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Randomised controlled trial of human derived breast milk fortifier versus bovine milk fortifier on body composition in very preterm babies

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ABSTRACT

Background: Preterm infants receiving a diet of exclusive human milk compared to predominantly preterm formula have lower weight and non-adipose tissue mass by term. Human milk fortification is recommended. However, it is not known if the protein source affects body composition.
Aims: To compare the effect of an exclusive human milk based diet (intervention) with a diet containing cow milk products (control) on body composition.
Participants: Infants born below 30 weeks gestation.
Study design: Randomised multicentre, open label, controlled trial. Infants preferentially received their own mother's milk. Infants were randomised to either an exclusive human milk diet (human milk formula to make up a shortfall in own mother's milk and human milk derived fortifier) or cow milk-based supplementation (preterm formula to make up a shortfall in own mother's milk and cow milk-based fortifier). Fortification began at an enteral intake of 150 ml/kg/day. Infants underwent whole-body magnetic resonance imaging at term.
Primary outcome: Body composition (adipose tissue (ATM) and non-adipose tissue mass (N-ATM)) at term.
Results: We randomly assigned 38 infants to intervention (n = 19) and control arms (n = 19). Primary outcomes were analysed in 15 infants in the intervention arm and 12 in the control arm. The estimates of the effect of the intervention following adjustment for length and sex, were non-significant (ATM (kg): 0.137, 95 % confidence interval (CI) -0.01, 0.29; N-ATM: −0.137; −0.01, 0.29).
Conclusions: We identified no clinically relevant differences in body composition in preterm babies <30 weeks gestation receiving a macronutrient-equivalent exclusive human milk diet compared with a diet containing cow milk products.

1. Introduction

Own mother's milk has short and longer-term advantages for infants although the full range of functional effects and causal pathways are not well defined. Not all mothers who deliver preterm are able to express sufficient milk to meet their infants' requirements. In this situation, options previously available were cow milk based formula or pasteurised human donor milk. Recently, new commercial products, both formula and fortifier prepared from pooled human milk purchased from lactating women, have become available. These have yet to be evaluated in adequately powered randomised controlled trials with respect of their effect on functional health outcomes [1,2] or cost-effectiveness across a range of different healthcare settings.

Some practitioners recommend macronutrient fortification of human milk, usually in the form of a commercial product. These "fortifiers" have hitherto only been available as cow milk derived product hence

Abbreviations: ATM, adipose tissue mass; N-ATM, non-adipose tissue mass; OMM, own mother's milk; AT, adipose tissue; NNRD, National Neonatal Research Database.

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infants receiving such fortification have not had an exclusive human milk diet. We have previously shown that preterm infants receiving an exclusive human milk diet have lower weight and non-adipose tissue mass at term compared to infants fed predominantly cow milk-based formula [3]. This may be a consequence of the lower protein content of human milk. However, it is not known if the source of protein affects body composition. We therefore undertook an exploratory study of the effect of an exclusive human milk diet versus one containing cow milk products, on body composition, gut bacteria and metabolism, with a view to obtaining data to inform a larger randomised controlled trial designed to detect important clinical and functional outcomes. Here we report on body composition outcomes.

2. Methods

2.1. Design

This was a randomised open-label, controlled trial in four UK centres of which two participated in the body composition evaluation.

2.2. Population

Between March 2018 and September 2019, preterm infants born below 30 weeks gestation were enrolled in the study if eligible and following parent informed consent. Exclusion criteria were major or life-threatening abnormalities, inability to randomise within 72 h of birth, exposure to bovine milk products prior to randomisation or likelihood of transfer to another hospital before 34 weeks postmenstrual age. The study was approved by the Health Research Authority, North East Tyne and Wear South Research Ethics Committee17/NE/0169. The trial was pre-registered (ISRCTN 16799022) where the full trial protocol may be accessed. The trial sponsor was Newcastle Hospitals NHS Foundation Trust and the funder was Prolacta Life Sciences. The sponsor and funder had no role in data analysis, presentation or decision to submit for publication.

2.3. Intervention and control

Infants received their own mother's milk (OMM) with randomisation to either exclusive human milk ('Ready to Feed Human Preterm 26' formula, RTF 26; Prolacta Biosciences) (intervention) or preterm formula (control) to make up any shortfall, until an enteral feed volume of 150 ml/kg/day was reached. In the exclusive human milk arm, human milk-based fortifier (Prolacta Biosciences) was added once an enteral feed volume of 150 ml/kg/day was reached, of which at least 50 ml was OMM. The maximum enteral intake was 165 ml/kg/day providing a protein intake of 3.7–4.6 g/kg/day, carbohydrate intake of 8.2–12.2 g/kg/day and fat intake of 8.2–9.2 g/kg/day, (total non-protein energy 115–141 kcal/kg/day). In the control arm, once an enteral feed volume of 150 ml/kg/day was reached of which at least 50 ml was OMM, standard cow milk based fortifier was added. The maximum enteral intake was 165 ml/kg/day providing a protein intake of 3.7–4.6 g/kg/day, carbohydrate intake of 13–14.7 g/kg/day and a fat intake of 6.3–6.4 g/kg/day (total non-protein energy 115–123 kcal/kg/day). Initiation and progression of enteral feeds were based on the neonatal unit protocol. The trial intervention ended at 34 weeks postmenstrual age.

2.4. Randomisation

Web-based randomisation (Sealed Envelope) was carried out by trained clinical staff using minimisation incorporating hospital site, gestation (two groups: ≥25 weeks and ≤24 weeks and 6 days), and multiple birth status. Multiples were randomised independently.

2.5. Outcomes

The study was designed to assess three outcomes, body composition, gut bacterial diversity, and gut derived metabolites. Body composition was assessed using whole body magnetic resonance (MR) imaging carried out in natural sleep at term using our well-established protocol [4]. Serial axial images were obtained (5 mm slice and inter-slice thickness) to quantify total adipose tissue (AT) volume as the sum of 6 discrete depots: superficial-subcutaneous abdominal, superficial-subcutaneous non-abdominal, deep-subcutaneous abdominal, deep-subcutaneous non-abdominal, internal-abdominal, and internal non-abdominal, also as previously described [4]. Image analysis was carried out using Slice-OMatic (Tomovision) undertaken independently and blinded to participant identity and group allocation by a commercial image analysis service (Vardis Group). AT volumes in litres were converted to AT mass in kg assuming AT density of 0.90 g/l. Non-AT mass was calculated by subtracting AT mass from the weight of the infant on the day of the MR scan using the following formula: [body weight (g) − [AT volume (cm^3) × 0.9]].

We assessed a number of additional outcomes to obtain indicative data. Feed related outcomes were total number of days on which feeds were withheld on any occasion after trial enrolment; age when enteral feeds ≥150 ml/kg/day were maintained for at least 3 days (coded as first day achieved); total days received OMM prior to 34 weeks; feeding mode at discharge home (breast, formula, mixed). Health care resource use measures were total length of stay (days); postmenstrual age at discharge; days in intensive, high-dependency and low-dependency care according to national definitions (British Association of Perinatal Medicine, 2011) [5]. Neonatal morbidities and clinical outcomes: were survival to discharge; retinopathy of prematurity (maximum stage and intervention); necrotising enterocolitis requiring surgery or leading to death; blood-culture positive sepsis; total days when any antibiotic was administered; chronic lung disease (oxygen requirement or need for any pressure support at 36 weeks postmenstrual age), peri-ventricular haemorrhage and/or presence of parenchymal damage. Definitions were based on national criteria (National Neonatal Audit Programme) [6]. Weight gain was described as a change in weight standard deviation score between birth and discharge home and between birth and MR scan.

2.6. Protocol amendments

After commencing recruitment, we extended the eligibility criteria from gestational age at birth <29 weeks to <30 weeks and extended the time from birth to randomisation from 48 to 72 h to enhance recruitment.

2.7. Trial oversight

We established a Trial Steering Committee to oversee study conduct. Trial data were obtained using a combination of paper case record forms and the National Neonatal Research Database (NNRD) [7]. This contains data (the Neonatal Data Set, an NHS Information Standard; SCCI 1595) extracted at regular intervals from the electronic patient records of infants admitted to UK neonatal units from 2007 to the present and quality-assured prior to deposition in the NNRD. The NNRD is a national Data Asset discoverable through the Health Data Research UK Alliance Innovation Gateway (https://www.healthdatagateway.org/) and is available for use by external investigators. A formal test of NNRD data quality showed <5 % discordance with equivalent items collected independently for a trial funded by National Institute of Health Research and performed to Good Clinical Practice standards [8].

2.8. Statistical considerations

We based sample size calculations on our previous work showing

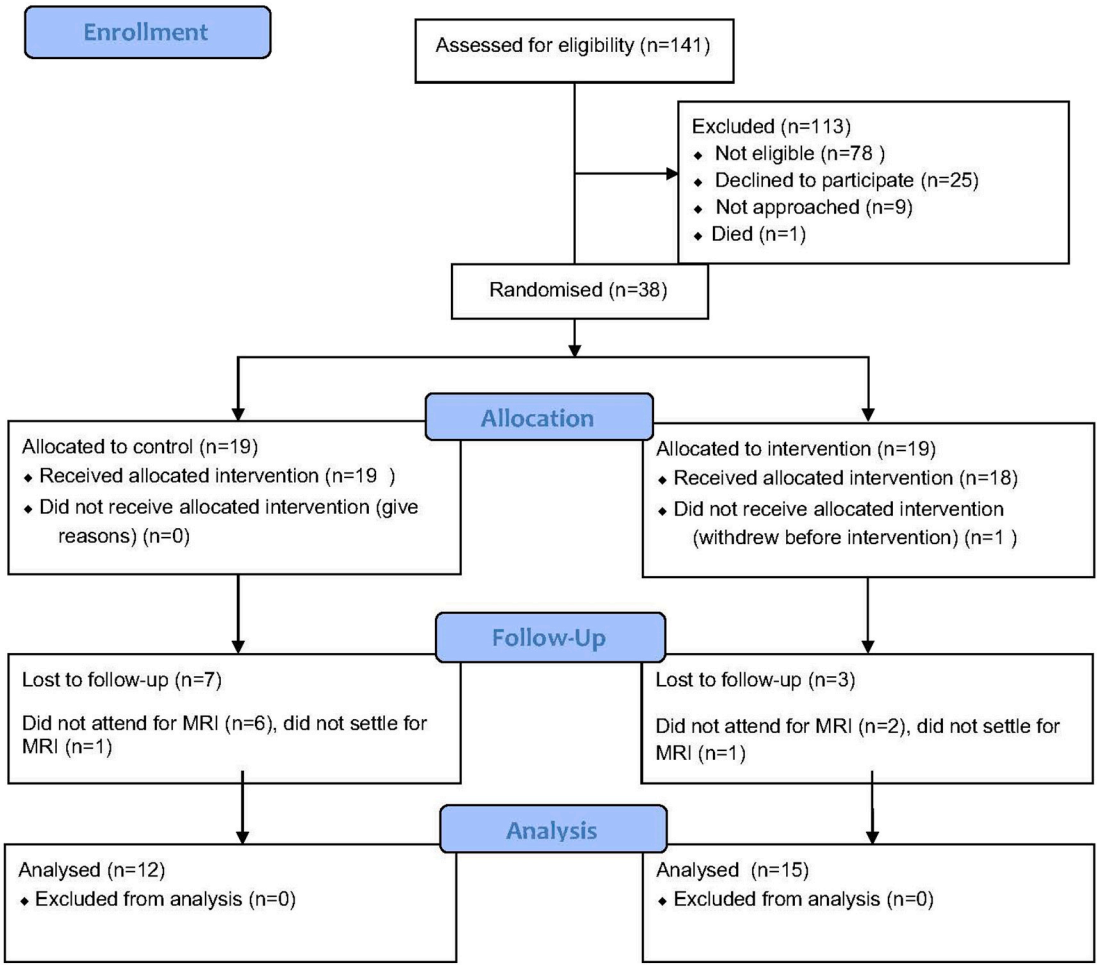

Fig. 1. CONSORT flow diagram.

Table 1
Baseline characteristics.

	Intervention n = 18	Control n = 19
	Exclusive human milk diet	Diet containing cow milk products
Gestational age at birth (weeks)	26.9 (25.8, 27.8)	27.1 (25.2, 28.9)
Birthweight (g)	926.5 (805, 1038.5)	978 (729, 1110)
Birthweight SDS	−0.29 (−0.58, 0.25)	−0.48 (−0.93, 0.18)
Sex (male) n (%)	11 (61)	8 (42)
Multiple birth n (%)	3 (17)	5 (26)
Antenatal steroids n (%)	Complete course: 16 (89) Incomplete course: 1 (6) Not known: 1 (6)	Complete course: 15 (79) Incomplete course: 2 (11) Not known: 2 (11)
Mode of delivery n (%)	Vaginal: 9 (50) In labour Caesarean: 4 (22) Pre-labour Caesarean: 5 (28)	Vaginal: 13 (68) In labour Caesarean: 1 (5) Pre-labour Caesarean: 5 (26)

Values are median and range or count.

mean (SD) non-AT mass of 2.41 (0.46) kg in very preterm babies [9]. We calculated that the enrolment of 20, 18 and 16 babies per group would enable the detection of 0.42 kg, 0.44 kg, and 0.47 kg respectively between group differences in non- AT mass (80 % power; 5 % significance level, 2-sided).

We compared total AT mass and non-AT mass using the permutation test. Adjustments were made for sex and length at the time of imaging using a regression model.

Continuous variables are presented as median and range, categorical variables are presented as numbers and percentages.

Analyses were carried out using R version 3.3.0.

3. Results

We enrolled 38 babies to the study, 19 each to intervention and control diets 0.113 were excluded because they did not meet the eligibility criteria, or parents declined consent or for other reasons (Fig. 1). Seven babies in the control group and three in the intervention group were lost to follow up either because they did not attend for the MR scan or did not settle for the scan.

Baseline characteristics are shown in Table 1.

There were no differences in feed tolerance, days of parenteral nutrition, exclusive breast-feeding at discharge, total length of stay on the neonatal unit, postmenstrual age at discharge from the neonatal unit, change in weight SDS between birth and discharge or any other core neonatal outcomes. There were no differences in total percentage or regional adiposity, Tables 2 and 3.

After adjustment for length and sex, the estimated effects of the intervention on the primary outcomes of ATM and N-ATM were not significant (ATM (kg): 0.137, 95 % confidence interval (CI) −0.01, 0.29; N-ATM: −0.137; −0.01, 0.29), Table 4.

Table 2
Body composition and anthropometry.

	Intervention n = 15	Control n = 12	P value
	Exclusive Human Milk Diet (Own Mother's Milk + Prolacta products)	Own Mothers Milk + Bovine Origin Fortifier + Preterm Formula	
Primary			
Non adipose tissue mass at term (kg)	2.25 (2.015, 2.492)	2.255 (2.105, 2.459)	0.97
Adipose tissue mass at term (kg)	0.8 (0.565, 1.05)	0.755 (0.66, 0.828)	0.27
Secondary			
% Adipose tissue mass	26.5 (21.1, 28.2)	25.6 (21.1, 27.4)	0.52
Non-adipose tissue to adipose tissue mass ratio	2.8 (2.6, 3.7)	2.9 (2.7, 3.8)	0.67
Total internal AT volume	0.106 (0.08, 0.14)	0.096 (0.09, 0.11)	0.04
Total SSC AT volume	0.783 (0.51, 0.98)	0.7 (0.62, 0.75)	0.13
Total DSC AT volume	0.025 (0.02, 0.04)	0.034 (0.02, 0.03)	0.67
Total internal abdominal AT volume	0.04 (0.03, 0.05)	0.038 (0.03, 0.04)	0.14
Total SSCA AT volume	0.151 (0.10, 0.20)	0.144 (0.12, 0.15)	0.15
Total DSCA AT volume	0.018 (0.01, 0.02)	0.02 (0.01, 0.02)	0.77
Weight (kg)[a]	2.920 (2.612, 3.600)	2.995 (2.730, 3.196)	0.82
Weight SDS	−1.3 (−1.8, −0.1)	−1.2 (−1.7, −0.9)	0.83
Head circumference (cm)[a]	34.2 (33.9, 35.9)	34.8 (34.1, 35.9)	0.51
Head circumference SDS	−0.222 (−0.9, 0.8)	−0.012 (−1.4, 1.0)	0.82
Length (cm)[a]	48.4 (46.7, 49.3)	48.2 (46.9, 49.5)	0.83
Length SDS	−1.6 (−2.4, −0.6)	−1.5 (−2.0, −0.8)	0.837
Change in weight Z-score, birth to MR scan	−0.87 (−1.8, −0.3)	−0.74 (−1.5, −0.4)	0.50

Values are median (IQR) or count.
AT volume in litres.
AT: adipose tissue; SSC: superficial subcutaneous; DSC: deep subcutaneous, SSCA: superficial subcutaneous abdominal; DSCA: deep subcutaneous abdominal.
[a] At MR scan.

4. Discussion

In this randomised controlled trial comparing an exclusive human milk-based diet with one containing cow milk products, with macronutrient fortification in both arms, we found no suggestion of important differences in body composition at term. Our sample size was small but the upper limit of the interval for the effect of the intervention was around 200 g for both non-AT and AT mass. Such an effect size might have clinical relevance as mean AT mass in this population is around 770 g.

The strengths of the study are the use of a gold standard method to assess body composition, image-analysis blind to treatment allocation, and excellent adherence to trial protocol. Study limitations are the sample size that provided limited power to detect small differences in outcomes and loss to follow up where parents declined or did not attend for study imaging, an understandable situation given the burden and stresses of very preterm birth.

Despite limitations, our study provides important preliminary data and raises intriguing questions. In a previous secondary analysis [3] of data obtained as part of a randomised controlled trial of parenteral nutrition we found greater non-AT mass and greater positive change in weight Z-score from birth at term in predominantly formula-fed

Table 3
Additional outcomes.

	Intervention n = 18	Control n = 19	P value
	Exclusive Human Milk Diet (Own Mother's Milk + pooled human milk derived products)	Own Mothers Milk + Cow Milk derived fortifier + preterm formula	
All infants recruited			
Number of days enteral feeds withheld on any occasion after trial enrolment to 34 weeks (days)	3 (0.5, 4.8)	3 (1, 4)	0.13
Postnatal age on first day when enteral feeds ≥150 ml/kg/day maintained for at least 3 days (days)	15 (12, 19)	14 (12, 18)	0.78
Days received PN (days)	16 (10.5, 20)	14 (12, 17.5)	0.13
Days any Own Mother's Milk received prior to 34 weeks postmenstrual age (days)	71 (55.5, 93.8)	59 (47, 83)	0.31
Postmenstrual age achieved suck feeds (weeks)	38.5 (36.1, 39.8)	37.3 (35.9, 38.9)	0.20
Infants receiving exclusive breast-feeding at neonatal unit discharge; n (%)	8 (44.4)	10 (52.6)	0.87
Mixed breast and formula feeding at neonatal unit discharge; n (%)	6 (33.3)	5 (26.3)	0.41
Total length of neonatal unit stay (days)	97 (74, 105)	88 (61, 113.5)	0.33
Postmenstrual age at neonatal unit discharge (weeks/days)	39.6 (38.6, 40.9)	39.6 (36.9, 40.9)	0.83
Proportion of neonatal unit stay in intensive care (%)	28.3 (17.9, 37.2)	19.5 (13.3, 29.6)	0.27
Proportion of neonatal unit stay in high dependency care (%)	44 (36.4, 53)	41.7 (27.5, 46.1)	0.34
Proportion of neonatal unit stay in special care (%)	25.2 (16, 45.7)	33.3 (23, 53.4)	0.09
Survival to discharge; n (%)	18 (100)	19 (100)	
Treated retinopathy of prematurity; n (%)	0 (0)	2 (10.5)	
Necrotising enterocolitis requiring surgery; n (%)	0 (0)	0 (0)	
Necrotising enterocolitis requiring surgery or resulting in death; n (%)	0 (0)	0 (0)	
Infants with 1 or more episodes of blood-culture positive sepsis; n (%)	3 (17)	4 (21)	0.91
Number of days antibiotics received as a proportion of length of stay (%)	18.4 (12.4, 25.3)	14.1 (11.7, 25.1)	0.24
Oxygen requirement or need for pressure support at 36 weeks postmenstrual age; n (%)	12 (66.7)	10 (52.6)	0.60
Periventricular haemorrhage and/or parenchymal damage; n (%)	0 (0)	0 (0)	
Change in weight Z-score, birth to neonatal unit discharge	−1.14 (−1.5, −0.7)	−1.22 (−2.2, −0.7)	0.751

Values are median (IQR).

Table 4
Regression analysis of effect of intervention on adipose tissue compartments adjusted for length and sex.

	Mean adjusted difference (95 % CI)	P value
Non adipose tissue mass (kg)	−0.035 (−0.21, 0.14)	0.70
Adipose tissue mass (kg)	0.137 (−0.01, 0.29)	0.06
Total internal AT volume	0.018 (−0.01, 0.04)	0.16
Total SSC AT volume	0.128 (−0.02, 0.27)	0.08
Total DSC AT volume	0.006 (−0.004, 0.016)	0.23
Total internal abdominal AT volume	0.006 (−0.01, 0.02)	0.28
Total SSCA AT volume	0.033 (−0.01, 0.07)	0.06
Total DSCA AT volume	0.004 (−0.0031, 0.01)	0.28

compared to exclusively human milk fed babies. We adjusted for macronutrient intake as approximately half the babies in the exclusive human milk group received cow milk based fortifier. The difference between the groups raised the possibility that differences in body composition might be attributable to the source of dietary protein. The authors of a literature review of feeding in preterm infants, concluded that in comparison with formula feeding, human milk feeds are associated with slower weight gain, and greater fat-free mass [10]. However the results of our study do not lend support to this conclusion.

The dietary ratio of protein to energy also affects body composition [11,12]. A low protein to energy ratio as found in human donor milk, leads to lower non-AT body mass with excess energy stored as fat. In the present study, both intervention and control groups received fortification and the ratio of protein to energy was similar.

Babies in the present study, irrespective of group allocation, had higher adiposity and lower non-AT mass to AT mass ratio in comparison with babies we studied between six and nine years ago [3]. The optimal ratio of non-AT mass to AT mass in very preterm babies at term is unknown. There is an association between body composition at term age equivalent and Bayley scores at 2 years of age in preterm infants, with fat free mass associated with higher Bayley composite motor and language scores at 2 years [13] In babies born at full-term this ratio is around 3 [14], and of note, babies in both intervention and controls arms of the present study were around this value at 2.8 and 2.9 respectively. Differences over time may reflect changes in nutritional practices.

In conclusion, we showed that a novel exclusive human milk diet was well-tolerated. We identified no indication of differences in body composition when compared with a diet containing cow milk products. The pressing and as yet unresolved issue for neonatal practice is whether an exclusive human milk diet results in functional benefits for very preterm babies. This will require a high-quality randomised controlled trial with power to detect clinically relevant differences in outcomes that are important to babies and their parents. Until such evidence are available, the optimal diet for very preterm babies remains uncertain.

CRediT authorship contribution statement

Sabita Uthaya: Conceptualisation, methodology, interpretation, first and final draft of manuscript.

Suzan Jeffries and Izabela Andrewsjewska: Recruitment, randomisation, data collection, review of final draft.

Vimal Vasu: Recruitment, oversight at trial site, review of draft and final draft.

Nicholas Embleton: Conceptualisation, methodology, review of draft and final draft.

Neena Modi: Conceptualisation, methodology, interpretation, reviewed and edited first and final draft of manuscript.

Declaration of competing interest

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SU, VV, IA, SJ declare no conflicts of interest relating to the work under submission.

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