Study Protocol

Short title

PREMFOOD: PREterM Formula Or Donor breast milk for preterm babies

Long Title

A pilot randomised controlled trial of fortified Human Donor Milk, unfortified Human Donor Milk or Preterm Formula in preterm babies, to make up any shortfall in the volume of Mother's Own Milk.

Clinical Trials.gov NCT: 01686477

Research Ethics Approval No: 12/LO/1391

Contents

1.Protocol summary

Title	A pilot randomised controlled trial of fortified Human Donor Milk (HDM), unfortified HDM, or Preterm Formula (PTF) in preterm babies, to make up any shortfall in the volume of Mother's Own Milk (MOM)
Study Centre	Chelsea and Westminster Hospital Neonatal Unit
Study Objectives	1. To assess the feasibility of a multicentre trial addressing short term (Necrotising Enterocolitis and Bloodstream Infection) and long term (growth and metabolic) outcomes 2. To address the null hypothesis: there are no differences in total body adiposity, as measured by whole body MRI, between any groups of infants randomised to receive unfortified MOM+/-HDM, fortified MOM+/-HDM or MOM+/-PTF 3. To assess the macronutrient content of HDM given to each infant in the HDM arms by analysis of HDM samples
Study Design	Single centre unblinded randomised controlled trial
Study Population	**Inclusion Criteria** • Preterm infants born between 25+0 to 31+6 weeks gestational age **Exclusion Criteria** • Major congenital or life threatening abnormalities or congenital abnormalities that preclude early milk feeding • Inability to randomise infant within 48 hours
Intervention	Either 1. HDM, fortified with standard commercial multicomponent breast milk fortifier, once enteral intake of 150ml/kg/d reached, to supplement MOM Or 2. Unfortified HDM to supplement MOM
Comparator	PTF to supplement MOM
Target number of patients	84 (28 in each group)
Randomisation	• Using minimisation • Stratifying factors are: gestational age (25-27+6 w and 28-31+6 w), birth weight z score (<-1.28, >-1.28) and sex
Primary outcomes	• Total body adiposity, as measured by whole body MRI, at term equivalent age
Secondary outcomes	• Total body adiposity, as measured by whole body MRI at term plus 6 weeks corrected gestational age • Regional adiposity, as measured by whole body MRI • Non adipose tissue, as measured by whole body MRI • Anthropometry (weight, length, and head circumference) • Intrahepatocellular Lipid, as measured by magnetic resonance spectroscopy • Blood pressure • Stool and urine metabolomic profile • MicroRNA profile • Fasting blood Quantitative Insulin Sensitivity Check Index at term equivalent age
Duration of study	Recruitment: 18 months End of trial: Up to 24 months

2. Background

2.1 Summary

Mother's Own Milk (MOM) is believed to confer many benefits for preterm babies. However, on average, mothers giving birth preterm are able to provide less than half their baby's milk requirements. In these circumstances standard clinical practice is to make up the shortfall in MOM with either pasteurised Human Donor Milk (HDM) or Preterm Formula (PTF). Pasteurisation reduces or destroys many biologically active components; human milk, unlike PTF, is very variable in composition and despite fortification, there are concerns that preterm macronutrient requirements are not met with HDM. Doctors who use HDM to supplement insufficient MOM, do so primarily in the hope that despite pasteurisation of HDM, this strategy will reduce two feared conditions, bloodstream infection and necrotising enterocolitis, a serious inflammatory disease involving bacterial invasion of the bowel. The mortality from these conditions is high and survivors are at substantially increased risk of neurodisability. Metabolic advantages of human milk for preterm infants may also exist. Follow-up of preterm babies recruited to landmark nutritional trials in the early 1980s suggest positive effects of human milk on insulin sensitivity, blood pressure, and other metabolic outcomes. Doctors who prefer PTF to supplement insufficient MOM, believe this will benefit growth, including brain growth, and long-term neurodevelopment. Which of these two approaches is more efficacious and effective is unknown.

In order to address this crucial question, central to preterm newborn care, a multicentre UK-wide study randomising 4000 preterm babies would be necessary to achieve sufficient power to evaluate the impact on the short-term outcomes necrotising enterocolitis and bloodstream infection, and establish cohorts with power to address long-term growth and metabolic outcomes.

The objective of this PhD proposal is to conduct a pilot study to inform an application to the NIHR or other major funding body to conduct a randomised controlled trial designed to address cardinal short-term (necrotising enterocolitis, bloodstream infection) and longer-term outcomes (growth, obesity, insulin resistance), that would require the recruitment of approximately 4000 babies across sites throughout the UK. This pilot trial will not involve any novel intervention, it will merely randomise babies to three existing, widely used, standard feeding practices.

2.2 The knowledge gap

2.2.1 Human milk versus preterm formula: Necrotising Enterocolitis

Human milk not only provides nutrition, but is rich in biologically active molecules with advantages to infant host defence. Large nutritional trials of MOM versus PTF in the late 1980's showed that infants who received MOM were 5-10 times less likely to develop necrotising enterocolitis (NEC) than infants fed with PTF, where both MOM and PTF were sole diet (1). A meta-analysis of seven trials in the 1970's and 1980's each comparing HDM with PTF for preterm infants (2), suggested that HDM given as a sole diet is associated with a lower risk of NEC compared with formula. However, in these trials, the total sample size was small, the methodological quality poor, and their nutritional protocols did not evaluate the currently widely used bovine human milk fortifiers, or contemporary preterm formulas. A more recent Cochrane meta-analysis has failed to show any such protection against NEC with HDM compared to PTF when used to supplement MOM (3). Furthermore, only one trial has ever compared bovine fortified

HDM with PTF, in which no significant differences in mortality or NEC was found (4). These uncertainties are reflected in the varying attitudes of staff, parents and countries to HDM (5). The lack of good evidence of efficacy of HDM led the National Institute for Clinical Excellence to refrain from advising on clinical indications in their guidance "Donor breast milk banks: the operation of donor milk bank services" (NICE 2010).

2.2.2 Human milk versus preterm formula: metabolic consequences

The association between low birth weight and later adult metabolic disease such as type-2 diabetes is now well established (6,7). Epidemiological studies show an association between low birth weight and decreased lean mass, increased total adiposity, and increased central adiposity in adults (8) ,a body composition which has consistently been linked with later insulin resistance (9). Third trimester and early postnatal growth appears to have a major impact on this later adult metabolic phenotype. Longitudinal growth data indicate that those born small for gestational age but then show catch up growth, specifically from birth to 3 months, are most at risk of later insulin resistance in young adulthood (10)

Prematurity, regardless of fetal growth, has been associated with later insulin resistance in adults (11). Using whole body MRI, our group has shown that the preterm born adult phenotype is characterised by increased total and abdominal adiposity compared to term born controls (12). As early as at term equivalent age we have shown that there is aberrant deposition of adipose tissue, with an excess of intra-abdominal adipose tissue, and reduced lean body mass in preterm infants in comparison to their term-born counterparts, with illness severity being the major determinant of adiposity (13). Although the neonatal morbidities associated with preterm birth may well contribute to long term metabolic health, mounting evidence highlights the impact of early growth velocity during postnatal third trimester growth.

Follow-up to early adulthood of preterm babies recruited to landmark nutritional trials in the early 1980s by Lucas and colleagues showed lower insulin resistance at adolescence in preterm infants randomised to receive standard formula compared to a growth promoting enriched formula (14) , those infants with the greatest growth velocity in the first 2 weeks of life having the greatest insulin resistance at adolescence. Consistent with these findings, Roteveel et al have recently shown that preterm infants who had highest early postnatal growth velocities had highest insulin resistance as young adults regardless of prior fetal growth (15).

In parallel trials Lucas and colleagues showed positive effects of human milk on insulin sensitivity, blood pressure, and other metabolic outcomes (16-19). Whether the observed metabolic advantages with human milk are due to its unique bioactive properties, or result from a protective slower early growth as compared to preterm formula, remain unclear (20). This pilot trial will begin to address this question by comparing fortified and unfortified HDM with PTF.

Increased adiposity could be a plausible mechanism by which manipulation of early postnatal nutrition may predispose to poor metabolic health in preterm born adults. Nutritional differences are key causal candidates for poor metabolic health: the diet of the preterm neonates in intensive care is high in fat and carbohydrate and low in protein. This is in marked contrast to third trimester intrauterine diet which is high in amino acids, moderate in glucose and low in lipid (21). But within this preterm diet, human milk may attenuate this adiposity: an observational study of an indirect measurement of body composition using dual energy x-ray absorptiometry (22) has shown increased fat mass in preterm babies at discharge when PTF was used compared to fortified HDM, in supplementing MOM. Using dual energy x-ray absorptiometry in a cohort of preterm infants recruited just before discharge, Cooke et al (23) showed that

infants randomised to receive PTF or term formula had increased total non-fat and fat mass but not percentage fat mass, at 3,6, and 12 months corrected age, compared to breast fed preterm controls. In contrast to the pattern of adiposity that we observed, changes in fat mass were explained by increased fat accretion in the legs rather than centrally. However, no studies to date have examined the impact of feeding preterm infants from birth, on changes in adiposity quantity and distribution, at discharge and post-discharge.

Emergence of insulin resistance has been shown to parallel that of central adiposity at age 2-4yrs in SGA infants who show catch up growth (24). Insulin resistance in this growth pattern correlates with growth velocity immediately after birth (10) and has been shown as early as 1yr of age, in a cohort of SGA infants who gained one birth centile in weight in the first year (25). The "gold standard" for measuring insulin resistance is the euglycaemic hyperinsulinaemic insulin-glucose clamp technique which cannot be used during infancy, but a surrogate marker of insulin resistance, a logarithmic transformation of the fasting insulin and glucose concentration (Quantitative Insulin-sensitivity Check Index, QUICKI (26) has been validated in children and adults, and has been measured in preterm infants shortly after birth in a handful of studies (27,28). Yet as far as we know there are no prospective studies assessing direct measurements of adipose tissue distribution with growth and markers of insulin resistance in the first few weeks and months of life.

Our group has also shown increased intrahepatocellular lipid (IHCL) content in preterm infants at term compared to healthy term infants (29). Raised IHCL is closely associated with abdominal adiposity (30), obesity, and dyslipidaemia. Recent work by Nobili et al (31), showed that breast feeding had a protective effect, in a dose dependent manor, on progression of non-alcoholic fatty liver disease in a sample of children diagnosed with the disease in a paediatric liver unit. Although there are several possible determinants of raised IHCL in the preterm infant, the impact of human milk warrants further investigation.

Metabolic "profiles" can now be measured using small volumes of biological tissues and fluids, such as dried blood spots, urine and stool. Analysis is carried out using high throughput nuclear magnetic resonance (NMR) spectroscopy, which provides the opportunity to analyse a large number of metabolites simultaneously and therefore offers extensive scope to study metabolic responses in relation to normal development, disease, physiological variation, nutritional intake and medications (32). A small number of reports describe metabolic profiling in newborns and demonstrate the feasibility of this technique in detecting differences in metabolic responses to preterm birth (33).

Yet there remains no randomised controlled enteral nutritional trial in preterm infants from birth to discharge and beyond with direct measures of body composition, QUICKI, IHCL or metabolomic profiling as the outcome. Furthermore, no long term follow up of such early preterm body composition, IHCL and metabolomic phenotypes exist, making any nutritional mechanistic pathways to poor adult metabolic health difficult to define.

2.2.3 MicroRNA profiling and metabolic disease

MicroRNAs (miRNAs) are small 22–25-nt-long noncoding RNA molecules that negatively regulate translation of target messenger RNAs. miRNAs normally bind to their target messenger RNA (mRNA) leading to translation inhibition and/or mRNA degradation (34). Over 500 miRNAs have been found in the human genome (35), and it has been estimated that they could regulate 74–92% of all protein coding mRNAs (36). By affecting gene regulation, miRNAs are likely to be involved in most biological processes, and aberrant expression of miRNAs has been implicated in numerous disease states including insulin resistance and type 2 diabetes. Several studies have identified

dysregulation of several miRNAs in animal and human type 2 diabetes, in insulin sensitive tissues such as adipose tissue (37). The discovery of circulating miRNAs has highlighted their potential as both endocrine signalling molecules and disease markers (38). MicroRNA expression has been implicated in the complex interplay between genetic susceptibility to disease and environmental factors. We aim to undertake the novel exploration into the impact of early nutrition on microRNA expression and correlate this with body composition, by global microRNA profiling at the time of discharge.

2.2.4 Human milk versus preterm formula: neurodevelopmental consequences

Accumulating evidence shows that poor early postnatal growth is associated with impaired neurodevelopment in preterm infants (39-42). Suboptimal nutritional intake and subsequent poor growth including brain growth has lasting consequences in this sensitive period. Follow-up studies showed that at 7 years of age, preterm infants randomised to receive standard formula from birth to discharge, demonstrated neurocognitive impairment with a significant reduction in IQ compared with infants randomised to receive enriched formula (39). Despite slower growth in breast milk fed preterm infants, there is a growing body of evidence which suggests that neurocognitive outcome is better. Long-term studies at 8 years of age through adolescence suggest that intelligence test results and white matter and total brain volumes are greater in subjects who had received human milk as infants in the neonatal unit.(39,43). Extremely preterm infants receiving the greatest proportion of human milk in the neonatal unit had significantly greater scores for mental, motor, and behaviour scores at ages 18 months and 30 months. (44,45) These data remain significant after adjustment for confounding factors, such as maternal age, education, marital status, race, and infant morbidities.

Evidence for neurodevelopmental advantage in the use of HDM used as sole diet or to supplement MOM, compared to PTF however, is less clear. In a meta-analysis of donor breast milk versus preterm formula for preterm infants, only one trial compared neurodevelopment as the outcome, and there were no significant differences in neurodevelopmental status at 9 or 18 months either used as sole diet or to supplement MOM (3). Indeed, the use of HDM, even with fortification, is consistently associated with slower growth compared to PTF (3, 4). The question of whether HDM has similar cognitive advantages as MOM, despite pasteurisation, and slower associated growth velocity compared to PTF, is worthy of further investigation.

2.2.5 Use of bovine fortifier for human milk

A Cochrane review (46) has shown that bovine multicomponent fortification of human milk in comparison to the unfortified human milk, improved short-term growth, but had no long-term advantage in terms of either growth or neurodevelopment, nor any clear effect on bone mineral content, and was not associated with clinically significant adverse effects. Fortification of human milk for preterm infants is now widely practiced, yet there exists widespread variation in its indication, method of initiation and indication for stopping (5). This reflects the lack of good evidence for optimal use.

Comparisons between fortified HM and PTF indicate that despite fortification, human milk fed preterm infants continue to grow slower than PTF fed infants (47, 48). Accumulating evidence suggests that a continuing protein deficit is primarily responsible for slow preterm growth (47,49) and there is growing concern that preterm protein requirements to maintain intrauterine growth rates and overcome catabolic deficits, is not being met (50). Recent ESPGHAN (2010) (51) guidelines have reflected this, recommending up to 4.5g/kg/d of protein for ELBW infants. Although a sole diet of preterm formula could match this intake, the assessment of protein intake from human milk is inherently difficult. The protein concentration of human milk decreases with gestational age and

duration of lactation but also varies unpredictably from sample to sample (52, 53). Furthermore, HDM is likely to have an even lower protein concentration than that from MOM as it is more likely to come from mothers of term infants. However, there is limited data on the macronutrient content of HDM, and we propose to analyse the macronutrient content of all HDM that each infant in the study receives, thus providing an accurate as possible nutritional intake for each interventional arm.

2.3 Bridging the gap

To begin to address these questions this feasibility pilot trial will assess growth and key candidate markers of long term metabolic health such as body composition, blood pressure, hepatic lipid content, QUICKI, metabolomic profile, in preterm infants randomised to receive PTF, fortified or unfortified HDM when there is a shortfall of MOM. An estimation of macronutrient content of HDM will be made by analysis of all HDM given to each infant in the HDM arm. To our best knowledge through wide professional and academic contacts, and searches of international registers, no trials similar to that which we propose are being undertaken in the UK or elsewhere.

2.4 Importance to the NHS

Infant nutrition studies involving randomisation, testing causality and unravelling biological mechanisms are rare. Yet infant nutrition is a cardinal candidate effector of long-term health. Current practice in feeding the preterm infant invariably involves the use of fortified Human Donor Milk or Preterm Formula. There are however no national standards or guidelines for enteral feeding of extremely preterm infants, and clinical practice is very variable.

Advocates of pasteurised HDM believe this is as efficacious as MOM in reducing two of the most feared conditions in newborn medicine, necrotising enterocolitis and bloodstream infection, and in improving long-term metabolic health. These result in major health services utilisation costs and a life-long burden of impairments on survivors.

An adequately powered trial is required to address these outcomes but poses two major hurdles, recruitment of large numbers of babies, and long-term follow-up. The organisation of NHS neonatal specialist care and the establishment of the Neonatal Data Analysis Unit that holds and archives national neonatal data at Chelsea & Westminster Hospital, and has received permission for the National Information Governance Board to link this to Hospital Episode statistics data, offers unique international opportunity to conduct this trial in the UK and achieve long term follow-up at low cost. Clarification of efficacy will advance clinical care, and enable economic evaluation and rational development of Human Milk Banking**.**

3. Aims and Hypothesis

Specific aims of this pilot trial are to:

A: Assess feasibility
1) test the acceptability of the trial design with parents and clinicians
2) test that recruitment to target is achievable, estimate consent and dropout rates
3) estimate the trial requirement for HDM from NHS Human Milk Banks
4) test that clinical outcome data can be retrieved from operational NHS electronic records, thus minimising the burden of data capture and facilitating the use of linked NHS records to achieve long-term follow-up at low cost.

B: Obtain data on the magnitude of effect at term equivalent age and again at term plus 6 weeks of age, on key mechanistic outcomes:

1) the primary outcome of total body adiposity, addressing the null hypothesis: there are no differences in total body adiposity, as measured by whole body MRI, between infants randomised to receive fortified MOM+/-HDM, unfortified MOM+/-HDM, or MOM+/-PTF.
2) the secondary outcomes of
   - Regional adiposity, as measured by whole body MRI
   - Non adipose tissue, as measured by whole body MRI
   - Total brain volume, as measured by MRI
   - Anthropometry (weight, length, and head circumference)
   - IHCL, as measured by magnetic resonance spectroscopy
   - blood pressure
   - Stool and urine metabolomic profile
   - Blood Quantitative Insulin Sensitivity Check Index (QUICKI) – at discharge

only between infants randomised to receive fortified MOM+/-HDM, unfortified MOM+/- HDM or MOM+/-PTF.

C: By using HDM with known macronutrient content, we aim to correlate macronutrient intake of each intervention with the above outcomes.

4. Methods

4.1 Design

This will be a pilot single centre randomised controlled trial. There will be no novel interventions, merely randomisation of infants to three existing, widely used, standard feeding practices. For the purposes of clarity, this study will be in two parts. The first part will involve an opt out of feeding randomisation. Eligible preterm infants will be randomised by 48 hours to receive unfortified Mother's Own Milk (MOM) +/- Human Donor Milk (HDM), fortified MOM +/- HDM, or MOM +/- Preterm Formula (PTF), where HDM or PTF are used to make up any shortfall in MOM. Anthropometric and blood pressure outcomes will be determined from routinely collected clinical data. The second part will involve informed consent for collection and analysis of urine, stool, and blood samples and magnetic resonance imaging scans. The flowchart illustrates exactly what is involved if an infant is in the study.

4.2 Study Flowchart

Please see page 12

4.3 Patient Population:

Preterm infants born between 25+0 and 31+6 weeks gestational age.

4.3.1 Inclusion Criteria

- Preterm infants whose mothers are resident within the north west neonatal network, born between 25+0 to 31+6 weeks gestational age

4.3.2 Exclusion Criteria

- Major congenital or life threatening abnormalities or congenital abnormalities that preclude early milk feeding
- Inability to randomise infant within 48 hours
- Infants less than 25+0 weeks or more than 31+6 weeks

4.4 Screening and enrolment

For part one of the study, we plan to randomise all eligible infants, unless explicitly requested by the parents for their baby not to take part. Where possible parents will be approached before delivery, and the study explained to them eg for mothers at risk of premature delivery. In all cases, the study will be explained to the parents by one of the clinical team or study investigators shortly after birth, and a written parent information leaflet (PIL pt 1) will be given to the parents. This process will be documented in the infant's medical notes by the person explaining the study, with a confirmation sticker. Randomisation to one of the three feeding arms will take place between 24-48hrs of age.

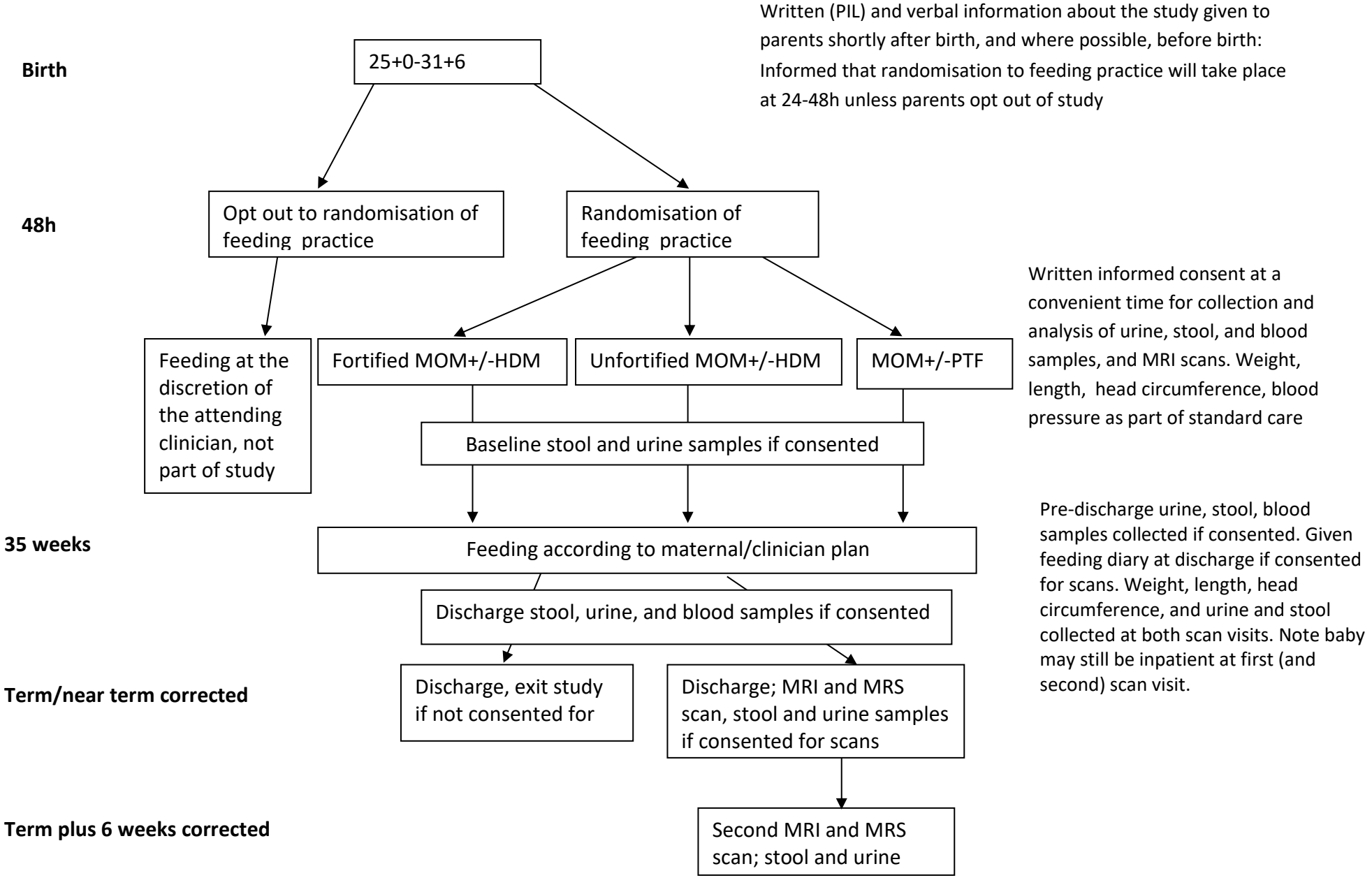
For part two of the study, parents will be approached by one of the clinical team or the study investigators after 48hrs of age, and part two of the study will be explained to them, with a written parent information leaflet (PIL pt 2) given to them. At a convenient time for the parents, informed consent will then be sought for part 2 of the study. All infants will be recruited from the Chelsea and Westminster Hospital Neonatal Unit.

4.5 Explanation and informed consent for the study

Explanation and informed consent requires individual discussion with the infant's parents about the nature of the research to be conducted in a language that is easy to comprehend. The parents will have the study verbally explained to them and also be given a written information sheet about the study. The parents will have sufficient time to think about the study and ask any further questions regarding it. It will be stressed to parents that their babies will follow one of three, widely used, standard feeding practices, and there will be no new treatments or interventions. The parents will also understand that refusal to participate in the study will not affect the quality of subsequent medical care and if they do consent to participate they may withdraw at any point without affecting their infant's care.

Before any trial-related procedures may be performed, informed consent must be obtained from the infant's parents by the investigator by means of a signed declaration. The investigator must record in the case report form to confirm that informed consent was obtained and store the original of the signed declaration of consent in the patient's notes. A copy will be given to the infant's parents and a copy filed in the investigator file.

4.1 PREMFOOD Flowchart



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4.6 Randomisation

Randomisation will be generated centrally using a computer program, with minimisation by infant sex, gestational age (25-27+6 wks, 28-31+6 wks) birth-weight Z score (<-1.28, >-1.28) Randomisation will take place between 24-48hrs of age. Infants will be randomised to receive either HDM or PTF to make up any shortfall in Mother's Own Milk (MOM) until 34+6 weeks postmenstrual age. Blinding of the intervention is not practicable because of the distinctive appearances of human milk and formula, and the method of administration to preterm babies by syringe and nasogastric tube. At 35 weeks, the infant will be close to discharge, and feeding will be directly from the breast or formula by bottle in accordance with maternal choice.

4.7 Withdrawals and protocol violations

4.7.1 Temporary discontinuation of feeds/alternative feeds

Infants in the trial may develop feed intolerance or suspected/confirmed necrotising enterocolitis (NEC) requiring the cessation of enteral feeds. The period of no feeding may vary from a few hours to a number of days. Once feeding is re-established, the infant will continue with the allocated interventional arm. However, there may be circumstances in which continuation of the allocated feed is deemed inappropriate by the clinical team. Examples would include feed intolerance and the use of hydrolysed or elemental feeds. Infants would remain in the study and included in the "intention-to-treat analysis."

4.7.2 Withdrawal of infant from trial procedures and incomplete follow-up

It is possible that parents may choose to withdraw their infant from trial procedures resulting in incomplete patient follow up and failure to capture outcome data. Likewise, if an infant dies before discharge or is still on the neonatal unit and considered too unstable at term equivalent age or term plus 6 weeks, they will not have their MRI scan.

4.8 Trial intervention

4.8.1 Description

The interventions will be fortified, or unfortified pasteurised Human Donor Milk (HDM) with the comparator Preterm Formula (PTF) to make up any shortfall in Mother's Own Milk (MOM).

4.8.2 Feeding schedule

The trial is designed to reflect current clinical practice. Preterm infants will be introduced to parenteral nutrition and enteral feeds within the first 24 hours of birth. Milk volumes will be increased up to 200ml/kg/day in accordance with a standard protocol modified if necessary in accordance with infant tolerance; parenteral intake simultaneously decreased. For infants randomised to fortified MOM± HDM, fortification will begin when a total enteral intake of 100ml/kg/day is reached and continued to 34+6 weeks postmenstrual age. At this point feeding will progress in accordance with maternal choice.

4.8.3 Pasteurised Human Donor Milk

The Chelsea and Westminster Hospital neonatal unit obtains all HDM from the Countess of Chester Human Milk Bank. The milk bank follows the recommendations for donor screening, pasteurisation, milk collection and storage published by NICE 2010 (54). All HDM supplied has been analysed for macronutrient content using infrared spectrometry done at the Countess of Chester Human Milk Bank. All bottles will be labelled with their macronutrient content and daily intakes recorded on the clinical case report form. If an infant is allocated to the HDM intervention, then the instruction only to use HDM to supplement MOM will be made on the infants feeding plan.

4.8.4 Fortified Human Milk

For infants randomised to receive fortified HDM to supplement MOM, all milk will be fortified once a total enteral intake of 150ml/kg/d is reached. This will be with a multicomponent bovine breast milk fortifier (Cow and Gate Nutriprem Breast Milk Fortifier). The amount used will be in accordance with current practice, namely two sachets per 100ml.

4.8.5 Unfortified Human Milk

For infants randomised to receive unfortified human milk the instruction to only use unfortified HDM to supplement unfortified MOM will be made on the infants feeding plan. For these infants, the situation may arise where the growth of the infants is deemed suboptimal by the clinical team. Therefore, weight gain will be assessed two weeks after a milk volume of 120ml/kg/d has been reached. At this point, if the infant has crossed down 3 centile bandwidths from birthweight centile bandwidth, or shows continued downward crossing if less than the 0.4th centile, using birthweight centile charts, then the

infant will discontinue the feeding intervention. There may be other causes of poor weight gain than inadequate enteral nutrition. These include for example, respiratory distress syndrome, infection, chronic lung disease, gastro-oesophageal reflux disease, anaemia, patent ductus arteriosus, or underlying congenital anomaly. Once these causes have been excluded or corrected, and the weight loss has been confirmed from at least two weights over a 72 hour period, fortification will be commenced. Infants will then receive fortified HDM, and undergo all outcome measures. Analysis will be as per protocol, and also for categories of overall intake (see statistical analysis).

This three centile drop cut off value is based on results of a longitudinal preterm growth reference by Cole et al (submitted). This is a study of routinely collected growth data (co-ordinated by the Neonatal Data Analysis Unit) from 113 neonatal units across the UK from 2006-2011. A growth reference for weight, length, and head circumference in preterm infants less than 32 weeks from birth to term/near term was constructed, stratified for birth gestation week, and compared to currently used birthweight centile charts. In this comparison, average growth curves for all infants by week of birth gestation, crossed down 2-3 birthweight centile bandwidths over a period of 3-4 weeks from birth, before becoming parallel to the birthweight centile bandwidth curves. Time taken to reach full milk feeds will vary depending on feeding tolerance, but typically will take from a few days to a couple of weeks. A period of two weeks after full milk feeding (120ml/kg/d and above) has been reached, is therefore expected to coincide with no further crossing down in birthweight centile bandwidth curves.

4.8.6 Preterm formula

For infants randomised to receive PTF, Nutriprem 1 will be used (Cow and Gate Nutriprem 1) as this is in standard use in the Chelsea and Westminster neonatal unit. If an infant is allocated to the PTF intervention, then the instruction only to use PTF to supplement MOM will be made on the infants feeding plan.

4.8.7 Assumed intakes

For comparison purposes the following table illustrates the macronutrient daily intakes for a 1kg baby on 180ml/kg/d for different milks, based on mean macronutrient content from a large number of samples (415 sequential samples from 273 donors) of banked donor milk (55) and the widely accepted values taken from the mean of a number of studies of milk from mothers of preterm infants (56). This is compared with the ESPGHAN 2010 recommended intakes (51).

Table 1. Assumed intakes compared with recommendations

	180ml of unfortified Mother's Own Milk (MOM)	180ml of unfortified Donor milk	180ml of fortified MOM	180ml of fortified Donor milk	180ml of Nutriprem 1	ESPGHAN 2010 Recommendation unit/kg/d
Protein (g)	2.7	2.2	4.7	4.4	4.7	4.0-4.5 * 3.5-4.0**
Carbohydrate (g)	12.4	14.0	17.3	18.9	15.1	11.6-13.2
Fat (g)	6.3	5.8	6.3	5.8	7.0	4.8-6.6
Energy (kcal)	117	117	144	144	144	110-135
Protein/Energy ratio (g/100kcal)	2.3	1.9	3.3	3.1	3.3	3.6-4.1* 3.2-3.6**

*For infants <1kg
** For infants 1-1.8kg

4.9 Trial observations, tests and investigations

4.9.1 Electronic data capture

The intention is to minimise the burden of data collection by utilising the National Neonatal Database; mortality, necrotising enterocolitis, blood-stream infection, growth, breast feeding at discharge and health resource utilisation (length of stay; intensive, high dependency and special care days, parenteral nutrition use) will be evaluated to discharge in all randomised babies; these data will be retrieved from the National Neonatal Research Database; this is created at the Neonatal Data Analysis Unit from electronic records captured at the point of care on all admissions to neonatal units nationally; the Neonatal Data Analysis Unit is based at Chelsea & Westminster Hospital and led by Professor Modi. Data will be retrieved electronically from the Neonatal Data Analysis Unit except that stated otherwise below.

4.9.2 Case report form

Randomisation data and certain clinical data will be collected on a case report form which will be stored securely at the neonatal data analysis unit. Only the research team will have access to the case report form.

4.9.3 Randomisation

- Study number
- Date and time of birth
- Gender
- Confirmation of eligibility (full eligibility check)
- Date of parental consent
- Name of person taking consent
- Gestational age (in weeks and days)
- Recruiting hospital
- Allocated trial intervention (HDM or PTF)

4.9.4 Baseline evaluation

- Birth weight (kg)
- Birth length (cm)
- Head circumference (cm)
- Ethnicity (NHS ethnicity)
- Mother's date of birth
- Mother's ethnicity
- Mother's height and weight (collected on case report form)
- Father's ethnicity
- Father's height and weight (collected on case report form)
- Mode of delivery
- Use of antenatal steroids
- Diastolic blood pressure
- Systolic blood pressure
- Medical conditions and perinatal medications
- Metabolomic profile (urine and stool) after informed consent

4.9.5 Daily evaluations

Performed daily until discharge from NICU

- Record nutritional intake (parenteral nutrition, daily volume of MOM, daily volume of trial feed – all collected on case report form)
- Record level of care (BAPM 2001 criteria)
- Record if infant is nil by mouth and reason for this (collected on case report form)
- Record of withdrawal information (collected on case report form)
- Weight (when on parenteral nutrition)

4.9.6 Weekly evaluations

Performed weekly until discharge form NICU

- Weight (when not on PN)
- Length
- Head circumference
- Diastolic blood pressure
- Systolic blood pressure
- Record any episode of suspected or confirmed NEC
- Record any episode of blood stream infection (blood culture positive)

4.9.7 Pre-discharge evaluations

- Metabolomic profile (urine and stool)

Performed with routine weekly bloods as close as possible to discharge and when on 3hrly feeds:

- Blood fasting (pre feeding) insulin and glucose (QUICKI), each 0.5ml volume.
- Blood for microRNA profiling 0.5ml in volume.

4.9.8 Magnetic Resonance Investigations (MRI and MRS)

Infants will undergo two magnetic resonance investigations, according to a well-defined and well established protocol (57-59). The first investigation will be as soon as possible after discharge. To investigate whether early feeding impacts on body composition

changes which persist beyond discharge, a second scan at around a corrected gestational age of term plus 6 weeks will be performed. This protocol has been used for over a decade in a large number of preterm and term infants. The MRI measurements are carried out during normal sleep without the need for sedation. All the MRI measurements (brain volume, body composition, and hepatic MRS) take a total of about 20-25 minutes. Both quantity and distribution of adipose and non-adipose tissue will be assessed. The infants will be monitored with pulse oximetry and a trained neonatal doctor will be present throughout the scan. Parents are invited to be present in the console room.

4.9.9 Scan visits

Data will be collected at each scan visit on the following:

- Weight
- Length
- Head circumference
- Feeding data (see below)
- Urine and stool for metabolomic profile

Urine samples (1 ml, from cotton wool placed in the nappy), and stool specimens (from the nappy) will be collected immediately after recruitment and at both scan appointments. All samples will be analysed at Imperial College using high throughput techniques employing in vitro NMR. This aspect of the proposed research will be supervised by Professor Elaine Holmes, a leading international authority in the field.

4.9.10 Feeding data

At each scan visit, data will be collected on breast/expressed breast milk and formula feeding from end of feeding intervention to time of scan. Breast feeding will be defined according to the criteria recommended by Labbock et al (60) and subsequently adopted by the World Health Organisation. Feeding will be defined as:

1. Full breast/expressed breast milk feeding (including exclusive and almost exclusive)
2. Partial - High breast/expressed breast milk feeding (>80%)
3. Partial - Medium breast/expressed breast milk feeding (20-80%)
4. Partial - Low breast/expressed breast feeding (<20%)
5. Full formula feeding (including token breast/expressed breast milk feeding)

To avoid recall bias, parents will be given a simple feed diary to complete during the study period. Furthermore date of first formula feed, when first predominantly formula fed and when exclusively formula fed will be recorded. Parents will be asked about method of feeding at both scan points and at all contact points throughout the study period (eg telephone contact to arrange appointment for MR studies).

4.9.11 Schedule of investigations

Table 2. Schedule of investigations

Evaluation	**Baseline**	**Daily**	**Weekly**	**Pre-discharge**	**Scan 1**	**Scan 2**
Informed Consent	**+**					
Eligibility	**+**					
Randomisation	**+**					
Weight	**#**	**#***	**#****		**+**	**+**
Length	**#**		**#**		**+**	**+**
Head circumference	**#**		**#**		**+**	**+**
Blood pressure	**#**		**#**			
Nutritional intake/feeding data	**+**	**+**	**+**		**+**	**+**
Efficacy						
Whole body MRI, MRS					**+**	**+**
Metabolomic Profile						
Urine and stool sample	**+**			**+**	**+**	**+**
QUICKI						
Fasting blood insulin and glucose				**+**		
microRNA						
Blood				**+**		

Key
+Research evaluation **#**Routine care evaluation ***When on parenteral nutrition
****When not on parenteral nutrition

4.10 Assessment of feasibility

4.10.1 Trial design peer review

The trial protocol has had extensive peer review from the Neonatal Clinical Studies Group at the National Institute of Health Research Medicines for Children Research Network, on two separate occasions. This process has involved trial redesign and further review, resulting in favourable support for this feasibility trial.

4.10.2 Trial acceptability to parents

Trial design has also been reviewed at the parent research forum in the 'Medicines for neonates' programme – a National Institute of Health Research funded Research Programme, led by Professor Neena Modi. This programme includes several interrelated projects, one of which is 'Data sharing in Neonatal Services': understanding parents' attitudes towards the use of NHS data for research purposes in the context of neonatal services. The parent research forum involved parents who have previously had preterm

babies in the neonatal unit, and their feedback has proved invaluable in the redesign of the protocol, parent information leaflet, and consent form.

4.10.3 Trial design feedback from other sources

Trial design has also been reviewed by experts in the field by the neonatal nutrition club, and peer review from grant, and training fellowship applications. Human donor milk banks and the charity BLISS (Breastfeeding and Lactation Information Support Source) have also been involved in the trial design.

4.10.4 Recruitment to target

This feasibility study will test if recruitment to target is achievable; a conservative estimate is that 50% of eligible preterm infants will be recruited; the intention is to demonstrate that 2-4 eligible babies per month will be recruited (this would enable recruitment of 4000 babies from 40 centres over 25-50 months).

4.10.5 Trial requirement for HDM from NHS Human Milk Banks

There are 17 human milk banks in the UK. A recent survey of human milk bank service provision in the UK (61) showed that the annual median number of donor mothers to the milk bank was 48, and the annual median number of litres of donor milk pasteurised by each milk bank was 206. It is anticipated that the provision of HDM from the Countess of Chester Human milk bank will match the demand for the purposes of this pilot trial. This Human milk bank currently provides all HDM for Chelsea and Westminster Hospital Neonatal Unit. This study will provide an estimate of trial annual requirement of HDM from NHS human milk banks from data collected on HDM volume requirements of each infant in the HDM feeding arm. This estimate could then be compared to the current service provision of UK human milk banks.

4.10.6 Electronic outcome data capture

Test that outcome data can be retrieved from operational NHS electronic records, thus minimising the burden of data capture and facilitating the use of linked NHS records to achieve long-term follow-up at low cost; data will be captured as detailed above in all randomised babies to discharge. The National Neonatal Research Database is created at the Neonatal Data Analysis Unit from electronic records captured at the point of care on all admissions to neonatal units nationally; the Neonatal Data Analysis Unit is based at Chelsea & Westminster Hospital and led by Professor Modi.

4.11 Statistical considerations

4.11.1 Sample size

Statistical considerations have been overseen by the Neonatal Data Analysis Unit Statistician Ms Shalini Santhakumaran. This study is primarily a feasibility pilot trial and has been designed as such. It is unknown what constitutes a clinically relevant difference in the primary outcome total body adiposity, as measured by whole body MRI, following HDM and PTF supplemented MOM in preterm infants from birth to discharge. Indeed, one of the aims of the study is to obtain data on the magnitude of effect on total body adiposity. However, an estimate of a clinically relevant difference has been made from one previous study using a different method of body composition measurement. In

an observational study, Pieltain et al (22) investigated change in body composition, measured using dual energy x-ray absorptiometry at birth and again at discharge, in preterm infants fed with fortified HDM or PTF to supplement MOM. Whole body composition was similar in the two groups at the initial measurement, but differed significantly at the time of the second measurement. They calculated that at the 5% significance level and 80% power, the minimal detectable difference in fat mass would be 86g or 96cc between groups for a total sample size of 40 (20 in each group).

Using our group's database of preterm infants at term age equivalent (July 2012), the mean (standard deviation) of directly measured total body adiposity, using whole body MRI is 0.677(0.243)l. Although the clinically relevant difference may be less than this, we have based our sample size on detecting a one standard deviation difference. A sample size of 22 infants in each group would enable us to detect a 0.243l difference in adiposity between groups with just over 80% power and at 5% significance level. Assuming 10% mortality prior to term and 10% drop out rate, 28 infants will be recruited in to each of the three groups or until 22 infants in each group has had their MRI and MRS at term age equivalent, a total of 66 scans. Thus, over a recruitment period of 18 months, with an estimated recruitment rate of 2-4 infants per month it is realised that the study will likely be underpowered for the outcome of total body adiposity. This sample size is based on single centre recruitment. In the eventuality of one or more centres being involved in the study, the following table has been constructed to demonstrate sample sizes and powers required for detecting a range of possible clinically significant differences in total body adiposity, for which the Bonferroni correction has been used for multiple testing.

Table 3. Power to detect possible clinically relevant differences in adiposity for different sample sizes; AT=adipose tissue; l=litre

Total n	Power (%) to detect 0.096(l) diff in AT	Power (%) to detect 0.243(l) (1 sd) diff in AT	Difference (l) in AT at 80% power
60	12.8	78.1	0.249
66	14.1	82.4	0.237
75	16.1	87.5	0.222
90	19.6	93.1	0.203
96	21	94.7	0.197
105	23.1	96.4	0.188
120	26.8	98.1	0.176
135	30.4	99.1	0.166
150	34	99.6	0.157
165	37.6	99.8	0.15
180	41.2	99.9	0.144
210	48	100	0.133
225	51.3		0.129
240	54.5		0.125

4.11.2 Statistical analysis

Outcomes will be analysed both for by intention to treat and as per protocol using multiple linear regression with adjustment for covariates where appropriate. Baseline covariates such as gender, gestational age, and birthweight z score, will be controlled for by randomisation stratification, while those such as ethnicity will be adjusted for. To address the null hypothesis differences between groups will be tested using ANOVA, with examination of the 3 pairwise differences for magnitude of differences. For the purposes of modelling randomised babies will be assigned to categories of overall intake (predominantly MOM, predominantly PTF, predominantly HDM). Results will be presented as estimates and 95% confidence intervals for differences in means.

4.12 Timeline

00-06 months: development of protocol, submission for research ethics approval, submission of PhD proposal
06-09 months: establishment of trial centres
09-27 months: recruitment
09-21 months: evaluation of design
27-30 months: delivery of research protocol completed for final recruits
30-36 months: final analyses and write-up

5. Regulatory and ethical considerations

5.1 Regulatory framework

This study is a randomised trial of fortified or unfortified HDM versus PTF to supplement MOM. The fortifier used is multicomponent breast milk fortifier which is classified as a food supplement for special medical purposes and is not an investigational medicinal product, and as such is not subject to the Medicines for Human Use (Clinical Trials) Regulations 2004. This study is registered with the clinicaltrials.gov online registry and results database.

5.2 Ethical approval and monitoring

The study protocol, patient information sheet and consent form will be submitted to the Research Ethics Committee for approval and subsequently to the R&D department of Chelsea and Westminster Hospital for site specific approval. As part of the ethics approval process, this study has been extensively peer reviewed by reviewers as outlined in the peer review process by the Joint Research Compliance Office, Imperial College London. As part of the PhD, the progression of the study and PhD will be assessed by the Progress Review Panel, department of medicine, Imperial College London.

5.3 Adverse Events

As this study is simply comparing standard feeding practices, there will be no expected adverse events related to the study. However, we are required by the sponsor to include the following information:

ADVERSE EVENTS
DEFINITIONS
Adverse Event (AE): any untoward medical occurrence in a patient or clinical study subject.
Serious Adverse Event (SAE): any untoward and unexpected medical occurrence or effect that:
? **Results in death**
? **Is life-threatening** – *refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe*
- **Requires hospitalisation, or prolongation of existing inpatients' hospitalisation**
? **Results in persistent or significant disability or incapacity**
? **Is a congenital anomaly or birth defect**
Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

REPORTING PROCEDURES
All adverse events should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the Chief Investigator in the first instance.

Non serious AEs
All such events, whether expected or not, should be recorded.
Serious AEs
An SAE form should be completed and faxed to the Chief Investigator within 24 hours. However, relapse and death due to conditions associated with prematurity and hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs.
All SAEs should be reported to the Fulham Research and Ethics Committee where in the opinion of the Chief Investigator, the event was:
? 'related', ie resulted from the administration of any of the research procedures; and
? 'unexpected', ie an event that is not listed in the protocol as an expected occurrence
Reports of related and unexpected SAEs should be submitted within 15 days of the Chief Investigator becoming aware of the event, using the NRES SAE form for non-IMP studies. The Chief Investigator must also notify the Sponsor of all SAEs.
Local investigators should report any SAEs as required by their Local Research Ethics Committee, Sponsor and/or Research & Development Office.

Contact details for reporting SAEs
Fax: 0161 625 7299 attention Shehnaz Ishaq
Please send SAE forms to:
Fulham REC Coordinator
4 Minshull Street
Manchester M1 3DZ
Tel: 0161 625 7821(Mon to Fri 09.00 – 17.00)

5.4 Indemnity

Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this study/ Imperial College Healthcare NHS Trust holds standard NHS Hospital Indemnity and insurance cover with NHS Litigation Authority for NHS Trusts in England, which apply to this study

5.5 Sponsor

Imperial College London will act as the main Sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in this study.

5.6 Funding

This study will be funded through CI core budget while grant funding is being sought.

5.7 Audit

The study may be subject to inspection and audit by Imperial College London under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the NHS Research Governance Framework for Health and Social Care (2nd edition).

5.8 Study Management

The day to day management of the study will be co-ordinated through the section of Neonatal Medicine, Imperial College London.

5.9 Publication Policy

Results of the study will be disseminated by publication in peer reviewed scientific journals, internal report, and conference presentation. All parents of participants will be sent a report of the study findings in the form of a letter. This will be sent to them once all data have been collected, analysed and the findings have been written up. A copy of the main publication will be provided to all study investigators. Investigators will be encouraged to share results with participants' parents as well.

5.10 End of Trial

The end of trial will be declared when the last infant recruited completes the last follow up visit ie MRI scan at term plus 6 weeks of age.

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