

Preterm Formula, Fortified or Unfortified Human Milk for Very Preterm Infants, the PREMFOOD Study: A Parallel Randomised Feasibility Trial

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Keywords

Clinical trial · Nutrition · Clinical outcomes

Abstract

Objective: Uncertainty exists regarding optimal supplemental diet for very preterm infants if the mother's own milk (MM) is insufficient. We evaluated feasibility for a randomised controlled trial (RCT) powered to detect important differences in health outcomes. **Methods:** In this open, parallel, feasibility trial, we randomised infants 25+0–31+6 weeks of gestation by opt-out consent to one of three diets: unfortified human milk (UHM) (unfortified MM and/or unfortified pasteurised human donor milk (DM) supplement), fortified human milk (FHM) (fortified MM and/or fortified DM supplement), and unfortified MM and/or preterm formula (PTF) supplement from birth to 35+0 weeks post menstrual age. Feasibility outcomes included opt-outs, adherence rates, and slow growth safety criteria. We also obtained anthropometry, and magnetic resonance imaging body composition data at term and term plus 6 weeks (opt-in consent). **Results:** Of 35 infants randomised to UHM, 34 to FHM, and 34 to PTF groups, 21, 19, and 24 infants

completed imaging at term, respectively. Study entry opt-out rate was 38%; 6% of parents subsequently withdrew from feeding intervention. Two infants met predefined slow weight gain thresholds. There were no significant between-group differences in term total adipose tissue volume (mean [SD]: UHM: 0.870 L [0.35 L]; FHM: 0.889 L [0.31 L]; PTF: 0.809 L [0.25 L], $p = 0.66$), nor in any other body composition measure or anthropometry at either timepoint. **Conclusions:** Randomisation to UHM, FHM, and PTF diets by opt-out consent was acceptable to parents and clinical teams, associated with safe growth profiles and no significant differences in body composition. Our data provide justification to proceed to a larger RCT.

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Introduction

Uncertainty exists regarding the optimal supplement for very preterm infants when the mother's own milk (MM) is insufficient. Pasteurised human donor milk (DM) and preterm formula (PTF) are options. Unlike

PTF, due to variable nutrient content of human milk, many practitioners also provide bovine-derived multi-component fortification. Current meta-analyses of preterm feeding trials have found no difference in necrotising enterocolitis (NEC) with fortified versus unfortified human milk [1], and a higher risk of NEC with formula versus DM as sole feed [2]. However, interpretation is limited by inadequate power for NEC as a primary outcome, variable use of bovine fortifier, and type of formula. Although faster short-term growth was associated with formula or fortifier in these trials, there was no difference in long-term anthropometry, nor many other long-term health outcomes in the few trials that have evaluated for these [1, 2]. Nevertheless, the increased risk of metabolic syndrome and cardiovascular disease [3] among very preterm survivors raises the possibility of early nutrition mediating these effects through rapid growth and/or altered body composition. Using whole-body magnetic resonance imaging (MRI), our group have previously shown that total and internal abdominal (visceral) adipose tissue (IAAT), a biomarker of long-term metabolic risk, is increased in young adults born preterm [4], with increases in IAAT seen as early as term equivalent age [5], compared with individuals born full term.

Clinical trials addressing these important uncertainties require sample sizes with adequate power to detect meaningful differences in important outcomes and establish cohorts for long-term follow up, with safe design and appropriate to maximise recruitment and completion of feed intervention. The objectives of this feasibility trial were to assess: (1) feeding intervention opt-out consent and adherence rates, (2) trial DM volume requirement, (3) anthropometry, and MRI body composition at term and term plus 6 weeks, and (4) to test that clinical outcome data can be retrieved from a national database of real-world clinical data.

Materials and Methods

We conducted PREMFOOD (PREterm Formula Or Donor milk for preterm babies), an open, multicentre, parallel, feasibility randomised controlled trial (RCT), preregistered at ClinicalTrials.gov (NCT01686477) and approved by the UK National Research Ethics Service (REC no: 12/LO/1391). Recruitment occurred from August 2013 to November 2017, at three London National Health Service hospitals.

Infants born between 25 + 0 and 31 + 6 weeks who were unlikely to be transferred to another hospital were eligible for inclusion. Infants with congenital abnormalities that precluded early milk feeding were excluded. The study was discussed with parents at the time of birth, with opportunity offered to opt-out; parents were

informed that they had the right to change a decision to participate at any time. Randomisation to feed intervention occurred postnatally within 48 h unless the parents opted-out. Opt-out consent was chosen for the comparative-effective component of the trial as this reduces the burden of decision making for parents at a stressful time, and is suitable for comparisons of routine care [6]. Additional opt-in consent was sought later for trial-related imaging procedures. Trial data were obtained from the National Neonatal Research Database (NNRD) [7], supplemented with prospectively recorded data on daily nutritional intake until discharge.

Randomisation was to one of three groups: (i) unfortified MM supplemented with unfortified DM (UHM group); (ii) fortified MM supplemented with fortified DM (FHM group); (iii) unfortified MM supplemented with PTF (PTF group). Allocation was computer generated centrally with randomisation to 1 of the 3 feeding groups in a 1:1:1 ratio with minimisation by infant sex, gestational age (25+0–27+6 weeks and 28+0–31+6 weeks), and small for gestational age status (birthweight *z* score <1.28 and ≥1.28). Twins were randomised individually. Feeding intervention continued until 35+0 weeks post menstrual age (PMA), after which they were transitioned to suck feeds by breast or bottle according to maternal choice with continuation of any fortification at the discretion of the attending clinician.

Pasteurised (Holder method) DM was supplied by the North-West UK Human Milk Bank analysed for macronutrient content pre-pasteurisation [8]. PTF was Cow and Gate nutripem 1 (Nutricia Ltd; 80 kcal/100 mL energy, 2.6 g/100 mL protein (non-hydrolysed whey: casein ratio: 60:40), 3.9 g/100 mL fat, 8.4 g/100 mL carbohydrate). Fortifier (Cow and Gate nutripem; 15 kcal/100 mL energy, 1.1 g/100 mL protein (extensively hydrolysed whey:casein ratio: 50:50), 0 g/100 mL fat, 2.7 g/100 mL carbohydrate, see online suppl. Table S1; for all online suppl. material, see <https://doi.org/10.1159/000535498>), was introduced in the FHM group once a total enteral feed volume of 100 mL/kg/d was reached.

Milk volumes were increased to a maximum of 200 mL/kg/d for UHM and FHM, and 165 mL/kg/d for PTF, as per recommendation, modified in accordance to infant tolerance, and parenteral nutrition weaned in accordance with standard guidelines. Safety criteria defined by slow growth were based on the UK Neonatal and Infant Close Monitoring growth chart 2009 [9, 10]: if after 2 weeks of reaching a milk volume of 120 mL/kg/d, the infant showed a 3 marked centile downward crossing (equating to approximately a 1.4–2.0 *z* score change from birthweight) fortification or formula was commenced. All infants were included in the intention-to-treat analysis unless withdrawn.

Study Outcomes

Feasibility outcomes included opt-out, and opt-in consent rates, triggering of safety criteria, adherence to feed intervention, estimation of trial DM requirement, and completeness and accuracy of data retrieval from the NNRD. A conservative 50% consent rate of parents approached was deemed acceptable for both stages. Nonadherence to feed intervention was expected in only a handful of cases. Accuracy and completeness of NNRD baseline clinical characteristic data and clinical morbidity data (see Table 4) including NEC were assessed by comparison with prospectively collected clinical data from individual patient case notes.

For anthropometry and body composition data, primary outcome was total body adipose tissue (TAT) volume at term (37–42 weeks PMA) as estimated by whole-body MRI. Secondary outcomes were (i) adipose tissue (AT) depot volumes (ii) non-adipose tissue mass (NATM) (iii) anthropometry (change in weight, length, and head circumference *z* score from birth to scan visits), at term and term plus 6 weeks, and (iv) TAT at term plus 6 weeks. Post-hoc comparisons between randomised groups of in-hospital change in anthropometry *z* scores, and macronutrient intake using reference values for MM [11] were also undertaken to aid interpretation of the primary and secondary outcomes.

We scanned infants in accordance with our well-established protocol [12]. TAT volume was quantified as the sum of six discrete depots (see online suppl. Fig. 1). Image analysis (Slice-OMatic V.4.2; Tomovision) was undertaken independently by Vardis Group, London, UK, blinded to participant identity and feeding group allocation. NATM was calculated as: body weight [g] – (TAT volume [cm³] × 0.9), assuming a value for the density of adipose tissue of 900 g/L. Since there are no reference data informing the impact of preterm diet on adiposity, we used our group's database of preterm at term body composition scans at the time, which did not have detailed information on diet, and based the sample size on our estimate that 22 infants in each group would provide 80% power (2-sided; 5% significance) to detect a 240-cm³ (1.0 standard deviation) difference in TAT volume.

Statistical Analysis

All analyses were performed with SPSS version 27. Statistical significance was defined as $p < 0.05$. Differences between unadjusted randomised groups were assessed using ANOVA with examination of the three pairwise differences. To allow for meaningful comparisons of relative adiposity, we adjusted for body size, using ponderal index at term and body mass index (BMI) at term plus 6 weeks [13], PMA at scan, and sex [14]. We used multiple linear regression for adjusted comparisons. We checked each outcome for normality, with natural log transformation of the dependent variable for non-normality. We report the adjusted mean difference with 95% confidence intervals. All analyses were intention-to-treat. Sensitivity analyses were undertaken for all outcomes for only those that completed their allocated feed intervention, and after excluding outcome outliers ($> \text{mean} \pm 3 \text{ SD}$).

Results

We randomised 103 infants, 35 to UHM, 34 to FHM, and 34 to PTF groups (Fig. 1). Baseline characteristics were comparable between groups (Table 1).

Of 165 parents approached, 103 (62%) chose not to opt-out; 93 out of 165 (56%) consented to both feeding intervention and MRI scans. Reasons for parental opt-out are provided in Figure 1. There were no instances in which the attending clinician felt randomisation was inadvisable. Fifteen infants did not complete feed intervention: there were 6 (6%), parental withdrawals from the study during feed intervention, all from the FHM arm (5

exclusively MM fed; 3 were twins); 5 were removed from feed intervention by the clinician (3 for slow weight gain [2 of which met rescue criterion]; 1 with a late diagnosis of duodenal atresia and 1 with feed intolerance); 2 died; and 2 were transferred to a nonparticipating hospital.

Two infants met the rescue criterion for poor weight gain. One, from the UHM group, received exclusive DM, with a weight *z* score change from birth to 31+1 weeks of -2.41 . The second, from the PTF group received exclusive MM, and had a weight *z* score change of -1.49 at 32+5 weeks. A third infant (UHM group; received 20% MM, 80% DM) was switched to formula at 34+4 weeks though weight *z* score change (-1.02) did not reach the prespecified threshold.

Feed intervention total enteral intake (online suppl. Table S3) and exclusive MM intake (UHM 50%; FHM 44%; PTF 39%) were comparable between groups. The mean volume of DM required to 35+0 weeks PMA for each infant randomised to UHM or FHM groups was 1.3 L. There were no significant differences in the volumes of UHM, FHM, and PTF from 35 weeks PMA to discharge between the 3 groups (online suppl. Table S3).

From the UHM, FHM, and PTF groups, 21, 19, and 24 infants completed imaging at term, and 14, 5, and 19 respectively, at term plus 6 weeks. There was no significant difference between groups in the unadjusted primary outcome, TAT volume at term (mean [SD]: UHM: 0.870 L [0.35 L]; FHM: 0.889 L [0.31 L]; PTF: 0.809 L [0.25 L], $p = 0.66$), nor in the adjusted TAT (Table 2). We found no significant between-group differences for any other body composition measure nor anthropometric measure at term (Table 2; Fig. 2) or term plus 6 weeks (online suppl. Tables S2; Fig. 2).

Post-hoc analyses revealed a fall in weight *z* score from birth to 35 weeks PMA occurring in all three groups (mean [SD] UHM: -1.20 [0.57]; FHM: -0.79 [0.46]; PTF: -0.79 [0.63]) and was most marked in the UHM group: UHM versus FHM ($p = 0.01$); UHM versus PTF ($p = 0.01$) (Table 2; Fig. 2). Total (parenteral and enteral) mean protein and protein:energy ratio from birth to 35 weeks PMA, but not energy or fat, were significantly higher in the FHM compared to either UHM and PTF groups (Table 3; online suppl. Table S3). Sensitivity analyses did not change the body composition, anthropometry, or nutritional intake findings.

Morbidity and mortality data by feed group are provided in Table 4. There were two deaths (Enterobacter sepsis and suspected sepsis with renal failure, from the UHM and PTF groups, respectively), both exclusively MM fed. Two infants, both in the UHM group, developed necrotising enterocolitis (NEC) $\geq$ Stage II (Bell's modified

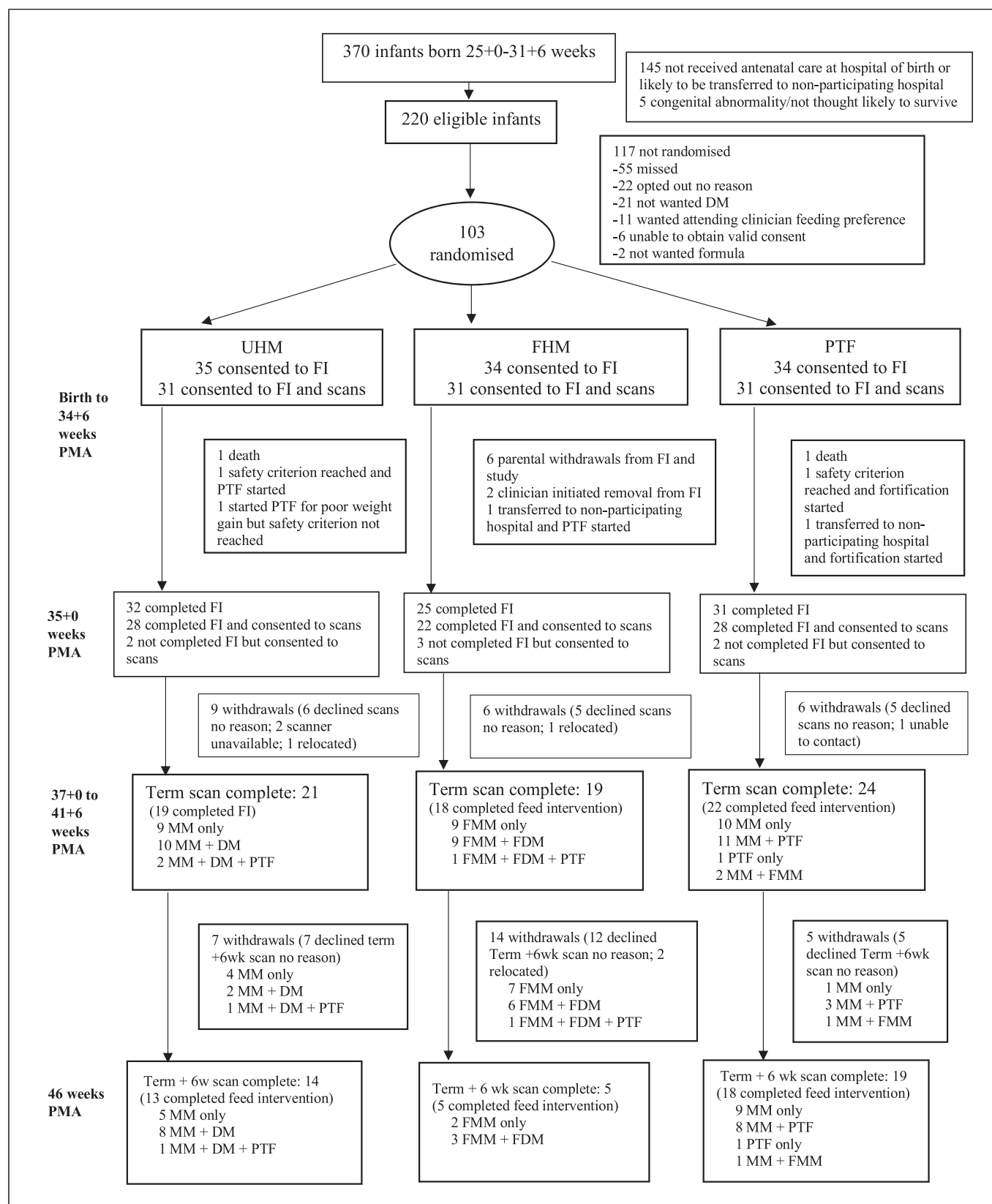


Fig. 1. Consort diagram for study infants; FI (Feed Intervention Birth to 34+6 weeks PMA).

Table 1. Baseline characteristics by randomised feed group

Characteristic	UHM (n = 35)	FHM (n = 34)	PTF (n = 34)
Sex, n (%)			
Boys	20 (57)	21 (62)	19 (56)
Girls	15 (43)	13 (38)	15 (44)
Gestational age (weeks) at birth, mean (SD)	29.4 (2.2)	29.6 (2.0)	29.7 (1.7)
Birthweight (g), mean (SD)	1,257 (323)	1,312 (333)	1,326 (334)
Birthweight z score, mean (SD)	−0.42 (0.94)	−0.40 (0.78)	−0.36 (0.88)
Birth length (cm), mean (SD)	37.5 (2.9)	38.2 (3.3)	38.6 (2.9)
Birth length z score, mean (SD)	−0.67 (1.15)	−0.60 (1.05)	−0.37 (1.12)
Birth OFC (cm), mean (SD)	26.7 (2.8)	27.2 (2.1)	27.5 (2.1)
Birth OFC z score, mean (SD)	−0.77 (1.40)	−0.40 (0.78)	−0.33 (1.03)
Multiple birth status, n (%)			
Singleton	17 (49)	21 (62)	21 (62)
Multiple	18 (51)	13 (38)	13 (38)
Small for gestational age (birthweight z score <1.28), n (%)	6 (17)	4 (12)	4 (12)
Received antenatal steroids, n (%)	31 (89)	31 (91)	33 (97)
Apgar score at 5 min, median (IQR)	8.0 (3.0)	9.0 (1.0)	9.0 (2.0)
Maternal age (years), mean (SD)	33.3 (6.0)	32.1 (5.6)	33.4 (6.0)
Maternal parity, n (%)			
1	28 (80)	24 (71)	23 (68)
>1	7 (20)	10 (29)	11 (32)
Maternal ethnicity, n (%)			
White	25 (71)	23 (68)	21 (62)
Mixed	3 (9)	2 (6)	2 (6)
Asian	1 (3)	1 (3)	7 (20)
Black	5 (14)	7 (20)	3 (9)
Other	1 (3)	1 (3)	1 (3)

criteria). One had surgical NEC and had received MM and DM; the other had NEC stage 2B and received only MM. Baseline characteristics and clinical morbidity data were recorded accurately in the NNRD with 100% agreement between NNRD and prospectively collected data from patient notes. Completeness of data recorded in the NNRD was 100% for clinical morbidity data and the majority of baseline characteristics with only a couple of domains scoring 93% and 94%.

Discussion

In this very preterm feeding feasibility trial, opt-out consent and randomisation to UHM, FHM, and PTF feeding interventions were acceptable to clinicians and parents, associated with safe growth profiles, with no statistically significant differences in anthropometry or body composition at term or term plus 6 weeks.

As far as we are aware, this is the first RCT to compare these feeding regimens which are in wide use globally. The strengths of the study lie in body composition evaluated using a direct gold-standard approach [15],

analyses of imaging data blind to feeding mode, and detailed data on macronutrient intake. Though separating an initial opt-out process from a subsequent opt-in process for non-clinical trial procedures maximises recruitment into all elements of the study, several parents declined the opt-in imaging, resulting in reduction in power for the body composition outcome. Inclusion criteria aimed to minimise loss of study infants from transfer to a non-participating hospital. A major barrier to recruitment was the high number of inborn eligible gestation infants which were not local to the hospital of birth (39%), which may have implications for generalisability.

Feeding intervention consent by opt-out method was 62%, which is higher than recruitment rates for other very preterm nutritional intervention studies using a single opt-in consent process [16, 17]. Using opt-out consent increases recruitment rate and generalisability of trial results [18]. There were no instances in which a clinician refused feeding intervention randomisation, and only one in which it was subsequently stopped (fortifier) due to tolerance concerns. We did not anticipate the 6% parental withdrawal rate from the FHM

Table 2. Term body composition and anthropometry outcomes by randomised feed group, data are mean (SD) for unadjusted comparisons and mean difference (95% CI) for adjusted comparisons

Unadjusted comparisons	Feed group			
Outcome	UHM, <i>n</i> * = 21	FHM, <i>n</i> * = 19	PTF, <i>n</i> * = 24	<i>p</i> [#] value
Δ Wt z score birth to 35 weeks	−1.20 (0.57), <i>n</i> = 34	−0.79 (0.46), <i>n</i> = 28	−0.79 (0.63), <i>n</i> = 33	<0.01 ^a
Δ Length z score birth to 35 weeks	−1.43 (0.73), <i>n</i> = 34	−1.12 (0.68), <i>n</i> = 27	−1.22 (0.70), <i>n</i> = 32	0.35
Δ OFC z score birth to 35 weeks	−0.18 (1.02), <i>n</i> = 34	−0.19 (0.76), <i>n</i> = 28	−0.21 (0.98), <i>n</i> = 33	0.99
PMA (weeks) at term MRI scan	40.9 (1.9)	40.2 (2.1)	40.9 (1.8)	0.36
Term MRI weight, g	3,295 (798)	3,185 (678)	3,153 (656)	0.79
Term MRI length, cm	50.0 (3.8)	49.5 (3.0)	49.6 (3.5)	0.90
Term MRI OFC, cm	35.9 (2.4)	35.0 (1.9)	35.4 (2.1)	0.46
Δ Wt z score birth to term	−0.51 (0.85)	−0.40 (0.75)	−0.75 (0.97)	0.41
Δ OFC z score birth term	1.02 (1.46), <i>n</i> = 20	0.69 (1.09)	0.41 (1.15), <i>n</i> = 23	0.28
Δ length z score birth to term	−0.49 (1.24)	0.07 (1.42)	−0.79 (1.18), <i>n</i> = 23	0.09
TAT at term, L	0.870 (0.35)	0.889 (0.31)	0.809 (0.25)	0.66
Non-ATM at term, g	2,511.8 (502.8)	2,385.7 (437.8)	2,424.7 (462.6)	0.68
%ATM at term	23.0 (4.7)	24.6 (4.5)	22.8 (3.4)	0.32
IAAT (L) at term	0.043 (0.02)	0.044 (0.02)	0.040 (0.01)	0.67
INAAT (L) at term	0.071 (0.03)	0.072 (0.03)	0.071 (0.02)	0.97
DSCAAT (L) at term	0.017 (0.01)	0.017 (0.01)	0.015 (0.01)	0.75
DSNAAT (L) at term	0.017 (0.01)	0.017 (0.01)	0.016 (0.01)	0.56
SSCAAT (L) at term	0.141 (0.07)	0.137 (0.06)	0.125 (0.05)	0.61
SSCNAAT (L) at term	0.579 (0.23)	0.601 (0.21)	0.543 (0.17)	0.63
Adjusted term body composition regression models	Feed group comparison			
outcome	PTF (<i>n</i> = 24) versus UHM (<i>n</i> = 21) (Ref)	FHM (<i>n</i> = 19) versus UHM (Ref)	PTF versus FHM (Ref)	
TAT (L) ¹	−0.064 (−0.235–0.108), <i>p</i> = 0.46	−0.012 (−0.193–0.179), <i>p</i> = 0.90	−0.052 (−0.228–0.124), <i>p</i> = 0.56	
TAT (L) ²	−0.063 (−0.199–0.074), <i>p</i> = 0.36	0.056 (−0.090–0.202), <i>p</i> = 0.45	−0.119 (−0.260–0.023), <i>p</i> = 0.10	
Non-ATM (g) ¹	−94.0 (−360.4–172.5), <i>p</i> = 0.48	−173.8 (−455.9–108.3), <i>p</i> = 0.22	79.9 (−193.1–352.8), <i>p</i> = 0.56	
Non-ATM (g) ²	−89.7 (−282.7–103.3), <i>p</i> = 0.36	−65.0 (−271.8–141.8), <i>p</i> = 0.53	−24.7 (−225.0–175.6), <i>p</i> = 0.81	
% ATM ³	−0.2 (−2.5–2.1), <i>p</i> = 0.87	2.2 (−0.3–4.7), <i>p</i> = 0.08	−2.2 (−4.8–0.3), <i>p</i> = 0.09	
IAAT (% difference) ¹	−2.7 (−22.4–21.9), <i>p</i> = 0.81	5.7 (−16.7–34.2), <i>p</i> = 0.64	−8.0 (−27.0–16.0), <i>p</i> = 0.48	
IAAT (% difference) ²	−2.5 (−20.2–19.1), <i>p</i> = 0.80	13.0 (−9.0–40.1), <i>p</i> = 0.26	−13.7 (−29.9–6.3), <i>p</i> = 0.16	
INAAT (L) ¹	−0.002 (−0.018–0.015), <i>p</i> = 0.84	−0.003 (−0.021–0.015), <i>p</i> = 0.73	0.001 (−0.016–0.018), <i>p</i> = 0.88	
INAAT (L) ²	−0.002 (−0.016–0.012), <i>p</i> = 0.82	0.003 (−0.012–0.018), <i>p</i> = 0.70	−0.004 (−0.019–0.010), <i>p</i> = 0.54	
DSCAAT (% difference) ¹	−0.9 (−28.2–36.8), <i>p</i> = 0.96	29.7 (−27.0–44.3), <i>p</i> = 0.88	−3.3 (−30.5–34.5), <i>p</i> = 0.84	
DSCAAT (% difference) ²	−0.9 (−24.3–29.8), <i>p</i> = 0.95	15.4 (−13.5–54.0), <i>p</i> = 0.32	−14.1 (−35.1–13.5), <i>p</i> = 0.28	
DSCNAAT (L) ¹	−0.002 (−0.006–0.004), <i>p</i> = 0.37	−0.0002 (−0.004–0.004), <i>p</i> = 0.92	−0.002 (−0.005–0.002), <i>p</i> = 0.44	
DSCNAAT (L) ²	−0.002 (−0.005–0.002), <i>p</i> = 0.32	0.001 (−0.003–0.004), <i>p</i> = 0.65	−0.003 (−0.006–0.001), <i>p</i> = 0.16	

Table 2 (continued)

Adjusted term body composition regression models	Feed group comparison		
	PTF (n = 24) versus UHM (n = 21) (Ref)	FHM (n = 19) versus UHM (Ref)	PTF versus FHM (Ref)
SSCAAT (L) ¹	−0.014 (−0.045–0.018), p = 0.38	−0.004 (−0.037–0.029), p = 0.81	−0.010 (−0.042–0.022), p = 0.55
SSCAAT (L) ²	−0.014 (−0.040–0.013), p = 0.31	−0.007 (−0.022–0.036), p = 0.64	−0.021 (−0.048–0.007), p = 0.14
SSCNAAT (L) ¹	−0.041 (−0.156–0.073), p = 0.47	−0.004 (−0.125–0.118), p = 0.95	−0.038 (−0.155–0.080), p = 0.52
SSCNAAT (L) ²	−0.041 (−0.132–0.050), p = 0.37	−0.041 (−0.056–0.139), p = 0.40	−0.082 (−0.177–0.012), p = 0.09

*Number of infants unless otherwise stated. ¹Model adjusted for Ponderal index at term scan (Weight [kg]/Length [M]³). ²Model adjusted as for model 1 plus post menstrual age (PMA) at scan, and Sex. ³Model adjusted for PMA at scan, and sex. Wt, weight; OFC occipito-frontal circumference; TAT, total adipose tissue; non-ATM, non-adipose tissue mass; IAAT, internal abdominal adipose tissue; INAAT, internal non-abdominal adipose tissue; DSCAAT, deep subcutaneous abdominal adipose tissue; SSCNAAT, deep subcutaneous non-abdominal adipose tissue; SSCAAT, superficial subcutaneous abdominal adipose tissue; SSCNAAT, superficial subcutaneous non-abdominal adipose tissue. #Between group ANOVA. ^aTukey post hoc test: PTF>UHM p = 0.01; FHM>UHM p = 0.01.

arm. We commenced fortification at 100 mL/kg/d, which is routine practice in some countries. However, in the UK, if used, fortification is typically started at a volume of 150 mL/kg/d [19]. Early use of fortifier may have introduced bias possibly influencing parent perspectives. Cluster randomisation would be one approach to reducing these biases and has been well received in our parent focus group discussions [20].

All but two of the infants had weight z score changes of no more than −2.0. One of these two infants had surgical NEC, and these data are in keeping with our large UK population cohort study showing weight change at term ranging from −1.3 to −1.8 z score, and those infants with mortality or serious morbidity having weight z scores up to 1.0 less than those without [21]. There were no statistically significant differences in severe clinical outcomes though the trial was not powered to detect clinically important differences. Data on birth characteristics and clinical course were accurately captured in the NNRD, thus demonstrating the potential for minimising the burden of data capture using linked electronic health care records to achieve long-term follow-up at low cost.

Our finding of no difference in total adiposity is consistent with three previous preterm feeding RCT's comparing diets of unfortified and fortified MM, fortified DM and PTF as supplements to fortified MM, and unfortified DM and PTF supplements to unfortified MM, finding no difference of fat or fat free mass using

different measurement techniques at term [22], 5.5 years [23], and adolescence [24], respectively. We also found no difference in regional adiposity between groups, and taken together, these findings suggest that term age [5] and adult adipose phenotype [4] in individuals born preterm is not mediated by these early diets. Nevertheless, a longer term programming effect cannot be excluded, and body composition follow up of this cohort is currently underway.

Consistent with significant differences in feeding intervention macronutrient intake, post hoc analyses revealed that weight gain at the end of the feeding intervention was lower in the UHM than either the PTF or FHM groups, with no significant differences in anthropometry subsequently. This suggests that infants may have self-regulated intake once transitioned to suck feeds, returning to a predestined growth trajectory.

Despite higher protein intake in the FHM group compared to UHM or PTF groups during feed intervention, there were no significant differences in NATM at term scan. Observational studies using reference values for macronutrient content of MM, have associated higher in-hospital protein intakes in very preterm infants, with increased fat free mass at term or near term [25, 26], yet with no significant differences between those infants receiving unfortified versus fortified human milk [26]. It is possible that variable macronutrient content in MM could explain the lack of difference seen in body composition between fortified and unfortified

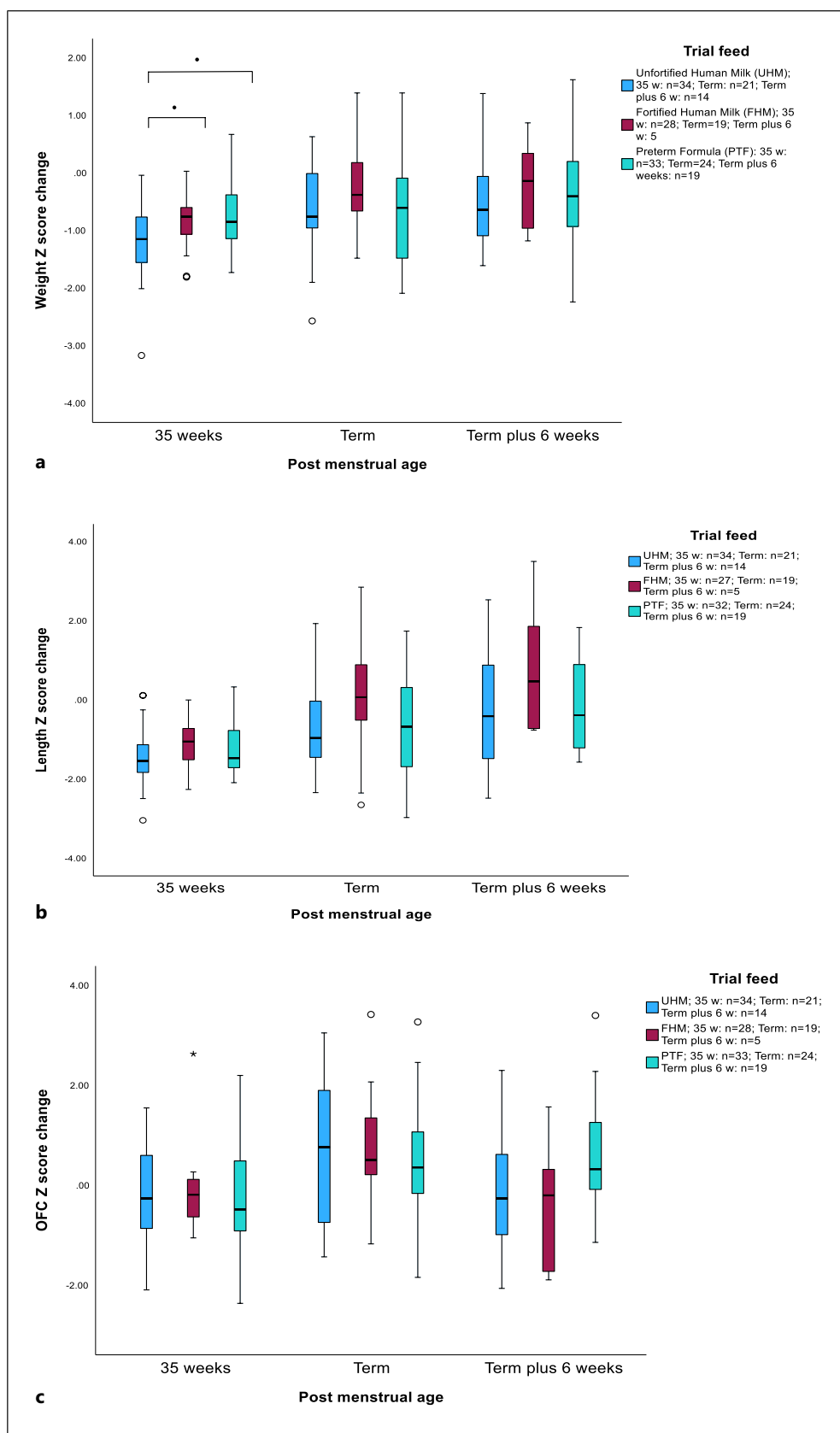


Table 3. Average daily nutritional intake (parenteral and enteral) for randomised feed groups to 35+0 weeks PMA

Parameter	UHM ^a	FHM ^b	PTF ^c	<i>p</i> * < 0.05
Day of age PN stopped	12 (7), <i>n</i> = 35	12 (8), <i>n</i> = 28	11 (7), <i>n</i> = 34	
% MM of total enteral intake	75.5 (32.1), <i>n</i> = 34	82.1 (27.5), <i>n</i> = 27	75.8 (31.3), <i>n</i> = 31	
UMM, mL/kg/d	90 (47), <i>n</i> = 34	31 (40), <i>n</i> = 27	94 (49), <i>n</i> = 27	a>b;c>b
UDM, mL/kg/d	29 (40), <i>n</i> = 34	4 (8), <i>n</i> = 27	0 (2), <i>n</i> = 28	a>b;a>c
FMM, mL/kg/d	8 (23), <i>n</i> = 34	73 (50), <i>n</i> = 27	3 (10), <i>n</i> = 27	b>a;b>c
FDM, mL/kg/d	0 (0), <i>n</i> = 34	13 (25), <i>n</i> = 27	0 (0), <i>n</i> = 28	b>a;b>c
PTF, mL/kg/d	4 (12), <i>n</i> = 34	3 (9), <i>n</i> = 27	31 (<i>n</i> = 27)	c>a;c>b
Total milk, mL/kg/d	130 (30), <i>n</i> = 34	124 (34), <i>n</i> = 27	128 (23), <i>n</i> = 27	
Total milk mL/kg/d from day reached full enteral feeds [#]	175 (35), <i>n</i> = 34	182 (18), <i>n</i> = 27	170 (23), <i>n</i> = 27	
Protein, g/kg/d	2.8 (0.6), <i>n</i> = 34	3.6 (0.6), <i>n</i> = 27	2.9 (0.4), <i>n</i> = 29	b>a;b>c
Energy, kcal/kg/d	110 (11), <i>n</i> = 34	121 (11), <i>n</i> = 27	109 (9), <i>n</i> = 29	b>a;b>c
Pro:En, g/100 kcal	2.5 (0.4), <i>n</i> = 34	3.0 (0.2), <i>n</i> = 27	2.6 (0.2), <i>n</i> = 29	b>a;b>c**
Fat, g/kg/d	5.0 (0.9), <i>n</i> = 34	5.1 (0.6), <i>n</i> = 27	5.1 (0.6), <i>n</i> = 29	
CHO, g/kg/d	13.2 (1.2), <i>n</i> = 34	15.1 (1.4), <i>n</i> = 27	12.8 (0.9), <i>n</i> = 29	b>a;b>c

Data are presented as the mean (SD). *Between group ANOVA post hoc Tukey test, all *p* values <0.001 except ***p* < 0.01; [#]Defined as day PN stopped; PN, parenteral nutrition; DM, pasteurised donor milk (unfortified or fortified); MM, mother's own milk (unfortified or fortified); UMM, unfortified mother's own milk; FMM, fortified mother's own milk; UDM, unfortified pasteurised donor milk; FDM, fortified pasteurised donor milk; PTF, preterm formula; Pro:En, protein:energy ratio; CHO, carbohydrate.

Table 4. Clinical course by randomised feed groups

Outcome*	UHM (<i>n</i> = 34)	FHM (<i>n</i> = 35)	PTF (<i>n</i> = 34)	<i>p</i> value ^a
Death	1 (2.9)	0	1 (2.9)	0.59
Late onset sepsis	6 (17.6)	8 (22.9)	5 (14.7)	0.68
NEC				
All stages	4 (11.8)	4 (11.4)	1 (2.9)	0.34
Bells stage ≥ II	2 (5.9)	0	0	0.13
Oxygen support at 36 weeks PMA	10 (29.4)	12 (34.3)	8 (23.5)	0.66
Serious brain injury ^b	4 (11.8)	5 (14.3)	1 (2.9)	0.25
Severe ROP ^c	0	0	0	
Percentage level 1 care ^d	11.3	12.8	13.4	0.75
Percentage level 2 care ^d	31.0	32.8	28.8	0.75

*Figures represent number or infants (%); ^a χ^2 2 × 3 for categorical variables; ANOVA for continuous variables; ^bDefined as echodense intraparenchymal lesions, periventricular leukomalacia, porencephalic cysts, or ventriculomegaly with or without intraventricular haemorrhage; ^cDefined as that requiring treatment; ^dAs classified by British Association of Perinatal Medicine (2011); NEC, necrotising enterocolitis; PMA, post menstrual age; ROP, retinopathy of prematurity.

groups. In support of this, we have previously shown in a secondary analysis of very preterm infants randomised to higher and lower protein content in parenteral nutrition [27] that infants predominantly fed the PTF had higher NATM compared to those exclusively human

milk fed. In this study, there was low intake of PTF and a high percent of MM intake across all groups at 75–82%.

In summary, our feasibility data provide justification to proceed to a larger RCT powered to detect differences in important health outcomes.

Statement of Ethics

This study protocol was reviewed and approved by the UK National Research Ethics Service, approval number [12/LO/1391]. As detailed in the main body of the text, opt-out and opt-in informed consent protocols were used for use of participant data for research purposes. These consent procedures were reviewed and approved by UK National Research Ethics Service, London – Fulham, approval number [12/LO/1391], December 10, 2012.

Conflict of Interest Statement

N.M. reports grants outside the submitted work from the Medical Research Council, National Institute for Health Research, Prolacta Life Sciences, Chiesi International, Bayer International, Westminster Children's Research Trust, European Health Data and Evidence Network, HCA International, Health Data Research UK, Shire Pharmaceuticals, and March of Dimes; N.M. accepts no personal remuneration for her role as a member of the Nestle International Scientific Advisory Board. The other authors have no conflicts of interest to disclose.

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Author Contributions

N.M. conceptualized and designed the study, funded the study through unrestricted funds, coordinated and supervised data collection and analyses, and contributed to writing the manuscript, and all revisions. L.M. drafted and revised the study protocol, recruited patients, collected data, carried out analyses, drafted the initial manuscript, and reviewed and revised the manuscript. S.S. contributed to and reviewed the statistical analysis plan, and reviewed the manuscript. K.E.C., R.E., and A.A. collected data, carried out the initial analyses, and reviewed the manuscript. I.A. helped in recruitment of patients, collected data, and reviewed the manuscript. R.N. and J.C. facilitated recruitment of patients, coordinated data collection, and reviewed the manuscript. S.U. coordinated and supervised data collection and initial analyses, critically reviewed the manuscript for important intellectual content, reviewed, and revised the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Data Availability Statement

All data generated or analysed during this study are included in this article and its supplementary material files. Further enquiries can be directed to the corresponding author.

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