**ADMINISTRATIVE INFORMATION**

Title: The Healthy Mums and Babies (HUMBA) Demonstration Trial Early Childhood Outcome Study

Short title: HUMBA Early Childhood Study

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Data Monitoring Committee: as per HUMBA Demonstration Trial

Safety Monitoring Committee: as per HUMBA Demonstration Trial

This study protocol follows the SPIRIT checklist.1,2

**CONTENTS**

[1 Introduction 4](#_Toc475045423)

[1.1 Background 4](#_Toc475045424)

[1.2 Overview of the HUMBA Demonstration Trial 5](#_Toc475045425)

[1.3 Study Design 8](#_Toc475045426)

[1.4 Objectives and Hypotheses 8](#_Toc475045427)

[1.4.1 Study Rationale 9](#_Toc475045428)

[1.4.2 Rationale for Infant objectives 9](#_Toc475045429)

[1.4.3 Rationale for maternal objectives 10](#_Toc475045430)

[2 Methods 10](#_Toc475045431)

[2.1 Participants, Interventions and Outcomes 10](#_Toc475045432)

[2.1.1 Study Setting 10](#_Toc475045433)

[2.1.2 Eligibility Criteria 10](#_Toc475045434)

[2.1.3 Study Cohort 10](#_Toc475045435)

[2.1.4 Infant Assessments 10](#_Toc475045436)

[2.1.5 Maternal Assessments 11](#_Toc475045437)

[2.1.6 Outcomes 11](#_Toc475045438)

[2.1.7 Sample Size 12](#_Toc475045439)

[2.1.8 Recruitment 12](#_Toc475045440)

[2.1.9 Blinding (masking) 12](#_Toc475045441)

[2.2 Data Collection, Management and Analysis 12](#_Toc475045442)

[2.2.1 Data Collection Methods 12](#_Toc475045443)

[2.2.2 Data Management 13](#_Toc475045444)

[2.2.3 Statistical Methods 13](#_Toc475045445)

[2.3 Monitoring 14](#_Toc475045446)

[2.3.1 Data Monitoring 14](#_Toc475045447)

[2.3.2 Harms 14](#_Toc475045448)

[3 Ethics and Dissemination 14](#_Toc475045449)

[3.1 Research Ethics Approval 14](#_Toc475045450)

[3.2 Locality Approval 14](#_Toc475045451)

[3.3 Protocol Amendments 14](#_Toc475045452)

[3.4 Consent or Assent 14](#_Toc475045453)

[3.5 Confidentiality 14](#_Toc475045454)

[3.6 Declaration of Interests 15](#_Toc475045455)

[3.7 Access to Data 15](#_Toc475045456)

[3.8 Dissemination Policy 15](#_Toc475045457)

[4 Study Management 15](#_Toc475045458)

[4.1 Steering Committee 15](#_Toc475045459)

[4.2 Management Committee 15](#_Toc475045460)

[5 Appendices 16](#_Toc475045461)

[5.1 Participant Information Sheet and Consent 16](#_Toc475045462)

[5.2 Case Report Forms 16](#_Toc475045463)

[5.3 Ethical and Locality Approval 16](#_Toc475045464)

[5.4 Protocol Amendments 16](#_Toc475045465)

[6 REFERENCES 17](#_Toc475045466)

# Introduction

## Background

*Obesity is a major health problem in New Zealand*

The economic and personal burden of obesity in New Zealand (NZ) is enormous with one-third of NZ children and two-thirds of adults currently overweight or obese.3 Obesity is a major risk factor for metabolic and cardiovascular disease, including type 2 diabetes, hypertension, ischaemic heart disease and stroke.4 Children who are overweight or obese are very likely to remain so as adults and are therefore on a trajectory towards developing these obesity-related complications.5 In addition, obesity has a social cost, being associated with low self-esteem, increased rates of mental illness, poor health-related quality of life and reduced employment opportunity.6-8

There is significant ethnic disparity in the prevalence of overweight and obesity, with rates two-fold higher among Māori and Pacific adults and children compared with those from European/other ethnic backgrounds.3 Among adults with obesity, metabolic complications such as type 2 diabetes, are more common among Māori and Pacific.9

*Obesity in Pregnancy*

In pregnancy, maternal obesity increases the risk of most obstetric complications including gestational diabetes mellitus (GDM), preeclampsia, stillbirth, shoulder dystocia and caesarean.10 Infants of obese mothers are more likely to be born large for gestational age (LGA) and experience birth trauma.11 They also have increased risk of *in utero* growth restriction, which often goes undetected and may contribute to a two-fold increase in the incidence of stillbirth among obese women.12,13

With increasing prevalence of overweight or obesity among the young women, maternal obesity is fast becoming one of the most important issues in maternity care. The latest New Zealand National Health Survey showed that 30% of women at child-bearing age are obese, and the trend is increasing.3 In the multi-ethnic Counties Manukau Health (CMH) region, this rate is even higher with 37% of pregnant women now obese in early pregnancy.14

*Maternal obesity contributes to childhood obesity and long-term risk of cardio-metabolic disease*

As rates of maternal obesity have increased there has been a marked increase in GDM, with prevalence in obese women reported between 15% and 30%, depending on criteria used and populations studied.15 Fetal exposure to an abnormal metabolic environment along with excessive nutrients, which occurs in maternal obesity and is compounded by GDM, results in accelerated growth, especially of adipose tissue.16 These infants have increased risk of childhood and adult obesity,17 diabetes 18,19 and dying prematurely from cardiovascular disease.20 This creates a vicious intergenerational cycle of obesity and cardio-metabolic disease, termed “developmental over-nutrition.”21

*Maternal obesity contributes to impaired childhood development*

Maternal obesity has been associated with increased risk of infant language delay,22 a decrease in school-age IQ of up to 6 points,23 and attention-deficit and hyperactivity disorder.24,25 Potential mechanisms in obese pregnancy that may adversely affect brain development include the altered metabolic and endocrine milieu, such as higher concentrations of oestrogen, cortisol, free fatty acids and pro-inflammatory cytokines;26,27 birth asphyxia;28 and reduced breastfeeding.29

*Excessive pregnancy weight gain compounds the effects of maternal obesity*

Based on a number of observational studies, the Institute of Medicine recommends 5 to 9 kg of gestational weight gain (GWG) in obese women, for optimal maternal and infant outcomes.30 Exceeding recommended GWG is associated with increased risk of GDM, preeclampsia, LGA, and emergency caesarean, independent of maternal body mass index (BMI).31,32 Mothers with excessive GWG are less likely to lose weight between pregnancies, and may enter further pregnancies even more overweight.33,34 Further, excessive GWG compounds the associations between maternal obesity and offspring metabolic dysfunction, cognitive impairment and infant mortality.23,35-37 However, implementation of GWG guidelines is challenging. Studies suggest that obese women are more likely to gain excessive weight during pregnancy than non-obese women,38 and may require more theoretically-designed interventions.39 There is also an increasing demand from prenatal care providers for effective and reproducible intervention guidance.40

*Pregnancy interventions for maternal obesity*

Lifestyle interventions during pregnancy may limit GWG;41 however, limited data are available to identify the key components of intervention(s) that contribute to positive outcomes.39 A systematic review reported that amongst diet, physical activity, and mixed approach interventions, dietary interventions were associated with the largest reduction in GWG (4 kg on average) and also with improved pregnancy outcomes.41 A UK multicentre trial of intensive behavioural intervention in obese pregnant women suggested that there is greater potential for change in dietary intake than in physical activity.42 However, several recent randomised controlled trials in obese women have found that lifestyle interventions alone have a limited effect on reducing GWG.43-45 Therefore, additional interventions in combination with lifestyle interventions are needed to control GWG. Two such adjunctive interventions are motivational texting and probiotics.

Mobile phone texting technologies are increasingly being used to assist with weight loss. A systematic review has shown that use of mobile phone technologies in non-pregnant populations can result in successful weight loss with increased physical activity and improved nutrition.46 There was a high level of acceptability and user satisfaction rating, with a significant number of participants stating they would recommend text messaging as a primary intervention to others.46 A New Zealand multi-ethnic study, in an obese non-pregnant cohort, confirmed the feasibility of using mobile phone technology together with behaviour change techniques and the authors reported successful weight loss in participants.47 A recent pilot study of 35 overweight and obese pregnant women reported a 2.7 kg mean GWG reduction with a texting intervention.48

Modification of the gut microbiome by ingestion of probiotics is a novel pathway for possible intervention to prevent metabolic disease. The microbiome influences energy extraction from food,49 and satiety, inflammation, and glucose and lipid metabolism,50-52 with potential to reduce obesity and type 2 diabetes.53 Probiotics are safe in pregnancy54,55 and provide a simple intervention in pill form. A randomised controlled trial of probiotics/placebo (*Lactobacillus rhamnosus GG* and *Bifidobacterium lactis* at 1010 colony-forming units/day) and nutritional advice in pregnant women in Finland showed an over 60% reduction in GDM, with a prevalence of 13% in the probiotic/nutrition group compared with 36% in placebo/nutrition group and 34% in controls.56 In addition, a reduction in maternal central adiposity at 6 months postpartum and a 127 g average reduction of birthweight were demonstrated in the probiotic treatment group.57,58 Besides the reported efficacy of these probiotic capsules in reducing GDM,daily probiotic yoghurt has been associated with a 40% reduction in preeclampsia,59 which is also a common complication in obese pregnant women. A RCT of probiotics/placebo aimed to prevent GDM in overweight and obese women has started in Brisbane, Australia,60 but no studies have been reported to date, confined to obese pregnant women or in combination with intensive dietary intervention, aimed to prevent excessive GWG.

## Overview of the HUMBA Demonstration Trial

We are undertaking the Healthy Mums and Babies Study (HUMBA), an innovative, randomised controlled demonstration trial of probiotics or placebo plus an intensive, culturally tailored multifaceted dietary intervention in obese pregnant women in South Auckland, New Zealand (**ACTRN12615000400561).** The HUMBA study is a single centre two by two factorial trial (parallel groups) designed according to CONSORT guidelines.61 We are investigating if an oral probiotic capsule consisting of *Lactobacillus rhamnosus GG* and *Bifidobacterium lactis BB12* at a dose of 7×109 colony-forming units per day each, or placebo, and a multifaceted dietary intervention or routine dietary advice, can reduce excessive GWG and optimise infant birthweight in obese pregnant women. Women are being recruited and randomised to each intervention between 120 and 176 weeks (Figure 1). Probiotic or placebo capsules are taken once daily from enrolment until birth. The research plan and assessments are summarized in Figure 2. Co-primary outcomes are excessive pregnancy weight gain (average >0.27 kg/week) and infant birthweight. A total of 220 participants will be recruited providing 80% power to detect a 25% reduction in excessive pregnancy weight gain from 80% to 60% and a 227g difference in birthweight with each intervention (allowing 10% loss to follow-up, alpha 0.025 for each primary outcome).

*Figure 1: HUMBA Demonstration Trial, Recruitment and Randomisation*

**Pregnant women at 12-17 6 weeks’ gestation considered to be eligible and approached to participate in HUMBA**

**Excluded:**

* **Declined consent**
* **Unable to contact**
* **Found to be ineligible**
	+ BMI<30
	+ Pre-existing diabetes or HbA1c ≥50
	+ Known congenital abnormality
	+ Taking probiotic capsules / supplements containing probiotics
	+ Multiple pregnancy
	+ Bariatric surgery
	+ Medications or medical conditions which alter glucose metabolism
	+ Severe hyperemesis

**Consented and Randomised**

**(Stratified by BMI)**

Routine care diet

Dietary intervention

Placebo capsule

Probiotic capsule

*Figure 2: HUMBA Demonstration Trial, Research Plan*

**120-176 weeks randomized**

**(Stratified by clinical site and BMI)**

**120-176 weeks**

**Baseline assessment by research assistant:**

* HbA1c, lipids (point-of-care test)
* Samples: non-fasting plasma, urine
* BP, weight, height, waist and mid-arm circumference
* Baseline data collected

**Questionnaires:**

* NZFFQ-SF (New Zealand Food Frequency Questionnaire Short Form)
* NZPAQ-SF (New Zealand Physical Activity Questionnaire-Short Form)
* EPDS (Edinburgh Postnatal Depression Scale)
* STAI*(*State-Trait Anxiety Inventory)
* SF-12 (12-Item Short-Form Health Survey)

**120-176 weeks**

Probiotic / Placebo commences

Dietary education scheduled if appropriate

**Monthly Telephone Contact**

Brief contact for provision of study capsules

**28-30 weeks**

**Assessment by research assistant:**

* Record OGTT results, HbA1c, lipids
* NZFFQ – SF (New Zealand Physical Activity Questionnaire Short Form)
* NZPAQ-SF (New Zealand Physical Activity Questionnaire - Short Form)
* Weight, BP, medical history

**36 weeks**

**Assessment by research assistant:**

* BP, weight, mid-arm circumference
* HbA1c, medical history
* Samples: urine, blood, stool, hair

**Questionnaires:**

* EPDS (Edinburgh Postnatal Depression Scale)
* STAI (State-Trait Anxiety Inventory)
* SF-12 (12-Item Short-Form Health Survey)

**Birth:**

* Collect maternal & birth outcomes
* Neonatal and maternal morbidity
* Baby – weight, length in neonatometer
* Baby body composition – skinfolds, Pea Pod
* Meconium Sample

**6 weeks post-delivery**

* Phone contact

**5 months post-delivery assessment by research assistant**

**Baby:**

* Feeding, diet, Baby Eating Behaviour Questionnaire (BEBQ)
* Anthropometry and body composition – skinfolds and Pea Pod
* General health, Infant Behaviour Questionnaire (IBQ)

**Mother:**

* BP, weight, waist, and mid-arm circumference, bioimpedance, HbA1c, lipids
* Lifestyle, physical activity & food frequency questionnaires

**12-15 months follow up assessment by research assistant**

**Baby:**

* 12M Tracking, 12M Cognitive Adaptive Test (CAT)
* 12M Feeding Questionnaires: Infant Feeding, Children’s Eating Behaviour (CEBQ), Infant Food Frequency
* Ages and Stages Questionnaire (ASQ): 12M, or 15M or 16M depending on age
* Infant anthropometry with bioimpedance (BIA)
* Infant abdominal subcutaneous and pre-peritoneal fat deposit thickness and aortic intima-media thickness by ultrasound (USS).

**Mother:**

• BP, weight, waist and mid-arm circumference, bio-impedance, HbA1c, lipids

• Lifestyle, mental health, physical activity & food frequency questionnaires (EPDS, STAI, SF-12, NZPAQ-SF, NZFFQ-SF)

##

## Study Design

This is a follow-up study of 1-year-old children and their mothers from the HUMBA Demonstration Trial, two by two factorial randomised controlled trial of a dietary intervention versus routine dietary advice, and probiotics versus placebo (double blind) in obese pregnant women.

## Objectives and Hypotheses

The primary objectives of this study are to determine if at 1 year post expected delivery date whether treatment of obese pregnant women with probiotics versus placebo, and a dietary intervention versus routine dietary advice:

* *For the infant* reduces risk factors for later obesity and cardio-metabolic disease, including large body size, rapid growth, excess adiposity, high food avidity (appetite), excess nutrient intake, aortic intima-medial thickening and low physical activity; and improves neurodevelopmental progress.
* *For the mother* reduces post-partum weight retention and increases healthy nutritional and physical activity behaviours, and measures of mental health and quality of life.

*Primary hypothesis*

* *For the infant:* At 1 year of corrected age, infants of women randomly allocated to probiotics or dietary intervention compared with placebo or routine dietary advice, respectively, will have decreased incidence of overweight (weight for length ≥90th centile) or rapid growth (conditional weight gain from birth ≥90th centile).
* *For the mother:* At 1 year post expected delivery date, women randomly allocated to probiotics or dietary intervention compared with placebo or routine dietary advice, respectively, will have decreased postpartum weight retention and adiposity (assessed by bio-impedance).

*Secondary hypotheses:*

* *For the infant:* By 1 year of corrected age, infants of women randomly allocated to probiotics or dietary intervention compared with placebo or routine dietary advice, respectively, will have decreased adiposity, appetite scores, energy intake, aortic intima-medial thickness and incidence of developmental delay, and increased physical activity
* *For the mother:* At 1 year post expected delivery date, women randomly allocated to probiotics or dietary intervention compared with placebo or routine dietary advice, respectively, will have improved positive nutritional and physical activity behaviours and quality of life scores, lower rates of depression,lower HbA1c concentration and improved lipid profile.

A secondary objective of this study is to evaluate access and engagement with healthcare in the first 12 months after birth, including contraception and family planning.

### Study Rationale

The HUMBA Demonstration Trial aims to not only improve pregnancy outcomes among obese women but also to reverse the longer-term consequences of obesity and developmental over-nutrition. Thus, longitudinal follow-up of this cohort is essential to fully evaluate the efficacy of the trial interventions.

### Rationale for Infant objectives

*Growth and body composition*

In developed countries, rapid weight gain in infancy is a key risk factor for later obesity and metabolic disease.62 Relative fat mass increases 2-fold by 4 months,63,64 and by 6 months of age there is modest tracking of subcutaneous fat throughout the preschool years.65 Thus, by 1 year of age high adiposity and growth in fat mass is predictive of longer-term risk of overweight and obesity.66 Offspring of women with high pre-pregnancy BMI continue to have higher fat mass throughout childhood, both overall and centrally, 35,67,68 and this association is strongest in boys.69

Observational studies suggest that limiting GWG in obese women may reduce childhood adiposity and cardiometabolic risk35,36,70,71 but there are currently few data from randomized trials. In unselected populations, probiotics appear to have a beneficial effect of restraining excessive infant weight gain.72 We will assess the effect of maternal probiotics and dietary intervention in obese women in pregnancy on infant body size and composition at 1 year of corrected age using anthropometry and bioelectrical impedance, including growth in weight and soft tissue mass from birth and 5 months’ corrected age. Recent findings from one study that associations between childhood adiposity and maternal BMI and GWG were ethnic-dependent73 emphasise the importance of local assessment of interventions, particularly in a multi-ethnic population such as South Auckland.

*Nutrition and appetite*

Rapid infant weight gain and subsequent childhood overweight is related to short duration of breast feeding74, higher energy and dairy protein intake,75-78 and early introduction of solids.79-81 Infants born to obese mothers consume more energy in the first 6 months and take larger feeds,82 which may relate to more vigorous feeding styles83 and high reinforcing values for food.84 Infant feeding behaviour is affected by fetal expression of hormones such as leptin and ghrelin, which are altered in obese pregnancy. We will compare feeding patterns, macronutrient intake and infant appetitive traits between infants exposed to either of the HUMBA trial interventions, using specially designed parental questionnaires.

*Neurodevelopment*

Offspring of obese mothers have higher rates of cognitive and attention problems up to school age.24,25 If the HUMBA interventions are successful in improving maternal metabolic control in pregnancy, there may be beneficial effects on brain development. We will assess cognitive function at 1 year of corrected age using standardised tests of development.85,86

Rapid infant growth may adversely affect motor development and psychomotor activity. For example, in a randomised trial, early introduction of solid foods was associated with delayed onset of crawling and walking.87 Conversely, reduced infant physical activity reduces energy expenditure and may promote greater gain in weight and fat.88,89 We will assess motor proficiency with a standardised developmental questionnaire of motor milestones.90

*Vascular development*

Associations between maternal obesity and offspring cardiovascular disease may be partly mediated by adverse effects on vascular development. There is increasing evidence that the pre-clinical atherosclerosis begins very early in life and increased aortic intima-media thickness has been demonstrated in infants following both fetal growth restriction and macrosomia.91 92 We will assess if the HUMBA interventions have beneficial effects on infant vascular development by measuring aortic intima-media thickness on ultrasound.

### Rationale for maternal objectives

Failure to return to pregnancy weight by 6 months post birth is associated with worsening obesity, adverse health outcomes and increased risk of obstetric complications in a subsequent pregnancy. The effect of antenatal interventions to limit GWG on post-natal weight retention is unclear as studies to date have shown mixed results.93 57 However, the individualised lifestyle and motivational approach used in HUMBA, combined with probiotics, may prove more effective. Reasons for post-natal weight retention are complex, and include psychological factors such as postpartum depression. We will assess a number of aspects of maternal health and wellbeing, which may be affected by participation in the HUMBA trial. We will also assess diet quality and activity scores to determine if the HUMBA interventions are associated with any sustained behavioural change. These outcomes are important not only for maternal health but also may impact on risk of childhood obesity.94

# Methods

## Participants, Interventions and Outcomes

### Study Setting

The HUMBA Demonstration Trial is being conducted in the multiethnic Counties Manukau Health region, which has approximately 8,000 births annually.14 Among women of childbearing age, over two-thirds are of Pacific (31%), Māori (21%) or Asian (22%) decent, and nearly two-thirds are overweight (27%) or obese (38%).14

### Eligibility Criteria

All surviving infants in the HUMBA Demonstration Trial, and their mothers, are eligible for this outcome study at 1 year of corrected age. Only women with a singleton pregnancy are eligible to participate in the HUMBA Demonstration Trial.

### Study Cohort

A total of 220 obese women with singleton pregnancy will be recruited to the HUMBA Demonstration Trial. It is expected that approximately 7% of births will be preterm.

### Infant Assessments

Infants, and their mothers, will be assessed at target age of 1 year corrected (±1 month), up to a maximum of 1.5 years.

*Anthropometry*

Length will be measured by infantometer to the nearest 0.5 cm. Head, chest, abdominal and left arm circumference will be measured to the nearest 1 mm by non-stretch lasso tape. Chest girth will be measured at the nipples and abdominal girth at the umbilicus. Left arm circumference will be measured at the mid-acromiale-radial.95 The left acromiale-radiale will be measured to the nearest 1 mm by non-stretch lasso tape. Subscapular and triceps skinfolds will be measured twice to the nearest 0.2 mm using Harpenden calipers, with a third measure if the difference between the first two measures is >0.6 mm. Weight will be measured to the nearest 10 g by electronic scale.

*Body composition*

Whole-body fat and lean mass will be measured by hand-to-foot bioimpedance analysis (Impedimed).96,97 Central fat distribution will be assessed by ultrasonographic measurement of abdominal subcutaneous and pre-peritoneal fat deposit thickness.98-100

*Nutritional intake and feeding patterns*

Feeding history including breast feeding, milk and fluid intake, solids and indicators of diet quality will be assessed by a feeding questionnaire. Nutrient intake will be estimated from a four-day food frequency record.

*Appetitive traits*

Appetitive traits will be assessed with the Children’s Eating Behaviour Questionnaire (CEBQ).101 Food approach and food avoid subscales will be calculated, which have shown consistent associations with weight gain in infancy and childhood.102

*Vascular health*

Abdominal aortic intima-media thickness will be measured by ultrasound.91,103,104

*Neurodevelopment*

Cognitive development will be assessed using the Cognitive Adaptive Test (CAT) which tests the visual motor developmental stream and the emergence of problem solving skills.85 A developmental quotient (DQ) <90, calculated as the CAT age divided by the test age, represents >1 SD below the normative mean.86 Children who cannot be tested due to presence of an underlying neurological disorder will be assigned a DQ of 65 (~3 SD below the normative mean). The CAT has been shown to have high positive predictive value and moderate negative predictive value for cognitive impairment compared with the Bayley Scales of Infant Development (BSID).105 Advantages of the CAT are that it is freely available, quick to administer (<10 minutes) and can be performed by any trained examiner.106

Developmental progress in communication, gross motor and personal-social interaction will be assessed by caregiver report using the Ages and Stages Questionnaire, version 3 (ASQ-3).107 Cut-offs are given for 2 SD (black zone) and 1 SD (grey zone) below the test mean. ASQ-3 is widely used for developmental screening and has modest agreement with BSID.108

### Maternal Assessments

Women will be assessed at the time of the infant visit for the following:

*General health and access to care*

Blood pressure using an aneroid sphygmomanometer

Questionnaire about access to health care

*Weight and body composition*

Weight by electronic scale

Waist and mid-arm circumference

Whole-body fat and fat-free mass using standing multi-frequency, hand-to-foot bioimpedance (BIA) analysis109

*Glucose and lipids*

HbA1c concentration and lipid profile measured by Cobas b101 machine

*Diet and physical activity*

NZFFQ –SF NZ (Food Frequency Questionnaire Short Form) 110

NZPAQ-SF (New Zealand Physical Activity Questionnaire) 111

*Mental health and wellbeing*

EPDS (Edinburgh Postnatal Depression Scale)112

STAI (State-Trait Anxiety Inventory)113

SF-12 (12-Item Short-Form Health Survey)114

### Outcomes

The primary infant outcome of this HUMBA Early Childhood Outcome Study is the incidence of infant overweight (weight for length ≥90th centile) or rapid growth (conditional weight gain from birth ≥90th centile) at 1 year of corrected age.

Secondary infant outcomes include**:**

* Individual components of the primary outcome and associated z-scores
* Weight, length, head circumference, mid-arm circumference, triceps and subscapular skinfold thickness and associated z-scores; chest and abdominal girth; arm muscle and fat area
* Growth (conditional SDS) in anthropometric measures from birth to 1 year, birth to 5 months and from 5 months to 1 year
* Whole-body fat and lean mass; abdominal subcutaneous and pre-peritoneal fat thickness; conditional z-scores for length calculated from the regression residuals
* Aortic intima-media thickness
* Food intake: frequency and amount for main food groups, average daily energy intake and macronutrient percentage from milks, non-milk fluids and foods; proportion of infants with minimum dietary diversity (e.g., receiving ≥4 of the following food groups: grains, root and tubers; legumes and nuts; dairy products; flesh foods; eggs; vitamin A rich fruits and vegetables; other fruits and vegetables)
* Appetitive traits (CEBQ): food responsiveness (FR, 25 points), enjoyment of food (EF, 20 points), emotional over-eating (EOE, 20 points), desire to drink (DD, 15 points), slowness in eating (SE, 20 points), satiety responsiveness (SR, 25 points), emotional under-eating (EUE, 20 points), food fussiness (FF, 30 points), food approach subscale (FR, EF, EOE, and DD, 80 points), food avoid subscale (SE, SR, EUE, and FF, 95 points), food approach and avoid ratio
* Visual motor and problem solving (CAT): developmental quotient and proportion <90 (>1 SD below the normative mean)86
* General development (ASQ-3): Communication, Gross Motor and Personal-Social subscale scores (60 points each) and proportion of children >1 SD below the test mean (grey zone)

The primary maternal outcomes are postpartum weight retention (defined as the difference between weight at HUMBA study recruitment and 1 year post expected delivery date) and whole-body percent fat.

Secondary maternal outcomes include:

* Diet quality
* Physical activity score
* Depression and anxiety and quality of life scores
* Blood pressure
* HbA1c concentration

### Sample Size

The study sample size is limited by HUMBA Demonstration Trial participant numbers (planned sample size 220). The background incidence of infant overweight or rapid growth at 12 months’ is expected to be approximately 36% (overweight 28%; additional 8% rapid growth).3,5,17,115 if 190 children are assessed at 12 months (lost to follow up ~ 15%), this study will have 80% power to detect a 50% reduction in the primary outcome from 36 to 18%. For continuous outcomes, the study will have 80% power to detect a standardized difference of 0.4.

### Recruitment

A contact database is being maintained, and women and families are being informed of possible longer term following, pending funding.

### Blinding (masking)

Assessors will be blinded to maternal dietary intervention and probiotic allocation.

## Data Collection, Management and Analysis

### Data Collection Methods

All data will be entered directly to electronic case record forms on the REDCap data collection system, housed on a local secure university server.116 REDCap is HIPAA compliant and includes unique user IDs, access level control and tracking logs.

### Data Management

Case record forms will be labelled with version number and dated, and any amendments documented in this protocol (appendix 5.2. Participant study data will be identified only by study ID, first and last initial, date of birth and estimated date of delivery. Identifiable data including national health index, name, address and phone number will be stored in a separate, password-protected REDCap project. Only single data entry will be performed but extensive range and logic checks will be used to identify data entry errors, and a data monitor, separate to the person entering data, will review each form, raise data queries and lock the form once all queries have been resolved.

### Statistical Methods

Statistical analysis will be performed with version 9.4 (SAS Institute).

*Derived variables*

Sex- and corrected-age-specific z-scores for weight, length, head circumference, body mass index and skinfold thickness, and sex- and length-specific z-scores for weight at 5 months and 1 year of corrected age will be calculated from the WHO 2006 Child Growth Standard,117 using the LMS method.118 Sex- and gestational-age-specific z-scores for weight, length and head circumference at birth will be calculated from the UK-WHO-preterm population reference.119 Left upper arm muscle area and fat area will be calculated from the arm circumference and triceps skinfold thickness.120,121 Growth in anthropometric measures will be assessed as conditional SD gain from birth to 1 year, birth to 5 months, and 5 months to 1 year, calculated either from the difference in population standard z-scores, accounting for the correlation of z-scores between time points,122 or the difference in z-scores calculated from the residuals of the regression of soft tissue mass on length.123

New Zealand Deprivation Index (NZDPI) will be used to indicate socioeconomic status, based on the maternal address at entry.124 If this is not available, NZDPI will be determined from the infant’s primary address at follow up.

*Descriptive statistics*

Categorical data will be presented as number and percent, and continuous data as mean and standard deviation or median and inter-quartile range, as appropriate. Count data will be presented as median and inter-quartile range or grouped into ordinal categories. Denominators will be given for all outcomes.

*Treatment effect*

Generalised linear models will be used to estimate the effects of dietary intervention and probiotics, compared to their respective control groups. Primary analyses will report only marginal effects for each randomised exposure, adjusted for body-mass-index at recruitment (stratification variable), co-intervention, ethnicity and sex (infant outcomes). Models for outcomes relating to infant body composition will also be adjusted for length, acromiale-radiale or lean mass, as appropriate.125,126 Treatment effect will be presented as risk ratio, odds ratio, count ratio, mean difference or ratio of geometric means (positively skewed data), as appropriate, with 95% confidence intervals. Analyses will follow the principle of intention-to-treat (ITT); patients will be analysed according to their assigned treatment groups at randomisation. For significance tests, alpha level will be set at 0.05.

*Secondary analyses*

The following exploratory analyses will be conducted for the primary outcome:

* An interaction test, to assess if the combination of interventions is more effective than either alone. It should be noted that the HUMBA Demonstration Trial has not been powered to detect interactions between the interventions.
* A sensitivity analysis to assess the effect of compliance with the study interventions, including those women who reported taking their probiotics more than 75% of the time, and those that attended at least 3 out of the 4 dietary education sessions.

*Missing Data*

Analyses will include only available data. Missing outcome data will not be imputed as they are unlikely to be missing at random. A sensitivity analysis may be performed to explore any potential impact of missing data.

## Monitoring

### Data Monitoring

A combined Data and Safety Monitoring Committee (DSMC) is in place for the HUMBA Demonstration Trial, that will monitor recruitment, sample size assumptions, completeness of data acquisition, and evidence for group differences in the main efficacy and safety outcome measures. The DSMC will advise the HUMBA Demonstration Trial Steering Committee annually on trial continuation or protocol modification. It is envisaged that the trial will be completed as planned. There will be no separate DMSC or interim analysis for this follow-up study.

### Harms

The HUMBA Demonstration Trial DSMC will monitor for serious adverse events, including maternal death, maternal admission to intensive care, fetal death, neonatal death or death up to primary hospital discharge, and stage 2-3 neonatal encephalopathy. Each serious adverse event will be reviewed by the DSMC to determine the likelihood of a causative association with the trial interventions. There will be no separate safety monitoring committee for this follow-up study.

# Ethics and Dissemination

## Research Ethics Approval

The HUMBA Demonstration Trial has been approved by the Southern Health and Disabilities Ethics Committee (14/STH/205), including this 1 year follow up.

## Locality Approval

Locality agreements are in place with ADHB and CMDHB. Amendments will be sought for the additional assessments in this study. It is envisaged that at 1 year of corrected age all infants and mothers in HUMBA will be followed up in the Counties Manukau region.

## Protocol Amendments

All amendments to the final version of this protocol will require review and approval of the Steering Committee, and will be submitted to HDEC and DHB Research Offices, as appropriate. All amendments, including approval date, will be recorded with this protocol (appendix 5.4.)

## Consent or Assent

Written informed consent will be obtained from each participant at the 12-month assessment (separate consent for maternal and infant assessments). The Participant Information Sheet will outline the assessments, data collection and processes to ensure confidentiality. Participants and their whanau will be given the opportunity to ask questions and will be provided with a copy of their written informed consent. They retain the right to withdraw from the study at any stage, without the need to provide a reason.

## Confidentiality

Electronic databases will be stored on secure local servers and access will be controlled by unique user ID and password, with full electronic tracking log. Case record forms will be identifiable only by study ID, first and last initial, date of birth and estimated date of delivery. Identifiable data including national health index, name, address and phone number will be stored in a separate, password-protected REDCap project. Extracted data files will contain date of birth and estimated date of delivery, as these are necessary for analysis, but participant initials will be removed. Study reports will contain only summary data and individual participant data will not be reported. Identifiable data will not be released to any third party. Research staff will be certified in best practice for clinical trials (ICH-GCP E6 and PHRP).

At the completion of the study, all electronic data will be permanently digitally archived on a secure university server and accessible only to the study investigators.

## Declaration of Interests

Investigators will declare any financial, intellectual or other potential conflicts of interest, as outlined by the ICMJE, to the Steering Committee.127 The Steering Committee will decide on how any conflicts of interest are to be managed.

## Access to Data

The Steering Committee will have access to the full dataset and oversee analysis, interpretation and reporting of results. Approval will be sought from the Steering Committee prior to publication of study data. Care will be taken to avoid duplication in reporting of results.

## Dissemination Policy

The results of this study will be submitted for peer-reviewed publication regardless of magnitude or direction of effect. All planned analyses, as outlined in this protocol, will be reported.

All named investigators will be included as authors in manuscripts reporting data specific to this study. Other individuals who fulfil authorship criteria may be included as authors, subject to approval of the Steering Committee.

# Study Management

## Steering Committee

The Steering Committee will take overall responsibility for all aspects of the study, meeting on a quarterly basis. Matters arising between meetings may be dealt with by email. The Principal Investigator will be responsible for maintaining a record of correspondence and minutes of meetings.

## Management Committee

A Management Committee will be appointed to oversee day-to-day running of the study, including the Principle Investigator, key research staff and one or more co-investigators.

# Appendices

## Participant Information Sheet and Consent

The following documents are to accompany this protocol:

|  |  |  |
| --- | --- | --- |
| Title | Version | Date |
| PIS: The HEALTHY MUMS and BABIES (HUMBA) Trial – 12-18 Months Follow-up | 1.0 | 20.10.2016 |
| CF: The HEALTHY MUMS and BABIES (HUMBA) Trial; 12-18 Month Follow-up | 1.0 | 6.10.2016 |
| Follow-up flyer |  |  |

##  Case Report Forms

The following case report forms (CRF) are to accompany this protocol:

|  |  |  |
| --- | --- | --- |
| Title | Version | Date |
| Infant Anthropometry |  |  |
| Feeding Questionnaire |  |  |
| Children’s Eating Behaviour Questionnaire |  |  |
| Food Frequency Questionnaire |  |  |
| Maternal healthcare questionnaire |  |  |
| Maternal assessment |  |  |
| NZFFQ –SF NZ  |  |  |
| NZPAQ-SF  |  |  |
| EPDS |  |  |
| STAI  |  |  |
| SF-12 |  |  |
| Ages and Stages Questionnaire (ASQ) |  |  |
| Infant ultrasound |  |  |

## Ethical and Locality Approval

The following letters of approval are to accompany this protocol:

|  |  |  |
| --- | --- | --- |
| Title | Reference | Date |
| Ethics letter of approval (amendment) – Southern HDEC  | 14/STH/205/AM05 | 2.11.2016 |
| CMH Research Office, locality approval - Shamshad Karatela | 1908 | 29.12.2016 |
|  |  |  |

## Protocol Amendments

|  |  |  |  |
| --- | --- | --- | --- |
| Protocol version, Date | Amendment(s) | Date accepted by Steering Group | Date ethics committee notified (or NA) |
| 1.1, 18.1.2017 | Remove infant physical activity outcome 2.1.16 (from previous draft). Added food intake as frequency and amount to outcomes. |  | NA (minor) |
| 1.2, 25.1.2017 | HbA1c and lipids added as a maternal outcome |  | NA (minor) |
| 1.3, 16.02.2017 | Grammatical revision, maternal abdominal and arm circumference added.  |  | NA (minor) |
| 1.4, 24.02.2017 | access to health care added as an objective |  | NA (minor) |
| 1.5, 21.03.2017 | Minor corrections | 17/3/2017 | NA (minor) |

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