



BMJ Open Double-blind RCT of fish oil supplementation in pregnancy and lactation to improve the metabolic health in children of mothers with overweight or obesity during pregnancy: study protocol

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ABSTRACT

Introduction Maternal obesity during pregnancy is associated with adverse changes in body composition and metabolism in the offspring. We hypothesise that supplementation during pregnancy of overweight and obese women may help prevent the development of greater adiposity and metabolic dysfunction in children. Previous clinical trials investigating fish oil supplementation in pregnancy on metabolic outcomes and body composition of the children have not focused on the pregnancies of overweight or obese women.

Methods and analysis A double-blind randomised controlled trial of fish oil (providing 3 g/day of n-3 polyunsaturated fatty acids) versus an equal volume of olive oil (control) taken daily from recruitment until birth, and in breastfeeding mothers, further continued for 3 months post partum. Eligible women will have a singleton pregnancy at 12–20 weeks' gestation and be aged 18–40 years with body mass index ≥ 25 kg/m² at baseline. We aim to recruit a minimum of 128 participants to be randomised 1:1. Clinical assessments will be performed at baseline and 30 weeks of pregnancy, including anthropometric measurements, fasting metabolic markers, measures of anxiety, physical activity, quality of life and dietary intake. Subsequent assessments will be performed when the infant is 2 weeks, 3 months and 12 months of age for anthropometry, body composition (dual-energy X-ray absorptiometry (DXA)) and blood sampling. The primary outcome of the study is a between-group difference in infant percentage body fatness, assessed by DXA, at 2 weeks of age. Secondary outcomes will include differences in anthropometric measures at each time point, percentage body fat at 3 and 12 months and homeostatic model assessment of insulin resistance at 3 months. Statistical analysis will be carried out on the principle of intention to treat.

Ethics and dissemination This trial was approved by the Northern A Health and Disabilities Ethics Committee, New Zealand Ministry of Health (17/NTA/154). Results will be published in a peer-reviewed journal.

Strengths and limitations of this study

- The study is a double-blind randomised controlled clinical trial.
- Use of an unoxidised fish oil supplement (independently verified) at a dose of eicosapentaenoic acid and docosahexaenoic acid previously reported to improve insulin sensitivity.
- Targeted to women with overweight or obesity, that is, whose children are at heightened risk of obesity and metabolic dysfunction and most likely to benefit. But as the body mass index inclusion criterion is calculated based on weight at randomisation, some women included in the study may not have been overweight prior to their pregnancy.
- Treatment will start prior to 20 weeks, before the onset of the increase in insulin resistance in the second half of the pregnancy, but not preconceptionally or throughout the entire duration of pregnancy.
- Fish oil treatments are difficult to blind, so that participants may come to believe they know which treatment they have been randomised to.

Trial registration number ACTRN12617001078347p; Pre-results.

INTRODUCTION

In New Zealand (NZ), consistent with most developed countries, the majority of men (71%) and women (60%) are overweight or obese.¹ Obesity-related non-communicable diseases comprise the greatest health problems, including the diseases associated with metabolic dysfunction: cardiovascular disease, type 2 diabetes and some malignancies.² Māori and Pasifika (representing 16.5% and 8.1% of the NZ population, respectively)³ are more severely impacted (75% and 87%

with overweight or obesity), as are people of lower socioeconomic status.⁴

Reflective of the burden in adulthood, up to 60% of women of reproductive age are also overweight or have obesity in developed countries.^{5 6} This is of utmost significance as maternal obesity during pregnancy is associated with adverse changes in body composition in the offspring, including macrosomia, greater adiposity in childhood and obesity in later life, perpetuating a cycle of obesity across generations.^{7–11} In addition, the offspring have greater insulin resistance,^{12 13} which is the major pathological process linking obesity to endothelial dysfunction, dyslipidaemia, greater blood pressure, and increased risk of type 2 diabetes mellitus, metabolic syndrome and cardiovascular disease.² Indeed, these are all more common in children whose mothers had obesity during pregnancy.^{7 12–15} There is a need for innovative treatments that can reduce the risk of disease in the offspring of women with obesity, breaking the cycle.¹⁰ Pregnancy therefore offers a unique window of opportunity where safe and effective treatments could have long-term benefits to the health trajectories of children.¹⁶

Obesity in pregnancy is associated with systemic inflammation¹⁷ and exaggeration of the normal insulin resistance that develops in the second half of pregnancy, which leads to excess delivery of lipid and glucose to the fetus.¹⁸ This, at least in part, underlies greater birth weight and body fat in the offspring, and is associated with alterations in gene expression mediated by epigenetic changes that increase risk of metabolic dysfunction and disease in later life.^{19–22} In addition, the systemic inflammation that occurs with maternal obesity increases the expression of fatty acid transporters in placenta^{23 24} which is associated with newborn adiposity,²⁵ and alters the commitment of fetal mesenchymal stem cells, from differentiation into skeletal muscle cells to adipocytes and fibrocytes.²⁶ Thus, an anti-inflammatory and insulin-sensitising treatment during pregnancy could be of great benefit to the metabolic and body compositional phenotype of the offspring.

Omega-3 fatty acids (n-3 polyunsaturated fatty acids (PUFAs)) are found in marine oils. They are anti-inflammatory, both because they compete with arachidonic acid for eicosanoid production,²⁷ and through direct effects on receptors²⁸ and transcription factors.²⁹ They are insulin sensitising in rodents,^{28 30} primarily through reducing adipose tissue inflammation.¹⁹ In obesity, insulin resistance develops because of adipose tissue inflammation that leads to inappropriate lipolysis and subsequent impairment of insulin action in muscle and liver.³¹ These effects are also likely to underlie the exaggerated insulin resistance that occurs in obese pregnancy.³² Recently, n-3 PUFAs have been reported to improve insulin sensitivity in adult patients with insulin resistance,^{33 34} and reduce adipose tissue inflammation in pregnant women with obesity,³⁵ suggesting they could potentially counter the consequences of insulin resistance in pregnancy. n-3 PUFAs also reduce circulating triglycerides.³⁶ Thus, n-3 PUFAs could have indirect benefits to the fetus through

reducing maternal systemic inflammation, improving insulin sensitivity and reducing excessive nutrient delivery to the fetus. In addition, direct effects on the fetus could also be important, as prostacyclin is an arachidonic acid-derived eicosanoid that increases proliferation and differentiation of adipose tissue,³⁷ and n-3 PUFAs compete for production of eicosanoids.²⁷ In a rat model of insulin-resistant pregnancy (maternal high-fat diet), supplementation with n-3 PUFAs during pregnancy prevented the offspring from becoming insulin resistant with age.³⁸ If this effect can be translated to obese human pregnancy, it could reduce the offspring's long-term risk of type 2 diabetes and cardiovascular disease.

Previous randomised clinical trials have assessed the effects of n-3 PUFA supplementation during pregnancy on body composition of the offspring.^{39–43} These studies and the recent Cochrane review did not show an effect on body composition.^{40 43–46} However, this is not surprising as while these studies often included some women with overweight or obesity, they predominantly focused on pregnant women with body mass index (BMI) within the normal range, and normal-weight mothers do not typically develop exaggerated insulin resistance or excessive systemic inflammation, and have children with a low risk of obesity.⁴⁷ n-3 PUFA supplementation in pregnancy is known to have persistent effects on DNA methylation in children.⁴⁸ However, in a recent study, the effects of maternal n-3 PUFA supplementation on methylation of a key metabolic gene (IGF-2) in children were opposite, depending on the body composition of the mother.⁴⁹ Together, these data suggest a need for studies focusing on women who are overweight or obese. The only trial⁴² that solely recruited women who were overweight or had obesity reported no effect on maternal metabolism or birth weight in babies. However, assessment of body composition was not carried out as this study focused on maternal outcomes. Notably, the rate of macrosomia was halved, but the study was inadequately powered to detect this difference.⁴² Thus, the potential for n-3 PUFAs to prevent programmed obesity and insulin resistance in children of pregnant women who are overweight or have obesity remains insufficiently tested.

Hypothesis

n-3 PUFA supplementation of women with overweight or obesity, during pregnancy (starting between 12 and 20 weeks' gestation) and lactation, will lead to lower percentage body fat in the infants at 2 weeks of age compared with supplementation with a control treatment.

METHODS AND ANALYSIS

Study design

This is a two-arm, double-blind randomised controlled clinical trial recruiting pregnant women who are overweight or have obesity in Auckland, NZ. Women will be randomised 1:1 to receive either unoxidised fish oil (to provide 3 g of n-3 PUFAs) or olive oil (control)

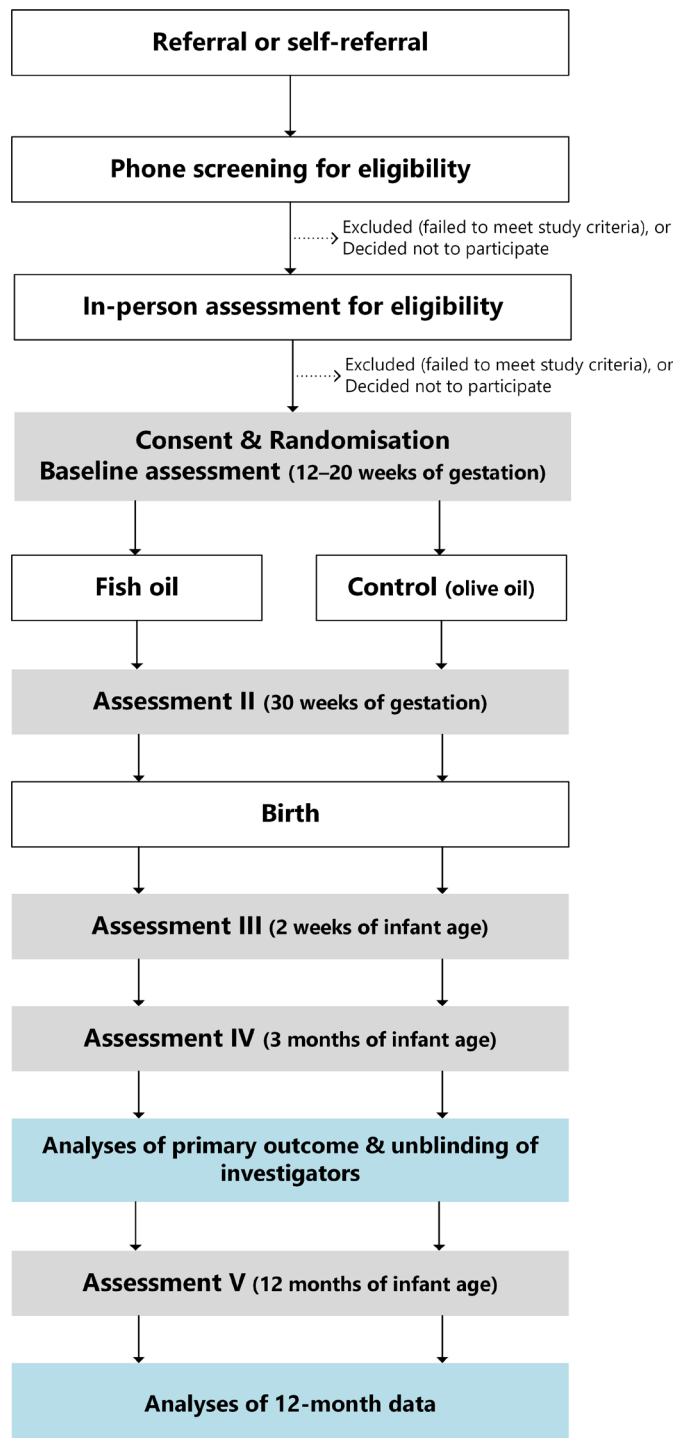


Figure 1 Design of the fish oil in overweight and obese pregnancy randomised controlled trial.

throughout pregnancy and the first 3 months of lactation, or until breast feeding is stopped if that occurs prior to 3 months. The trial protocol has been reported as per the Standard Protocol Items: Recommendations for Interventional Trials guidelines (figure 1).⁵⁰

Eligibility criteria

Eligible women will be from the Auckland region, pregnant with a singleton, at 12–20 weeks of gestation, aged 18–40 years with BMI $\geq 25 \text{ kg/m}^2$ at the baseline

assessment. Exclusion criteria are: (1) current tobacco use; (2) regular use of e-cigarettes or recreational drugs; (3) regular use of medications that influence blood pressure, lipid metabolism or insulin sensitivity; (4) any form of diabetes; and (5) malignancies or systemic inflammatory diseases.

Study intervention

Women will be randomised to take unoxidised fish oil capsules (a concentrated supplement to provide 3 g/day of n-3 PUFAs, produced from sardine, anchovy, mackerel and menhaden; New Zealand Health Manufacturing, Auckland, NZ) or an equal volume of standard olive oil (control; New Zealand Health Manufacturing), to be taken each day of pregnancy. Women who breast feed will continue the supplement for the first 3 months of lactation. Olive oil has the same energy content as fish oil, but contains no long-chain n-3 PUFAs and has been used as a control treatment in metabolic studies.⁵¹ n-3 PUFAs of 3 g/day have been shown to improve insulin sensitivity in insulin-resistant adults.^{33 34} Standard olive oil in this dose range has not been shown to influence metabolism. The fish oil and olive oil capsules will have the same size, colour and odour when intact.

Study capsules will be stored in airtight, opaque containers in a dark refrigerator to avoid oxidation. At the time the trial oils were procured, analyses of their peroxide values were carried out independently of the manufacturer. Both were below the recommended limits (fish oil: 4.5 meq/kg, olive oil: 1.4 meq/kg).⁵² Participants will be asked not to consume other oil supplements. No other aspect of pregnancy care is directed by the study. Pregnancy and healthcare will be provided by the participant's lead maternity carer (midwife or obstetrician) and other health professionals unrelated to the study.

Outcome measures

Primary outcome

- ▶ Total body fat percentage (excluding head) of the infants at 2 weeks of age as determined using whole-body dual-energy X-ray absorptiometry (DXA).

Maternal secondary outcomes

- ▶ Weight and BMI (30 weeks of pregnancy, 2 weeks after birth and 3 months after birth).
- ▶ Cardiometabolic: sitting blood pressure (30 weeks of pregnancy, 2 weeks and 3 months after birth), insulin, glucose, homeostatic model assessment of insulin resistance (HOMA-IR) and triglyceride concentrations (30 weeks of pregnancy).
- ▶ Well-being: depression score and health-related quality of life (from questionnaires) (30 weeks of pregnancy, 2 weeks after birth and 3 months after birth).
- ▶ Breast milk analysis: eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) concentrations (2 weeks after birth and 3 months after birth).

Infant secondary outcomes

- ▶ Anthropometry to assess body weight and linear growth (birth, 2 weeks, 3 months and 12 months).
- ▶ Metabolic: insulin, glucose and lipid profile to assess infant metabolism (3 months).
- ▶ Body composition: DXA scan (3 months), skinfold thickness—triceps and subscapular (12 months) and bioimpedance spectroscopy analysis (12 months).
- ▶ Infant feeding: complementary food frequency questionnaire (12 months).
- ▶ Fetal stress: hair cortisol measurement (2 weeks).
- ▶ Gut microbiome analysis (2 weeks).

Additionally, we will also assess maternal PUFA intake during pregnancy and physical activity using questionnaires at 30 weeks of pregnancy, 2 weeks after birth and 3 months after birth. These factors are potential confounders that could affect the primary outcome.

Timing of assessments

Antenatal assessments will be performed at baseline (12–20 weeks of pregnancy) and at 30 weeks of pregnancy. Postnatal assessments will take place at 2 weeks, 3 months and 12 months of infant age. Participants will be contacted by email, text messages or telephone to arrange the follow-up visits (table 1).

Maternal assessments

Medical history

Participants' medical history and previous pregnancies will be recorded at the baseline assessment. Participants will be asked about the progress of their current pregnancy, use of medications and supplements at every assessment.

Anthropometry

Height will be measured to the nearest millimetre using a Harpenden stadiometer. Participants will be asked to stand with their feet together, with their back straight and their heels in the same upright plane as the back of the head. Gentle upward traction on the mastoid process will be applied to straighten out the spine. At the 2-week postnatal assessment, height will be measured three times and the mean will be used in all BMI calculations throughout the study. Weight was measured to the nearest 5 g using a Wedderburn scale (WM206). Participants were asked to remove their shoes and loose or heavy clothing, but not to fully undress.

Blood pressure

Resting systolic and diastolic blood pressures will be measured in the sitting position, after a 5 min rest, using an oscillometric digital blood pressure monitor (ri-champion N; Riester, Jungingen, Germany) with an appropriately sized cuff.

Fasting blood sample

A 16 mL fasting blood sample will be drawn into plain, EDTA and lithium heparin blood tubes taken at baseline assessment and at 30 weeks of gestation to measure

omega-3 index⁵³ (a marker of compliance and absorption), fasting glucose, insulin and triglyceride levels. In addition, the first 40 participants will be offered to be part of an ancillary study examining DNA methylation of cell-free fetal DNA within maternal blood and one-carbon metabolites. Those involved will have an additional 6 mL of maternal blood drawn into a cell-free DNA collection tube at both baseline and the 30-week assessment.

Dietary n-3 PUFA intake

This will be estimated during pregnancy and at the 2-week and 3-month assessments using the NZ PUFA semiquantitative food frequency questionnaire.⁵⁴ These data will be reported in order to show whether participants alter their n-3 PUFA intake, or whether there are between-group differences.

Other questionnaires

The Edinburgh Postnatal Depression Scale⁵⁵ will be used to screen for depression and as a marker of psychological distress. Physical activity levels will be quantified using the International Physical Activity Questionnaire-long form.⁵⁶ Health-related quality of life will be assessed using the 12-item Short Form questionnaire.⁵⁷

Cord blood samples

Women who are planning to give birth in hospital will be provided with a blood collection pack containing a prelabelled lithium heparin blood tube and a laboratory form to take with them when they are in labour. Where possible, the attending midwife will take the blood sample, which will be processed in the local laboratory and stored until collected by the research team. Plasma glucose, insulin and C-peptide will be measured, and the omega-3 index determined.

Breast milk samples

At 2 weeks and 3 months postnatally, mothers who are breast feeding will be asked for a blood and breast milk sample. Women who agree to provide the breast milk samples will empty the milk from one breast using a breast pump (Medela Symphony, Switzerland) for a maximum of 10 min. Five millilitres will be sampled, and the remainder will be returned to the mother. EPA and DHA concentrations will be analysed.

Infant assessments

Infant health

Participants will be asked about the birth history and the infants' health.

Anthropometry and body composition

Infants will undergo measurement of weight, length, and abdominal and head circumferences at all assessments. Ponderal index will be calculated as per Röhler's formula:

$$\text{ponderal index (g/cm}^3\text{)} = \frac{100 \times \text{weight}}{\text{length}^3}$$

Body composition will be assessed with DXA scans (Lunar Prodigy and Lunar iDXA, GE Medical Systems, Chicago, Illinois, USA) at 2 weeks and 3 months. Body composition

Table 1 Timing of assessments

	Antenatal		Postnatal			
	Baseline	30 weeks	Birth	2 weeks	3 months	12 months
Maternal						
Medical history and examination	✓	✓	–	✓	✓	✓
Assessments						
Height	✓	–	–	✓	–	–
Weight	✓	✓	–	✓	✓	✓
BMI	✓	✓	–	✓	✓	✓
Blood pressure	✓	✓	–	✓	✓	–
Questionnaires						
NZ PUFA food frequency questionnaire	✓	✓	–	✓	✓	–
Edinburgh Postnatal Depression Scale	✓	✓	–	✓	✓	✓
SF-12	✓	✓	–	✓	✓	–
IPAQ-long form	✓	✓	–	✓	✓	–
Samples						
Fasting blood sample	✓	✓	–	–	–	–
Breast milk+paired blood sample	–	–	–	✓	✓	–
Infant						
Medical history and examination	–	–	–	✓	✓	✓
Anthropometry						
Weight	–	–	–	✓	✓	✓
Length	–	–	–	✓	✓	✓
Head circumference	–	–	–	✓	✓	✓
Abdominal circumference	–	–	–	✓	✓	✓
Chest circumference	–	–	–	–	–	✓
Mid-arm circumference	–	–	–	–	–	✓
Body composition						
DXA scan	–	–	–	✓	✓	–
Skinfold thickness—triceps, subscapular	–	–	–	–	–	✓
Bioimpedance spectroscopy analysis	–	–	–	–	–	✓
Samples						
Cord blood sample	–	–	✓	–	–	–
Hair sample	–	–	–	✓	–	–
Stool sample	–	–	–	✓	–	–
Blood sample	–	–	–	–	✓	–
Questionnaires						
Complementary food frequency questionnaire	–	–	–	–	–	✓

BMI, body mass index; DXA, dual-energy X-ray absorptiometry; IPAQ, International Physical Activity Questionnaire; NZ, New Zealand; PUFA, polyunsaturated fatty acid; SF-12, 12-item Short Form.

will again be measured at 12 months of age using a bioimpedance spectroscopy device (ImpediMed, version SFB7, Queensland, Australia) (DXA at 12 months of age is impractical due to the need for the infant to remain still). Additionally, at 12 months they will undergo measurements of chest and mid-arm circumferences along with triceps

and subscapular skinfold thicknesses (Holtain skinfold callipers, Holtain, Crosswell, Crymch, UK).

Blood sample

A heel-prick blood sample will be collected at the 3-month assessment for measurement of glucose and insulin and

calculation of HOMA-IR 2.⁵⁸ As it is inappropriate to fast infants for a prolonged period, participants will be asked to feed their infant 3 hours before the assessment, and fast until the blood sample is collected to standardise the metabolic state at the time of collection.

Hair and stool samples

A hair sample to measure hair cortisol and a stool sample to assess gut microbiome will be taken from the infant at the 2-week assessment.

Dietary intake

This will be measured using the infant complementary food frequency questionnaire⁵⁹ at 12 months.

Sample size and power calculation

This was calculated for the primary outcome, that is, percentage body fat at 2 weeks of age, measured by DXA. This 2-week time point reflects the birth and neonatal periods and allows sufficient time for the infant to regain the normal early loss of birth weight (considering that DXA scanning at birth is not practical). Importantly, larger babies have a greater percentage body fat,⁶⁰ which tracks through life, with greater BMI through childhood, adolescence and young adulthood.⁶¹ The absolute difference in infant percentage body fat between obese and normal pregnancies is 1.9%–2.4%.^{62 63} A sample size of 64 infants per group will be able to detect an absolute difference in percentage body fat of 1.4% (~63 g) between groups at 2 weeks of age (with 90% power and $\alpha=0.05$), based on an SD of 2.43% (from DXA scan data in seventy-four 2-week-old infants, unpublished in-house data). This difference would indicate partial amelioration of the effect of obesity during pregnancy on infant adiposity. Thus, we intend to recruit a minimum of 128 participants.

Recruitment

Recruitment is through contacts in primary care, the district health boards in Auckland, specialty obstetric and midwifery services, as well as direct advertising to potential participants using both traditional (eg, radio and television interviews) social media (eg, Facebook and Instagram). In NZ, people of Māori and Pacific ethnicity and those of lower socioeconomic status are frequently under-represented in clinical studies. To improve recruitment of these populations, social media will be used as it is a free platform available to anyone with a mobile phone and focused advertisements will be placed in regions with a higher density of these populations. Māori and Pacific midwifery networks will assist in recruitment. Further, clinical space within the community will be used where possible, transport costs will be reimbursed and weekend assessment will be available.

After a referral or a self-referral is received, an investigator will contact the participant. The study is explained including its rationale, randomised double-blind nature, and the assessments and time commitment. Eligibility is determined, and eligible participants will be invited to a baseline assessment between 12 and 20 weeks of

pregnancy. At the first assessment, eligibility is reassessed, and for those eligible, written informed consent will be obtained, at which time the participant is considered recruited and the baseline assessment will be conducted. Patient information sheets and consent forms are provided as online supplemental appendices 1–5.

Randomisation and masking

Participants will be randomised in a 1:1 ratio to either the treatment or placebo group, stratified by self-reported ethnicity (Māori vs Pasifika vs all other ethnicities) using computerised random number generation. Investigators and participants will also be blinded to group allocation, with the code held by a third party in a password-protected spreadsheet. The third party will obtain and label appropriate study capsules, which are deidentified as to content and labelled for the participant.

Unblinding of the investigators will occur after completion of analysis of the primary outcome (ie, percentage body fat of 2-week-old infants), which will occur when the last participant has completed the 3-month assessment.

When the infant is 12 months of age, the mothers will be asked which treatment they think they received. This will be used to determine the success of treatment blinding using Bang's Blinding Index (BBI).⁶⁴ Blinding success will be determined using the following thresholds as per Moroz *et al*: BBI ≥ 0.2 (unblinded); BBI >-0.2 but <0.2 (random guesses); and BBI ≤ -0.2 (opposite guesses).⁶⁵

Compliance

Participants will be overissued with capsules and will be asked to return all unused capsules. These will be counted to determine the number of capsules taken throughout the study, so that compliance with the treatment protocol can be assessed.

Retention

To optimise retention, visits will be booked at times suitable to participants, including during the weekend, and petrol vouchers and parking will be provided. Off-site research space will be used as appropriate to improve convenience for participants who live farther from the clinical research unit. Participants will be contacted well in advance of planned assessments and encouraged to contact the research team if they have problems with the study or intervention capsules, or there are important events in their pregnancy. If the participants have adverse effects from the study interventions that they cannot tolerate, we will allow them to reduce their capsule dose, and remain in the study. In addition, if a participant cannot attend a visit, we will offer a telephone interview, with internet-based questionnaires and request that infants have the key measurements performed by their maternity carer and shared with the investigators. Where a participant wishes to exit the study, permission will be sought to access birth and laboratory data from their maternity carer, to maximise the data that can be collected. Participants who exit the study will still be included in the primary analysis as their missing data will be imputed.

Data management

Data will be entered into a custom-built database using a secure web platform (REDCap V.9.4.1, Vanderbilt University, Nashville, Tennessee, USA). After each set of data is entered, it will be checked by a second investigator to ensure accuracy. Data will be exported from this database for interim analyses as required by the Data Monitoring Committee (DMC), and then for final analyses.

Data analyses

The data on the primary outcome will be analysed using the intention-to-treat principle. Missing data will be imputed by multiple imputations, assuming that data were missing at random. Sensitivity analyses will also be performed on observed data only. If there are differences in dietary n-3 PUFA intake between groups, then an exploratory analysis will be performed correcting for dietary intake.

Generalised linear regression models will be used to evaluate the main treatment effects on the primary outcome, adjusting for maternal BMI at study entry, gestational age at birth and offspring sex. A similar approach will be adopted to examine potential treatment effects on continuous secondary outcomes (eg, offspring weight, length, ponderal index and insulin levels). Models looking at potential treatment effects on maternal anthropometry, blood pressure and well-being will also include the baseline value of the respective parameter as a covariate.

The interaction between treatment and offspring sex will be assessed⁶⁶; differences between groups may be compared and presented separately for each sex. In addition, subgroup analysis may be carried out on the offspring of mothers who did and did not develop gestational diabetes, and mothers who did and did not fully breast feed. Exploratory analyses will be performed to assess the relationships between dietary n-3 PUFA intake and the gestational age at study entry/initiation of treatment, with the effect of the intervention.

For categorical secondary outcomes and rates of adverse events, χ^2 tests, Fisher's exact tests or generalised linear regression models will be used as appropriate. Per-protocol analyses will be carried out considering treatment compliance.

Where appropriate, continuous outcomes with a highly skewed distribution will be log transformed to approximate a normal distribution prior to analyses. Statistical analyses will be conducted using SAS V.9.4 (SAS Institute), IBM SPSS V.26 (IBM) and/or Minitab V.16 (Pennsylvania State University, State College, Pennsylvania, USA). All statistical tests will be two tailed and maintained at a 5% significance level, without adjustment for multiple comparisons.

Safety monitoring

This study has two levels of oversight. The Steering Committee includes clinicians from different medical specialties (paediatrics, general practice and obstetrics) and has both Māori and Pasifika representation. Clinical

concerns will be discussed within this group as they arise. The study also has oversight by the Health Research Council DMC, who will monitor safety of the trial and ensure that the study is successfully carried out. Minor adverse events will be reported at regular meetings to the DMC, but severe adverse events (ie, stillbirth, maternal death or a neonatal death) will be reported to the DMC within 5 days.

Adverse events will be collected during the assessments at 30 weeks of pregnancy, and when the infant is 2 weeks, 3 months and 12 months of age. However, participants are also provided with a contact card and encouraged to contact the research team if they have an adverse event. In addition, their medical professionals are asked to share information with the research team if a significant adverse event is to occur.

In the event that the participants suffer an injury as part of the study, they will be eligible to apply for compensation from Accident Compensation Corporation, NZ.

Study status

Recruitment of participants began in March 2017 and recruitment is expected to finish in early 2020. The last 3-month assessment is likely to be conducted by September 2020, with the final 12-month follow-up finishing around July 2021.

Ethics and dissemination

Ethical approval

This trial was approved by the Northern A Health and Disabilities Ethics Committee (Ministry of Health, NZ; approval number 17/NTA/154). An amendment was granted to offer the first 40 participants to also be part of an ancillary study, where additional maternal blood was sampled during pregnancy for cell-free DNA analysis, and to enable measurement of one-carbon metabolites. Informed consent will be obtained from all participants prior to undergoing any assessment or intervention, and a separate consent form signed for the ancillary study. This study will be conducted in accordance with all appropriate institutional, national and international guidelines and regulations for medical research, in line with the principles of the Declaration of Helsinki.⁶⁷

Confidentiality will be kept strictly, except where the participant gives permission for data to be shared—for example, if an illness is identified that her general practitioner or midwife should be informed about.

Dissemination of trial results

The individual results for the participants (women and their babies) in this study will be presented and explained to them. The findings of our study will be published in international peer-reviewed journals and presented at reputable conferences. Authorship will be attributed based on the principles of the International Committee of Medical Journal Editors.⁶⁸ The results will also be communicated to the general public via media through our communications manager. Relevant findings will be

shared with the community in a culturally appropriate manner.

Patient and public involvement

The study design was reviewed by the Northern A Health and Disability Ethics Committee in open meetings, where public representatives gave input into the study design. These representatives included clinical and laypersons, as well as those of Māori and Pacific ethnicity. In addition, meetings were held with many community midwifery groups, including Ngā Māia Ki Tamaki Makaurau and Pasifika Midwives Tamaki Makaurau, and the New Zealand College of Midwives, where feedback into study design was taken, including ensuring that procedures and materials were culturally appropriate. Information about the study was made available on a public website and social media. In addition, the study was publicised with interviews on radio, television and print media, and in public lectures.

Trial registration

This study is registered with the Australian New Zealand Clinical Trials Registry. In addition, the Universal Trial Number, WHO, has been obtained (U1111-1199-5860).

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