point. If the interaction term is not significant ($p\geq0.05$), the model will be re-fit without the interaction and one overall odds ratio for treatment will be reported. If the proportional odds assumption is not met, fit a repeated measures multinomial logistic GEE model.

8.6.3 Number of Unused Capsules

Outcome: Mother level count outcome. Number of unused capsules at end of intervention period (F.6) minus the number expected. The expected number of capsules returned is calculated as:
[Total number of bottles dispensed (CRF reference A.6) x 90 (number of capsules per bottle)] - [3 x (min(Date at 34 weeks based on GA as defined in Section 8.1, CRF reference J.5 date of birth) CRF reference A1.1 Consent Date)]

Analysis: Fit a log Poisson GEE model to estimate the ratio of mean number of unused capsules (omega-3 LCPUFA relative to placebo).

8.6.4 Treatment Cessation

Outcome: Mother level binary outcome. Treatment is considered to have ceased if the patient completely stops taking the capsules at any time prior to 34 weeks gestation or birth, whichever occurs first, recorded at 2 weeks post randomisation (CRF reference D.3), 28 weeks gestation (E.3) or 34 weeks gestation (F.3, F.5).

Analysis: Fit a repeated measures log binomial GEE model to estimate the relative risk of ceasing treatment (omega-3 LCPUFA relative to placebo). An interaction term between treatment and time (2 weeks post randomisation, 28 weeks and 34 weeks) will be included in the initial model. If the interaction term is significant ($p<0.05$), relative risks for treatment will be reported for each time point. If the interaction term is not significant ($p\geq0.05$), the model will be re-fit without the interaction and one overall relative risk for treatment will be reported.

8.6.5 Blood Fatty Acid Concentrations

Outcome: Mother level continuous outcomes. Blood concentration of the following fatty acids at 34 weeks, as provided by the Waite laboratory:

- Docosahexaenoic acid (22:6n-3)
- Linoleic acid (18:2n-6)
- Arachidonic acid (20:4n-6)
- Eicosapentaenoic acid (20:5n-3)
- Docosapentaenoic acid (22:5n-3)

Analysis: Fit separate linear GEE models to estimate the difference in mean blood fatty acid concentration at 34 weeks (omega-3 LCPUFA minus placebo).

8.6.6 Use of Dietary Supplements

Outcome: Mother level binary outcome. Reported use of any other dietary supplements containing fish oil, DHA or EPA, recorded at 2 weeks post randomisation, 28 weeks and 34 weeks gestation (CRF references D.5, D.6, E.5, E.6, F.8, F.9).

Analysis: Fit a repeated measures log binomial GEE model to estimate the relative risk of use of other dietary supplements (omega-3 LCPUFA relative to placebo). An interaction term between treatment and time (2 weeks post randomisation, 28 weeks and 34 weeks) will be included in the initial model. If the interaction term is significant ($p<0.05$), relative risks for treatment will be reported for each time point. If the interaction term is not significant ($p\geq0.05$), the model will be re-fit without the interaction and one overall relative risk for treatment will be reported.

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ORIP Protocol Date and Version Identifier

Current Protocol Issue Date: 14th September 2015
Protocol Amendment Number: 04

Revision Chronology:

. . . 2013-Mar-11	Original
. . . 2013-Sep-13	Amendment 01. Protocol v2.: Throughout the document: Replacement of the term 'placebo' with 'control' Page 6: Design: Revised from 'Randomised placebo-controlled multi-centre trial' to 'Double blind, randomised controlled multi-centre trial'. Page 6: Participants and Page 13: Section 5.2: Number of participants: Increase in the total number of participants from 5,510 to 5,540, i.e. 2770 per randomised group, will be included in the study. Page 8: Section 2.4.2: Data Monitoring Committee: Renaming of the 'Trial Monitoring Committee' to the 'Data Monitoring Committee'. Page 13: Section 5.4: Primary and secondary outcome measures; bullet point 4 and Page 19: Section 8.5: At birth and discharge from hospital after birth; bullet point 5: Addition of jaundice, hypoglycaemia, brain injury (including intra-ventricular haemorrhage (IVH)) and retinopathy of prematurity as neonatal complications as a secondary outcome measure. Page 14: Section 6.1.1: Description: Change of □-3 LCPUFA content in each capsule from 320mg to 300mg Page 16: Section 7.2: Exclusion criteria: The exclusion criteria relating to women who are taking dietary supplements containing LCPUFA has been revised to replace >200mg/day with >150mg/day. Women taking dietary supplements containing LCPUFA at screening will be considered eligible on the basis they agree to stop taking these dietary supplements at randomisation and throughout the duration of the study. A new exclusion criteria has been included to reflect this. Page 17: Section 7.3: Randomisation procedures: Stratification will be by centre and supplements (containing no LCPUFA vs ≤150

	LCPUFA/day prior to randomisation). The amount of LCPUFA has been decreased from <200 LCPUFA/day to ≤150 LCPUFA/day. Page 18: Section 8.3: At the second follow up call (28 weeks gestation): Inclusion of an additional telephone contact point at 28 weeks' gestation in between the 2 weeks' post randomisation telephone contact and 34 weeks' gestation. Page 18: Section 8.5: At birth and discharge from hospital after birth: Additional text has been included. Page 22: Section 10.2: Data analysis: Inclusion of a single planned interim analysis to be performed after data collection is completed for the first 50% of the target number of randomised participants Page 29 and 30: Appendix 1: Inclusion of the actual Certificate of Analysis for the active and control capsules.
. . . 2014-Apr-04:	Amendment No.2: Protocol V3 Page 13 Section 5.4: Primary and secondary outcome measures; bullet point 5: At the end of the bullet point, the following sentences have been added: 'Intraventricular haemorrhage is defined as intraventricular haemorrhage seen on either side of the head by imaging or post mortem examination; Grade 1 − Sub ependymal germinal matrix haemorrhage. Grade 2 − Intraventricular haemorrhage with no ventricular distension. Grade 3 − Intraventricular haemorrhage with ventricle distended with blood. Grade 4 − Intraparenchymal haemorrhage. Sepsis is defined as a clinical picture consistent with sepsis, and either a positive bacterial or fungal culture of blood and/or cerebrospinal fluid, or a positive urine culture by sterile collection only.' Page 14 Section 5.4: Primary and secondary outcome measures; bullet point 8 and Page 20: Section 9.1: Serious Adverse Event (SAE): Revised 'SAEs (deaths; maternal or infant admissions to intensive care; and major congenital anomalies)' to 'SAEs [maternal or fetal deaths (stillbirth), fetal loss (miscarriage)]; maternal admissions to intensive care; admissions to intensive care for infants born after 34 weeks; and major congenital anomalies]'. Page 18 Section 8.3: Between 22 and 26 weeks' gestation: Addition of a blood sample via venepuncture and a urine sample collection at this time. Page 19 Section 8.6 At birth and discharge from hospital after birth; bullet point 4: Revised from 'small for gestational age (defined as below the 10th percentile for corresponding gestation age and sex)' to 'small

	for gestational age (defined in two different ways: below the 10th centile and below 3rd centile for corresponding gestation age and sex)'. Page 19 Section 8.5: 34 weeks' gestation; bullet point 3: Revised from 'Collection of maternal blood sample (finger prick) for fatty acid analysis' to 'Collection of maternal blood sample (finger prick) for fatty acid analysis at 34 weeks appointment or birth whichever occurs first.'
. . . 2015-Sep-14	Amendment 3; Protocol v4 Page 1: Design: Principal Investigator changed from to Dr Shao (Jo) Zhou to Primary Contact Professor Maria Makrides Page 8: Addition of Dr K Best as Chief Investigator Page 9: Addition of Study Locations: Joondalup Health Service, Brisbane Mater Mothers Hospital, Werribee Mercy Hospital, Northern Adelaide Health Service (Lyell McEwin Hospital and Modbury Hospital) Page 23: Emergency contact details: Changed from to Dr Shao (Jo) Zhou to Professor Maria Makrides

The ORIP Trial

Participant Information Sheet and Informed Consent Form

Scientific Title: Omega-3 fats to Reduce the Incidence of Prematurity – the ORIP Trial

You are invited to participate in a study to determine whether increasing the amount of a special fat called DHA in the diet of pregnant women lowers the risk of preterm birth. This study is being conducted by Dr Jo Zhou and her colleagues from the South Australian Health and Medical Research Institute (SAHMRI) and the University of Adelaide.

Why are we doing this study?

Most infants are born at term (around 40 weeks of pregnancy) but unfortunately some infants are born prematurely (before 37 weeks of pregnancy). Although we have made tremendous improvements in the care of premature infants there are still many health risks of being born early including developmental delay, chronic lung disease and even death in rare cases. These risks are greater the earlier the baby is born, particularly in those born very premature (less than 34 weeks). This study will investigate whether taking a natural dietary supplement, a fish oil extract rich in the omega 3 fat called DHA, will help prevent very premature delivery.

DHA is found in fish and fish oils and is thought to have a number of health benefits including helping to maintain a pregnancy to full term. We have found evidence to support this when we conducted a study of DHA supplementation in over 2,000 pregnant women to see if DHA supplementation in pregnancy benefits the development of children (the DOMInO trial). We observed that women who took DHA-rich fish oil supplements from 20 weeks until birth had pregnancies that were 1-2 days longer than women in the control group. We also saw that fewer women in the DHA-rich fish oil group gave birth to very premature babies. However, this was offset to some extent by an increase in the number of women who had gone over their due date and were induced or had Caesarean-sections.

In the ORIP trial, we will examine the effects of taking DHA supplements in pregnancy more carefully and focus on the rate of preterm birth. We will use the same treatment as in the DOMInO trial, but we will start the supplementation earlier and stop the supplements at 34 weeks. We hope that this can prevent very premature birth without increasing the need for inducing birth or Caesarean section.

What is involved?

You will be randomly assigned (like tossing a coin) to one of two groups. One group will be asked to take capsules with DHA-rich fish oil and the other group will take capsules containing a blend of vegetable oils (canola, sunflower and palm oils) with a small amount of fish oil with DHA to best match what is typical in the Australian diet and to aid masking. Neither you, nor the research team, will be able to choose which group you are in or know which type of capsules you have been assigned to take. You will be asked to take 3 capsules per day that are easy to swallow from enrolment until 34 weeks pregnancy or the birth of your baby, whichever comes first. The total amount of fat from the 3 capsules is tiny – only 1.5 gms per day which is negligible in terms of energy.

The dose of DHA in the DHA-rich fish oil capsules is the same as that used in DOMInO and has been shown to be safe. The only side effect was some women reporting fishy burps. The other

capsules will contain DHA at a level that approximates many DHA fortified foods, for example, 2 slices of DHA fortified bread.

You can be part of this study if you are under 20 weeks of pregnancy. If you are on multivitamin supplements containing fish oil you can still take part in the study if you are willing to stop taking those supplements or change to ones that do not contain fish oil.

What will happen during the study?

1. At enrolment, we will collect some information about you, including contact details, age, weight, height, education, occupation, smoking status and pregnancy related information.
2. A tiny blood sample by finger prick will be taken at enrolment and again at 34 weeks of pregnancy to assess fatty acid levels.
3. We will also contact you 2 weeks after enrolment and at 28 weeks of pregnancy to monitor your progress, ask a few questions about compliance and answer any questions you may have.
4. Following the birth of your baby, details about your pregnancy and birth as well as your infant will be collected from your medical records and your baby's medical records.

Optional blood and urine collection for an additional study related to the ORIP trial

The following samples are optional; if you decide not to have these samples taken you can still participate in the study. For those who choose to have these samples taken, they may be used to look at how fats work in the body (mechanisms of action) and how they might interact with other agents. The collection and analysis of these optional additional samples will help to understand how DHA may improve immune function and lower the risk of preterm birth. Your data (without any way to identify you) may be used in pooled results from different studies so we can gain a more complete understanding. Following analysis for the ORIP trial remaining samples may be stored for use in future research studies that may or may not be related to the original research project.

These samples include:

- An extra 20mL of blood collected via venepuncture once at the start and at mid-pregnancy between 22-26 weeks.
- A urine sample collected at mid-pregnancy between 22-26 weeks
- A cord blood and placenta sample collected at birth. The cord blood collected for this study will preclude families from using it for other purposes in the future.

We will reimburse you with $15 for your travel costs should your enrolment and / or 34 week appointments at the hospital fall outside your regular antenatal visits.

Possible benefits

You may or may not directly benefit by your participation in this study. However, participants taking the capsules containing the higher dose of DHA may be at less risk of giving birth to a very premature baby.

Possible risks

We have already shown in the DOMInO trial that DHA at the dose to be used in the study is safe. At extremely high doses (12 times higher than the dose in this study) DHA rich fish oil may result in an approximately 10% increase in blood clotting time and a 10% decrease in blood pressure, neither of which is considered dangerous. Taking blood may cause brief discomfort or pain. All blood samples will be taken by trained, experienced staff to minimise any discomfort.

Future follow-up studies

DHA has a number of potential health benefits. We may conduct follow-up studies to determine the potential benefits. If you consent to participate in this trial it does not mean you have agreed to any future studies. We would contact you again to see if you are willing to take part as each study begins.

Alternate Contacts

We recognise that people often change their telephone number and address, and therefore cannot be contacted by researchers. To help keep in contact with you we are asking you to provide us with the names and contact details of persons who would be able to let us know your new contact details. These people are usually friends or relatives and are called alternate contacts. If we needed to use one of the alternate contacts we would call them, explain who we are and that you were involved in a study and have given us their contact details so that they can put us in touch with you.

Your rights

Participation in any research project is voluntary and you are free to withdraw from the study at any time without affecting yours or your child's care in any way. If you decide to withdraw, please let any member of the research team know. All information gathered will be treated with confidence and no information that could identify you or your baby will be released to any person not associated directly with the study. These results may eventually be published in medical journals or at professional meetings, but you or your child will not be identified in any way.

Your information will remain confidential except in the case of a legal requirement to pass on personal information to authorised third parties. This requirement is standard and applies to information collected both in research and non-research situations. Such requests to access information are rare; however we have an obligation to inform you of this possibility.

Any questions?

If at any time during the study you have any problems regarding appointments or have any other queries, please ring our office on **8161 7512** and leave a message on our answering machine. One of our staff will return your call as soon as possible. Alternatively, you can email us at orip@sahmri.com. If you have a problem and would like to talk to us immediately please ring **0474 333 001** and one of our research team members will answer your call.

Study Updates

Detailed information about the ORIP study and other Child Nutrition Research Centre studies and trials can be found on the SAHMRI Healthy Mothers, Babies and Children web page: www.sahmri.com/our-research/themes/healthy-mothers-babies-children/theme/theme-overview. More information about the Child Nutrition Research Centre and the work we do can be found on Facebook at www.facebook.com/CNRCAdelaide.

Study approval

This study has been reviewed and approved by the Human Research Ethics Committee at the Women's & Children's Health Network. If you wish to discuss the approval process with someone not directly involved, or have any concern or complaint, you may contact the Research Secretariat, Ms Brenda Penny on: (08) 8161 6521.

WOMEN'S & CHILDREN'S HEALTH NETWORK (WCHN)
HUMAN RESEARCH ETHICS COMMITTEE (HREC)

CONSENT FORM

The ORIP trial

SCIENTIFIC TITLE: Omega-3 Fats to Reduce the Incidence of Prematurity - the ORIP trial

I _____

hereby consent to my involvement in the research project entitled:

"Omega-3 Fats to Reduce the Incidence of Prematurity"

1. The nature and purpose of the research project described on the attached Information Sheet has been explained to me. I understand it and agree to taking part.
2. I understand that I may not directly benefit by taking part in this study.
3. I acknowledge that the possible risks and/or side effects, discomforts and inconveniences, as outlined in the Information Sheet, have been explained to me.
4. I understand that I can withdraw from the study at any stage and that this will not affect medical care or any aspects of my own or my child's relationship with this healthcare service.
5. I understand that there will be no payment to me for taking part in this study but that I will be reimbursed for travel costs should my enrolment and / or 34 week appointments at the hospital fall outside my regular antenatal visits.
6. I have had the opportunity to discuss taking part in this research project with a family member or friend, and/or have had the opportunity to have a family member or friend present whilst the research project was being explained by the researcher.
7. I am aware that I should retain a copy of the Consent Form, when completed, and the Information Sheet.
8. I consent to the following:

   1) To take the assigned capsules from trial entry until 34 weeks of pregnancy or the birth of my baby, whichever comes first.

   2) At trial entry and at 34 weeks pregnancy or at birth whichever comes first, have a finger prick blood sample taken from me.

   3) To be contacted 2 weeks after enrolment and at 28 weeks of pregnancy to monitor my progress, ask a few questions about compliance.

9. I additionally consent to: (please circle)

1) Have my blood collected from me at the start of the study and in mid-pregnancy (between 22-26 weeks) Yes / No

2) Provide a urine sample in mid-pregnancy (between 22-26 weeks) Yes / No

3) Have a cord blood sample collected at birth Yes / No

4) Have a placenta sample collected at birth Yes/ No

10. I do / do not consent to the samples being used in any other research project, provided the project has the approval of the Women's & Children's Health Network Human Research Ethics Committee.

11. I agree to the accessing of my and my child's medical records at the Women's and Children's Health Network and any other hospitals my baby and I may be transferred to and from, for the purpose of this study.

12. I understand that the alternate contacts provided by me may be used to contact me as explained in the information sheet for study related purposes.

13. I am aware that I may be contacted regarding future follow-up studies.

14. I understand that my and my child's information will be kept confidential as explained in the information sheet except where there is a requirement by law for it to be divulged.

Signed: .....

Relationship to Patient: .....

Full name of patient: .....

Dated:.....

I certify that I have explained the study to the parent mother and consider that she understands what is involved.

Signed: ..... Title: .....

Dated: .....

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	Yes
Protocol version	3	Date and version identifier	Supp.
Funding	4	Sources and types of financial, material, and other support	20
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1
	5b	Name and contact information for the trial sponsor	1
	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	N/A
	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)	16

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
	6b	Explanation for choice of comparators	5
Objectives	7	Specific objectives or hypotheses	10, 11
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)	8
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	8
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)	8, 9
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)	N/A
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	10
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A
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	10, 11
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)	12 & Fig. 2

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	12, 13
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	13
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	13
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	13, 14
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	14
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	14
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, 15
	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	14, 15

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	15, 16
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	15, Supp. SAP
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	16
	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)	16
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	16, 17
	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	16
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	14, 15
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	17
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)	17

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	13
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
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	18
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	20
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	N/A
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	Supp. PICF
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	18
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Supp. PICF
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	Supp. PICF

*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

Study protocol for a randomized controlled trial evaluating the effect of prenatal omega-3 LCPUFA supplementation to reduce the incidence of preterm birth: The ORIP trial

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Study protocol for a randomized controlled trial evaluating the effect of prenatal omega-3 LCPUFA supplementation to reduce the incidence of preterm birth: The ORIP trial

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Keywords: Pregnancy, preterm birth, premature birth, docosahexaenoic acid, omega-3 long chain polyunsaturated fatty acids, prevention, maternal diet

Abbreviations

AA, arachidonic acid; ACTRN, Australia and New Zealand Clinical Trial Registry; CRF, case report form; DOMInO, DHA to Optimise Mother and Infant Outcome; DHA, docosahexaenoic acid; DMAC, data management and analysis centre; EPTB, Early preterm birth; EPA, eicosapentaenoic acid; EDD, estimated date of delivery; GA, gestational age; Good Clinical Practice; GMP, code of good manufacturing practice; HREC, Human Research Ethics Committee; ID, Identifier; LA, linoleic acid; LCPUFA, long-chain polyunsaturated fatty acids; mg, milligram; ω-3, omega-3; ω-6, omega-6; ORIP, the acronym is derived from 'Omega-3 fatty acids to reduce the incidence of prematurity'; NHMRC, National Health and Medical Research Council of Australia; RR, Relative Risk; SAE, Serious Adverse Event.

ABSTRACT

Introduction: Preterm birth accounts for more than 85% of all perinatal complications and deaths. Seventy-five percent of early preterm births occur spontaneously and without identifiable risk factors. The need for a broadly applicable, effective strategy for primary prevention is paramount. Secondary outcomes from the Docosahexaenoic Acid (DHA) to Optimize Mother Infant Outcome (DOMInO) trial showed that maternal supplementation until delivery with omega-3 long chain polyunsaturated fatty acid (LCPUFA), predominantly as DHA, resulted in a 50% reduction in the incidence of early preterm birth, but also an increase in the incidence of post-term induction or post-term pre-labour caesarean section due to extended gestation. We aim to determine the effectiveness of supplementing the maternal diet with omega-3 LCPUFA until 34 weeks' gestation on the incidence of early preterm birth.

Methods and analysis: This is a multicentre, parallel group, randomised, blinded and controlled trial. Women less than 20 weeks' gestation with a singleton or multiple pregnancy and able to give informed consent are eligible to participate. Women will be randomised to receive high DHA fish oil capsules or control capsules without DHA. Capsules will be taken from enrolment until 34 weeks' gestation. The primary outcome is the incidence of early preterm birth, defined as delivery before 34 completed weeks' gestation. Key secondary outcomes include length of gestation, incidence of post-term induction or pre-labour caesarean section and spontaneous early preterm birth. The target sample size is 5540 women (2770 per group), which will provide 85 % power to detect an absolute reduction in the incidence of preterm birth of l.16% (from 2.45% to 1.29%) between the DHA and control group (two sided α =0.05). The primary analysis will be based on the intention-to-treat principle.

Trial Registration: Australia and New Zealand Clinical Trial Registry Number: 2613001142729

INTRODUCTION

Background

Preterm birth, or birth before 37 completed weeks of pregnancy, is the leading cause of death in newborns, accounting for more than 85% of all perinatal complications and death.[1] It is estimated that 10% of births (13 million babies each year worldwide) are preterm and 20% of these preterm births occur before 34 weeks, these being referred to as early preterm birth (EPTB). It is EPTB that is the major cause of perinatal mortality, serious neonatal morbidity and moderate to severe childhood disability in developed countries.[2-4] Effective, broadly applicable primary prevention strategies to reduce the risk of EPTB are lacking.

Prostaglandins and other lipid mediators derived from omega-6 (ω-6) and omega-3 (ω-3) fatty acids play essential roles in normal and pathologic initiation of labour.[5 6] The feto-placental unit is supplied with long chain polyunsaturated fatty acids (LCPUFA) from the maternal circulation, which is influenced by maternal LCPUFA intake and endogenous synthesis. Prostaglandins derived from ω-6 arachidonic acid (AA) are countered by those derived from ω-3 LCPUFA within the same tissues. The balance between the metabolites of ω-3 and ω-6 fatty acids plays an important role in the maintenance of normal gestation length and is a critical element in cervical ripening and the initiation of labour.[7] If local production of ω-6 derived prostaglandins within the feto-placental unit is too high, or local accumulation of ω-3 LCPUFA is too low, the cervix may prematurely ripen and uterine contractions increase, which may in turn lead to preterm birth or EPTB.

Current Western diets are low in ω-3 LCPUFA.[8] The World Health Organization recommends an intake of 300 mg/d of ω-3 LCPUFA for pregnant women, however, the median intake in Australian women of child bearing age is only 29 mg/day[9] compared with 1000mg/d in women from nations with high-fish consumption such as Japan, Korea and Norway. The intake of ω-3 LCPUFA is further reduced among pregnant women due to a reduced intake of all fish

following advisories for pregnant women to avoid specific long-lived predatory fish due to the concern about mercury contamination.[10] The level of ω-3 LCPUFA intake estimated to achieve normal rises in the feto-placental unit for the maintenance of a full term pregnancy is well above the intake of many pregnant women, highlighting a potential insufficiency for the uterus and feto-placental unit. Optimising ω-3 LCPUFA intake in pregnancy may restore the balance between ω-3 and ω-6 fatty acids and reduce the risk of preterm birth.

At the time the trial commenced, only 2 of the 6 included trials in the Cochrane review[11] of marine oil supplementation in pregnancy reported the effect on EPTB. Both trials[12 13] were conducted in high risk pregnancies designed to assess the effect of ω-3 LCPUFA supplementation to prevent reoccurrence of intrauterine growth restriction, preterm birth or pregnancy induced hypertension. From these two relatively small trials (total of 860 women), it was seen that women allocated to receive fish oil had a lower risk of EPTB compared with control (relative risk (RR) 0.69, 95% CI 0.49 to 0.99). Systematic reviews[11 14 15] have shown no difference in the incidence of antepartum hospitalisation, caesarean section, eclampsia or other serious maternal morbidity between treatment (ω-3 LCPUFA supplements) and control groups. The relative risk of miscarriage, stillbirth and neonatal death also did not differ between the groups[11 14 15]. None of the included trials reported the effect of ω-3 LCPUFA supplementation on EPTB in the general population of pregnant women.

The strongest evidence to support the efficacy of ω-3 LCPUFA supplementation to reduce EPTB comes from our Docosahexaenoic Acid [DHA] to Optimize Mother Infant Outcome [DOMInO] trial[16], the largest trial to date of ω-3 LCPUFA supplementation in pregnancy. DOMInO was designed to assess the effect of ω-3 LCPUFA supplementation during the last half of pregnancy on the prevalence of postnatal depression in women and on early childhood neuro-developmental outcomes. We enrolled 2399 women from 5 perinatal centres around Australia who were randomly assigned to receive identical looking capsules containing either

fish oil concentrate (900 mg ω-3 LCPUFA/day) or a blend of vegetable oils (no ω-3 LCPUFA) from 20 weeks' gestation until birth. In a pre-specified secondary analysis, there were fewer EPTB in the ω-3 LCPUFA supplemented group compared with control (1.09% vs. 2.25%, adjusted RR 0.49, 95% CI 0.25 to 0.94, p=0.03) and fewer admissions to level three intensive care (1.75% vs. 3.08%, RR 0.57, 95% CI 0.34 to 0.97, p=0.04). Similar findings were reported in a smaller US trial (n=301), the Kansas University DHA Outcomes (KUDOS) Study[17], which showed that supplementation with 600 mg of DHA daily from mid-pregnancy until birth resulted in a reduction in the incidence of EPTB (0.6% vs. 4.8%, p=0.025). The effect of DHA supplementation in pregnancy in lowering the risk of EPTB observed in both the DOMInO and KUDOS trials is consistent with the Cochrane review[18] published before these two trials. Despite these encouraging findings, results have been viewed with caution by researchers and clinicians because EPTB was a secondary outcome of the DOMInO and KUDOS trials. A recent update to this Cochrane review (publication pending) includes 54 randomised trials with a total of >15,000 participants. Results show a clear reduction in EPTB (RR 0.61, 95% CI 0.46 to 0.81; 9 RCTs; n = 4351) for supplementation compared with placebo[19]. Our DOMInO trial supports the safety data from previous systematic reviews, however there were more post-term births requiring obstetric intervention (induction or caesarean section) in the ω-3 LCPUFA supplemented group compared with control (17.59% vs. 13.72%, adjusted RR 1.28, 95% CI 1.06 to 1.54, p=0.01). To determine both efficacy and safety of this intervention, we therefore plan to undertake a further trial with adequate power to assess the effect of ω-3 LCPUFA supplementation in pregnancy on EPTB as the primary outcome, with a unique design ceasing the intervention at 34 weeks' gestation to reduce the risk of post-term birth.

Hypotheses

Optimising ω-3 LCPUFA intake in pregnancy will lead to lower risk of EPTB.

METHODS

Trial design

The ORIP trial is designed as a randomised, controlled, clinician, researcher and participant/family blinded, multicentre trial with two parallel groups and a primary outcome of incidence of EPTB, defined as delivery before 34 completed weeks' gestation.

Participating Centres

The sponsoring institution and Trial Coordinating Centre is the South Australian Health and Medical Research Institute based at the Women's and Children's Hospital, Adelaide, South Australia. The trial is being conducted in the following six centres in Australia: Women's & Children's Hospital, Flinders Medical Centre and Lyell McEwin Hospital in South Australia; Joondalup Health Campus, Western Australia; Werribee Mercy Hospital, Werribee, Victoria; and Mater Mothers Hospital, Brisbane, Queensland.

Study Population

Participants are pregnant women less than 20 weeks' gestation.

Eligibility Criteria

Pregnant women are approached by trained clinical trial personnel in the antenatal clinic of participating centres.

Inclusion Criteria

Women must meet both the following criteria to be enrolled in this study:

- Singleton or multiple pregnancy and less than 20 weeks' gestation.
- Able to give informed consent.

Exclusion criteria

Women must not have any of the following criteria to be enrolled in this study:

- Fetus with a known abnormality.
- Taking dietary supplements containing LCPUFA > 150mg/day.
- Taking dietary supplements containing LCPUFA ≤150mg/day and are not willing to stop.
- Bleeding disorders where fish oil is contraindicated or on anticoagulant therapy.
- History of drug or alcohol abuse.

Study treatments

Participating women will be randomised to high-DHA fish oil capsules or vegetable oil capsules. The study capsules are identical in colour, size, shape and packaging and are iso-caloric. Women will be asked to consume three capsules per day.

DHA Capsules

Each 500mg DHA enriched fish oil capsule contains approximately 300mg of ω-3 LCPUFA. Women will be asked to take three capsules per day orally, which will provide approximately 1g of ω-3 LCPUFA (800mg of DHA and 100 mg of eicosapentaenoic acid [EPA]) per day. The remaining portion of oil consists of monounsaturated and saturated fatty acids.

Control Capsules

The control capsules (also 500mg in weight) contain a blend of vegetable oils (canola, sunflower and palm oils) with a trace of tuna oil (12.6mg ω-3 LCPUFA) to assist with blinding. This blend of oil matches the fatty acid composition of the typical Australian diet.

Manufacture of study capsules

The active and control capsules are manufactured in a licensed facility (licensed according to the Code of Good Manufacturing Practice (GMP)) and have been donated to the trial by Efamol Ltd/Wassen International Ltd. The capsules are packaged and labelled in accordance with GMP including an individual product identifier (ID), batch number, expiry date and the statement "for clinical trial use only". The pharmacist or the investigator's designee maintain

accurate records of the receipt of all study capsules, including dates of receipt. Unused study capsules will be destroyed in compliance with applicable regulations.

Monitoring adherence to study treatment

Research personnel in each centre will maintain regular contact with participating women to monitor and encourage capsule adherence and study compliance and answer any questions as they arise. Women are asked to return unused capsules at the final study visit (34 weeks' gestation) and the proportion of capsules returned serves as an additional measure of compliance. A finger prick blood specimen collected from the woman at this final visit will be used in fatty acid analysis as an independent biomarker of adherence. All clinical care for women involved in this trial will be undertaken by her designated obstetric/midwifery care provider.

Outcome Measures

The primary outcome of this trial is the incidence of EPTB, defined as delivery before 34 completed weeks' gestation. Gestational age (GA) in days at delivery for primary and secondary outcomes will be determined from the estimated date of delivery (EDD) using the equation [280 - (EDD - date of birth)]. EDD will be determined using a combination of maternal menstrual dating and ultrasound dating in accordance with established obstetric practice, as outlined in Figure 1.

Key Secondary outcomes:

- Length of gestation
- Post-term induction or post-term pre-labour caesarean section
- Preterm birth (<37 completed weeks)
- Spontaneous EPTB,
- Birth weight
- Low birth weight (< 2500g).

- Admission to Neonatal Intensive Care Unit
- Perinatal Death (stillbirth and death within first 28 days of birth)

Other Outcomes

- Spontaneous preterm birth, pre-labour rupture of membranes, pre-labour, preterm rupture of membranes.
- Birth length, birth head circumference, small for GA, very small for GA, large for GA, very large for GA.
- The length of hospital stay of infants and mothers, length of admission in Neonatal Intensive Care Unit.
- Pregnancy and labour outcomes including; gestational diabetes, pregnancy induced hypertension, pre-eclampsia, eclampsia, maternal use of antibiotics, caesarean section, estimated blood loss at birth and post-partum haemorrhage.
- Incidence of Serious Adverse Event (SAE) defined as; maternal, neonatal or fetal deaths (still birth); fetal loss (miscarriage); maternal admissions to intensive care; admissions to intensive care for infants born after 34 weeks' gestation; and major congenital anomalies.
- Neonatal complications including jaundice (requiring phototherapy), hypoglycaemia (requiring intravenous dextrose infusion), neonatal convulsion, brain injury (including intra-ventricular haemorrhage), retinopathy of prematurity, sepsis, necrotising enterocolitis and duration of supplemental oxygen. The definitions and classifications of these neonatal complications are in accordance with the Australian and New Zealand Neonatal Network Data Dictionary.[20]
- Other side effects and tolerability of DHA supplementation assessed through data collected in the case report form.

Participant Timeline

Women will be randomised and commence study treatment capsules from enrolment (<20 weeks' gestation) until 34 weeks' gestation or birth, whichever occurs first. At enrolment, following informed consent and prior to commencement of study treatment, research personnel will collect a finger prick blood sample for baseline fatty acid status. The woman's height and weight will be measured and baseline clinical and demographic data collected. Research personnel will contact the participant two weeks following the enrolment visit and again when the woman is 28 weeks' gestation to ascertain capsule adherence and record adverse events. At 34 weeks' gestation participating women will attend a clinic appointment for collection of a finger prick blood sample for fatty acid analysis and unused capsules are returned and counted. Six weeks following delivery, research personnel will extract details of pregnancy, labour and birth from the woman and her baby's medical records for primary and secondary outcome data; see Figure 2 for a summary of the ORIP trial schema.

Sample Size

Our previous trial of DHA supplementation of pregnant women, the DOMInO trial[16], included women with singleton pregnancies and treatment resulted in a 1.16% absolute reduction (from 2.25% to 1.09%) in the incidence of EPTB. This reduction occurred in the presence of non-adherence resulting from women assigned to the intervention failing to take the trial supplements as directed, and control women taking prenatal supplements containing low dose ω-3 LCPUFA. Similar levels of non-adherence are expected in the ORIP trial. Inclusion of women with multiple pregnancies in the ORIP trial is expected to result in a small increase in the incidence of EPTB in both treatment groups, since EPTB is more common in multiple pregnancies. To demonstrate an absolute reduction in the incidence of EPTB of 1.16% (from 2.45% to 1.29%) with 85% power and overall two-sided alpha=0.05 (alpha=0.049 at the final analysis), a sample size of 2631 per group (i.e. 5262 total) is required. Allowing for a loss to follow up of 5%, a total of 5540 women is required to ensure adequate power for the primary

outcome. Since EPTB is a mother level outcome, the sample size calculations define the number of mothers that should be recruited and no adjustment for clustering due to multiple pregnancies is required for this outcome.

Recruitment

Women will be approached to enter the trial by research staff at the time of attending their routine antenatal visit at one of the centres involved. Pregnant women will also be informed about the trial by display of approved advertising material which will be circulated in hard copy and via social media avenues. A participant information sheet describing the purpose of the study, the procedures to be followed, and the risks and benefits of participation will be explained to interested women. Following a screening process to ensure inclusion and exclusion criteria are met, the investigator, or nominee will conduct the informed consent discussion and will check that information provided is understood and answer any questions about the study. A record of all women screened and their enrolment status will be maintained to adhere to the consolidated standards for the reporting of randomised controlled trials.[21] Women may withdraw their involvement in the trial at any time and wherever possible the reason for withdrawal will be recorded.

Randomisation procedures

Once the consent process has been documented by signing of the written consent form, the participant will be randomised using a web-based randomisation service. Stratification will be by centre and current supplement use (no LCPUFA vs ≤150mg LCPUFA/day prior to randomisation). Allocation follows a computer-generated randomisation schedule with balanced variable blocks, prepared using ralloc.ado version 3.7.5 in Stata version 12.1 by an independent statistician who is not involved with trial participants or data analysis. A unique six-digit study ID and a study pack with a unique product ID are assigned. The study ID

identifies the randomised woman and the product ID identifies a pack of five bottles of either active or control capsules, pre-packaged corresponding to the randomisation schedule.

Blinding

Participants and their family, care providers, data collectors, outcome assessors, research personnel and data analysts will all be blinded to randomisation group. The intervention and control capsules are identical in shape, size, colour, packaging and labelling and uniquely identified only by the product ID. In the DOMInO trial, we identified that more women in the intervention group correctly guessed their group allocation. We therefore added a small amount of tuna oil (5%) to the control capsules to enhance blinding of study participants. The randomisation code for an individual participant may be un-blinded in an emergency if the Site Investigator decides a participant cannot be adequately treated without knowing their study treatment allocation. The Principal Investigator will be notified and must contact the Data Management and Analysis Centre, where the randomisation list is securely stored. The time, date, study ID of the participant and reason for un-blinding must be documented and events leading to the emergency un-blinding will be recorded.

Data collection and trial management

A purpose-built web-based clinical trial management information system (CTMS) with password protection and defined user-level access is used to facilitate trial management. A record of all women approached, screened for eligibility, and consented is recorded in real time in the CTMS. Once consented and randomised, the CTMS automatically calculates study milestones for each participant. This information is readily available for clinical trial staff to enable scheduling of appointments and sample collection. Data entered by individual study centres is routinely scrutinized by the Coordinating Centre to monitor protocol adherence and study progress. Summary reports including screening data, enrolment, appointment attendance, sample collection, serious adverse events and study completion are generated from the CTMS

and reviewed at monthly trial steering committee meetings. Site monitoring visits to ensure compliance with good clinical practice and the study protocol are conducted at site start-up and then six monthly or as required to ensure the integrity of the trial.

Data are collected by trained clinical trial staff at each participating centre onto carbon copy paper case report forms (CRF). Carbon copies of the completed CRF remain on site at participating centres with original copies being mailed to the Data Management and Analysis Centre at the University of Adelaide for data management including data entry and data cleaning. Data validity checks are completed by data entry personnel and data queries posted to the CTMS for resolution by participating centres. Data queries generated by statisticians during regular blinded reviews of data quality and batch checks are also managed through the CTMS. Electronic data are stored on secure servers at the Data Management and Analysis Centre and released only to persons authorised to receive those data. Following study completion, copies of study documents are retained at each study site or in archives. Documents held at the Coordinating Centre will be retained for at least 30 years after study completion in line with the data retention schedules for research involving minors[22]. At the completion of this time, documentation will be destroyed using confidential document disposal. Electronic data will be stored indefinitely on secure servers with access only granted to authorised study personnel.

Statistical methods

All participants will be analysed according to the group to which they were randomised (intention-to-treat principle) for the primary analysis. The primary outcome of EPTB will be compared between intervention and control groups using a log binomial regression model. Adjustment will be made for the stratification variables centre and supplements, and results will be presented as a relative risk with confidence interval and 2-sided p-value. Statistical significance will be assessed at an alpha of 0.049 to account for the pre-specified interim analysis (see below) using the O'Brien-Fleming approach. Key secondary and other outcomes

will be compared between treatment groups using log binomial regression models for binary outcomes and linear regression models for continuous outcomes with no adjustment for multiple comparisons. Clustering due to multiple pregnancies and repeat participation in the trial will be taken into account where applicable using generalised estimating equations. Secondary analyses will be performed for the primary outcome and related key secondary and other outcomes to test for evidence of effect modification by pregnancy status (single vs multiple pregnancy), history of preterm birth and baseline DHA. Missing data will be addressed using multiple imputation under a missing at random assumption. Sensitivity analyses will be performed using the original un-imputed data. A secondary per-protocol analysis will be performed for the primary outcome including only women who were compliant with the protocol. Two separate definitions of compliance will be used. First, women will be considered compliant if they returned their remaining capsules at the end of the trial and consumed at least 75% of the number of capsules expected to be consumed. Second, women will be considered compliant if they had the 28-week telephone contact and reported they had missed less than 7 capsules in the last week out of the recommended 21 capsules.

All analyses will follow a pre-specified statistical analysis plan. There is a single planned interim analysis of the primary outcome to be performed after data collection is complete for the first 50% of the target number of randomized participants.

Data monitoring

An independent Data Monitoring Committee (DMC) has been set up to review the progress of the trial and provide feedback to the Trial Steering Committee. The DMC reviews general study progress (recruitment, compliance, loss to follow-up), the EPTB event rate and key secondary/safety outcomes. The committee meets annually or as required and consists of a neonatologist, obstetrician and statistician with clinical trials expertise. The DMC will be responsible for reviewing the results of the interim analysis.

An independent (blinded) SAE Committee has been established to review SAEs. There are no SAEs which would be anticipated as a unique consequence of participation in the trial, however all deaths, admissions to an intensive care unit of mothers or of infants born after 34 completed weeks' gestation, and major congenital abnormalities are to be reported. The SAE Committee includes experts in obstetrics, neonatology, pathology and midwifery who are not involved in the trial. The primary role of the SAE Committee is to review all SAEs and determine whether there is any likelihood that involvement in the trial could have contributed to the event. Determinations of causality will be made from medical records retrieved for this purpose. This committee meets quarterly, or as required.

ETHICS AND DISSEMINATION

Research ethics approval

This protocol and the informed consent and participant information document have been approved by the Human Research Ethics Committee (HREC) of each study site. Women's and Children's Health Network HREC (Coordinating HREC & Women's and Children's Hospital, Adelaide; Southern Adelaide Clinical HREC, Adelaide, South Australia); Mercy Health HREC (Werribee Mercy Hospital, Werribee, Victoria); Mater Mothers' Hospital, Brisbane, Queensland), University of Notradame, Joondalup Health Campus, Joondalup, Western Australia. Any subsequent modifications will be reviewed and approved by the HREC and governance of each study site. The study will be conducted in compliance with the current approved version of the protocol. Any change to the protocol document or informed consent form that affects the scientific intent, study design, patient safety, or may affect a participant's willingness to continue participation in the study will be considered a major amendment. All such amendments will be submitted to the HREC for approval. Any other changes to the protocol (such as administrative changes to dates and study personnel) will be considered minor amendments and will be notified to the HREC as appropriate. Study findings will be

disseminated via peer-reviewed publication, conference presentations, informing participants with a lay summary and the wider community through social media.

Confidentiality

Participant confidentiality is strictly held in trust by the participating investigators, research staff and their agents. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participants. The study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorised third party, without prior written approval of the Coordinating Centre. The Coordinating Centre and regulatory authorities may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records and pharmacy records for the infants in this study subject to individuals having obtained approval/clearance through State/National Governments and HREC as required by local laws. The study site will permit access to such records. Clinical information will not be released without written permission of the parent/guardian, except as necessary for monitoring by HREC or regulatory agencies.

Dissemination plan

Study findings will be submitted for peer-reviewed publication and for presentation at appropriate local and international conferences. In addition, study findings will be disseminated to participants through a one-page lay summary. Results will be made available to the wider community through social media avenues and the South Australian Health & Medical Research Institute website.

Trial status

This enrolment phase of this trial completed on 27th April 2017 with successful attainment of sample size (n=5544). Completion of study follow up appointments and collection of labour and birth data from participant medical records will be completed by December 2017.

Strengths and limitations of this study

- This study is the largest randomised controlled trial with adequate power that is designed to assess the effect of ω-3 LCPUFA supplementation in pregnancy on the incidence of early preterm birth as the primary outcome.
- The study uses an innovative intervention strategy, which is unique compared with other trials in this field. The intervention targets the period before and during the time when early preterm birth is likely to occur and it is ceased at 34 weeks' gestation, at which time early preterm birth has been prevented. This strategy may reduce the risk of post-term birth.
- The study assesses maternal LCPUFA status at baseline and at the end of the intervention period, providing an independent biomarker of compliance, in addition to maternal self-report and capsule back count.
- The study is conducted in an industrialised setting where intake of ω-3 LCPUFA is low. Findings from this study may not be applicable in populations with high intake of ω-3 LCPUFA.

Acknowledgements

We thank the research and clinical teams at all participating study centres.

Authors' contributions

MM, JQ, RAG, AJM and SJZ conceived and designed the study with KPB contributing to ongoing study implementation and management. LY provided statistical expertise. SJZ and KPB wrote the first draft of the manuscript. All authors contributed to refinement of the study protocol and approved the final manuscript.

Funding statement

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Competing Interests

The authors declare: financial support for the submitted work from the National Health and Medical Research Council (NHMRC) Australia (Project Grant 1050468). RAG serves on a scientific advisory board for Fonterra; MM serves on scientific advisory boards for Nestle and Fonterra. Associated Honoraria for RAG and MM are paid to their institutions to support conference travel and continuing education for postgraduate students and early career researchers. The authors declare that they have no competing interests.

Additional authors and members of the ORIP Investigative Team

The following are members of the ORIP (Omega-3 fatty acids to Reduce the Incidence of Preterm birth) Investigative team: Gus Dekker (Northern Adelaide Health Service [Lyell McEwin Hospital and Modbury Hospital], Adelaide, South Australia. Elinor Atkinson (Flinders Medical Centre, Adelaide, South Australia), Jodie Dodd (Women's and Children's Hospital, Adelaide, South Australia), Jacqueline van Dam & Nisha Khot (Mercy Hospital, Werribee, Melbourne, Victoria), Huda Safa (Mater Mothers' Hospital, Brisbane, Queensland).

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Figure 1. Flow chart for determining estimated date of delivery (EDD)

LMP, last menstrual period; GA, gestational age; U/S ultrasound; w, weeks

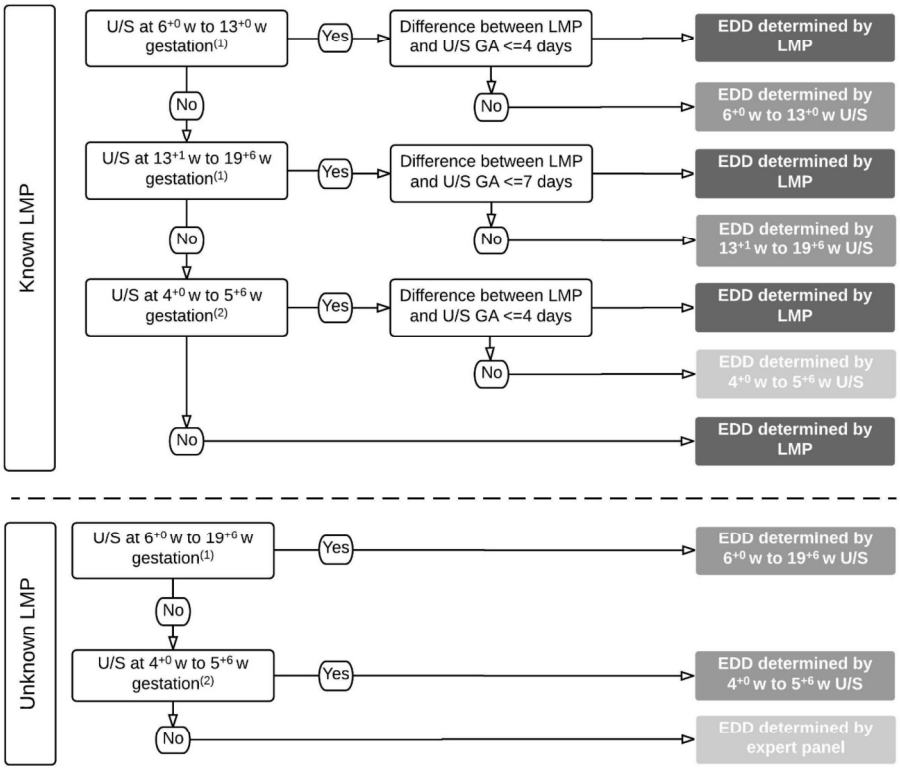
(1) Where multiple ultrasounds are performed within a given GA range, the earliest ultrasound will be used to determine gestational age.
(2) Where multiple ultrasounds are performed within a given GA range, the latest ultrasound will be used to determine gestational age.

Figure 2. ORIP Trial Schedule

***-t1 = <20 weeks' gestation; t1 = 2 weeks' post randomization and allocation; t2 = 28 weeks' gestation; t3 = 34 weeks' gestation or birth, whichever occurs first; t x= 6 weeks post estimated due date.*

Figure 1. Flow chart for determining estimated date of delivery (EDD)

LMP, last menstrual period; GA, gestational age; U/S ultrasound; w, weeks



(1) Where multiple ultrasounds are performed within a given GA range, the earliest ultrasound will be used to determine gestational age.

(2) Where multiple ultrasounds are performed within a given GA range, the latest ultrasound will be used to determine gestational age.

229x248mm (300 x 300 DPI)

Figure 2. ORIP Trial Schedule

***-t1 = <20 weeks' gestation; t1 = 2 weeks' post randomization and allocation; t2 = 28 weeks' gestation; t3 = 34 weeks' gestation or birth, whichever occurs first; t x= 6 weeks post estimated due date.*

	Enrolment	Allocation	Post-allocation			Completion
TIMEPOINT**	$-t_1$	0	t_1	t_2	t_3	t_x
ENROLMENT:						
Eligibility screening	X					
Informed consent	X					
Randomisation	X					
Allocation		X				
INTERVENTION:						
Treatment: 3x500mg capsules ω-3 LCPUFA per day						
OR						
Control: 3x500mg capsules blended vegetable oils						
ASSESSMENTS:						
Fatty Acid Analysis (finger prick)	X				X	
Maternal clinical & demographic details	X					
Compliance & side effects			X	X	X	
Labour & birth data extracted from medical records						X

218x225mm (300 x 300 DPI)

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	Yes
Protocol version	3	Date and version identifier	Supp.
Funding	4	Sources and types of financial, material, and other support	20
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1
	5b	Name and contact information for the trial sponsor	1
	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	N/A
	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)	16

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
	6b	Explanation for choice of comparators	5
Objectives	7	Specific objectives or hypotheses	10, 11
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)	8
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	8
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)	8, 9
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)	N/A
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	10
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A
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	10, 11
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)	12 & Fig. 2

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	12, 13
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	13
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	13
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	13, 14
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	14
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	14
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, 15
	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	14, 15

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	15, 16
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	15, Supp. SAP
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	16
	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)	16
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	16, 17
	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	16
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	14, 15
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	17
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)	17

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	13
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
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	18
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	20
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	N/A
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	Supp. PICF
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	18
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Supp. PICF
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	Supp. PICF

*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.