Protocol

Protocol for: Alexander T, Asadi S, Meyer M, et al. Nutritional support for moderate-to-late–preterm infants — a randomized trial. N Engl J Med 2024;390:1493-504. DOI: 10.1056/NEJMoa2313942

This trial protocol has been provided by the authors to give readers additional information about the work.

DIAMOND trial Supplement

This supplement contains the following items:

The DIAMOND trial

DIfferent **A**pproaches to **MO**derate & late preterm **N**utrition: **D**eterminants of feed tolerance, body composition and development

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1. INTRODUCTION

Babies born at moderate-late preterm gestations account for >80% of all preterm births. Although survival is excellent, these babies are at increased risk of adverse neurodevelopmental outcomes. They also are at increased risk of adverse long-term health outcomes, such as cardiovascular disease, obesity and diabetes. There is little evidence guiding optimal nutritional practices in these babies; practice therefore varies widely. Areas of uncertainty include whether to provide parenteral nutrition, whether to provide milk supplements until sufficient amounts of mother's milk is available and how to improve tolerance of feeds. All of these factors may impact upon growth, gut microbiome, development of adiposity and later metabolic health. The factorial design clinical trial will address the role of parenteral nutrition, milk supplementation and exposure of the preterm infant to smell and taste with each feed on the time to tolerance of full feeds, adiposity, gut microbiome population and activity, and neurodevelopment at 2 years.

2. BACKGROUND

Of the ~5,000 babies born preterm in NZ each year[1], >80% are born at moderate- to late-preterm (MLPT) gestations between 32+0 and 36 completed weeks'[1] and these babies constitute a much larger proportion of the health care burden related to prematurity than do extremely preterm babies[2]. Although survival of MLPT babies is excellent, they are at risk of impaired later development. MLPT babies have a 36% increased risk for developmental delay or disability at pre-school ages and a 50% increased risk of special education needs at school[3]. MLPT birth also carries increased risk of adverse long-term health outcomes, including obesity, hypertension and diabetes, even by the 3rd and 4th decades of life[4,5]. This metabolic risk is substantially related to increased adiposity. Late preterm babies demonstrate a 182% increase in fat mass between birth and term-corrected age, by which time they have ~50% greater percentage body fat than term-born controls[6]. This appears to be due to preserved development of fat mass but impaired accretion of lean mass, indicative of inadequate protein intake between birth and term corrected age[6].

There is good reason to believe that nutritional practices in early life impact upon later metabolic health. A period of relative undernutrition will be accompanied by faltering growth and an accumulating negative nitrogen balance[7] which is followed by later accelerated growth, when nutrition is restored. Enhanced growth in infancy may protect the infant from cognitive impairment but also is linked to childhood adiposity, persisting through adulthood[8]. Different nutritional practices will affect the gut microbiome which also is associated with later adiposity[9]. Data such as these have led to the suggestion that there may be a trade-off in preterm babies whereby providing enhanced nutrition to prevent postnatal growth faltering results in better brain growth and cognitive outcomes, but accelerates weight gain and alters the microbiome, thus increasing the risk of later metabolic and cardiovascular disease[8].

In MLPT babies, there is inevitably a delay between birth and the establishment of full enteral feeds. Preterm babies have immature suck / swallow / breathe coordination and immature gut motility, so it can be days to weeks before they are able to tolerate full feeds without gastric residuals or vomiting. Supply of breast milk is also often delayed, as their mothers may have difficulty expressing due to the underlying condition leading to preterm birth. Further, hepatic glycogen stores double between 36 and 40 weeks' gestation, so MLPT babies cannot rely on this stored energy source. During these first days to weeks, almost all preterm babies experience a degree of protein undernutrition and incur a nitrogen (protein) deficit that is then very difficult to overcome. Thus, optimal nutritional support during this critical period may substantially improve later metabolic and developmental outcomes.

Practices around early nutritional support for MLPT babies vary widely as there is little high quality evidence. Usual practice is to provide intravenous fluids while gradually increasing the volumes of milk given by gastric tube until full enteral feeds are tolerated, and then transitioning to sucking feeds as suck / swallow / breathe coordination matures. However, there are many variations within this general approach. There are no data on whether it is better to start supplemental milk early, either formula or donor milk (if available, and which is of lower nutritional value than fresh breast milk or formula) or to wait until the mother's breast milk is available. While waiting for mother's milk or for feeds to be tolerated, there are no data on whether provision of 10% dextrose alone is sufficient, despite the inevitable catabolism and accumulating nitrogen deficit, or whether babies should receive parenteral nutrition containing protein in an attempt to prevent catabolism. All of these approaches are in use in NZ. In the two tertiary neonatal units in Auckland, one routinely commences infant

formula, the other 10% dextrose. Parenteral nutrition use is dependent upon physician preference. A study of nutritional support of 33-35 week gestation late-preterm infants in 10 California and Massachusetts hospitals similarly found the rate of intravenous nutrition use varied from 4.7 – 66% and the rate of discharge with an enriched formula varied from 4.5 – 71%[10].

Smell and taste may also be important in food tolerance. Even before ingestion of food, smell and taste initiate metabolic processes through secretion of hormones such as insulin and ghrelin[11]. However, the role these senses are not usually considered in the care of preterm infants despite preterm infants having functional taste receptors from 18 weeks' gestation and flavour perception from around 24 weeks' gestation[12]. Changes in brain tissue oxygenation have been detected by near-infrared spectroscopy (NIRS) in preterm infants >32 weeks' gestation in response to odours, with differential responses to odours rated as pleasant or unpleasant[13]. Taste receptors in the mouth relay a signal to the brainstem and higher centres through the gustatory nerve fibres, leading to activation of the cephalic phase response and the release of appetite hormones in saliva[14]. These salivary hormones are postulated to play a role in metabolism[14]; indeed, impaired oral nutrient sensing is associated with increased energy intake and a greater body mass index[15].

We have conducted a pilot trial of exposing very preterm infants to the smell and taste of milk before each tube-feed by placing a cotton wool bud soaked in milk against lips and in front of the infant's nose. Infants in the control group were not exposed to the smell and taste of milk. Infants in the intervention group tolerated enteral feeds earlier than infants in the control group, reached full enteral feeds at an earlier gestational age (median (interquartile range (IQR)) 29.0 (27.7-30.0) vs 29.9 (29.3-30.4) weeks, (P<0.05) and tended to have the nasogastric tube removed at an earlier gestational age, perhaps indicating earlier attainment of full sucking feeds. These data suggest that the simple intervention of providing taste and smell stimuli before gastric tube feeds may enhance feed tolerance. It is not known if exposing the baby to smell and taste before each gastric tube feed, with the consequent secretion of metabolic hormones, will improve feed tolerance and reduce time to full sucking feeds in MLPT babies.

3. AIM(S) OF STUDY

Our aims are to investigate the impact of different feeding strategies currently in use in NZ on feed tolerance, body and microbial composition, and on developmental outcome in MLPT babies. We will address these aims through a factorial design clinical trial, enabling us to assess the effects of each intervention separately, whilst also exploring the effects of interactions. For the questions to be asked, this is a more appropriate and more efficient design than a multi-arm, parallel randomised controlled trial.

4. OBJECTIVES

Our overall objective is to provide robust evidence to inform feeding practices in moderate- to late-preterm (MLPT) infants that will optimise their growth, metabolic outcomes and development.

To determine the role of (i) parenteral nutrition, (ii) supplementary milk feeds and (iii) the role of taste and smell in MLPT infants whilst waiting for full enteral nutrition with mother's own milk to be established on feed tolerance, growth, body composition and development.

5. HYPOTHESIS

Because MLPT infants are relatively large (compared with extremely preterm babies) and reach full enteral feeds via nasogastric tube within 7-10 days, their early nutritional requirements are often overlooked and it is common for them to receive only 10% dextrose whilst enteral feeds are graded up and an adequate supply of expressed breast milk eventuates. This exposes MLPT infants to a period of catabolism that then may be followed by accelerated or "catch-up" growth. Our hypothesis is that this disordered pattern of growth has several consequences. First, the period of catabolism occurs at a time of extremely rapid brain growth, potentially compromising neurodevelopment. Secondly, the accelerated growth results in altered body composition, with deposition of fat rather than lean mass. Thirdly, the lack of exposure to taste and smell, with the powerful signals these senses send to the appetite regulatory centers of the brain, compounds the metabolic dysregulation MLPT infants experience. Fourthly, altered feeding patterns in the first days following birth will impact upon the microbiome, which is known to interact with the host to regulate metabolism. Fifthly, the pattern of growth, lack of sensory input accompanying meals and altered microbiome all contribute to the increased risk of developing diabetes and hypertension in adulthood.

1. Early nutrition avoiding protein deficit an altered growth pattern will lead to:
   a. Body composition at 4 months' corrected age similar to that of term-born children
   b. The same, or a trend towards improved, neurodevelopmental outcomes
2. Exposure of MLPT babies to smell and taste before each feed prior to establishment of full breast feeds will decrease time to full enteral feeds and to full sucking feeds
3. Feeding practices in MLPT babies in the period immediately following birth and before establishment of full breast feeding will affect microbiome composition and activity

6. STUDY DESIGN

Multi-site, randomised, factorial design, clinical trial. See table for conditions.

Condition	Parenteral nutrition (i)	Milk supplement (ii)	Taste/smell (iii)
1	+	+	+
2	+	-	+
3	+	+	-
4	+	-	-
5	-	+	+
6	-	-	+
7	-	+	-
8	-	-	-

Table 1: Factorial design randomisation table

7. STUDY SETTING/LOCATION

The Neonatal care unit at Auckland District Health Board and Counties Manukau Health.

8. STUDY POPULATION

Babies 32^{+0} to 35^{+6} weeks' gestation whose mothers intend to breast-feed.

9. ELIGIBILITY CRITERIA

9a. Inclusion criteria

- Babies born between 32^{+0} and 35^{+6} weeks' gestation
- Babies whose Mothers intend to breastfeed
- Babies admitted to the Neonatal Care Unit
- Babies requiring insertion of intravenous lines on admission

9b. Exclusion criteria

- Babies in whom a particular mode of nutrition is clinically indicated
- Babies admitted to the postnatal wards
- Babies with a congenital abnormality that is likely to affect growth, body composition or neurodevelopmental outcome

10. STUDY OUTCOMES

10a. Primary Outcome

For parenteral nutrition (i) and milk supplement (ii) factors: % fat mass at 4 months' corrected age when infant adiposity is predictive of childhood fat mass[16] measured by air displacement plethysmography (ADP).

For smell/taste (iii) factor time to full enteral feeds defined as 150 $ml.Kg^{-1}.day^{-1}$.

10b. Secondary Outcome(s)

- Feed tolerance assessed by time to full enteral feeds (120 $mL.Kg^{-1}.d^{-1}$);
- Time to full sucking feeds (defined as removal of nasogastric tube);
- Body composition by ADP at 10 days' postnatal age and at term;
- Growth: length, weight and head circumference Z scores and Z-score change from birth to 4 months' corrected age and at 2 years;
- Development assessed by Ages and Stages Questionnaire at 4 months

- Bayley Scales of Infant Development Edition III at 24 months of age;
- Fully breast fed rates at 4 months' corrected age;
- Nutritional intake from birth to NICU discharge;
- Gut Microbiobial composition and activity at 4 months' corrected age (determined by metatranscriptomics (10 million 400 bp reads sample^{-1}, Ion Proton™ System) and analysed by MetaPhlAn[18] and HUMAnN[19]. Inter-sample comparisons between the phenotype, treatment, microbial composition and activity will be performed using the LDA Effect Size algorithm[20] to identify biomarkers of change. The microbial impact on human buccal cell gene expression will be determined by RNA-sequencing and analysis with Cufflinks [21].)

In a subset:

- Gastric emptying assessed by ultrasound[17];
- Cerebral blood flow in response to taste and smell assessed by near infra-red spectroscopy[13];

11. STUDY PROCEDURES

11a. Recruitment of participants

Researchers will be in regular contact with antenatal unit and delivery suite to identify potential eligible babies that fit into the inclusion criteria. These eligible families will be approached either on delivery suite or antenatal unit prior to delivery (where appropriate) or when they are first admitted to the neonatal unit to gain consent. This will involve providing both verbal and written information about the Diamond trial by a member of the research team or medical team; an opportunity to ask questions or liaise with whanau members will also be provided. Formal written consent will be required prior to babies entering the study. Consented babies who are admitted to the neonatal unit and require an intravenous line will be immediately randomised to one of eight conditions (table 1). Randomisation must occur within 24 hours of birth. If parents decline consent, nutritional care will be according to the plan of the attending physician.

We require a total of 530 (265 babies per intervention). Counties Manukau Health and Auckland District Health Board each admit > 350 eligible babies per year, we therefore anticipate full recruitment to take approximately 1 year.

11b. Randomisation

Once written consent has been obtained babies will randomised into one of eight conditions (as per the following table) and stratified by gestation (32^{+0} to 33^{+6}; 34^{+0} to 35^{+6} weeks), site and sex using a computer-generated, web-based system. The Neonatal nurse specialist, neonatologist, register, charge nurse or research team will be in charge of generating the computer based randomisation at the time of admission.

Condition	Parenteral nutrition (i)	Milk supplement (ii)	Taste/smell (iii)
1	+	+	+
2	+	-	+
3	+	+	-
4	+	-	-
5	-	+	+
6	-	-	+
7	-	+	-
8	-	-	-

Table 1: Factorial design randomisation table. + means the baby receives this intervention; - means the baby does not.

Parenteral nutrition: if randomised to receive PN the baby will receive an amino acid solution (according to local hospital practice) intravenously, either by peripheral or central line as deemed appropriate. Administration of lipid is at the discretion of the clinical team, as is administration of any supplementary fluids, such as 10% dextrose. Babies not randomised to PN will receive 10% dextrose only.

Milk supplement: if randomised to receive milk supplement, the the baby will receive donor breastmilk or infant formula (according to local practice) whilst waiting for breastmilk to meet prescribed fluid amounts. Babies with a birthweight < 2 kg will receive preterm infant formula; babies with a birthweight > 2 kg will receive standard infant formula. Babies not randomised to receive milk supplement will only receive breastmilk as available.

Taste and smell: if randomised to receive taste and smell, the baby will be exposed to the taste and smell of the milk feed prior to every enteral feed. If the baby is receiving both breast milk and supplementary formula, the smell and taste will be of breast milk. If only receiving formula due to a lack of breast milk, formula will be used. The milk will be administered by placing a cotton bud in a small amount of milk and placing it under baby's nose and another cotton bud to the baby's lips immediately prior to administering the tube feed.

The goal for all babies is to transition to full feeds of expressed breastmilk as soon as possible.

Due to the nature of the study it is not possible to blind researchers or patients. Each baby will have their specific set of interventions printed out and placed on laminated, colour card and stuck to the front of the heat table or incubator so everyone is aware that they are in the trial and what interventions they should be receiving throughout their admission.

11c. Study procedure

At the time of birth; weight, length and head circumference will be measured and recorded. During admission on the Neonatal unit weight will be measured twice weekly using an infant weight scale

and recorded. Weekly length and head circumference will be measured using a neonatometer and a SECA tape respectively.

During admission participants will undergo an abdominal ultrasound at .
A near infra-red spectroscopy will be done at...

The Mother will be asked to provide a stool sample from their baby's nappy at

Body composition using air displacement plethysmography (APD) using the Peapod will be measured at 10 days postnatal age, term corrected age and at 4 months corrected gestational age when infant adiposity is predictive of childhood fat mass. Babies discharged prior to 10 days postnatal age will have an ADP measurement as close to discharge as is feasible. 4 months corrected gestational age and parents will be given taxi chits or petrol vouchers if required to ensure attendance as this is the primary objective of the study.

At 4 months corrected gestational age when families return for body composition measurements they will also be given a verbal questionnaire regarding breastfeeding, and ages and stages questionnaire will also be administered and growth measurements (weight, length and circumference) will be completed. Attendance will be encouraged through the offer of petrol vouchers and parking costs.

At 6 months corrected gestational age families will be given a phone call and the breastfeeding questionnaire completed at 4 months corrected gestation will be replicated verbally over the phone.

At 2 years of age families will be contacted and requested to attend a clinic appointment this will involve growth measurements (weight, length and head circumference) and the Bayley Scales of Infant Development Edition III, gross motor functional classification score will be completed by an experienced and trained researcher.

11d. Measurement tools used

All hardcopy data will be collected by a member of the research team and stored in a locked filing cabinet.

An individualized data collection sheet will be designed for each baby to include all relevant data relating to primary and secondary outcomes; % fat mass and % fat free mass at relevant time points, growth measurements, nutritional intake during hospital admission (including use of probiotics), time to full enteral feeds, abdominal ultrasound report, infra-red spectroscopy results and microbiome analysis.

3 questionnaires will be filed with the patient data collection form: breastfeeding questionnaire at 4 and 6 months corrected gestation and the ages and stages questionnaire completed at 4 months corrected gestation. The results of the Bayley Scales of Infant Development Edition III from 2 years corrected gestation will also be kept in the patients file.

11e. Safety considerations/Patient safety

Data forms will contain the patient's hospital sticker which has identifiable information on it e.g. name, NHI, address, phone number these will all be stored in a locked filing cabinet only accessible to researchers. The information will be stored for 10 years after which time they will be disposed of securely and appropriately.

Any adverse events related to intravenous lines ie. Intravenous line tissuing, burns or CLAB events will be recorded.

11f. Data monitoring

Trial procedures will be in accordance with the CONSORT guidelines, including the appointment of a Trial Steering Committee, an independent Data Monitoring Committee, and an independent Safety Monitoring Committee, each with appropriate terms of reference

The principle investigators, Tanith Alexander, Frank Bloomfield, research nurse and data entry clerk will be responsible for data collection, recording and quality of the data. Data will be entered into a database. Double data entry and cross validation methods will be used to ensure validity and quality of data. Security/storage of data will be by password encrypted storage with locked filing cabinets for hard copy.

12. STATISTICAL CONSIDERATIONS AND DATA ANALYSIS

12a. Sample size and statistical power

Unlike multi-arm, parallel RCT or comparative experiments, factorial experiments are designed to estimate main effects and their interactions[22]. Each main effect and interaction analysis is therefore based upon the total sample size which is chosen to be large enough to detect all primary outcomes[22]; having more factors does not increase total sample size[22]. We have based our estimate on 90% power, overall type 1 error rate of 5%, alpha per main effect = 0.0167; estimated 10-15% loss to follow-up managed by multiple imputation. For interventions (i) and (ii): to detect a minimal clinically significant difference in % fat mass of 3% (lower 95% confidence interval) with a standard deviation of 4% and assuming a distance of 1% or more from the mean difference between the two groups with and without the intervention of interest requires 140 babies in the intervention and 140 babies without the intervention. The expected effect size is based on an estimated 3% increase in % fat mass in moderate to late preterm infants compared to term infants[6] and an estimated 27% fat mass in term infants at 4 months of age[23]. There are no good data on % fat mass beyond 4 months of age; therefore, this age has been used for the primary outcome. For (iii) To decrease time to full enteral feeds from 10 to 7 days (hazard ratio 1.43), requires 265 babies in the intervention and 265 in the non-intervention arms. Sample size is therefore 2 x 265=530. This sample size also provides >80% power on a one-sided test with an alpha of 0.05 to detect a decrease in the proportion of 2 year olds surviving free from neurodisability from 80% to 70%.

Randomisation will be stratified by gestation (32^{+0} to 33^{+6}; 34^{+0} to 35^{+6} weeks), site and sex.

12b. Statistical methods

Statistical analyses will be performed on an intention-to-treat basis. Regression analysis appropriate to continuous and categorical outcomes will be used to estimate the effect of intervention, adjusting for stratification factors and important baseline confounding variables.

13. ETHICAL CONSIDERATIONS

Informed consent will be obtained by providing written information that will be easy to ready and use lay language, spoken information about the study will provide a brief summary in everyday language detailing what the study involves including follow-up requirements after discharge. Family members will be encouraged to ask questions to researchers and the consent form will only be requested to be signed if the family agree to understanding all the information provided. If the families have limited English an interpreter will be arranged and all information will be given through the hospital interpreter service. For Māori and Pacific Island families they will be asked if they would like to have a cultural support worker present at any point of the informed consent process.

An ethical issue that may arise is that in order for a baby to be randomised to the study the parents will need to provide consent for the use of formula as their baby may be randomised to a group that receives formula however the primary end goal for every baby is to transition to full feeds of expressed breastmilk as soon as possible.

Parents will be requested to provide stool samples for microbiome analysis

The health information of prospective participants will be screened by an assigned research nurse or one of the research team who will adhere to confidentiality standards as stated in their nursing registration and employment contracts.

All paper hardcopies will be stored in a locked filing cabinet for 10 years after which time the data will be securely destroyed. Hardcopy information will be scanned and kept electronically on a password protected computer that can only be accessed by members of the research team. The electronic information will be kept for 10 years after which time it will be deleted. All data collection forms will have patient labels assigned to them to ensure the correct data is collected for the correct patient they will also be assigned a unique study number after data has been recorded and electronically entered the identifiable information will be removed from hardcopy and electronic copies of the data.

14. OUTCOMES AND SIGNIFICANCE

This factorial design clinical trial will enable us to develop a package of care that will have maximum benefit and if clinically successful will not only be cost-effective and economically sustainable but also have the potential to improve long-term health outcomes.

This research has the opportunity to provide robust evidence to inform feeding practices in moderate-to late-preterm infants that will optimise their growth, metabolic outcomes and development across New Zealand and Australia.

As both preterm birth and later metabolic disease (obesity and diabetes) are more common in Māori[1,25], this research has the potential to contribute to reducing inequalities.

This research is designed to address that gap and will address several goals and priorities of the *Health and Wellbeing* and *Improving Outcomes for Acute and Chronic Conditions* investment streams by preventing later disease and contributing to improved outcomes in individuals born preterm. The interventions are inexpensive and if clinically effective will also be cost-effective and economically sustainable. This research also is aligned with the National Science Challenges A *Better Start* and *Healthier Lives*. The research team are active clinicians caring for these babies and also have national profile, serving on college and Ministry committees. They are, therefore, ideally placed to contribute to the translation of evidence into practice

15. REFERENCES

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The DIAMOND trial

DIfferent **A**pproaches to **MO**derate & late preterm **N**utrition: **D**eterminants of feed tolerance, body composition and development

Registration: Australian New Zealand Clinical Trials Registry ACTRN12616001199404

Lead Investigator: Professor Frank Bloomfield

Issue date: 21 February 2022 Version 5

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Co-Investigator (B): Dr Jane Alsweiler
Co-Investigator (C): Dr Michael Meyer
Co-Investigator (D): Dr Yannan Jiang
Co-Investigator (E): Dr Clare Wall
Co-Investigator (F): Dr Justin O'Sullivan

FUNDING

Health Research Council of New Zealand Programme grant application to Professor Frank Bloomfield
ID number: 16-605

- awarded June 2016
- term: 01/10/16-30/09/22
- $4,999,704

Title: *Feeding preterm babies for life-long health*

Counties Manukau Health Te Rangahau Puawai grant to Tanith Alexander

- Awarded November 2016
- Term: 09/01/16 – 09/01/23
- $199,173.32

CLINICAL TRIAL NETWORK ENDORSEMENT

This trial has been endorsed by the Perinatal Society of Australia and New Zealand's IMPACT Network (Interdisciplinary Maternal Perinatal Australasian Collaborative Trials Network).

STUDY SITES

Newborn Services, Auckland City Hospital, Auckland District Health Board

Neonatal Unit, Kidz First, Middlemore Hospital, Counties Manukau Health

Special care baby unit, North Shore Hospital, Waitemata District Health Board

Special care baby unit, Waitakere Hospital, Waitemata District Health Board

Neonatal Unit, Palmerston North Hospital, MidCentral District Health Board

ROLES AND RESPONSIBILITIES

Lead Investigator and Principal Investigator

Design and conduct of DIAMOND

Protocol development and revisions

Preparation of Case Report Forms

Organising steering committee meetings and management meetings

Managing locality offices

Publication of study reports

Liaison with Data and Safety Monitoring Committees

Steering Group Committee

A Trial Steering Committee will be formed to supervise the conduct of the study and will meet bi-monthly. The terms of reference will be agreed at the first meeting (before the trial begins). Trial procedures will be in accordance with the CONSORT guidelines (Moher et al., 2012; Schulz, Altman, & Moher, 2010).

Members: Tanith Alexander, Professor Frank Bloomfield, Professor Jane Harding, Dr Jane Alsweiler, Dr Michael Meyer, Dr Yannan Jiang, Dr Clare Wall

Provide academic oversight by advising on study design and protocols and approving major changes as needed

Provide ethics oversight by ensuring all required approvals are in place and up to date, all requirements are complied with, and reports provided as required.

Oversee and approve all student projects related to the project (e.g. scope, possible overlaps, etc.) in accordance with the agreed student policy

Put in place and maintain a data management framework

Oversee data ownership and publications in accordance with data access agreements and agreed authorship policy

Oversee funding arrangements for the study, and support funding applications as required

Management Committee

A subcommittee of the steering committee will be formed to monitor the trial on a bi-monthly basis.

Study planning
Organisation of steering committee meetings
Provide annual reports to ethics committee and HRC
Budget administration and contractual issues with individual centres
Advice for lead investigators
Data verification
Management of research staff workforce

Data Management

Building of data management database
Maintenance of IT system and data entry
Data verification

Data Monitoring Committee

Report to Chair of the Trial Steering Committee
Monitoring recruitment, aggregate safety data and trial conduct
Provide Steering Committee with recommendations regarding study modification, continuation or termination
Monitor protocol adherence and patient withdrawal
Safeguard the interests of the study participants

Safety Monitoring Committee

Report to the Chair of the Trial Steering Committee
Monitor human subject safety
Monitor adverse and serious adverse events
Provide expert advice on likelihood of an adverse event being related to a study intervention

This study protocol follows the SPIRIT checklist (Chan, Tetzlaff, Altman, et al., 2013; Chan, Tetzlaff, Gøtzsche, et al., 2013).

Signed: 

Date: 02/03/2022

Professor Frank Bloomfield
Lead Investigator

Signed: 

Date: 01/03/2022

Tanith Alexander
Principal Investigator

Protocol Revision History

The DIAMOND Trial protocol:

Bloomfield FH, Harding JE, Meyer MP, Alsweiler JM, Jiang Y, Wall CR, Alexander T on behalf of the DIAMOND Study Group. The DIAMOND trial – Different Approaches to Moderate & Late Preterm Nutrition: Determinants of feed tolerance, body composition and development. Protocol of a randomised trial. BMC Pediatrics 2018 Jul;18:220. DOI: https://doi.org/10.1186/s12887-018-1195-7

Protocol Version	**Date**	**Sections affected**	**Rationale**
Version 3	22 October 2017	Published version.	Not applicable.
Version 4 (Amendment 01)	24 September 2018	A. Study Sites	Addition of two further recruiting centres.
		B. Section 2.1.1	Addition of Waitemata District Health Board
		C. Section: 2.1.3. Revision. Last paragraph beginning "The goal for..." Page 15.	Revised wording for clarity.
		D. Removal of 'Gastric Emptying'. All sections and statements.	Test not done.
		E. Participant Timeline. Page 16.	Revised as per section F
		F. Section: 2.3.1 Revisions: ○ Page 18: 'Taste and Smell' ○ Page 19: 'Body Composition' ○ Page 20: Cerebral blood flow: 'Sensory Stimulation' ○ Page 21: 'Saliva analysis' ○ Page 21 'Microbiome' ○ Page 21: 'Breast-milk analysis'	Revised for clarity, revised sample collection time-points and to add previously omitted detail regarding sensory stimulation.
Version 5 (Amendment 02)	14 February 2022	A. Study Sites	Addition of a further recruiting centre
		B. Abstract: Methods/design	Revised as per section D and F
		C. Section 2.1.1 and 3.2	Addition of MidCentral District Health Board
		D. Removal of 'Saliva analysis' and 'Maternal body composition'. All sections and statements.	Tests not done
		E. Participant Timeline. Page 16	Revised as per section D and to add previously omitted skinfold measurement
		F. Section: 2.3.1 Revisions: ○ Page 19: 'Body Composition' ○ Page 19: 'Monitoring of Nutritional Intake' ○ Page 21: Breast milk analysis	Revised for correction: P 19: due to inability to calculate nutritional intake accurately once there is significant breastfeeding

			p21: change of breast milk analysis method

ABSTRACT

Background

Babies born at moderate-late preterm gestations account for >80% of all preterm births. Although survival is excellent, these babies are at increased risk of adverse neurodevelopmental outcomes. They also are at increased risk of adverse long-term health outcomes, such as cardiovascular disease, obesity and diabetes. There is little evidence guiding optimal nutritional practices in these babies; practice, therefore, varies widely. Areas of uncertainty include whether to provide parenteral nutrition, whether to provide milk supplements until sufficient mother's milk is available and how to improve tolerance of feeds. All of these factors may impact upon growth, gut microbiome, development of adiposity and later metabolic health. It also is unknown whether preterm girls and boys should be fed differently; currently, until breast-feeding is established, girls and boys are fed the same despite the fact that they grow differently. It is not known whether breast milk composition differs according to the sex of the baby. This factorial design clinical trial will address the role of parenteral nutrition, milk supplementation and exposure of the preterm infant to taste and smell with each feed on the time to tolerance of full feeds, adiposity, gut microbiome population and activity, and neurodevelopment at 2 years. It also will investigate whether breast milk composition differs according to sex of the baby.

Methods/design

Design: Multi-site, randomised, factorial design, clinical trial.

Inclusion criteria: Babies born between 32^{+0} and 35^{+6} weeks' gestation, whose mothers intend to breastfeed and who are admitted to the Neonatal Care Unit, who have insertion of intravenous lines based on clinical need and whose parents / caregivers have given written informed consent.

Exclusion criteria: Babies in whom a particular mode of nutrition is clinically indicated; babies with a known chromosomal or genetic abnormality, or congenital disorder affecting growth, body composition or neurodevelopmental outcome.

Trial entry and randomisation: Parents will be given a written information sheet about the study antenatally (where possible), which will be reviewed with them by a member of the study team. If birth occurs without time for this to happen, information will be given to the parents as soon as is feasible after birth and reviewed with them by a member of the study team. Within 24 hours of birth, eligible babies of consenting parents will be randomised to one of eight conditions. Randomisation will be stratified by gestation, recruitment centre and sex in balanced blocks of variable size. Twins and Triplets will be randomised as separate babies.

Study groups and blinding: The factorial design will explore three factors (i) parenteral nutrition, (ii) milk supplements and (iii) taste/smell. Babies will be randomised to one of eight conditions to receive either an intravenous nutrition amino acid solution or dextrose, supplemental milk whilst waiting for expressed breastmilk feeds or exclusively breastmilk, and taste / smell given prior to gastric tube feeds or no taste / smell prior to gastric tube feeds. Due to the nature of the trial it is not possible to blind the babies' families, clinicians or researchers.

Primary study outcome: For parenteral nutrition (i) and milk supplement (ii) factors: body composition assessment at 4 months' corrected age when infant adiposity is predictive of childhood fat mass. For smell/taste (iii) factor time to full enteral feeds defined as 150 $ml.Kg^{-1}.day^{-1}$ or exclusive breastfeeding.

Secondary outcomes: Days to full sucking feeds (defined as removal of nasogastric tube); days in hospital; body composition before discharge; growth: length, weight and head circumference Z scores and Z-score change from birth to 4 months' corrected age and at 2 years; development assessed by Bayley Scales of Infant Development

Edition III at 24 months' corrected age; fully breast fed rates at 4 months' corrected age; nutritional intake in the first two weeks after birth; gut microbial composition and activity at 10 days of age and 4 months' corrected age; maternal milk composition during the first week after birth and at 4 months' corrected age. In a subset, cerebral blood flow in response to taste and smell assessed by near infra-red spectroscopy. (Revised per Amendment 01 and 02)

Sample size: A total of 480 babies (n=240 per intervention arm) will provide ≥90% power at an overall type 1 error rate of 5% to detect a minimal clinically significant difference in % fat mass at 4 months' correct age of 3% (lower 95% confidence interval) for parental nutrition and milk supplement interventions, or to detect a reduction in median time to full enteral feeds from 10 to 7 days (hazard ratio 1.43) with smell/taste intervention. Allowing for 10% loss to follow up, we aim to recruit 528 babies (n = 66 per randomised condition).

Statistical analysis: Treatment evaluation will be performed on the principle of intention-to-treat, adjusting for gestation, recruitment centre and sex (stratification factors), and for the non-independence of multiple births. For the primary outcomes, % fat mass at 4 months' correct age will be analysed using generalised linear regression with the model-adjusted mean difference tested between groups. Time to full enteral feeds will be analysed using Cox proportional hazards model. A full statistical analysis plan will be developed to guide final data analysis.

Discussion

This multi-centre, factorial design clinical trial aims to assess different feeding strategies of moderate to late preterm infants and the effects on body and microbial composition, feed tolerance and neurodevelopmental outcome. Until data from large, well-designed randomised trials are available to assess the effects of current feeding strategies on outcomes it is difficult to develop and recommend evidence-based nutrition guidelines. A conclusive trial outcome will provide the first direct evidence to inform feeding practices in moderate- to late-preterm infants that will optimise their growth, metabolic outcomes and development across New Zealand, Australia and Internationally.

This factorial design clinical trial will enable us to develop a package of care that will have maximum benefit and if clinically successful will not only be cost-effective and economically sustainable but also have the potential to improve long-term health outcomes.

Contents

1. Introduction

1.1 Background

Of the ~5,000 babies born preterm in NZ each year (Ministry-of-Health, 2014), >80% are born at moderate- to late-preterm (MLPT) gestations between 32^{+0} and 36 completed weeks' (Ministry-of-Health, 2014) and these babies constitute a much larger proportion of the health care burden related to prematurity than do extremely preterm babies (Petrou & Khan, 2012). Although survival of MLPT babies is excellent, they are at risk of impaired later development. MLPT babies have a 36% increased risk for developmental delay or disability at pre-school ages and a 50% increased risk of special education needs at school (MacKay, Smith, Dobbie, & Pell, 2010). Because there are so many more MLPT babies born than extremely preterm babies, they account for almost 10 times as many children with neurodisability than do extremely preterm babies (Blencowe et al., 2013). MLPT birth also carries increased risk of adverse long-term health outcomes, including obesity, hypertension and diabetes, even by the 3rd and 4th decades of life (Crump, Winkleby, Sundquist, & Sundquist, 2011b, 2011a). This metabolic risk is substantially related to increased adiposity. Late preterm babies demonstrate a 182% increase in fat mass between birth and term-corrected age, by which time they have ~50% greater percentage body fat than term-born controls (Johnson, Wootton, Leaf, & Jackson, 2012). This appears to be due to preserved development of fat mass but impaired accretion of lean mass, indicative of inadequate protein intake between birth and term-corrected age (Johnson et al., 2012).

There is good reason to believe that nutritional practices in early life impact upon later metabolic health. A period of relative undernutrition will be accompanied by faltering growth and an accumulating negative nitrogen balance (Corpeleijn, Kouwenhoven, & van Goudoever, 2013), which is followed by later accelerated growth when nutrition is restored. Enhanced growth in infancy may protect the infant from cognitive impairment but also is linked to childhood adiposity, persisting through adulthood (Belfort, Gillman, Buka, Casey, & McCormick, 2013). Different nutritional practices will affect the gut microbiome which also is associated with later adiposity (Graham, Mullen, & Whelan, 2015). Data such as these have led to the suggestion that there may be a trade-off in preterm babies whereby providing enhanced nutrition to prevent postnatal growth faltering results in better brain growth and cognitive outcomes, but accelerates weight gain and alters the microbiome, thus increasing the risk of later metabolic and cardiovascular disease.

In MLPT babies, there is inevitably a delay between birth and the establishment of full enteral feeds. Preterm babies have immature suck / swallow / breathe coordination and immature gut motility, so it can be days to weeks before they are able to tolerate full feeds without gastric residuals or vomiting. Supply of breast milk is also often delayed, as their mothers may have difficulty expressing due to the underlying condition leading to preterm birth. Further, hepatic glycogen stores double between 36 and 40 weeks' gestation, so MLPT babies cannot rely on this stored energy source. During these first days to weeks, almost all preterm babies experience a degree of protein undernutrition and incur a nitrogen (protein) deficit that is then very difficult to overcome. Thus, optimal nutritional support during this critical period may substantially improve later metabolic and developmental outcomes.

Practices around early nutritional support for MLPT babies vary widely as there is little high-quality evidence. Usual practice is to provide intravenous fluids while gradually increasing the volumes of milk given by gastric tube until full enteral feeds are tolerated, and then transitioning to sucking feeds as suck / swallow / breathe coordination matures. However, there are many variations within this general approach. There are no data on whether it is better to start supplemental milk early, either formula or donor milk (if available, and which is of lower nutritional value than fresh breast milk or formula) or to wait until the mother's breast milk is available. While waiting for mother's milk or for feeds to be tolerated, there are no data on whether provision of

intravenous dextrose solution alone is sufficient, despite the inevitable catabolism and accumulating nitrogen deficit, and the provision of nutrition that does not have a balanced energy: protein ratio, or whether babies should receive parenteral nutrition containing protein in an attempt to prevent catabolism. All of these approaches are in use in New Zealand. In the two tertiary neonatal units in Auckland, one routinely commences infant formula, the other 10% dextrose. Parenteral nutrition use is dependent upon physician preference. A study of nutritional support of 33-35 week gestation late-preterm infants in 10 California and Massachusetts hospitals similarly found the rate of intravenous nutrition use varied from 5 – 66% and the rate of discharge with an enriched formula varied from 5 – 71% (McCormick, Escobar, Zheng, & Richardson, 2006). Taste and smell may also be important in food tolerance. Even before ingestion of food, taste and smell initiate metabolic processes through secretion of hormones such as insulin and ghrelin (Teff, 2011). However, the role these senses play is not usually considered in the care of preterm infants despite preterm infants having functional taste receptors from 18 weeks' gestation and flavour perception from around 24 weeks' gestation (Lipchock, Reed, & Mennella, 2011). Changes in brain tissue oxygenation have been detected by near-infrared spectroscopy (NIRS) in preterm infants >32 weeks' gestation in response to odours, with differential responses to odours rated as pleasant or unpleasant (Bartocci et al., 2000). Taste receptors in the mouth relay a signal to the brainstem and higher centres through the gustatory nerve fibres, leading to activation of the cephalic phase response and the release of appetite hormones in saliva (Zolotukhin, 2013). These salivary hormones are postulated to play a role in metabolism (Zolotukhin, 2013); indeed, impaired oral nutrient sensing is associated with increased energy intake and a greater body mass index (Stewart et al., 2010).

We have conducted a pilot trial of exposing very preterm infants to the taste and smell of milk before each tube-feed by placing a cotton wool bud soaked in milk against lips and in front of the infant's nose. Infants in the control group were not exposed to the taste and smell of milk. Infants in the intervention group tolerated enteral feeds earlier than infants in the control group, reached full enteral feeds at an earlier gestational age (median (interquartile range (IQR)) 29.0 (27.7-30.0) vs 29.9 (29.3-30.4) weeks, ($P<0.05$) and tended to have the nasogastric tube removed at an earlier gestational age, perhaps indicating earlier attainment of full sucking feeds (Beker, Opie, Noble, Jiang, & Bloomfield, 2017). These data suggest that the simple intervention of providing taste and smell stimuli before gastric tube feeds may enhance feed tolerance. It is not known if exposing the baby to taste and smell before each gastric tube feed, with the consequent secretion of metabolic hormones, will improve feed tolerance and reduce time to full sucking feeds in MLPT babies.

Although it has been recognised for centuries that girls and boys grow differently, experience different metabolic and endocrine milieux, and have different cognitive and health outcomes, little attention has been paid to the potential to improve outcomes following preterm birth by treating girls and boys differently. This is despite that fact that it is well recognised that preterm boys have higher mortality and morbidity (Glass et al., 2015), and are more likely to have adverse developmental and educational outcomes, than preterm girls. It also is well recognised that perinatal insults can result in different adult phenotypes in males and females. For example, in animal studies across many different species after a wide variety of prenatal insults, males are more likely than females to exhibit adverse effects in later life such as impaired renal function, hypertension, insulin resistance, altered HPA axis function and altered growth (Aiken & Ozanne, 2013). The reasons for this sex difference in vulnerability to early environmental perturbations are not well understood, but may include faster growth and hence greater substrate demands in males, altered tempo of maturation, different exposure to sex steroids and sex-specific epigenetic mechanisms (Aiken & Ozanne, 2013). Unfortunately, most clinical studies have not reported findings separately by sex and are not adequately powered to do so. Further, because the majority of animal experiments are done in polytocous species, prenatal and postnatal sex effects cannot be separated in

mixed-sex litters. However, we recently have shown in term newborn lambs that nutritional supplementation for just two weeks leads to increased β-cell mass in males, but not in females (Jaquiery et al., 2016).

If there are important differences in early nutrient requirements between girls and boys, then one might expect the composition of breast milk to differ accordingly (biological plausibility). The composition of preterm breast milk is different from that of term breast milk, particularly in the first few days after birth (Gidrewicz & Fenton, 2014), with protein content of colostrum up to 35% higher. However, after the first week, differences are minimal (Gidrewicz & Fenton, 2014). Breast milk composition is also highly variable, even within individuals (de Halleux & Rigo, 2013), and can be impacted by maternal factors such as body composition (Kugananthan et al., 2017; Panagos et al., 2016). Breastmilk also contains volatile components (measured in the metabolome) (Cesare Marincola, Dessi, Corbu, Reali, & Fanos, 2015; Marincola et al., 2012) that stimulate the olfactory system and may contribute to tolerance, digestion, appetite regulation and satiety with breast-feeding in a similar way that components of saliva do (Zolotukhin, 2013).

There is no consensus on whether breast milk composition varies according to the sex of the offspring (Stam, Sauer, & Boehm, 2013). Richer milk for boys compared with girls has been found in economically sufficient Kenyan women (Fujita et al., 2012) and in a study in Massachusetts (Powe, Knott, & Conklin-Brittain, 2010). However, sample sizes were small and in the Kenyan study the opposite was found in poorer women, with richer milk for girls (Fujita et al., 2012). A study in Filipino women did not find any difference in macronutrients according to sex of the offspring (Quinn, 2013). There are also reports in the animal literature that milk composition differs according to the sex of the offspring (Gallego et al., 2009; Hinde, 2009). However, there are no data on sex differences in preterm breast milk. In a recent pilot study, we found higher concentrations of the stress hormones cortisol and cortisone in breast milk expressed for preterm boys than for girls, and these differences persisted beyond hospital discharge, suggesting altered maternal hormonal regulation of breast milk (Pundir, S., Thorstensen, E. B., Linderborg, K. M., Lagström, H., Fraser, K., Wall, C. R., & Cameron-Smith, 2016).

Because MLPT infants are relatively large (compared with extremely preterm babies) and reach full enteral feeds via nasogastric tube within 7-10 days, their early nutritional requirements are often overlooked and it is common for them to receive only intravenous dextrose solution whilst enteral feeds are graded up and an adequate supply of expressed breast milk eventuates. This exposes MLPT infants to a period of catabolism that then may be followed by accelerated or “catch-up” growth. Our hypothesis is that this disordered pattern of growth has several consequences. First, the period of catabolism occurs at a time of extremely rapid brain growth, potentially compromising neurodevelopment. Secondly, the accelerated growth results in altered body composition, with deposition of fat rather than lean mass. Thirdly, the lack of exposure to taste and smell, with the powerful signals these senses send to the appetite regulatory centres of the brain, compounds the metabolic dysregulation MLPT infants experience and delays the establishment of full sucking feeds. Fourthly, altered feeding patterns in the first days following birth will impact upon the microbiome, which is known to interact with the host to regulate metabolism. Fifthly, the pattern of growth, lack of sensory input accompanying meals and altered microbiome all contribute to the increased risk of developing diabetes and hypertension in adulthood.

1.2 Hypothesis

1. Early nutrition supplementation including protein will prevent a protein deficit leading to
   a. Body composition at 4 months' corrected age similar to that of term-born children, and
   b. Improved neurodevelopmental outcomes
2. Exposure of MLPT babies to taste and smell before each feed prior to establishment of full breast feeds will decrease time to full enteral feeds and to full sucking feeds
3. Feeding practices in MLPT babies in the period immediately following birth and before establishment of full breast feeding will affect microbiome composition and activity
4. Breast-milk composition will vary by sex of the infant.

Aims

Our aims are to investigate the impact of different feeding strategies currently in use in New Zealand on feed tolerance, body and microbial composition, and on developmental outcome in MLPT babies. We will address these aims through a factorial design clinical trial, enabling us to assess the effects of each intervention separately, whilst also exploring the effects of interactions.

Objectives

Our overall objective is to provide robust evidence to inform feeding practices in moderate- to late-preterm (MLPT) infants that will optimise their growth, metabolic outcomes and development.

To determine the role of (i) parenteral nutrition, (ii) supplementary milk feeds and (iii) the role of taste and smell in MLPT infants whilst waiting for full enteral nutrition with mother's own milk to be established on feed tolerance, growth, body composition and development.

1.3 Study Design

Multi-site, randomised, factorial design, clinical trial.

2. Methods

2.1 Participants, Interventions and Outcomes

2.1.1 Study setting

The neonatal intensive care units at Auckland District Health Board, Counties Manukau Health, Waitemata District Health Board (Added as per Amendment 01) and MidCentral District Health Board (Added as per Amendment 02).

2.1.2 Eligibility criteria

Inclusion criteria

- Born between 32^{+0} and 35^{+6} weeks' gestation
- Mother intends to breast-feed
- Admitted to the neonatal care unit or special care baby unit
- Requires insertion of intravenous line for clinical reasons

Exclusion criteria

- A particular mode of nutrition is clinically indicated
- A congenital abnormality that is likely to affect growth, body composition or neurodevelopmental outcome

2.1.3 Interventions

(i) Parenteral nutrition vs intravenous dextrose solution;

(ii) Supplemental milk (donor breast milk if available, else infant formula) vs only mother's own milk as available;

(iii) Infant exposed to taste and smell of milk prior to every tube feed vs no exposure (milk administered only via gastric feeding tube).

All babies will receive nutrition according to individual neonatal intensive care unit practices. The first two interventions only apply until the baby is established on full enteral feeds with mothers' own milk, which remains the primary nutritional goal. Babies randomised to receive taste and smell prior to tube feeds will continue to receive this intervention until the baby is no longer receiving any gastric tube feeds.

Parenteral nutrition: If randomised to receive parenteral nutrition the baby will receive an amino acid solution (according to local hospital practice) intravenously, either by peripheral or central line as deemed appropriate. Administration of lipid is at the discretion of the clinical team, as is administration of any supplementary fluids, such as 10% dextrose. Babies not randomised to parenteral nutrition will receive dextrose solution with electrolytes as clinically indicated but no protein or lipid. The randomised intravenous fluid will be continued until full enteral feeding is established.

Supplemental milk: If randomised to receive milk supplement, the baby will receive donor breastmilk or infant formula (according to local practice) whilst waiting for mother's breastmilk to meet prescribed fluid amounts. If formula is used, babies with a birthweight < 2 Kg will receive preterm infant formula; babies with a birthweight > 2 Kg will receive standard infant formula. Babies not randomised to receive milk supplement will only receive mother's breastmilk as available.

Taste and smell: If randomised to receive taste and smell, the baby will be exposed to the taste and smell of the milk feed prior to every enteral feed. If the baby is receiving both breast milk and supplementary formula, the taste and smell will be of breast milk if available, but if there is insufficient breastmilk, then taste and smell can be of formula. However, if the baby is randomised to not receive supplementary infant formula then taste and smell can only be provided with breastmilk and taste should be given in preference to smell. To administer taste, give 0.2 mL of milk from a syringe directly to the tip of the baby's tongue. To administer smell, apply 0.1 – 0.5 mL of milk to a piece of gauze or cotton swab and place by the baby's nose. This will remain in place until completion of the enteral feed. Both taste and smell should be given immediately prior to administering the tube feed.

Gastric emptying: (Removed. Amendment 01).

The goal for all babies enrolled in the study is to transition to full feeds of expressed breastmilk as soon as possible. If at any time the responsible clinician feels that any of the randomised interventions is no longer appropriate, they may deviate from the allocated intervention for clinical reasons. They will be encouraged to discuss this with a member of the trial Lead Investigator, Principal Investigator or another member of Steering Group before making the decision. The baby will be considered to have a protocol deviation and remain in the allocated condition group for the purposes of analysis (intention-to-treat principle). (Revised per Amendment 01).

2.1.4 Outcomes

Primary Outcomes

For parenteral nutrition (i) and supplemental milk (ii) factors: body composition assessment at 4 months' corrected age when infant adiposity is predictive of childhood fat mass (Gishti et al., 2014) measured by air displacement plethysmography (ADP) or skin-fold thickness measurements. For smell/taste (iii) factor, time to full enteral feeds defined as 150 ml.Kg^{-1}.day^{-1} or exclusive breastfeeding if this occurs prior to enteral feeds of 150 ml.Kg^{-1}.day^{-1} being reached.

Secondary Outcomes

- Time to full sucking feeds (defined as removal of nasogastric tube with no enteral feed top ups given) (Revised as per Amendment 02).;
- Number of days in hospital;
- Body composition measurement as close to discharge as feasible;
- Growth: length, weight and head circumference Z scores and Z-score change from birth to 4 months' corrected age and at 2 years;
- Neurodevelopmental disability and its components at 24 months' corrected age;
- Fully breastfeeding rates at 4 and 6 months' corrected age;
- Nutritional intake for the first two weeks after birth (Revised as per Amendment 02);
- Gut microbiome composition and activity at 10 days of age and 4 months' corrected age;
- Breastmilk composition in the first 10 days after birth and at 4 months' corrected age;
- Hormone saliva concentrations (Removed as per Amendment 02)
- Any confirmed central line associated blood stream infection (CLABSI)
- Late onset sepsis

In a subset:

- Gastric emptying (Removed as per Amendment 01).
- Cerebral blood flow in response to taste and smell assessed by near infra-red spectroscopy (Bartocci et al., 2000)

2.1.5 Participant timeline (Revised as per Amendment 01 and 02)

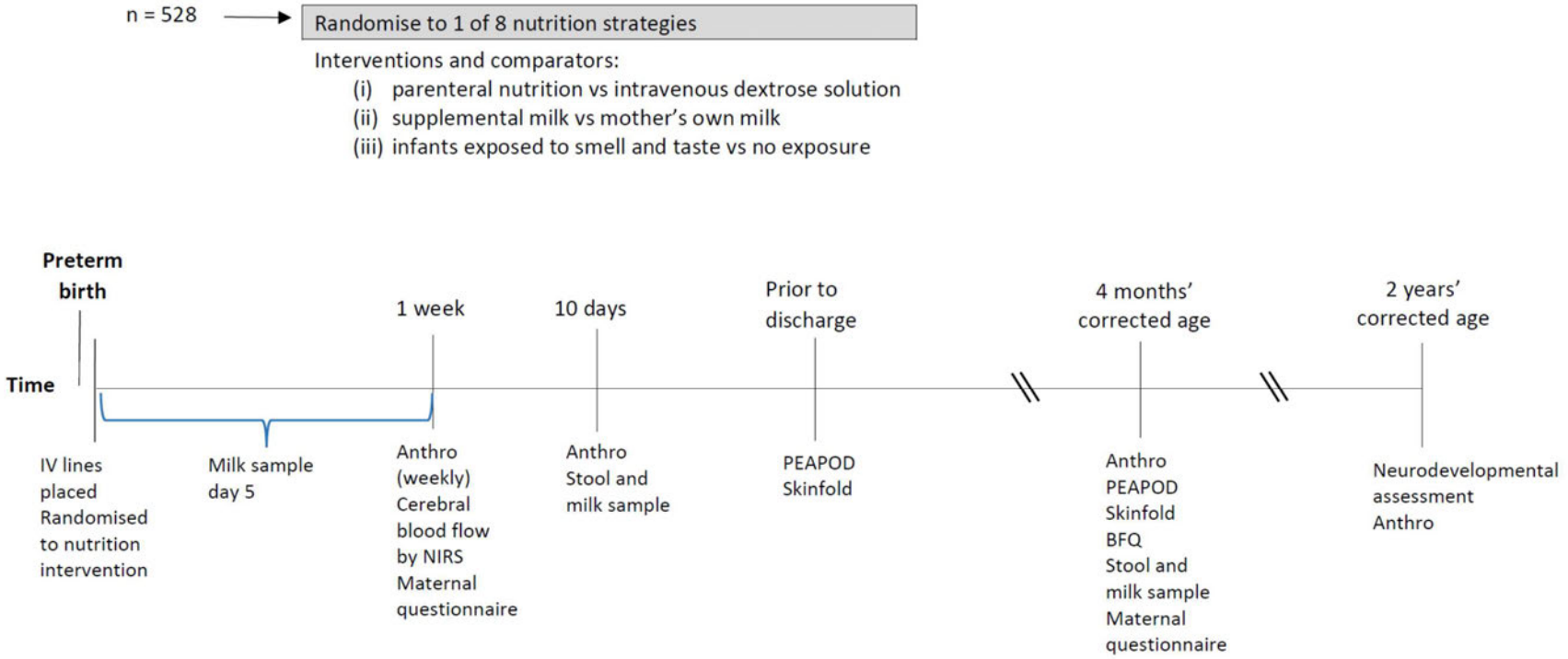

Figure 1: Flow chart of the DIAMOND study from birth to 2 years' corrected age.
Anthro = Anthropometry, BFQ = Breastfeeding questionnaire, PEAPOD = air displacement plethysmography, NIRS = Near-infrared spectroscopy.

2.1.6 Sample size

Unlike multi-arm, parallel RCT or comparative experiments, factorial experiments are designed to estimate main effects and their interactions (Collins, Dziak, Kugler, & Trail, 2014). Each main effect and interaction analysis is, therefore, based upon the total sample size which is chosen to be large enough to detect all primary outcomes (Collins et al., 2014); having more factors does not increase total sample size (Collins et al., 2014). A total of 480 babies (n=240 per intervention arm) will provide ≥90% power at an overall type 1 error rate of 5% to detect a minimal clinically significant difference in % fat mass at 4 months' correct age of 3% (lower 95% confidence interval) for parental nutrition and milk supplement interventions, or to detect a reduction in median time to full enteral feeds from 10 to 7 days (hazard ratio 1.43) with smell/taste intervention. This sample size has assumed a standard deviation of 4% in % fat mass, with Bonferroni corrections to each of the three tests (i.e. alpha per main intervention effect = 0.0167). Allowing for 10% loss to follow up, we aim to recruit 528 babies (n = 66 per randomised condition). The expected effect size is based on an estimated 3% increase in % fat mass in moderate to late preterm infants compared to term infants (Johnson et al., 2012) and an estimated 27% fat mass in term infants at 4 months of age (Gianni et al., 2014). There are no good data on % fat mass beyond 4 months of age; therefore, this age has been used for the primary outcome.

2.1.7 Recruitment

Parents of eligible babies will be approached by a member of the research team for recruitment antenatally where appropriate; if antenatal recruitment is not possible than families will be approached after birth upon admission to the neonatal unit. Recruitment will need to occur within 24 hours after birth for the baby to be randomised.

2.2 Assignment of Interventions

2.2.1 Allocation sequence generation

Within 24 hours of birth, once written consent is obtained, babies will be randomised into one of eight conditions (Table 1) via a secure web-based interface. Randomisation will be stratified by gestation (32^{+0} to 33^{+6}; 34^{+0} to 35^{+6} weeks), recruitment centre (each centre has different nutrition practices) and sex (this influences growth and body composition), using variable block sizes of 8 or 16. Twins and Triplets will be randomised as separate babies.

Condition	Parenteral nutrition (i)	Milk supplement (ii)	Taste/smell (iii)
1	+	+	+
2	+	-	+
3	+	+	-
4	+	-	-
5	-	+	+
6	-	-	+
7	-	+	-
8	-	-	-

Table 1: Factorial design randomisation table.

+ means the baby receives this intervention; - means the baby does not.

2.2.2 Allocation of concealment mechanism

The secure web-based interface will be maintained and concealed by an independent database controller.

2.2.3 Implementation

Members of the medical team caring for the baby or a member of the research team will enrol the participant, generate the allocation sequence from the web-based interface and assign the participants to the allocated interventions. The website allocation page will be printed and placed in the notes to ensure that the condition the patient has been randomised to receive is accessible and available to all members of the team to view.

2.2.4 Blinding

Due to the nature of the study it is not possible to blind researchers, clinical staff or families. Each baby will have their specific set of interventions printed out and placed on laminated, coloured card and stuck to the front of the heat table or incubator so everyone is aware that they are in the trial and what interventions they should be receiving throughout their admission. Researchers involved in the follow-up assessments at 4 and 6 months' corrected and at 2 years' corrected age will be blinded to the allocation that the infant received during their admission.

2.3 Data Collection, Management and Analysis

2.3.1 Data collection methods

Taste and smell

Taste and smell data will be collected based on the total number of times taste and smell intervention was administered when a full tube was given (purple sticker) over the total number of full tube feeds with no oral component. Tube feeds where an oral feed (bottle or breast) was attempted prior to a tube feed will not be counted in the totals as the baby will be receiving a taste and smell naturally themselves. In the first few days

of life when there is limited breastmilk and milk is given orally (i.e. 0.3 mL) this will be recorded as a smell/taste intervention even if there was no enteral feed administered due to lack of milk volume available. (Added as per Amendment 01).

Body composition

Body composition will be measured at 4 months' corrected age when infant adiposity is predictive of childhood fat mass (Gishti et al., 2014). Measurement using air displacement plethysmography (APD) system PEA POD, will occur as close to discharge as is feasible and at 4 months' corrected age as the preferred method for determining body composition. Subscapular, triceps, biceps, thigh and suprailiac skinfold thickness (mm) will also be measured in duplicate by trained personnel at discharge and 4 months' corrected age using standardised skinfold calipers. If the difference between measures is >0.4 mm, a third measurement will be taken. (Revised as per Amendment 01 and 02).

Anthropometry

Weight, length and head circumference will be measured at birth and every week up till discharge, and at 4 months' corrected age. Crown-heel length will be measured with a neonatometer or a length board. Babies will be weighed naked using electronic scales accurate to ± 10 g. Head circumference will be measured using a non-stretch tape measure (Shaw & McCarthy, 2007). All staff taking anthropometric measurements will be trained to ensure standardisation and accuracy. All growth data Z-scores will be calculated individually for each baby using Fenton 2013 normative data (Fenton & Kim, 2013; Fenton et al., 2013), transitioning to WHO growth standards at 50 weeks' post-conceptional age (de Onis, Garza, Onyango, & Martorell, 2006).

At the 4 months follow up appointment maternal weight will be measured using a standard clinical scale and standing height will be measured by stadiometer. (Revised as per Amendment 02)

Monitoring of nutritional intake

Total enteral and intravenous intakes will be recorded daily until discharge for maximum of 28 days or until baby begins receiving breastfeeds with less than full tube feed top-ups as the quantity of breastmilk received cannot be quantified. Mean daily protein and energy intakes will be calculated based on actual intakes. Full enteral feeds will be defined as 150 $mL.Kg^{-1}.d^{-1}$. Energy and protein intakes will be calculated using mature breastmilk composition (72 kcal and 1.2 g protein/100 ml). For all reporting of neonatal nutrition and growth outcomes we will use the StRoNNG checklist (Cormack et al., 2016). Time to full sucking feeds will be defined as removal of the nasogastric tube, when we assume that given the opportunity to be with their mothers 24 hours a day the baby would exclusively breastfeed. Breastfeeds in the Neonatal unit are coded by the nurse and mother and indicate the quality and quantity of the breastfeed. (Revised as per Amendment 02)

Questionnaires

At 4 months' corrected age when families return for body composition measurements there will be an interviewer administered questionnaire regarding breastfeeding. Maternal factors influencing breastmilk composition will be assessed using validated questionnaires. These include maternal stress, depression, home stability and sleep quality using the Perceived Stress Scale (PSS) Edinburgh Postnatal Depression Scale (EPDS) questionnaires. These will be administered at the 4 months' corrected age visit as well as prior to hospital

discharge (between day 7-10) (Cohen, Kamarck, & Mermelstein, 1983; Cox, Holden, & Sagovsky, 1987). Travel and parking costs will be reimbursed. At 6 months' corrected age families will receive a phone call and the breastfeeding questionnaire will be administered again over the phone.

Gastric emptying (Removed as per Amendment 01).

Cerebral blood flow

Near-infrared spectroscopy (NIRS) is a bedside, non-invasive methodology to detect concentration changes of natural chromophores like oxygenated Hb [Hb O_2] and deoxygenated Hb [Hb H] during cortical activation (Bartocci et al., 2000). Newborn infants, including preterm infants, demonstrate changes in cerebral oxygenation in response to odours, with increased orbito-frontal oxygenation in response to pleasant odours (Bartocci et al., 2000) and decreased oxygenation in response to unpleasant odours (Bartocci et al., 2001). Oxygenation increases to a greater degree in response to the odour of breast-milk than to formula (Aoyama et al., 2010), indicating that neonates can distinguish these different milks by smell and suggesting that different odours may have different effects upon cerebral responses to feeding. (Revised as per Amendment 01).

A NIRO 200 (Hamamatsu Photonics, Hamamatsu, Japan) device will be used. Changes in the concentration of oxyhemoglobin [Hb O_2] and deoxyhemoglobin [Hb H] will be monitored, starting from an arbitrary zero point. The changes in the total hemoglobin ([Hb tot] = [Hb O_2] + [Hb H]), reflecting changes in cerebral blood volume (CBV) will also be calculated (Wyatt, Delpy, Cope, Wray, & Reynolds, 1986).

Smell stimulus.

After a 5 minutes period of baseline data recording, a gauze soaked with milk will be placed close to the baby's nose, moving the gauze slowly from one nostril to the other at a distance of approximately 1–2 cm. The tip of the gauze will not touch the infant's nose. Smell exposure will last 1 minute. Trigger signals will be marked on the NIRO 200 at the beginning and end of the stimulation to identify the period of exposure to smell. Recording of NIRS data will continue until 1 minute after the end of the exposure. (Revised as per Amendment 01).

Taste stimulation

After the exposure to smell, 1 minute of resting period will be recorded between exposures. Then, a syringe containing 0.2-0.5 ml of milk will be placed on the tip of baby's tongue or opening of the mouth. Taste exposure will last 1 minute. Trigger signals will be marked on the NIRO 200 at the beginning and end of the stimulation to identify the period of exposure to taste. Recording of NIRS data will continue until 1 minute after the end of the exposure. Tube feeding should start 1 minute after taste stimulation has finished and trigger signals will be marked on the NIRO 200 at the beginning and end of the feed. (Added as per Amendment 01).

Control group

After a 9 minute period of baseline data recording, trigger signals will be marked on the NIRO 200 at the beginning of tube feed to identify the period of assessment. The cerebral monitoring will go on for 9 minutes before the feeding starts and throughout the feeding. (Added as per Amendment 01).

Saliva analysis (Removed as per Amendment 02)

Microbiome

We will collect stool samples from all babies enrolled in the DIAMOND trial at 10 days of age, when the majority of babies should be established on enteral nutrition, and again at four months' post-conceptional age. Two stool samples will be collected from the same dirty nappy. The first sample we be collected using a sterile tube, the second sample will be collected in a sterile tube with RNAlater. Both samples will be immediately placed in the freezer (-20^{o} C) and transported to the Liggins Institute laboratory within 5 days after collection after collection on cold chain transport. DNA and RNA will be extracted upon reaching the Liggins laboratory using an AllPrep DNA/RNA mini kit (Qiagen) and stored at - 80^{o} C. A metatranscriptomics analysis (Jayasinghe TN Chiavaroli V Derraik JG Hofman PL O'Sullivan JM Cutfield W & editors, 2015) will be undertaken to identify microbial markers of the different treatment regimens. Samples from 10 randomly chosen babies of each sex from each of 4 out of the 8 conditions (see table 1), based upon differences in the primary outcome (fat mass) at 4 months of age. RNA samples will be sequenced on an Illumina platform. Comparisons between the treatment groups will be undertaken using the LDA Effect Size algorithm (Segata et al., 2011) to identify the microbial species and pathways that are characteristically identified and hence are biomarkers of the treatment regimen. Inter-sample comparisons between the phenotype, treatment, microbial composition and microbial activity metadata will be performed using MaAsLin (Weingart, 2014) to identify statistically significant correlations that suggest interactions between these variables. We will store the remaining stool samples for later analysis should this be indicated by our findings and should funding be available. (Revised as per Amendment 01).

Breast-milk analysis

We will collect breast milk samples on days 5 and 10 after birth and at 4 months' corrected age from mothers who consent to this part of the study. The breastmilk sample will be collected preferably between 10 am and 12 pm and 2-3 hours after the mother has last expressed or breastfed. All samples will be collected from the right breast only which will need to be completely emptied in the expression or breastfeed 2 – 3 hours before the sample will be taken. The milk sample must be collected until the full breast is empty with a Medela symphony breast pump into sterile disposable plastic containers and the total volume of milk recorded. The sample will be vortexed for 2 minutes at high speed and 2 mL of the total volume are drawn from the disposable bottle by using an enteral syringe. Four aliquots of 500 µL will be stored in lobind protein microtubes and placed into the freezer at -20 ^{o}C and transported for storage at -80 ^{o}C within 2 weeks' of collection. Any remaining milk will be returned to the mother for feeding of the infant.

We will undertake a comprehensive analysis of breast milk, including hormones (steroids and adipokines) volatile compounds, and metabolites. Steroids, including *glucocorticoid hormones, and metabolites* will be measured using methodology we have developed by Liquid Chromatography tandem Mass Spectrometry (LC-MS) (Pundir et al., 2017). Adipokines will be measured by enzyme-linked immunosorbent assay. *Volatile*

compounds of milk will be analysed by head space coupled to gas chromatography-mass spectrometry (Toso, Procida, & Stefanon, 2002), providing an untargeted analysis of volatile (and odourant) compounds. Breast milk metabolites will be quantified on a Q-Exactive Orbitrap Liquid Chromatography-Mass Spectroscopy (LC-MS) using the Biocrates AbsoluteIDQ® p400 HR kit. This is a standardised and automated method that allows the detection and quantification of up to 408 metabolites (including amino acids, biogenic amines, acylcarnitines, di- and tri-glycerides, phospholipids, sphingolipids, etc) covering a wide range of biological functions. Breast milk fatty acid composition will be analysed by Gas Chromatography-Mass Spectroscopy (GC-MS) using direct transesterification protocol for esterification of fatty acids (Lepage & Roy, 1986; Cruz-Hernandez et al, 2013). This method allows the detection and quantification of large range of fatty acids (from 6 to 24 carbons, including mono and polyunsaturated fatty acids). Breastmilk composition will be analysed using univariate and multivariate statistical tools to determine significant differences associated with the sex of the infant, gestational age at birth and postnatal outcomes. (Revised as per Amendment 01).

Two year assessments

All surviving children will be assessed formally at two years' corrected age by trained assessors who can administer the Bayley Scales of Infant Development, Edition III (BSID III) and undertake a structured assessment of neurodevelopment and growth. The assessment will include a neurological examination to diagnose cerebral palsy (loss of motor function and abnormalities of muscle tone and power). The severity of gross motor problems will be classified using the Gross Motor Function Classification System (GMFCS) (Palisano et al., 1997). The psychological assessment will include the cognitive, motor and language scales of the BSID-III (Bayley, 2006). Psychological test scores will be recorded as a standardised normal score [derived from test score - mean/standard deviation (SD)]. Children with severe developmental delay who are unable to complete the psychological assessment will be given a standardised score of - 4 SD. Children will be considered blind if visual acuity in both eyes is worse than 6/60, and deaf if their hearing loss is sufficient to require hearing aid(s), or worse. The child's height (length if height not possible), weight, head circumference and waist circumference will be measured. Weight will be measured on a paediatric digital scale, height will be measured on a stadiometer, head circumference measured around the largest area of the head (occipital frontal circumference) using a non-stretch tape measure (Shaw & McCarthy, 2007) and waist circumference to be measured at just above the uppermost lateral border of the right ilium, at the end of a normal expiration, recorded to the nearest millimetre using a non-stretch tape measure (Fernández, Redden, Pietrobelli, & Allison, 2004). All staff taking anthropometric measurements will be trained to ensure standardisation and accuracy. All measurement Z-scores will be calculated individually using UK-WHO normative data (Cole, Wright, & Williams, 2011).

Categorisation of neurodevelopmental disability

Children will be considered to have a neurodevelopmental impairment if they have cerebral palsy, blindness, deafness or developmental delay (any of the language, cognitive or motor scores Bayley Scale scores more than 1 SD below the mean (<−1 SD)). The neurodevelopmental disabilities imposed by the various neurodevelopmental impairments will be classified as severe, moderate or mild (Doyle, 2004) (Table 2).

Table 2 Neurodevelopmental disability classifications

I Severe disability	Any severe cerebral palsy (child non-ambulant and likely to remain so; GMFCS level 4 or 5), severe developmental delay (standardised score <−3 SD) or blindness
II Moderate disability	Moderate cerebral palsy (child non-ambulant at 2 years of age but who is likely to ambulate subsequently; GMFCS level 2 or 3), or deafness, or moderate developmental delay (standardised score from −3 SD to <−2 SD)
III Mild disability	Mild cerebral palsy (child walking at 2 years of age with only minimal limitation of movement (GMFCS level 1), or suspect developmental delay (standardised score from −2 SD to <−1 SD)
IV No neurodevelopmental disability	Children without any neurodevelopmental impairment

2.3.2 Data management

Data will be collected prospectively from patients' records, observation charts, feeding plans and electronic medical notes by research staff and the investigators. Double data entry and cross validation methods will be used to ensure validity and quality of data. The principal investigators, Tanith Alexander, Professor Frank Bloomfield, research nurse and data entry clerk will be responsible for data collection, recording and quality of the data. Security/storage of data will be by password encrypted storage with locked filing cabinets for hard copy. The information will be stored for 10 years after maturity (26 years) after which time they will be disposed of securely and appropriately.

2.3.3 Statistical methods

Statistical analyses will be performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). The main intervention effects will be evaluated on an intention-to-treat basis. All eligible infants will be analysed according to the assigned condition at randomisation, adjusting for stratification factors and the non-independence of multiple births. Other baseline confounders that are closely associated with the outcomes will be considered in the model if there is evidence of group imbalance by chance (≥ 10%). For the primary outcomes, % fat mass at 4 months' correct age will be analysed using generalised linear regression with the model-adjusted mean difference. Time to full enteral feeds will be analysed using Cox proportional hazards model with the adjusted hazard ratio. The between group difference will be estimated with 95% confidence interval and p-value. An overall type I error rate of 5% will be maintained controlling for multiple comparisons. Secondary outcomes will be evaluated using regression models appropriate to their distributions with similar model adjustment.

Primary analyses will focus on the main effect of each intervention against its comparator, controlling for co-intervention in the same condition. Secondary analyses will test for possible interactions between the main effects. Additional, per protocol analyses will be conducted on those babies without protocol deviations. Missing data will not be imputed on the study outcomes, as the key assumption of missing at random is

unlikely to hold in the analysis populations. Sensitivity analyses will be conducted, however, using a multiple imputations method to explore the potential impact of missing data on the primary outcome.

2.4 Monitoring

2.4.1 Data monitoring

An independent Data Monitoring Committee (DMC) will be formed. The terms of reference will be agreed at the first meeting. Planned interim summaries of death and other serious adverse events will be supplied, in strict confidence, to the DMC by the trial statistician. The Trial Steering Committee will meet within a month of all Data Monitoring Committee meetings to consider their recommendations.

2.4.2 Harms

An independent Safety Monitoring Committee (SMC) will also be formed. The SMC will review individual reports of adverse events. Group allocation will not be revealed to the Safety Monitoring Committee or the investigators. Should the SMC rule that the intervention may have impacted on the adverse outcome, this will be immediately reported to the Chair of the Trial Steering Committee. The Steering Committee will decide on the actions to be taken and advise the investigators.

Adverse events

Adverse events for this trial have been defined by the steering group committee as:

- Intravenous line extravasation requiring clysis
- Any non-elective removal of a central line
- Any confirmed central line-associated blood stream infection (CLABSI):
  A central line-associated blood stream infection is a laboratory-confirmed bloodstream infection (BSI) in a patient who had a central line within the 48 hour period before the development of the BSI and that is not related to an infection at another site. The CLABSI must meet one of the following criteria (CDC - Center for Disease Control and Prevention, 2016):
  - Criterion 1: Patient has a recognised pathogen cultured from one or more blood cultures *and* organism cultured from blood is not related to an infection at another site.
  - Criterion 2: Patient has at least one of the following signs or symptoms: fever (>38°C), chills, or hypotension *and* signs and symptoms and positive laboratory results are not related to an infection at another site *and* common skin contaminant is cultured from two or more blood cultures drawn on separate occasions.
  - Criterion 3: Patient < 1 year of age has at least one of the following signs or symptoms: fever (>38°C core) hypothermia (<36°C core), apnoea, or bradycardia *and s*igns and symptoms and positive laboratory results are not related to an infection at another site *and* common skin contaminant is cultured from two or more blood cultures drawn on separate occasions.
- Late-onset sepsis (48 hours or more after birth): To be a "significant positive culture", these conditions must apply: Isolation of an organism from at least one culture of a normally sterile bodily fluid or identification via PSR in CSF and, after consideration of the clinical and laboratory evidence, a decision is made to give antibiotics with therapeutic intent against this organism.

  The following conditions must not apply:

  - Mixed coagulase negative staphylococci or other skin flora contaminant

- Same organism isolated from blood or CSF during previous 14 days – repeat isolate

All data relating to any of the adverse events will be collected routinely as part of trial data collection forms, they will be reported to the Safety Monitoring Committee after every 100 babies or every 6 months whichever occurs first.

Serious adverse events

Serious adverse events for this trial have been defined by the steering group committee as:

- Any death
- Proven necrotising enterocolitis, defined as Bell stage II and above
- Any gastrointestinal surgery

3. Ethics and Dissemination

3.1 Research Ethics Approval

The Northern A Health and Disability Ethics Committee has given ethical approval for this study 22nd July 2016**,** approval number 16/NTA/90. Locality approval will be sought from each centre.

3.2 Locality Approval

Locality approval has been gained from Auckland District Health Board, Counties Manukau Health, Waitemata District Health Board and MidCentral District Health Board and has been authorised by each locality in the Health and Disability Ethics online forms.

Auckland District Health Board has given research approval for the trial number A+7241.

Counties Manukau Health has given research approval for the trial number 150.

Waitemata District Health Board has given research approval for the trial number RM13787.

MidCentral District Health Board has given research approval for the trial number 2019.06.003.

3.3 Consent

Parents will be given a written information sheet and verbal information about the study antenatally (where possible), which will be reviewed with them by a member of the study team. If birth occurs without time for this to happen, information will be given to the parents as soon as is feasible after birth and reviewed with them by a member of the study team to provide additional information and answer any queries about the study. Verbal information will be provided in everyday language detailing what the study involves including follow-up requirements after discharge. Family members will be encouraged to ask questions of researchers, who will endeavour to ensure that parents / guardians understand the study prior to giving them the consent form. If parents / guardians have limited English an interpreter will be arranged and all information will be given through the hospital interpreter service. Māori and Pacific Island families will be asked if they would like to have a cultural support person present at any point of the informed consent process. Formal written consent will be required prior to babies entering the study. If parents decline consent, nutritional care will be according to the plan of the attending physician.

3.4 Participant withdrawal

Participants will be withdrawn from the trial on parental request or if the medical team caring for the baby believe it is essential for the overall well-being of the baby. Clinical team members will be encouraged to discuss the situation with a member of the study Steering Committee before making the decision to withdraw the baby. Consent will be sought to continue to access routinely collected clinical data for study purposes, and to approach the family about follow-up.

3.5 Confidentiality

All study-related information will be stored securely at the study site or sent directly to the Liggins Institute for data entry. All participant information will be stored in locked filing cabinets in areas with limited access. All data collection forms will include a ten-digit identification number rather than identifiable information to maintain participant confidentiality. All records that contain names or other personal identifiers, such as contact forms and informed consent forms, will be stored separately from data collection forms identified by code number. The electronic database will be secured with password-protected access systems.

3.6 Declaration of Interests

There are currently no conflicts of interest declared.

3.7 Access to Data

The Liggins Institute Data Access Committee will oversee data sharing process. The Principal Investigators will be given access to the cleaned data sets and all data sets will be password protected. To ensure confidentiality, data distributed to any team members will be blinded of any identifying participant information.

3.8 Dissemination Policy

The steering group committee will be responsible for the appropriate and timely dissemination of research results to participants, health care professionals, the public and other relevant groups. Each paper or abstract, must be submitted to the steering group committee for review prior to submission for publication or conference. The research protocol will be submitted for publication within 9 months of commencing recruitment. Every attempt will be made to reduce to a minimum the interval between the completion of data collection and the release of the study results.

4. Appendices

4.1 Participant Information Sheet and Consent Form

4.2 Case Report Forms

4.3 Ethical and Locality Approval

4.4 Protocol Amendments

4.5 Study Committee Terms of Reference

5. References

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The DIAMOND trial

DIfferent **A**pproaches to **MO**derate & late preterm **N**utrition: **D**eterminants of feed tolerance, body composition and development

Statistical Analysis Plan

Draft

October 2017, Version 1

1. Background

Babies born at moderate-late preterm gestations account for >80% of all preterm births. Although survival is excellent, these babies are at increased risk of adverse neurodevelopmental outcomes. They also are at increased risk of adverse long-term health outcomes, such as cardiovascular disease, obesity and diabetes. There is little evidence guiding optimal nutritional practices in these babies; practice, therefore, varies widely. This factorial design clinical trial will address the role of parenteral nutrition, milk supplementation and exposure of the preterm infant to smell and taste with each feed on time to tolerance of full feeds, adiposity, and neurodevelopment at 2 years.

2. Hypothesis

We aim to investigate the impact of different feeding strategies currently in use on feed tolerance, body composition, and on developmental outcome in babies born moderate- to late-preterm (MLPT). The research hypotheses are:

1. Early nutrition supplementation including protein will prevent a protein deficit leading to
   a. Body composition at 4 months' corrected age similar to that of term-born children, and
   b. Improved neurodevelopmental outcomes
2. Exposure of MLPT babies to smell and taste before each feed before establishment of full breast feeds will decrease time to full enteral feeds and full sucking feeds

3. Study Design

The DIAMOND trial is a multi-centre, factorial, randomised, controlled clinical trial.

3.1 Eligibility Criteria

Inclusion criteria

Babies born between 32^{+0} and 35^{+6} weeks' gestation, whose mothers intend to breastfeed, who are admitted to the neonatal intensive care unit within 24 hours of birth, and require insertion of intravenous lines for clinical reasons.

Exclusion criteria

Babies in whom a particular mode of nutrition is clinically indicated or babies with a congenital abnormality that is likely to affect growth, body composition or neurodevelopmental outcome.

3.2 Randomisation and Blinding

Within 24 hours of birth, once written consent is obtained, eligible babies will be randomised into one of eight treatment conditions (Table 1) that include a combination of each of the three interventions whilst waiting for full enteral feeds with mother's milk to become established. The interventions are: (i) intravenous amino acid

solution vs. dextrose 10% until full milk feeds established; (ii) milk supplement vs. exclusive breastmilk, and (iii) taste/smell given before gastric tube feeds or not.

Randomisation will be stratified by gestation (32^{+0} to 33^{+6}; 34^{+0} to 35^{+6} weeks), recruitment centre (each centre has different nutrition practices) and sex (this influences growth and body composition), using variable block sizes of 8 or 16. Twins and triplets will be randomised as separate babies. Randomisation lists will be prepared by the trial statistician, maintained and concealed by an independent database controller till the point of randomisation using secure web-based interface.

Table 1: Factorial design randomisation table.

Condition	Parenteral nutrition (i)	Milk supplement (ii)	Taste/smell (iii)
1	**+**	**+**	**+**
2	**+**	**-**	**+**
3	**+**	**+**	**-**
4	**+**	**-**	**-**
5	**-**	**+**	**+**
6	**-**	**-**	**+**
7	**-**	**+**	**-**
8	**-**	**-**	**-**

+ means the baby receives this intervention; - means the baby does not.

Due to the nature of the study, it is not possible to blind researchers, clinical staff or families. Researchers involved in the follow-up assessments at 4 and 6 months' corrected and at 2 years' corrected age will be blinded to the allocation that the infant received during their admission.

3.3 Interventions and comparators

All babies will receive nutrition according to individual neonatal intensive care unit practices. The first two interventions only apply until the baby is established on full enteral feeds with mothers' milk, which remains the primary nutritional goal. Babies randomised to receive smell and taste before tube feeds will continue to receive this intervention until the baby is no longer receiving any gastric tube feeds.

Parenteral nutrition: If randomised to receive parenteral nutrition the baby will receive an amino acid solution (according to local hospital practice) intravenously, either by peripheral or central line as deemed clinically appropriate. Administration of lipid is at the discretion of the clinical team, as is the administration of any supplementary fluids, such as 10% dextrose. Babies not randomised to parenteral nutrition will receive 10% dextrose only. The randomised intravenous fluid will be continued until full enteral feeding is established.

Milk supplement: If randomised to receive milk supplement, the baby will receive donor breastmilk or infant formula (according to local practice) while waiting for mother's breastmilk to meet prescribed fluid amounts. Babies not randomised to receive milk supplement will only receive mother's breastmilk as available.

Taste and smell: If randomised to receive taste and smell, the baby will be exposed to the taste and smell of the milk feed before every enteral feed. If the baby is receiving both breast milk and supplementary formula, the smell and taste will be of breast milk if available, but if there is insufficient breastmilk, then smell and taste can be of formula. However, if the baby is randomised to not receive supplementary infant formula, then only the smell and taste of breastmilk will be provided with taste given priority if supply is limited.

The goal for all babies enrolled in the study is to transition to full feeds of expressed breastmilk as soon as possible.

3.4 Study Outcomes

Primary Outcomes

For parenteral nutrition (i) and milk supplement (ii) factors: body composition assessment at 4 months' corrected age when infant adiposity is predictive of childhood fat mass.

For smell/taste factor (iii), time to full enteral feeds defined as 150 $ml.Kg^{-1}.day^{-1}$ or exclusive breastfeeding if this occurs prior to enteral feeds of 150 $ml.Kg^{-1}.day^{-1}$ being reached.

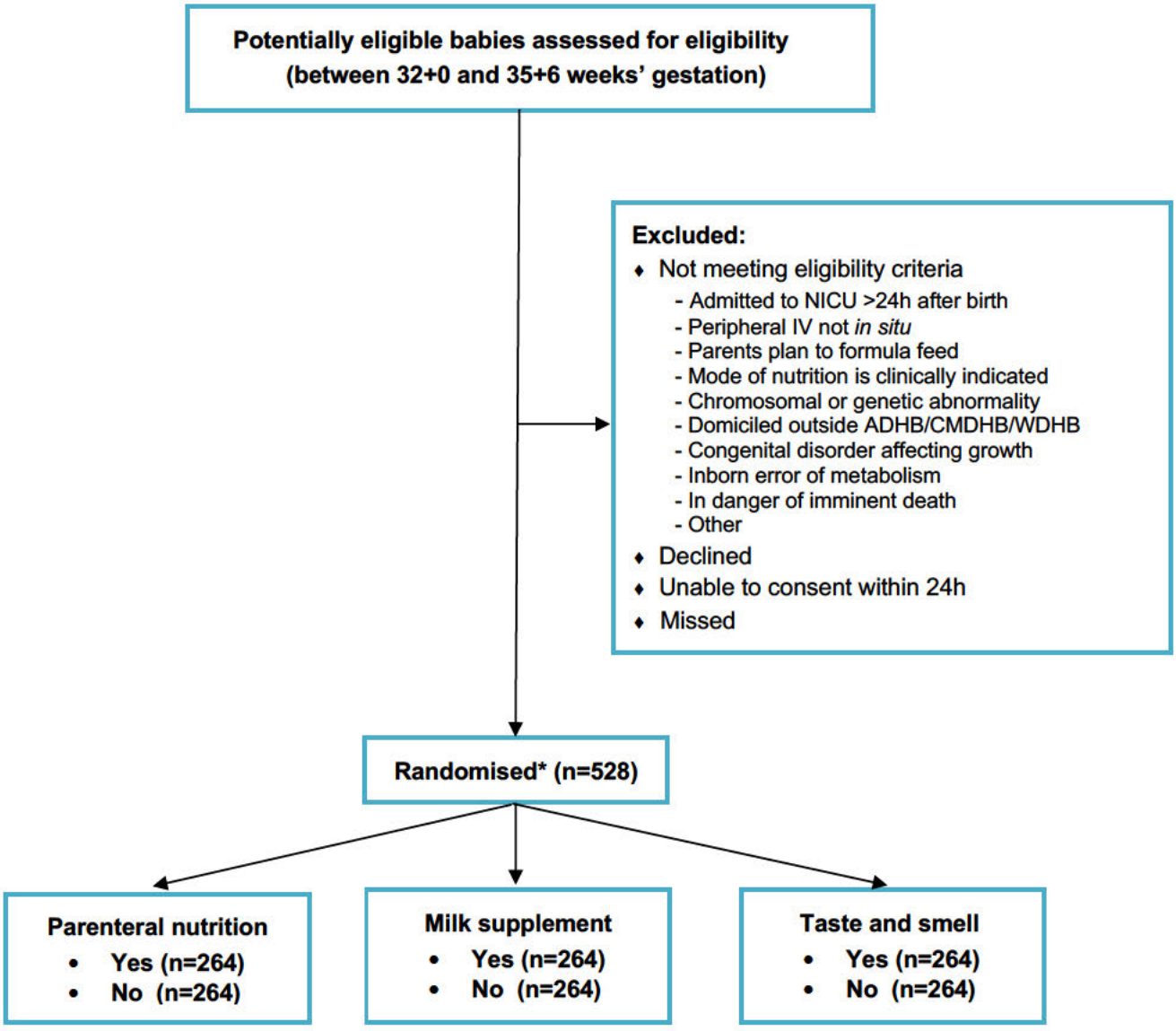
Secondary Outcomes

Time to full sucking feeds defined as removal of the nasogastric tube for at least 24 hours or until discharge, whichever is the sooner; number of days in hospital; body composition at discharge; growth: length, weight and head circumference z-scores and change in z-score from birth to 4 months' corrected age and at 2 years; developmental assessment at 4 months' and 2 years corrected age; breast feeding rates; nutritional intake from birth to full enteral feeds or for maximum of 28 days.

3.5 Sample size

Unlike multi-arm, parallel RCT or comparative experiments, factorial experiments are designed to estimate main effects and their interactions. Each main effect and interaction analysis are, therefore, based upon the total sample size which is chosen to be large enough to detect all primary outcomes; having more factors does not increase total sample size. A total of 480 babies (n=240 per intervention arm) will provide ≥90% power at an overall type 1 error rate of 5% to detect a minimal clinically significant difference in % fat mass at 4 months' correct age of 3% (lower 95% confidence interval) for parental nutrition and milk supplement interventions, or to detect a reduction in median time to full enteral feeds from 10 to 7 days (hazard ratio 1.43) with the smell/taste intervention. This sample size has assumed a standard deviation of 4% in % fat mass, with Bonferroni corrections to each of the three tests (i.e. alpha per main intervention effect = 0.0167). Allowing for 10% loss to follow-up, we aim to recruit 528 babies (n = 66 per randomised condition).

CONSORT Flow Diagram



* Eligible babies are randomised into one of eight conditions, with half of the babies allocated to receive each type of intervention, and stratified by study centre, gestation (32^{+0} to 33^{+6}; 34^{+0} to 35^{+6} weeks) and sex.

4. Statistical Analysis

Statistical analysis will be performed at the end of the trial. No interim analysis is planned. All study data will be imported from secure ACCESS database to SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) for analysis. Statistical tests will be two-sided and maintained at 5% significance level.

4.1 Analysis Populations

The primary analysis population will be based on the intention to treat (ITT) principle, including all randomised babies as representing the group they were randomly allocated to, whether or not they completed or indeed received that treatment. Babies would be excluded from the ITT population if they failed to satisfy entry criteria (i.e. randomised in error), withdrawn from the study and consent to use their data, or were lack of any data post randomisation.

The secondary analysis population will include a subset of the ITT population with babies who have no major protocol deviations. A protocol deviation form will be used to record all cases during the trials, and reviewed by the Steering Group members before the final analysis to determine the Per Protocol (PP) population for analysis.

Due to the nature of the interventions tested in the study, an exploratory analysis population may be considered including the randomised babies with treatment as actually received, rather than as prescribed in random assignment. The findings from this As Treated (AT) analysis must be interpreted with caution, as it may produce inconsistent or counter-intuitive results that deviate from the principal ITT analysis.

All defined primary and secondary outcomes will be conducted on the ITT population. If the proportion of babies excluded from the PP analysis is greater than 10%, the study outcomes will also be analysed on the PP population. The AT analysis will be considered on the study outcomes when more than 10% of randomised babies fail to take the allocated treatment or are switched to a different study treatment.

4.2 Missing Data

Missing data will not be imputed on the study outcomes, as the key assumption of missing at random is not likely to hold in the analysis populations. Sensitivity analyses will be conducted, however, using multiple imputations method to explore the potential impact of missing data on the primary outcome, if the proportion of missing is greater than 10%. The characteristics of those participants with missing data will be compared between the intervention groups.

4.3 Baseline Characteristics

Demographics and birth anthropometry collected from all randomised babies in the ITT population will be summarised descriptively by each type of intervention and treatment condition. Continuous variables will be summarised as the numbers observed and missing (if any), mean, standard deviation (SD), median, inter-quartile range (IQR), minimum and maximum (range). Categorical variables will be summarised as frequency (n) and percentage (%).

Baseline imbalance will not be formally tested between randomised groups as suggested by the CONSORT 2010 statement, however, important baseline confounders that are closely associated with the outcomes will be considered in the model if there is evidence of group difference by chance (≥ 10%).

4.4 Primary Outcome Analyses

The primary outcomes defined for the first two interventions (% fat mass at 4 months' corrected age) and the smell/taste before tube feeds (time to full enteral feeds or exclusive breastfeeding whichever comes first), will be first summarised descriptively by the intervention group. The number of babies with the outcomes measured or missing will be described together with mean, SD, median, IQR and range.

Primary analyses will focus on the main effect of each intervention against its comparator, controlling for co-intervention(s) in the same condition. Secondary analyses will test for possible interactions between the main effects.

Parenteral Nutrition

For babies randomised to receive parenteral nutrition (intervention I) versus 10% dextrose (comparator) until full milk feeds is established, linear regression model will be used to test the effect of intervention on the primary outcome adjusting for gestational age, sex and other important baseline confounders identified in baseline comparison. The non-independence of multiple births will be controlled in the model using a random cluster effect. Model-adjusted mean difference between groups will be estimated with 95% confidence interval and associated p-value. When the interaction effect with co-intervention in the same condition is present, model-adjusted means will be estimated and compared between each combination of treatments.

Milk Supplement

Same outcome analysis will be conducted for babies randomised to receive milk supplement (intervention II) versus exclusive breastmilk (comparator).

Taste and Smell

For babies randomised to receive taste and smell of the milk feed before gastric tube feeds (intervention III) versus those who are not, time to full enteral feeds or exclusive breastfeeding will be analysed using Cox proportional hazards model. The model will adjust for gestational age, sex and other important baseline confounders identified in baseline comparison, and control for the non-independence of multiple births using a cluser effect. Adjusted hazard ratio (HR) will be reported with 95% confidence interval and associated p-value. When the interaction effect with co-intervention in the same condition is present, model-adjusted HRs will be estimated and compared between each combination of treatments.

4.5 Secondary Outcome Analyses

Secondary outcomes will be evaluated using regression models appropriate to their distributions. Continuous outcomes will be analysed in the same way as the primary outcomes.

For categorical outcomes, descriptive summaries will be first presented by the intervention group using frequency and percentage. Generalised linear regression model will be used to test the effect of intervention adjusting for gestational age, sex and other important baseline confounders identified in baseline comparison. The non-independence of multiple births will be controlled in the model using generalised estimating equation (GEE). Adjusted odds ratio (OR) will be reported with 95% confidence interval and associated p-value. When the interaction effect with co-intervention in the same condition is present, model-adjusted ORs will be estimated and compared between each combination of treatments.

4.6 Subgroup Analyses

Pre-defined subgroup analyses will be conducted on the primary and key secondary outcomes to test the main effect of each intervention against its comparator by:

1. Moderate (32^{+0} to 33^{+6} weeks) and Late (34^{+0} to 35^{+6} weeks) preterm babies
2. Girls and Boys

Same regression analyses will be conducted on each subgroup to explore the main effect of each intervention against its comparator. The consistency of intervention effects between subgroups will be tested using an interaction term between intervention arm and subgroup. The subgroup analyses will be conducted for (1) and (2) separately. Due to the size of subgroups, potential interactions between the main effects will not be explored.

5. Data Collections

The following case record forms (CRFs) will be used to collect the data after birth at scheduled visits.

7 day forms	Form X	Trial entry and randomisation	
	Form Y	Trial consent	14 days after randomisation
	Form Z	Contact details	
	Form A	Demographics & birth anthropometry	
Discharge forms	Form B	Growth measurements	
	Form C	Clinical outcomes	
	Form F	Lines	14 days after discharge
	Form G	Nutrition	
	Form H1 & H2	28 day Intravenous and enteral intakes	
	Form M	Samples	
	Form P	Maternal questionnaire	
4-month follow-up forms	Form K	Four-month follow-up	14 days after follow-up appointment
	Form N	Breastfeeding questionnaire	
	Form P	Maternal questionnaire	
6-month follow-up form	Form N	Breastfeeding questionnaire	14 days after follow-up appointment
1-year follow-up form	Form L	Age & Stages Questionnaire	14 days after follow-up appointment
2-year follow-up forms	*To add*	*To add*	14 days after follow-up appointment
Other forms	Form D	Serious adverse event	24 hours of event
	Form E	Withdrawal from study	14 days after withdrawal
	Form J	Protocol deviation	14 days of event

Demographics and birth anthropometry

Babies' birth status (plural, birth order), sex, gestational age and birth anthropometry (weight, length and head circumference) will be collected. Maternal age, ethnicity, education will also be collected at baseline. Antenatal corticosteroids and whether the mother has diabetes and caesarean section will be recorded.

Growth measurements and body composition

Weight, length and head circumference will be measured every week up till discharge and at 4 months' corrected age. Measurement using air displacement plethysmography (APD) system PEA POD, will occur as close to discharge as is feasible and at 4 months' corrected age as the preferred method for determining body composition. The Peapod measurements include percentage fat mass and fat free mass, fat mass and fat free mass, and body mass. Skinfold measures (in mm) will also be measured at 4 months' corrected age using standardised skinfold calipers. Up to three measures will be taken on each skinfold thickness, and the average value will be used.

Nutritional intakes

Total enteral and intravenous intakes will be recorded daily until discharge for a maximum of 28 days or until baby begins receiving breastfeeds with less than full tube feed top-ups. Daily macronutrient intakes will be calculated based on actual intake (in mL) and standardised to baby's weight (in kg). Intravenous fluids include amino acid, dextrose, lipid and other IV fluids. Enteral fluids include expressed breastmilk, fortified expressed breastmilk, preterm formula, other formula and nutrient supplements (in g/mL). Smell and taste provided on each day will also be recorded.

Questionnaires

Maternal questionnaire will be completed by the mother at discharge and 4 months' corrected age, providing feedbacks on how they have felt in the past 7 days and month. They will also complete a questionnaire regarding breastfeeding at 4 months and 6 months' corrected age. Development will be assessed using the Ages and Stages Questionnaire (ASQ) at 1-year corrected age.

Two-year assessments

All surviving children will be assessed formally at two years' corrected age by trained assessors who will administer the cognitive, motor and language scales of the Bayley Scales of Infant Development, Edition III (BSID III) and undertake a structured assessment of neurodevelopment and growth. The assessment will include a neurological examination to diagnose cerebral palsy (loss of motor function and abnormalities of muscle tone and power). The severity of gross motor problems will be classified using the Gross Motor Function Classification System (GMFCS). BSID III test scores will be recorded as a standardised normal score, derived from (test score – mean)/standard deviation (SD). Children with severe developmental delay who are unable to complete the assessment will be given a standardised score of - 4 SD.

Other forms

For randomised babies, all serious adverse events (SAE) will be recorded during the trial and referred to the independent Safety Monitoring Committee (SMC) within 24 hours of notification to the Principal Investigator. A

SMC report will be recorded with clinical synopsis, recommendations and whether it is likely to be trial related. The SAEs include death, necrotising enterocolitis (NEC), any gastrointestinal surgery and other serious adverse events that the principal or local investigator believes should be referred to SMC.

A study withdrawal form will be filled for the baby who was randomised but withdrawn from any part of the study, including withdrawal from breastmilk, stool or saliva samples; any of the allocated interventions; peapod measurements or other. Reasons for participant withdrawal will also be recorded including parents or clinician's decision, or due to SAE. Ongoing consent to collect data from routine neonatal intensive care and follow up will be sought from the mother or main caregiver, as the baby may still be included in the ITT population if the consent is given.

A protocol deviation form will be recorded during the trial, if any of the randomised babies have the following protocol deviations: randomised in error, consent error, incorrect intervention administered, or other deviations that the principal or local investigator believes should be recorded.

6. Data Monitoring Committee

An independent Data Monitoring Committee (DMC) will be formed to monitor the overall conduct and safety of the interventions during the trial. Aggregate reports of recruitment rate by centre, study withdrawal and protocol deviations, serious adverse events and SMC reports will be supplied in a blinded manner, in strict confidence, to the DMC by the trial statistician. A letter reporting the DMC's recommendations will be sent to the Trial Steering Committee (TSC) within 2 weeks of each DMC meeting, and the TSC will meet within a month of the DMC meeting to consider their recommendations.

7. Results and Reports

Statistical analysis will be conducted at the end of the trial, when all randomised babies completed their follow up at 2 years' corrected age. Regular data quality checks will be conducted during the trial, in a blinded manner, by the trial statistician and data manager. The final efficacy and safety results will be presented in tables and figures, and the templates will be prepared by the trial statistician and sent to the TSC for approval before the data lock. The final study report will follow the guidelines in the CONSORT 2010 statement for randomised controlled trials, with modification to the factorial design.

The DIAMOND trial

DIfferent **A**pproaches to **MO**derate & late preterm **N**utrition: **D**eterminants of feed tolerance, body composition and development

Statistical Analysis Plan

Version 5

19 October 2022

Registration: Australian New Zealand Clinical Trials Registry
ACTRN12616001199404

Preface

The purpose of this Statistical Analysis Plan (SAP) is to provide an overview of the intended statistical analyses that will be performed on data from the DIAMOND main trial. This document is intended to stand alone from the protocol and adhere to the main points in the analysis summary specified in the protocol. However, it is envisaged that the SAP can undergo revision outside of the protocol.

The following documents have been reviewed in preparation of this SAP:

- Published study protocol (2018 Bloomfield *et al.* BMC Pediatrics 18:220)
- DIAMOND Protocol SPIRIT v6
- DIAMOND study case record forms (CRFs)
- Consort 2010 statement: updated guidelines for reporting parallel group randomized trials (2010 Schulz *et al.* BMJ 340:c332)
- Guidelines for the content of statistical analysis plans in clinical trials (2017 Gamble *et al.* JAMA 318(23):2337-2343)

Revision History

SAP version	Date	Sections affected	Rational
Version 1	October 2017		
Version 2	8 February 2022	Major revision to the first SAP draft in 2017	Remove secondary outcomes not specified in the published study protocol; incorporate any revisions to the CRFs
Version 3	11 February 2022	Secondary outcomes to be included in the SAP	Outcomes at 2 years of age are not included in this SAP and will be reported at a later date
Version 4	07 March 2022	CONSORT flow chart, outcome definitions and result tables	Address SG comments
Version 5	19 October 2022	Added footnote to table 3	To specify that the two primary outcome measures also will be reported for the factor(s) for which each measure is not the primary outcome
		Specify body composition parameters	Clarify detail of the body composition measures and that fat and lean mass indices will be calculated
		Add in Table 5	Include skin fold measurements for consistency with protocol
			Results to be presented as adjusted Relative Risk rather than adjusted Odds Ratios

Signature Page

Name:	Signature:	Date:
Professor Frank Bloomfield Lead investigator Director, Liggins Institute University of Auckland	[redacted]	20 October 2022
Tanith Alexander Principal investigator Liggins Institute University of Auckland	[redacted]	20 October 2022
Dr Yannan Jiang Co-investigator, Statistician Department of Statistics University of Auckland	[redacted]	20 October 2022

Contents

1. Background

Babies born at moderate-late preterm gestations account for >80% of all preterm births. Although survival is excellent, these babies are at increased risk of adverse neurodevelopmental outcomes. They also are at increased risk of adverse long-term health outcomes, such as cardiovascular disease, obesity and diabetes. There is little evidence guiding optimal nutritional practices in these babies; practice, therefore, varies widely. This factorial design clinical trial will address the role of parenteral nutrition, milk supplementation and exposure of the preterm infant to smell and taste with each feed on time to tolerance of full feeds, adiposity, and neurodevelopment at 2 years.

2. Hypothesis

We aim to investigate the impact of different feeding strategies currently in use on feed tolerance, body composition, and on developmental outcome in babies born moderate- to late-preterm (MLPT). The research hypotheses are:

1. Early nutrition supplementation including protein will prevent a protein deficit leading to
   a. Body composition at 4 months' corrected age similar to that of term-born children, and
   b. Improved neurodevelopmental outcomes
2. Exposure of MLPT babies to smell and taste before each feed prior to establishment of full breast feeds will decrease time to full enteral feeds and to full sucking feeds
3. Feeding practices in MLPT babies in the period immediately following birth and before establishment of full breast feeding will affect microbiome composition and activity
4. Breastmilk composition will vary by sex of the infant.

3. Study Design

The DIAMOND trial is a multi-centre, factorial, randomised, controlled clinical trial.

3.1 Eligibility criteria

Inclusion criteria

Babies born between 32^{+0} and 35^{+6} weeks' gestation, whose mothers intend to breastfeed, who are admitted to the neonatal nursery, and require insertion of intravenous lines for clinical reasons.

Exclusion criteria

Babies in whom a particular mode of nutrition is clinically indicated or babies with a congenital abnormality that is likely to affect growth, body composition or neurodevelopmental outcome.

3.2 Randomisation and blinding

Within 24 hours of birth, once written consent is obtained from parents or caregivers, eligible babies will be randomised at equal allocation ratio into one of eight treatment conditions (Table 1) that include a combination of each of the three interventions whilst waiting for full enteral feeds with mother's milk to become established. The interventions are: (i) parenteral nutrition vs. intravenous dextrose solution; (ii) supplemental milk (donor

breast milk if available, else infant formula) vs. only mother's milk, and (iii) exposure to taste and smell of milk before every gastric tube feed vs. no exposure (milk adminstered only via gastric feeding tube).

Randomisation is stratified by gestation (32^{+0} to 33^{+6}; 34^{+0} to 35^{+6} weeks), recruitment centre (each centre has different nutrition practices) and sex (this influences growth and body composition), using variable block sizes of 8 or 16. Twins and triplets are randomised as separate babies. Randomisation lists are prepared by the trial statistician, maintained and concealed by an independent database controller till the point of randomisation using secure web-based interface.

Table 1: Factorial design randomisation table.

Condition	Parenteral nutrition (i)	Milk supplement (ii)	Taste/smell (iii)
1	**+**	**+**	**+**
2	**+**	**-**	**+**
3	**+**	**+**	**-**
4	**+**	**-**	**-**
5	**-**	**+**	**+**
6	**-**	**-**	**+**
7	**-**	**+**	**-**
8	**-**	**-**	**-**

+ means the baby receives this intervention; - means the baby does not.

Due to the nature of the study it is not possible to blind researchers, clinical staff or families. Researchers involved in the follow-up assessments will be blinded to the allocation that the infant received during their admission.

3.3 Interventions and comparators

All babies will receive nutrition according to individual neonatal unit practices. The first two interventions only apply until the baby is established on full enteral feeds with mothers' milk. Babies randomised to receive taste and smell before tube feeds will continue to receive this intervention until the baby is no longer receiving any gastric tube feeds. The goal for all babies enrolled in the study is to transition to full feeds of mother's breastmilk as soon as possible.

Parenteral nutrition: If randomised to receive parenteral nutrition, the baby will receive an amino acid solution (according to local hospital practice) intravenously, either by peripheral or central line as deemed clinically appropriate. Administration of lipid is at the discretion of the clinical team, as was the administration of any supplementary fluids, such as 10% dextrose. Babies not randomised to parenteral nutrition will receive dextrose solution with electrolytes as clinically indicated but no protein or lipid. The randomised intravenous fluid will be continued until full enteral feeding was established.

Milk supplement: If randomised to receive milk supplement, the baby will receive donor breastmilk or infant formula (according to local practice) while waiting for mother's breastmilk to meet prescribed fluid amounts. Babies not randomised to receive milk supplement will only receive mother's breastmilk as available.

Taste and smell: If randomised to receive taste and smell, the baby will be exposed to the taste and smell of the milk feed before every gastric tube feed. If the baby is receiving both breastmilk and supplementary formula, the taste and smell will be of breast milk if available, but if there is insufficient breastmilk, then taste and smell can be of formula. However, if the baby is randomised to not receive supplementary infant formula, then only the taste and smell of breastmilk will be provided with taste given priority if supply is limited.

3.4 Study outcomes

Primary outcomes

For (i) parenteral nutrition and (ii) milk supplement factors: % fat mass at 4 months' corrected age when infant adiposity is predictive of childhood fat mass. For (iii) taste/smell factor, time to full enteral feeds defined as 150 $mL.Kg^{-1}.day^{-1}$ or exclusive breastfeeding if this occurs prior to enteral feeds of 150 $mL.Kg^{-1}.day^{-1}$ being reached.

Secondary outcomes

- Time to full sucking feeds
- Number of days in hospital
- Nutritional intake from birth to full enteral feeds or until 28 days of age
- Breastfeeding rates at discharge and 4 months' corrected age
- Body composition at discharge and 4 months' corrected age
- Growth measurements and z-scores from birth to discharge, at 4 months' corrected age, and at 2 years' corrected age
- Developmental assessment at 2 years' corrected age

Note that outcomes at 2 years of age are not included in this SAP and will be reported at a later date, although using the same general analytical approaches.

3.5 Sample size

Unlike multi-arm, parallel RCT or comparative experiments, factorial experiments are designed to estimate main effects and their interactions. Each main effect and interaction analysis is, therefore, based upon the total sample size which is chosen to be large enough to detect all primary outcomes; having more factors does not increase total sample size. A total of 480 babies (n=240 per intervention arm) will provide ≥90% power at an overall type 1 error rate of 5% to detect a minimal clinically significant difference in % fat mass at 4 months' correct age of 3% (lower 95% confidence interval) for parental nutrition and milk supplement interventions, or to detect a reduction in median time to full enteral feeds from 10 to 7 days (hazard ratio 1.43) with the taste/smell intervention. This sample size has assumed a standard deviation of 4% in % fat mass, with Bonferroni corrections to each of the three tests (i.e. alpha per main intervention effect = 0.0167). Allowing for 10% loss to follow-up, we aim to recruit 528 babies (n = 66 per randomised condition).

The expected effect size is based on an estimated 3% increase in % fat mass in moderate to late preterm infants compared to term infants and an estimated 27% fat mass in term infants at 4 months of age. There are no good data on % fat mass beyond 4 months of age; therefore, this age has been used for the primary outcome.

4. Statistical Analysis

Statistical analysis will be performed at the end of the trial. No interim analysis is planned. All study data will be imported from secure ACCESS database to SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) for analysis. Statistical tests will be two-sided and maintained at 5% significance level.

4.1 Analysis populations

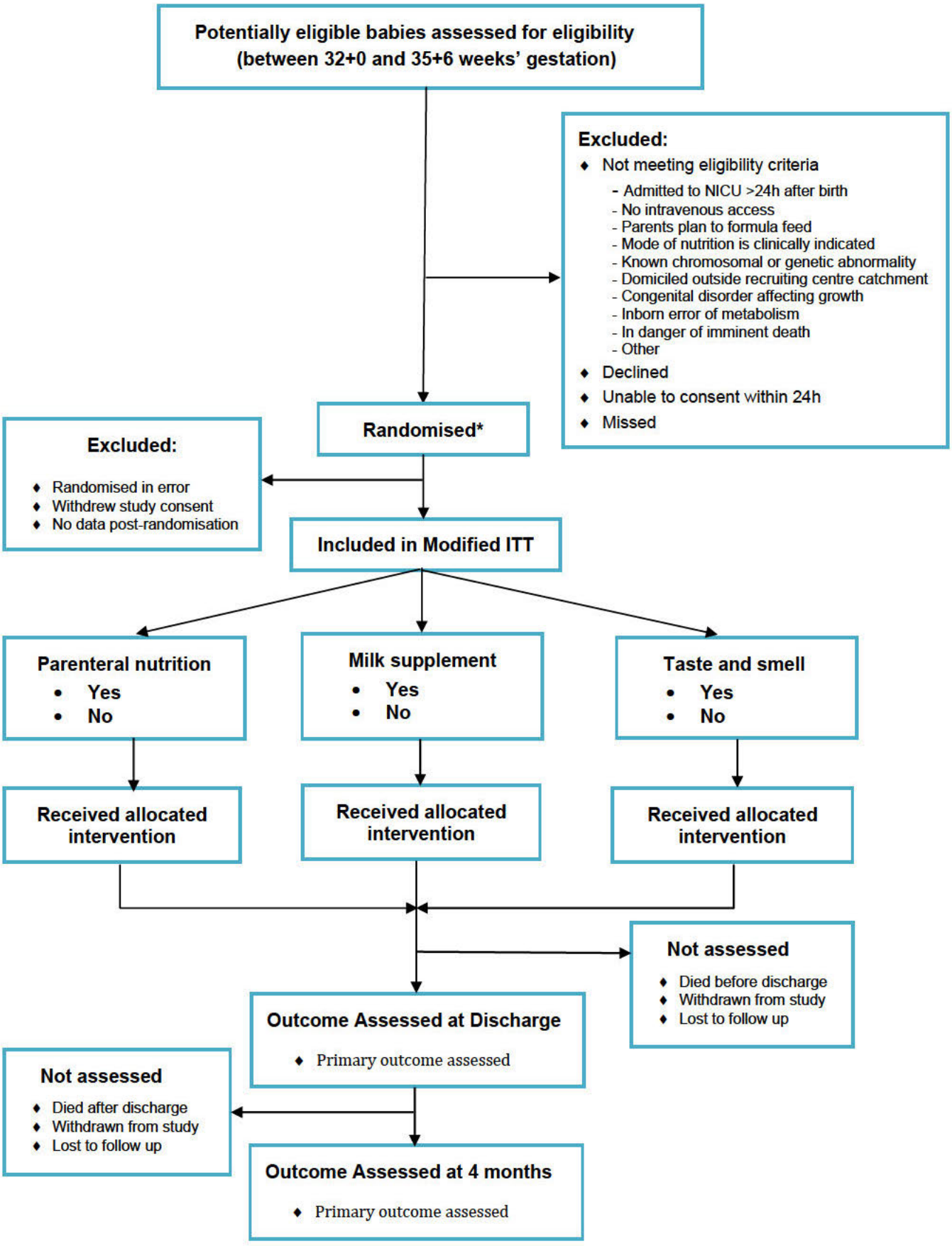
The primary analysis population will be based on the modified intention-to-treat (ITT) principle, including all randomised babies as representing the group they were randomly allocated to, whether or not they completed or indeed received that treatment. Babies will be excluded from the ITT population if they failed to satisfy entry criteria (i.e. randomised in error), withdrew from the study and consent to use their data, or provided no data post randomisation. The CONSORT flow diagram will be presented (Figure 1).

The secondary analysis population will include the subset of the ITT population who have no major protocol deviations (Per Protocol (PP) analysis). All protocol deviation forms will be reviewed by the Steering Group members before the final analysis to determine the PP population for analysis.

Due to the nature of the interventions tested in the study, an exploratory analysis population will be undertaken including the randomised babies with treatment as actually received, rather than as prescribed in random assignment. The findings from this As Treated (AT) analysis must be interpreted with caution, as it may produce inconsistent or counter-intuitive results that deviate from the principal ITT analysis.

The analysis populations will be applied to primary and secondary outcomes as appropriate.

Figure 1. CONSORT Flow Diagram



* Eligible babies are randomised into one of eight conditions at equal allocation ratio to receive each type of intervention, and stratified by study centre, gestation (32^{+0} to 33^{+6}; 34^{+0} to 35^{+6} weeks) and sex.

4.2 Missing data

Missing data will not be imputed on the study outcomes, as the key assumption of missing at random is not likely to hold in the analysis populations. Babies with missing time to full enteral feeds data will be treated as censored. Sensitivity analyses will be conducted, however, using multiple imputation to explore the potential impact of missing data on % fat mass (primary outcome). The characteristics of those participants with missing data will be compared with those without missing data.

4.3 Baseline characteristics

Demographics and birth anthropometry collected from all randomised babies in the modified ITT population will be summarised descriptively by each type of intervention and treatment condition. Continuous variables will be summarised as the numbers observed and missing, mean, standard deviation (SD), median, inter-quartile range (IQR), minimum and maximum. Categorical variables will be summarised as frequency (n) and percentage (%).

Baseline imbalance will not be formally tested between randomised groups as suggested by the CONSORT 2010 statement. However, important baseline confounders that are closely associated with the outcomes will be considered in the model if there is evidence of group difference by chance (≥ 10%).

4.4 Primary outcome analyses

The primary outcomes defined for the first two interventions (% fat mass at 4 months' corrected age) and the taste/smell before gastric tube feeds (time to full enteral feeds), will be first summarised descriptively by the intervention group. The number of babies with the outcomes measured or missing will be described together with mean, SD, median, IQR and range.

Primary analyses will focus on the main effect of each intervention against its comparator, controlling for co-intervention(s) in the same condition. Secondary analyses will test for possible interactions between the main effects.

Parenteral nutrition

For babies randomised to receive parenteral nutrition (intervention I) versus dextrose solution (comparator) until full enteral feeding is established, linear regression models will be used to test the effect of intervention on the primary outcome adjusting for stratification factors (hospital, gestation, sex). Other baseline confounders that are closely associated with the outcome will be considered in the model if there is evidence of group difference by chance (≥ 10%). The non-independence of multiple births will be controlled in the model using a cluster effect. Model-adjusted mean difference between groups will be estimated with 95% confidence interval and associated p-value. When the interaction effect with co-intervention in the same condition is present, model-adjusted means will be estimated and compared between each combination of treatments.

Milk supplement

The same outcome analysis will be conducted for babies randomised to receive milk supplement (intervention II) versus exclusive breastmilk (comparator).

Taste and smell

For babies randomised to receive taste and smell of the milk feed before gastric tube feeds (intervention III) versus those who are not, time to full enteral feeds will be analysed using Cox proportional hazards model. The model will adjust for stratification factors (hospital, gestation, sex) and controlled for the non-independence of multiple births using a cluster effect. Adjusted hazard ratio (HR) will be reported with 95% confidence interval and associated p-value. When the interaction effect with co-intervention in the same condition is present, model-adjusted HRs will be estimated and compared between each combination of treatments.

4.5 Secondary outcome analyses

Secondary outcomes will be evaluated using regression models appropriate to their distributions. Descriptive summaries will be first presented by intervention group. Continuous variables will be summarised as the numbers observed and missing (if any), mean, SD median, IQR, minimum and maximum (range). Categorical variables will be summarised as frequency (n) and percentage (%). Generalised linear regression models will be used to test the effect of interventions adjusting for stratification factors and other important baseline confounders identified in baseline comparison. The non-independence of multiple births will be controlled in the model using a cluster effect. For categorical outcomes, adjusted relative risk (aRR) will be reported with 95% confidence interval and associated p-value. When the interaction effect with co-intervention in the same condition is present, model-adjusted ORs will be estimated and compared between each combination of treatments.

4.6 Subgroup analyses

Pre-defined subgroup analyses will be conducted on the primary and secondary outcomes to test the main effect of each intervention against its comparator by:

1. Moderate (32^{+0} to 33^{+6} weeks) and late (34^{+0} to 35^{+6} weeks) preterm babies
2. Girls and boys

Regression analyses will be conducted on each subgroup separately to explore the main effect of each intervention against its comparator. The model will be adjusted for hospital and stratification factor(s) not used to define the subgroup. The consistency of intervention effects between subgroups will be tested in the main model using an interaction term between intervention arm and subgroup. The subgroup analyses will be conducted for (1) and (2) separately. Due to the size of subgroups, potential interactions between the main effects will not be explored.

5. Data Collections and Variable Definitions

The following case record forms (CRFs) will be used to collect the data from birth to 4 months' corrected age.

7 day forms	Form X	Trial entry and randomisation
	Form Y	Trial consent
	Form Z	Contact details
	Form A	Demographics & birth anthropometry

Discharge forms	Form B	Growth measurements
	Form C	Clinical outcomes
	Form F	Lines
	Form G	Nutrition

	Form H1 & H2	28 day Intravenous and enteral intakes

Four-month forms	Form K	Four-month follow-up
	Form N	Breastfeeding questionnaire

Other forms	Form D	Serious adverse event
	Form E	Withdrawal from study
	Form J	Protocol deviation
	Form I	Log of transfer

5.2 Baseline demographics

Babies' birth status (plural, birth order), sex, gestational age will be collected. Maternal age, ethnicity, education will be collected at baseline. Antenatal corticosteroids (any) and whether the mother had diabetes (Yes/No) or a caesarean section (Yes/No) are recorded.

5.3 Anthropometry

Babies' weight, crown-heel length and head circumference will be measured at birth and every week up till discharge, and then at 4 months' corrected age. All growth data will be standardised to z-scores using Fenton 2013 normative data, and transitioning to WHO growth standards after 50 weeks' postmenstrual age. Waist and mid-arm circumferences will be collected at 4 months' corrected age only.

5.4 Body composition and skinfold measurements

Body composition (fat mass and fat free mass, %fat mass, %lean mass, fat mass index and lean mass index) will be measured at discharge and 4 months' corrected age, using air displacement plethysmography (ADP) system PEA POD. Fat mass and fat free mass z-scores will be calculated using the mean and standard deviation in male and female infants at birth and 4.5 months of age reported by Carberry et al. (2010). Subscapular, triceps, biceps, abdominal, thigh and suprailiac skinfold thickness (mm) will be measured in duplicate at discharge and 4 months' corrected age using standardised skinfold calipers. If the difference between measures is >0.4 mm, a third measurement will be taken, and the median value will be used.

5.5 Nutritional intakes

Total enteral and intravenous intakes will be recorded daily until discharge for maximum of 28 days or until baby begins receiving breastfeeds with less than full tube feed top-ups, as the quantity of breastmilk received cannot be quantified. Full enteral feeds will be defined as 150 $mL.Kg^{-1}.day^{-1}$ or exclusive breastfeeding. Time to full sucking feeds will be defined as until removal of the nasogastric tube for at least 24 hours or until discharge home, whichever is the sooner. Nutrient intakes were calculated using recommended values (Cormack 2016), manufacturers' composition data and estimates of expressed breastmilk composition for the first week after birth (57.1 kcal and 1.9 g protein/100 mL) and for weeks 2–8 (65.6 kcal and 1.27 g protein/100 mL) with separate values for donor breastmilk milk (Boyce 2016; Cooper 2013).

Daily macronutrient intakes will be calculated based on actual intake (in mL) per day divided by the most recent weight (in Kg) to give the nutrient intake per Kg per day. The birthweight will be used until the most recent weight is higher than the birthweight. Intravenous fluids (IV) include amino acid, dextrose, lipid and other IV fluids.

Enteral fluids include expressed breastmilk, fortified expressed breastmilk, preterm formula, other formula and nutrient supplements (in g/mL).

For each week, only those babies with at least 3 valid days of nutrition data were included in the analysis. Valid days were defined as a minimum intake of fluid depending on the postnatal day, to account for increasing fluid intakes in the days after birth. The total fluid intake recorded at or above the following thresholds will be used to define a valid day:

- Day 1-2: 40 $mL.Kg^{-1}.day^{-1}$
- Day 3: 60 $mL.Kg^{-1}.day^{-1}$
- Day 4: 75 $mL.Kg^{-1}.day^{-1}$
- Day 5: 90 $mL.Kg^{-1}.day^{-1}$
- Day 6: 105 $mL.Kg^{-1}.day^{-1}$
- From Day 7: 120 $mL.Kg^{-1}.day^{-1}$

Total energy or carbohydrate must be provided on a valid day as nutrition cannot be provided without energy or carbohydrate (whereas it is possible a baby receives zero protein and / or zero fat). Other nutrient composition data will be treated as zero if daily intake was not recorded on that day. Intakes on the day of birth and day of discharge or death will be excluded as these are unlikely to represent a full 24-hour intake.

Initial cohort analysis on a sample of babies showed that, since the fluid intakes could not be recorded when babies started to breastfeed, about half of the babies did not provide data for at least 3 valid days after the first two weeks. Therefore, we will only assess nutritional intake in week 1 and week 2.

Taste and smell data will be collected based on the total number of times taste and smell intervention was administered when a full tube feed was given (recorded by a purple sticker) over the total number of full tube feeds with no oral component. Tube feeds where an oral feed (bottle or breast) was attempted prior to a tube feed will not be included in the totals as the baby will have received a smell and taste exposure during the oral component of the feed. In the first few days after birth when there is limited breastmilk and a very small amount of milk is given orally (eg 0.3 mL), this will be recorded as a smell/taste.

For all reporting of neonatal nutrition and growth outcomes, we will use the StRoNNG checklist (Standardised Reporting of Neonatal Nutrition and Growth) (Cormack 2016).

5.6 Study Withdrawal

A study withdrawal form was completed for any baby who was randomised but withdrawn from the study. Reasons for participant withdrawal include parents or clinician's decision, or due to serious adverse event. Ongoing consent to collect data from routine neonatal intensive care and follow up will be sought from the mother or main caregiver. The baby will be included in the ITT population if consent is given.

5.7 Protocol Deviation

A protocol deviation form will be recorded during the trial if any of the randomised babies have the following protocol deviations: randomised in error, consent error, incorrect intervention administered, deviation from

allocated intervention(s) based on medical team and/or parental decisions, and other deviations that the principal or local investigator believes should be recorded.

The DIAMOND Trial Steering Group have agreed that starting additional nutrition (formula or P100) in babies not randomised to those conditions which occurs on Day 5 or beyond will not be considered a protocol deviation as, at some point, all babies who do not receive sufficient breastmilk will require supplementation and this is a pragmatic trial. Therefore, these will instead be considered appropriate clinical decisions. Day 5 has been chosen as the cut-off based on data from a recent survey of clinicians' practices around nutrition in moderate-late preterm babies, including their willingness to wait for sufficient breastmilk in babies for whom breastmilk supply does not match demand before charting additional nutrition (Alexander and Bloomfield 2018).

6. Results and Reports

Statistical analysis will be conducted at the end of the trial, when all randomised babies completed their follow up at 4 months' corrected age (primary outcome). Regular data quality checks will be conducted during the trial, in a blinded manner, by the trial statistician and data manager.

The final trial results will be presented in tables and figures, and the templates will be prepared by the trial statistician and sent to the trial steering committee for approval before the data lock. The final study report will follow the CONSORT 2010 guidelines for randomised controlled trials, with modification to the factorial design.

7. Result Tables

Table 1. Baseline demographics and clinical characteristics of mothers and babies

	Parenteral nutrition		**Milk supplement**		**Taste and smell**	
	Yes	**No**	**Yes**	**No**	**Yes**	**No**
For Mother:						
Maternal age (in years)						
Maternal ethnicity						
Māori						
Pacific Islander						
Asian						
Caucasian or other						
Maternal education						
Lower secondary education or lower						
Upper secondary education/post-secondary						
University degree						
Other/Unknown						
NZ Deprivation Index (2018)						
Quintile 1						
Quintile 2						
Quintile 3						
Quintile 4						
Quintile 5						
Antenatal corticosteroids (any)						
Maternal diabetes						

Caesarean section						
For Baby:						
Hospital						
Auckland						
Middlemore						
Palmerston North						
North Shore						
Waitakere						
Gestation (in weeks)						
Preterm						
Moderate (32^{+0} to 33^{+6})						
Late (34^{+0} to 35^{+6})						
Sex						
Female						
Male						
Plural						
Singleton						
Twin						
Triplet						
Birthweight (g)						
Birthweight z-score						
Birth length (cm)						
Birth length z-score						
Birth head circumference (cm)						
Birth head circumference z-score						

*Data are n, means (SD) or numbers (%).

Table 2. Protocol deviations in eligible babies recruited

	Parenteral nutrition		**Milk supplement**		**Taste and smell**	
	Yes	**No**	**Yes**	**No**	**Yes**	**No**
Incorrect intervention administered in error						
Deviation from allocated intervention(s) based on medical team decision						
Deviation from allocated intervention(s) based on parental decision						
TOTAL						

Table 3. Primary and related outcomes

Body composition (Peapod) at 4 months' corrected age	**Parenteral nutrition**			**Milk supplement**		
	Yes	**No**	**Treatment Difference**	**Yes**	**No**	**Treatment Difference**
% fat mass						
Fat mass (Kg)						
Fat mass z-score						
Fat mass index (Kg/m^2)						

% fat free mass						
Fat free mass (Kg)						
Fat free mass z-score						
Fat free mass index (Kg/m^2)						

* Numbers observed, mean (SD) are reported for each group; Treatment difference is reported as mean difference with 95% confidence interval and p-value, adjusting for co-intervention(s), hospital, gestational age and sex.
** The same outcome measures will be analysed for the intervention not included in the primary analysis (taste and smell).

Table 3. Primary and related outcomes (cont.)

Feeds	**Taste and smell**		
	Yes	**No**	**Treatment Difference**
Time to full enteral feeds, days			
Time to full sucking feeds, days			

* Numbers observed, median (IQR) are reported for each group; Treatment difference is reported as hazard ratio with 95% confidence interval and p-value, adjusting for co-intervention(s), hospital, gestational age and sex.
** The same outcome measures will be analysed for the interventions not included in the primary analysis (parenteral nutrition and milk supplement).

Table 4. Secondary outcomes

	Parenteral nutrition			**Milk supplement**			**Taste and smell**		
	Yes	**No**	**Treatment Difference**	**Yes**	**No**	**Treatment Difference**	**Yes**	**No**	**Treatment Difference**
Number of days in hospital									
Type of feeding at discharge:									
Exclusively breastfed									
Exclusively bottle fed									
Complete tube feeds									
Complete intravenous nutrition									
Partially breastfed and partially bottle/cup fed									
Partially breastfed and partially tube fed									
Partially bottle/cup fed and partially tube fed									
Partially tube, bottle/cup and breast fed									
Partially intravenous nutrition and partially other feeds									
Breastfeeding at 4 months									
Body composition (Peapod) at discharge:									
% fat mass									
Fat mass (Kg)									
Fat mass z-score									
Fat mass index (Kg/m^2)									
% fat free mass									
Fat free mass (Kg)									

Fat free mass z-score									
Fat free mass index (Kg/m^2)									
Growth measurements:									
At Day 7:									
Weight (g)									
Weight z-score									
Change in weight z-score from birth									
Length (cm)									
Length z-score									
Change in length z-score from birth									
Head circumference (HC) (cm)									
HC z-score									
Change in HC z-score from birth									
At Day 14:									
Weight (g)									
Weight z-score									
Change in weight z-score from birth									
Length (cm)									
Length z-score									
Change in length z-score from birth									
Head circumference (HC) (cm)									
HC z-score									
Change in HC z-score from birth									
At Day 21:									
Weight (g)									
Weight z-score									
Change in weight z-score from birth									
Length (cm)									
Length z-score									
Change in length z-score from birth									
Head circumference (HC) (cm)									
HC z-score									
Change in HC z-score from birth									
At Day 28:									
Weight (g)									
Weight z-score									
Change in weight z-score from birth									
Length (cm)									
Length z-score									
Change in length z-score from birth									
Head circumference (HC) (cm)									
HC z-score									
Change in HC z-score from birth									
At Discharge:									
Weight (g)									
Weight z-score									
Change in weight z-score from birth									
Length (cm)									
Length z-score									
Change in length z-score from birth									

Head circumference (HC) (cm)									
HC z-score									
Change in HC z-score from birth									
At 4 months' corrected age:									
Weight (g)									
Weight z-score									
Change in weight z-score from birth									
Length (cm)									
Length z-score									
Change in length z-score from birth									
Head circumference (HC) (cm)									
HC z-score									
Change in HC z-score from birth									
Waist circumference (WC) (cm)									
Mid-arm circumference (AC) (cm)									
AC z-score									

Table 4. Secondary outcomes (cont.)

	Parenteral nutrition			**Milk supplement**			**Taste and smell**		
	Yes	**No**	**Treatment Difference**	**Yes**	**No**	**Treatment Difference**	**Yes**	**No**	**Treatment Difference**
Total nutritional intake:									
Total fluid (mL.Kg^{-1}.day^{-1})									
Week 1									
Week 2									
Energy (kcal.Kg^{-1}.day^{-1})									
Week 1									
Week 2									
Protein (g.Kg^{-1}.day^{-1})									
Week 1									
Week 2									
Fat (g.Kg^{-1}.day^{-1})									
Week 1									
Week 2									
Carbohydrate (g.Kg^{-1}.day^{-1})									
Week 1									
Week 2									
Intravenous nutritional intake:									
Energy (kcal.Kg^{-1}.day^{-1})									
Week 1									
Week 2									
Protein (g.Kg^{-1}.day^{-1})									
Week 1									
Week 2									
Fat (g.Kg^{-1}.day^{-1})									
Week 1									
Week 2									
Carbohydrate (g.Kg^{-1}.day^{-1})									

Week 1									
Week 2									
Enteral nutritional intake:									
Energy (kcal.Kg^{-1}.day^{-1})									
Week 1									
Week 2									
Protein (g.Kg^{-1}.day^{-1})									
Week 1									
Week 2									
Fat (g.Kg^{-1}.day^{-1})									
Week 1									
Week 2									
Carbohydrate (g.Kg^{-1}.day^{-1})									
Week 1									
Week 2									
Intravenous fluids:									
Amino acid (mL.Kg^{-1}.day^{-1})									
Week 1									
Week 2									
Dextrose (mL.Kg^{-1}.day^{-1})									
Week 1									
Week 2									
Lipid (mL.Kg^{-1}.day^{-1})									
Week 1									
Week 2									
Other IV fluids (mL.Kg^{-1}.day^{-1})									
Week 1									
Week 2									
Enteral fluids:									
Smell and taste provided (feeds per day)									
Week 1									
Week 2									
Birth to discharge									
Donor EBM (mL.Kg^{-1}.day^{-1})									
Week 1									
Week 2									
Mature Fortified EBM (mL.Kg^{-1}.day^{-1})									
Week 1									
Week 2									
Transitional Fortified EBM (mL.Kg^{-1}.day^{-1})									
Week 1									
Week 2									
Preterm formula (mL.Kg^{-1}.day^{-1})									
Week 1									
Week 2									

Term formula ($mL.Kg^{-1}.day^{-1}$)									
Week 1									
Week 2									

Table 5: Skinfold measurements

	Parenteral nutrition			Milk supplement			Taste and smell		
	Yes	**No**	**Treatment Difference**	**Yes**	**No**	**Treatment Difference**	**Yes**	**No**	**Treatment Difference**
At discharge:									
Abdominal Skinfold (mm)									
Biceps Skinfold (mm)									
Subscapular Skinfold (mm)									
Suprailiac Skinfold (mm)									
Thigh Skinfold (mm)									
Triceps Skinfold (mm)									
At 4 months' corrected age:									
Abdominal Skinfold (mm)									
Biceps Skinfold (mm)									
Subscapular Skinfold (mm)									
Subscapular Skinfold z-score									
Suprailiac Skinfold (mm)									
Thigh Skinfold (mm)									
Triceps Skinfold (mm)									
Triceps Skinfold z-score									

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