Protocol

The HEALTHY MUMS and BABIES

(HUMBA)

DEMONSTRATION TRIAL

Universal Trial Number (UTN): U1111-1155-0409

ANZCTR registration number: ACTRN12615000400561

Version 5

31 March 2017

List of Abbreviations

BP	Blood pressure
BMI	Body Mass Index
CHW	Community Health Worker
CMH	Counties Manukau Health
GDM	Gestational Diabetes Mellitus
HAPO	Hyperglycemia and Adverse Pregnancy Outcome
HbA1c	Haemoglobin A1c
IADPSG	International Association of Diabetes in Pregnancy Study Groups
LGA	Large for gestational age
OGTT	Oral Glucose Tolerance Test
RCOG	Royal College of Obstetricians and Gynaecologists
RCT	Randomised controlled trial
SPRING	Study of Probiotics IN the prevention of Gestational diabetes
UPBEAT	UK Better Eating and Activity Trial

Contents

1. General Information

Full title: A two by two factorial randomised controlled demonstration trial of a multifaceted dietary intervention versus routine dietary advice; and probiotic versus placebo capsules in obese pregnant women in the Counties Manukau Health region.

Short Title: Healthy Mums and Babies (HUMBA) Demonstration Trial

Name and Contact Details of Principal Investigator: Professor Lesley McCowan, Department of Obstetrics and Gynaecology, University of Auckland, New Zealand.

Study Duration: Two years

Study Site: This is a single centre study in which recruitment will be undertaken in the Counties Manukau Health region in South Auckland, New Zealand.

2. Introduction

2.1 Main Study Objective

To conduct a two by two factorial design randomised controlled demonstration trial of: 1) a culturally appropriate, affordable and sustainable dietary intervention compared with routine dietary advice; and 2) probiotic versus placebo capsules in obese pregnant women in South Auckland, New Zealand.

2.2 Primary Objectives

The primary objectives are to determine if the dietary intervention and/or probiotic capsules:

1. Reduce excessive pregnancy weight gain (PWG)
2. Reduce infant birthweight

2.3 Secondary Objectives

To explore:

1. Whether the dietary intervention or probiotics improve maternal glucose metabolism (as assessed by OGTT parameters at 26-28 weeks' and HBA1c at 28 and 36 weeks'), reduce pregnancy induced hypertension and Caesarean birth.
2. Whether the dietary intervention improves diet quality and healthy eating index at 28 weeks' gestation and 5 months postpartum.
3. Whether probiotics or the dietary intervention influences maternal quality of life, including measures of depression, anxiety, functional health and well-being scores at 36 weeks' and 5 months postpartum.

4. Whether the dietary intervention or probiotics are associated with reduced maternal adiposity, lower HbA1c and altered maternal lipid profile at 5 months postpartum.
5. Whether the dietary intervention or probiotics influence neonatal body composition, initiation and establishment of breast feeding, and neonatal complications (e.g., hypoglycaemia).
6. Whether the dietary intervention or probiotics influence infant growth, body composition, feeding behaviour, nutritional intake, and general health up to 5 months of age.
7. The relationship between HbA1c at recruitment (12-17^{6} weeks' gestation) and PWG and birthweight; and additional pregnancy outcomes (glucose metabolism, gestational diabetes mellitus [GDM], pregnancy induced hypertension, and infant adiposity).
8. The relationship between HbA1c at 28 and 36 weeks' gestation and changes in HBA1c between recruitment and 28 and 36 weeks' and pregnancy outcomes (glucose metabolism, GDM, pregnancy induced hypertension,PWG, birthweight and infant adiposity).

3. HUMBA Demonstration Trial Overview and Rationale

The economic and personal burden of obesity in New Zealand is enormous with one-third of NZ children and two-thirds of adults currently overweight or obese [1, 2]. In 2012, 43% of Māori and 51% of Pacific 2-14 year olds were overweight or obese compared with 25% of children from European/other ethnic backgrounds [2]. Many New Zealand children are therefore on a trajectory to developing chronic metabolic diseases as a consequence of their increased weight. These overweight young girls are the mothers of tomorrow. The rates of obesity are also much higher in Māori and Pacific women (45% and 64% respectively vs 26% in European) as are obesity related health complications (e.g. type 2 diabetes) [1]. Obese pregnant women have increased rates of most pregnancy complications including GDM, preeclampsia and caesarean sections. Their infants are at higher risk of congenital abnormalities, stillbirth, being born large for gestational age (LGA) [3], becoming obese themselves [4], and dying prematurely from cardiovascular disease [5]. Forty percent of pregnant women who reside in the multi-ethnic Counties Manukau Health (CMH) region are obese in early pregnancy [6]. More than 10% of all New Zealand babies (>7000 annually) are born in the CMH region where the birthing population comprises 36% Pacific, 24% Māori, 17% Indian/other Asian, and 23% European/other [6, 7]. Interventions in this high-risk population are urgently required to improve maternal and child health.

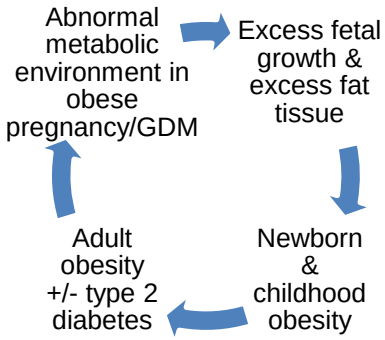


Figure 1. Vicious cycle: maternal obesity and GDM.

As rates of maternal obesity have increased there has been a marked increase in GDM [8] with the prevalence in obese women reported between 15 and 30% [9, 10], depending on criteria used and populations studied. 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 [11]. The infants are more likely to be born LGA with consequent increased rates of traumatic birth [11, 12]. This creates a vicious intergenerational cycle, termed "developmental over-nutrition" [13]. Larger infants with increased fat mass are more likely to become obese children [14] and adults with subsequent diabetes themselves [15] (**Figure 1**). This cycle promotes health inequalities in the next generation as Pacific and Māori women have rates of obesity [1] and GDM which are substantially higher than European [6, 16]. Importantly, a strong continuous relationship between increasing maternal glucose levels on the 24-32 week OGTT (below those diagnostic of GDM) and risk of LGA, neonatal hypoglycaemia and caesarean delivery have recently been demonstrated [17] implying that smaller reductions in maternal glucose levels may be beneficial.

Many women exceed recommendations for optimum pregnancy weight gain [18] with potential lifelong consequences for mother and child, which are independent of the effects of maternal BMI. Risks for the offspring include a four-fold increase in LGA [19] and an alarming increase in BMI, blood pressure, and abnormal metabolic profile in childhood and adulthood [20-22]. Mothers with excessive weight gain have increased risk of GDM, preeclampsia and caesarean delivery [18, 19]. In addition, they are more likely to retain the weight and enter further pregnancies more obese with consequences for future children. Of further concern, obese women are more likely to gain excessive weight in pregnancy than non-obese women [18, 23]. Obesity cannot be reversed during pregnancy, however dietary interventions during pregnancy may limit pregnancy weight gain [24]. A recent systematic review of trials of dietary interventions reported a 4 kg mean reduction in pregnancy weight gain, a 60% reduction in GDM and fewer traumatic births, suggesting that dietary interventions may have also reduced adiposity in the infants [24]. A recent report from a large randomised controlled trial (RCT) of lifestyle interventions (LIMIT study) in overweight and obese pregnant women from Adelaide (Australia) reported reduced rates of infants with birthweight >4 kg born to women who received the lifestyle interventions [25], as well as improved diet and increased physical activity [26].

Mobile phone texting technologies are increasingly being used to assist with weight loss [27]. A recent systematic review of studies of texting and smart phone apps to assist with weight loss reported significant improvements in at least one of the following outcomes: weight loss, decreased waist circumference, increased physical activity, or reduced intake of sugar-sweetened beverages [27]. Feedback from participants was also favourable [27]. Data from an Auckland pilot

RCT, with a high proportion of Māori and Pacific participants, confirmed the feasibility of using mobile phone technology in an ethnically diverse obese non-pregnant population. Furthermore, the texting intervention was associated with successful weight loss [28]. The large majority of pregnant women in CMH (98% in a recent survey) have a mobile phone. The high penetrance of mobile phones in low socioeconomic communities makes texting a useful tool to complement dietary interventions.

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 [29], and satiety, inflammation, and glucose and lipid metabolism [30-32], with potential to reduce obesity and type 2 diabetes [33]. Probiotics are safe in pregnancy [34] and provide a simple intervention in pill form. A small RCT of probiotic/placebo capsules in 256 Finnish women reported a marked reduction in GDM, with probiotics (*Lactobacillus rhamnosus GG* and *Bifidobacterium lactis* at 10^{10} colony-forming units/day) combined with dietary advice [35]. Participants were randomised at 14 weeks' to: dietary advice + probiotic; dietary advice + placebo; or control group (standard care + placebo). The dietary advice comprised counselling by a nutritionist at recruitment, 24 and 34 weeks' who promoted food intake consistent with Finnish guidelines [36]. Probiotic capsules were associated with a >60% reduction in GDM by Finnish criteria [37]: a prevalence of 13% in those in the probiotics/nutrition group compared with 36% in the placebo/nutrition arm and 34% in controls (p= 0.003). There was no reduction in GDM with the dietary advice alone but only two of the three dietary counselling sessions occurred before the OGTT. A 144 g reduction in birthweight was also observed with probiotic [38] as well as significant reductions in maternal waist circumference 6 months postpartum [39].

Besides the reported efficacy of these probiotics in reducing GDM, *L rhamnosus* use has been associated with increased weight loss in a RCT of obese men and women [40] while increased stool *Bifidobacteria* concentrations have been associated with weight reduction [41]. *Bifidobacterium* genera are healthy commensal bacteria, which are less common in the gut microbiome of obese mothers and those who gain excessive weight in pregnancy [42]. Daily probiotic yoghurt has been associated with a 40% reduction in preeclampsia [43] suggesting potential for reduction in hypertensive pregnancy disorders, which are also increased in obese women. A RCT (SPRING study) of probiotics/placebo aimed to prevent GDM in overweight and obese women has started in Brisbane (n=540, no nutrition intervention included) [44], but no studies have been undertaken in a population of obese women nor in a New Zealand setting .

Pregnancy is described as a "teachable moment" providing a finite window during which women are more likely to undergo behavioural change if there are perceived benefits for their offspring [45]. Accordingly, we are planning an innovative, two by two factorial design, randomised

controlled demonstration trial of probiotics or placebo plus an intensive, culturally appropriate dietary intervention to be undertaken in obese pregnant women in the Counties Manukau Health (CMH) region in South Auckland, New Zealand.

We hypothesise that in obese pregnant women the interventions will reduce: 1) the incidence of excessive pregnancy weight gain; and/or 2) infant birthweight.

4. Study Population

4.1 Setting

Counties Manukau Health region which has more than 7000 births yearly, comprising >10% of all New Zealand (NZ) births.

4.2 Target Population

Obese pregnant women who comprise over forty percent of the yearly births in the Counties Manukau Health region (>2800 births in obese women annually). Recruitment will commence in April 2015 with the goal of achieving complete recruitment in mid 2016.

4.3 Inclusion Criteria

Women with a BMI greater than or equal to 30 kg/m^2, singleton pregnancy, between 12^0 and 17^6 weeks of gestation and able to provide informed written consent.

4.4 Exclusion Criteria

Pre-existing diabetes or HbA1c at booking ≥50 [46], taking probiotic supplements, known congenital abnormality, medications or medical conditions which alter glucose metabolism, multiple pregnancy, bariatric surgery, and severe hyperemesis.

5. Study Design and Procedures

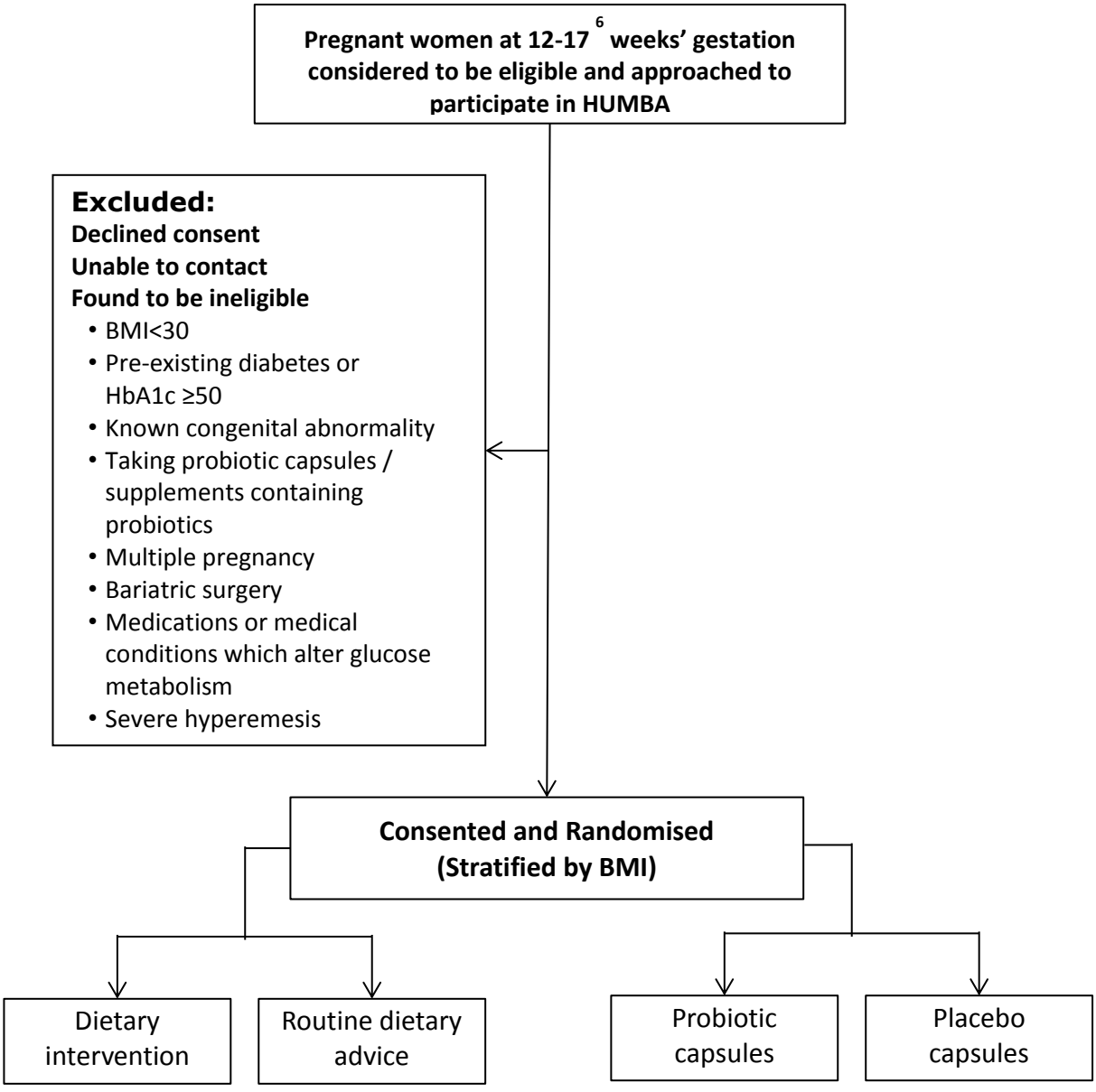
This is a randomised placebo controlled (for probiotics) two by two factorial demonstration trial, designed according to CONSORT guidelines [47]. We will investigate the role of an oral probiotic capsule consisting of *Lactobacillus rhamnosus GG* and *Bifidobacterium lactis BB12* at a dose of $7*10^9$ colony-forming units per day each or placebo, and a multi-faceted dietary intervention or routine dietary advice. Women will be randomised at recruitment to each intervention (**Figure 2**). Probiotic or placebo capsules will be taken once daily from enrolment between 12^0 and 17^6 weeks of gestation until birth.

5.1 Engagement of Pregnant Women

We will have a multi-pronged approach to optimise recruitment including through lead maternity caregivers (self-employed midwives), community ante-natal clinics, general practitioners, practice

nurses, ultrasound clinics, and community contacts. Our research team will provide educational sessions for the health care providers to maximise reach for recruitment. Lead maternity caregivers will notify the research team about contact details of eligible women who are interested in participating. The research assistant will arrange a time to meet the woman in a suitable location to explain the study, confirm eligibility and obtain informed consent.

Figure 2: Overview of recruitment and randomisation



In order to exclude women with previously unrecognised Type 2 diabetes, HbA1c will be measured in all participants (using the *Roche cobas b 101* point-of-care system) prior to randomisation. If HbA1c is ≥50, women will be ineligible and considered to have undiagnosed diabetes [46] .They will be referred to the Diabetes in Pregnancy Service at CMH. For the large

majority of participants (those with HbA1c <50) routine testing with a 75 g OGTT will be performed at 26-28 weeks. The HUMBA trial *Roche cobas b 101* HbA1c results in women with HbA1c <50 will not be revealed.

5.2 Randomisation Procedure

Randomisation will be undertaken using a web-based protocol, randomize.net (http://randomize.net), using random block sizes (minimum 4; maximum 8). For randomisation purposes, each research midwife will serve as a proxy for 'clinical site' (this will enable each research midwife to be able to dispense the randomised study capsules at recruitment). Participants will be stratified by 'clinical site' (n=2) and BMI category (BMI of 30-34.9 or BMI >=35 kg/m2) and randomly allocated to dietary intervention or routine dietary advice; and to probiotic or placebo (**Figure 2**).

5.3 Assessment at Study Entry

Comprehensive information will be obtained from the woman and her clinical records, including:

- Demographic, socioeconomic, educational and employment data
- Medical history
- Obstetric history: parity, method of conception, gestation and accuracy of estimated date of delivery, previous pregnancy complications (GDM, hypertensive disease, pre-term delivery, caesarean section)
- Family history of diabetes, hypertension and cardiovascular disease
- History of smoking, alcohol and other drug use
- Medications and nutritional supplements
- Probiotic food ingestion
- Maternal anthropometrics: weight, height, waist and mid-arm circumference
- Blood pressure (BP)
- Finger-prick blood lipid testing (*Roche cobas b 101* point-of-care system)
- Samples: non-fasting blood and urine specimen for biobank
- Questionnaires include: NZ Food Frequency Questionnaire - Short Form [48], NZ Physical Activity Questionnaire [49], Edinburgh Postnatal Depression Scale [50], State-Trait Anxiety Inventory (STAI) [51], and the 12-Item Short-Form Health Survey (SF-12) [52]. It is estimated that it will take 30 minutes to complete these questionnaires.

5.4 Study Interventions

5.4.1 Dietary Intervention Study

5.4.1.1 Dietary intervention

This group will receive the multifaceted dietary intervention comprising of the following components**:**

5.4.1.1.1 Encounters with nutrition advisor

A review of the literature suggests that at least 4 encounters with a nutrition advisor are required to effect change in eating patterns and limit weight gain in pregnancy [53, 54]. Adherence to a schedule of four visits with a nutritional advisor also seems to be achievable [55]. The nutrition advisors will be community health workers (CHW), who comprise an integral part of the health workforce in Counties Manukau, usually come from Pacific or Māori ethnic backgrounds, and are experienced in engaging hard-to-reach women in maternity care. They have been trained in the Pacific Heart Beat Certificate of Nutrition (Pacific Heart Beat Course by the National Heart Foundation) [56] complemented by extra training in pregnancy nutrition. A New Zealand Registered Dietitian provides professional supervision and continuing education to support the CHWs. In-depth education will be provided by the CHW to women in the dietary intervention groups on healthy eating including: portion control, healthy food and drink choices, limiting energy dense foods, healthy recipes, label reading and managing cravings. Each participant will have an initial 1 to 1.5 hour educational session (on average at about 14 weeks' gestation) with the CHW. Three further 30 to 60 minute face-to-face sessions will be planned with the CHW at two weekly intervals and be completed before the 26-28 week OGTT. Compliance with the dietary intervention will be assessed by the number of educational sessions participants attend with the CHW.

5.4.1.1.2 Behaviour Change Techniques

CHWs will also receive training in evaluation methodology and counselling techniques, including healthy conversations [57]. Behaviour change techniques will be incorporated in the nutrition education sessions including identifying barriers, self-monitoring, goal setting, and providing regular feedback [58]. Targets will be set for optimal pregnancy weight gain at the first visit with the CHW. Weight will be measured and plotted on a personalised pregnancy weight gain chart, in the participant handbook, at each subsequent encounter.

5.4.1.1.3 Physical Activity Advice

Physical activity advice which is included in the dietary intervention will incorporate information from the Te Wai o Rona program (http://www.sportwaikato.org.nz/te_wai_o_rona_resources.cfm)

and the Royal College of Obstetricians and Gynaecologists (RCOG) guidelines for recreational exercise in pregnancy [59]. In Te Wai o Rona, there are four key physical activity messages which are relevant to pregnant women:

- Look for ways to be active everyday
- Increase daily exercise
- Move more, add more steps
- Reduce sedentary leisure time

The RCOG guideline recommends that previously sedentary women begin with 15 minutes of exercise focusing on walking three times weekly and gradually increasing to 30 minute sessions four times weekly [59].

5.4.1.1.4 Motivational texting

Three times weekly motivational texting, which reinforces the educational content covered in the face-to-face meetings with the CHW, will be implemented for those participants in the dietary intervention with cell phones (98% in a recent survey of pregnant women at CMH). Text messaging will continue until birth and incorporate messages about diet and physical activity.

5.4.1.2 Routine dietary advice

These women will be provided with a pamphlet produced by the NZ Ministry of Health (*Eating for Healthy Pregnant Women*) which contains dietary advice that follows current New Zealand nutrition guidelines [60]. They will also receive a pamphlet providing information about healthy weight gain and physical activity in pregnancy [61].

Although it will not be possible for clinical and research staff to be blinded to the dietary intervention allocation, the key health outcomes including pregnancy weight gain, infant birthweight and results from the OGTT, are not subject to bias.

5.4.2 Probiotic study

5.4.2.1 Probiotic group

Participants randomised to this arm will receive probiotic capsules containing *Lactobacillus rhamnosus GG* and *Bifidobacterium lactis BB12* (Chr. Hansen A/S, Horsholm, Denmark) at a dose of $7*10^9$ colony-forming units per day each. This is the equivalent probiotic combination that was used by Luoto et al in the Finnish study [35] and also being used in the ongoing study in Queensland [62].

5.4.2.2 Placebo group

Participants randomised to this group will receive identical placebo capsules containing microcrystalline cellulose and dextrose anhydrate, also supplied by Chr. Hansen A/S, Horsholm, Denmark.

5.4.2.2.1 Allocation Concealment, Blinding and Compliance

Christian Hansen has provided identically packaged canisters of placebo and probiotic capsules, containing 31 capsules each. AnQual Laboratories (School of Pharmacy, University of Auckland) have labeled the canisters using a pre-allocated random list. The kit list used to label the canisters was generated by the Project Manager and AnQual, using the Excel random function. This list has secure password protection and is stored with AnQual.

The Project manager is the only HUMBA staff member un-blinded to probiotic/placebo allocation,

Compliance with probiotic/placebo will be assessed by the research team via participant self-report and counting and documenting capsules remaining in canisters when repeat supplies are provided at monthly intervals.

5.5 Optimizing Engagement and Retention

All women will receive antenatal care, from their usual maternity care provider. Members of the research team will meet participants at pre-specified intervals during pregnancy (**Figure 3**) to collect outcome data, provide further supplies of probiotic/placebo and check compliance.

5.6 Comprehensive 28-30 week Research Assessment

This appointment will be scheduled after the woman has had her OGTT at 26-28 weeks'. Weight, mid-arm circumference and BP will be measured. The food frequency and physical activity questionnaires and an updated medical history will be obtained by the research midwife (**Figure 3**). The OGTT results will be reported to the research team with fasting, one- and two-hour results enabling a diagnosis of GDM by the International Association of Diabetes in Pregnancy Study Groups (IADPSG) criteria [63]. The maternity care provider will receive fasting and two-hour glucose results from the OGTT reported using the New Zealand Society for the Study of Diabetes (NZSSD) criteria [64] as per usual clinical practice. Women diagnosed with GDM by these NZSSD criteria will be referred by their maternity care provider to the Diabetes in Pregnancy Service (as per usual practice) and managed according to local guidelines including postpartum testing.

HbA1c and lipids will be measured using the *Roche cobas b 101* point-of-care system.

5.7 Comprehensive 36 week Research Assessment

Weight, mid-arm circumference, BP, HbA1c and lipids (Cobas b101) will be measured. Lifestyle questionnaires [50, 51] will be repeated, functional health and well-being (SF-12) [52] assessed, and data collected on pregnancy complications. Maternal urine, blood, stool and hair samples will be collected from consenting women for basic science studies.

5.8 Post-Delivery Evaluation Assessment

Maternal and neonatal outcomes will be collected within 72 hours of birth by a research midwife. Infants will be weighed at birth and detailed anthropometric measurements obtained by research midwives within 72 h of birth, including crown-heel length by neonatometer, head, left mid-arm, chest and abdominal circumferences using a lasso tape, and subscapular, triceps and suprailiac skinfolds by Harpenden calipers (average of 2 measurements, or median of 3 if initial measurements differ by >0.4 mm) [65].

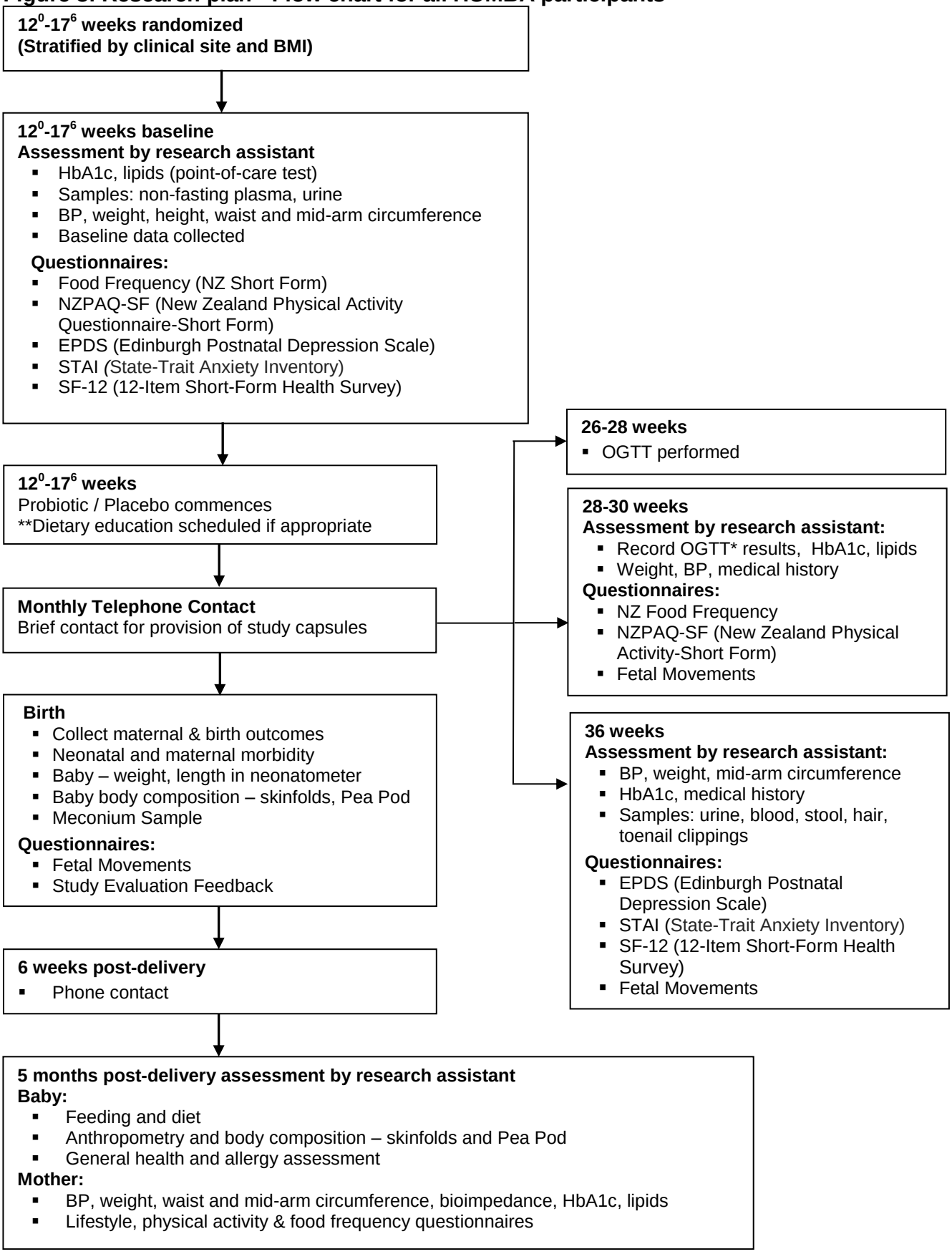
Among consenting participants, infant body composition will be measured in the Pea Pod as soon as practical after birth. In these infants, anthropometry will be performed at the same time. A sample of meconium will be collected for microbiome analysis.

A short survey will also be administered to assess feedback about participation in HUMBA and the interventions.

5.9 Assessment at 5 months post delivery

A follow up appointment with the research midwife and a paediatrician will be scheduled at 5 months (±2 weeks) postpartum. Lifestyle and food frequency questionnaires, and infant health and well-being questionnaires will be completed. Measurements of maternal blood pressure, weight, height, waist and mid-arm circumference, and body composition by Bioelectrical Impedance Analysis (BIA) [66] will be undertaken. Data will be collected on infant feeding, allergies, health and anthropometry (weight and length; head, left mid-arm, chest and abdominal circumference; subscapular, triceps and suprailiac skinfolds) and body composition by Pea Pod. Infant feeding behaviour will be assessed using the Baby Eating Behaviour Questionnaire [67], and infant eating patterns and nutritional intake will be determined by a food frequency questionnaire. For infants born preterm (<37 weeks' gestation), timing of assessment will be based on corrected age. Consent will be obtained for ongoing contact when funding is obtained for further follow up.

Figure 3: Research plan - Flow chart for all HUMBA participants



* Fasting, one-hour and two-hour glucose after 75 gm glucose load.
** Women randomised to the dietary intervention will be seen 4 times at 2-3 weekly intervals between recruitment and the 26-28 week OGTT.

6. Investigational material – not applicable

7. Statistics

7.1 Primary outcomes

- Proportion of women with excessive pregnancy weight gain [18] - defined as mean weekly weight gain >0.27 kg between recruitment and 36 weeks' (or closest pregnancy weight if delivery occurs <36 weeks') [18].
- Infant birthweight

7.2 Secondary outcomes

7.2.1 Maternal:

Continuous variables:

- Pregnancy weight gain adjusted for gestation
- Maternal pregnancy glucose metabolism (as assessed by OGTT parameters at 26-28 weeks' [68] and HBA1c at 28 and 36 weeks' and differences in HBA1c between recruitment and 28 and 36 weeks' and 5 months postpartum)
- Changes in diet quality and dietary patterns assessed by NZ Food Frequency Questionnaire between recruitment, 28-30 weeks' and 5 months postpartum [48]
- Functional health and well-being (SF-12) at 36 weeks and 5 months postpartum [52]
- Depression and anxiety scores at 36 weeks' and 5 months postpartum [50, 51]
- Maternal HBA1c and lipid profile at 5 months postpartum
- Maternal adiposity at 5 months postpartum (assessed by BMI, waist and arm circumference and fat mass measured by bioimpedance)

Categorical variables

- GDM by NZSSD criteria [64]
- Pregnancy Induced hypertension (preeclampsia and gestational hypertension) [69]
- Mode of birth
- Weight and body composition by bio-impedance at 5 months postpartum
- HbA1c and blood lipids at 28-30 and 36 weeks' gestation and 5 months postpartum

7.2.2 Infant:

Continuous variables:

- Neonatal anthropometry
  - Birthweight, head circumference, length (Z-scores) [70]
  - Birthweight adjusted for length
  - Girths (chest, arm, abdominal) adjusted for length
- Neonatal body composition
  - Fat mass and lean mass (Pea Pod), adjusted for length
  - Fat mass adjusted for lean mass
  - Subscapular, triceps and suprailiac skin fold, adjusted for length
  - Arm muscle area, adjusted for arm length
- Gestational age at birth
- Infant nutritional intake

- Infant anthropometry and body composition (as above), feeding behaviour as assessed by Baby Eating Behaviour Questionnaire (BEBQ scores) [71]
  - Infant body composition as measured by Pea Pod at 5 months of age

Categorical variables:

- Large for gestational age - customised [72] and population [70] LGA
- Small for gestational age - customised [72] and population [70] SGA
  - Admission to neonatal care unit (and reason)
  - Neonatal composite morbidity, including birth trauma (fracture, brachial plexus injury, cephalhaematoma, subgaleal haematoma), hypoxic ischaemic encephalopathy, sepsis, respiratory distress requiring positive pressure support, hypoglycaemia requiring dextrose treatment.
  - Initiation and establishment of breast feeding
    - Feeding in first two postnatal weeks (collected by phone call at 6 weeks).
    - Infant feeding, feeding behaviour, health and anthropometry at 5 months of age.

7.2.3 Other secondary outcomes

- Process/intervention outcomes
- Attendance at study visits
- Adherence to probiotic/placebo regimen
- Cost effectiveness of the intervention
- Maternal feedback about being in the study (survey at post birth visit)

Secondary outcomes relating to HbA1c- observational studies

- Glucose metabolism (parameters on the 26-28 weeks' OGTT), birthweight and infant skinfold thickness, lean mass and fat mass (adjusted for length or lean mass)

- Rates of GDM and pregnancy induced hypertension.

7.3 Sample size calculation and power

A total of 220 participants will be recruited. With 80% power and 100 subjects remaining in each main intervention group (allowing 10% lost to follow-up) we can detect:

- 25% reduction in excessive pregnancy weight gain from 80% to 60% (based on an 80% rate of excess weight gain in obese participants in the SCOPE study) [19], and
- 227 g difference in mean birthweight (based on CMH data; mean=3,638, SD=521) [7].

To allow for the two primary outcomes an alpha of 0.025 is used for the power calculations (Bonferroni approach).

7.4 Statistical Analyses and Data Management

Statistical advice has been provided on the study design, sample size and planned data analyses from Dr John Thompson, Department of Paediatrics, University of Auckland, who is a member of the research team.

Analyses will follow the principle of intention-to-treat (ITT). Participants will be analysed according to the assigned treatment group at randomisation. Statistical models will adjust for the key randomisation stratification variable, BMI at recruitment.

Binary endpoints will be analysed using logistic regression to estimate odds ratios for each of the interventions (dietary intervention and probiotics). Continuous outcomes will be modelled using generalised linear models to estimate any changes in outcomes with the interventions (dietary intervention and probiotics) in relation to their respective control groups. Multivariable analyses will control for potential confounders including ethnicity and infant sex.

Primary analyses will report only marginal effects for each randomised exposure, with adjustment for co-intervention. Secondary analyses will test for interactions between the main effects (primary outcomes only), though this pilot trial has been powered only for the main effects (see below).

Secondary analyses involving maternal data collected at recruitment, 36 weeks and 5 months will be analysed using mixed methods to allow for the repeated measures of these outcomes over time.

Given there are two primary outcomes, two-sided p-values of less than 0.025 will be used in determining statistical significance for each primary outcome (Bonferroni adjustment). For secondary outcomes, two-sided p-values of less than 0.05 will be used in determining statistical significance, and corrections will be made to allow for multiple testing.

The proportion of women with excessive pregnancy weight gain, will be modelled using logistic regression to estimate the effects of dietary intervention and probiotics, adjusting for BMI at recruitment (randomisation variable) and other potential confounders (ethnicity). We will additionally test for an interaction between dietary intervention and probiotics, though this study has been powered only to detect main effects.

The mean infant birthweight will be modelled using generalised linear models to estimate the effects of dietary intervention and probiotics, adjusting for maternal BMI at recruitment (randomisation variable) and other potential confounders (ethnicity, infant sex). We will additionally test for an interaction between interventions, as above.

Measures of infant body composition (whole-body fat and fat-free mass, skinfold thickness and arm muscle area) will be adjusted for infant length, sex and ethnicity. For birth data, analyses will be adjusted for gestational age at birth (wk). We will explore for an interaction between intervention effect and infant sex.

8. Safety Aspects and Data Monitoring

The research team will oversee and manage the project. A *Trial Steering Committee (TSC)* will be established that contains a representative group of the named investigators who are responsible for the conduct of the trial and will follow consort guidelines [73]. An independent committee will be appointed with established terms of reference to serve as the combined *Data Safety and Monitoring Committee* (DSMC) ,(see Section 10), which will assess the progress of the trial and review reports of serious adverse events and adverse events. The functions will be combined in the same committee due to the small size of the HUMBA demonstration trial.

Serious adverse events will be defined as:

- Maternal death
- Maternal admission to intensive care unit
- Fetal death
- Neonatal death or death up to primary hospital discharge
- Stage 2-3 neonatal encephalopathy
- Any other serious adverse event that the principal or local lead investigator believes should be referred for independent review by the DSMC

The DSMC will classify SAE as: to the likelihood of a causative association with the study intervention*:* no, unlikely, possible, or probable.

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A systematic review has confirmed the safety of probiotics in pregnancy [74]. Data will be collected about any adverse events that are thought to be attributable to use of probiotics (e.g. sepsis confirmed to be due to *Lactobacillus rhamnosus* or *Bifidobacterium lactis* in mother or newborn). Rates of pregnancy complications will be compared between participants receiving probiotics and placebo.

9. Compliance Statements

The study will be conducted in line with the Principles of the Declaration of Helsinki (1996) and according to best practice as detailed in the HUMBA demonstration trial standard operating procedure manuals. Ethics approval to undertake this study has been obtained from the Health and Disability Ethics Committees (HDEC), Ministry of Health, New Zealand (http://ethics.health.govt.nz/). The researchers will respect the principles of partnership, participation and protection in the Treaty of Waitangi (the founding document of New Zealand). In addition, the HUMBA demonstration trial will follow the 2012 *Ethical Guidelines for Intervention Studies* published by the National Ethics Advisory Committee, Ministry of Health (http://neac.health.govt.nz/streamlined-ethical-guidelines-health-and-disability-research).

The HUMBA demonstration trial is registered with the Australian New Zealand Clinical Trials Registry (ACTRN12615000400561). A Universal Trial Number (UTN) has been obtained from the World Health Organization (http://www.who.int/ictrp/unambiguous_identification/utn/en/).

10. Quality controls and quality assurance

10.1 Confirmation of the quality and safety of the probiotic

Chr. Hansen A/S make the Probio-TecÒ BG-Vcap-6.5 capsules from library colonies which are meticulously DNA fingerprinted to ascertain the presence of *Bifidobacterium BB12* and *Lactobacillus rhamnosus GG* only. The packaging and storage of the probiotics will comply with company specifications ensuring the quality of the product.

10.2 Data handling

Consent forms and participant data that contain identifying information will be stored separately. Most study data will be collected electronically on a tablet but when paper forms or questionnaires are completed these will be coded using study ID numbers; this will ensure confidentiality for each participant. All data forms will be stored securely in locked cabinets for 10 years and all identifiable computer data will be accessible only using a password. Access to the data forms and computer data will be restricted to researchers directly involved with the study.

10.3 Data monitoring

The independent data safety and monitoring committee will include experts (with no conflict of interest) in the fields of obstetrics, neonatology and epidemiology/statistics with experience in perinatal trials. Terms of reference will be established including reporting procedures. The committee will review data prepared by the trial statistician on trial conduct and safety and report to the trial steering committee.

11. Summary

This two by two factorial design randomised controlled demonstration trial aims to reduce excessive pregnancy weight gain and optimise infant birthweight in a multi-ethnic sample of obese pregnant women. If successful, either one or both of the interventions are applicable to clinical practice.

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