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A novel human milk fortifier supports adequate growth in very low birth weight infants: a non-inferiority randomised controlled trial

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ABSTRACT

Objective To compare growth, tolerance and safety parameters in very preterm infants receiving human milk (HM) fortified with a multicomponent cow's milk-based HM fortifier (HMF; control) versus a novel HMF-containing lipids (including docosahexaenoic acid and arachidonic acid), higher protein and lower carbohydrate levels (test). Our hypothesis was that weight growth velocity in the test group would be non-inferior to that in the control group.

Design Double-blind, randomised controlled trial.

Setting Nine European neonatal intensive care units.

Patients HM-fed infants born at <32-week gestational age.

Interventions Fortification of HM with Test or Control HMF for a minimum of 21 days.

Primary outcome Weight growth velocity between baseline and intervention day 21.

Results From March 2018 to July 2020, 102 and 103 infants were enrolled in the test and control groups, respectively. Weight growth velocity during the first 21 days in the test group (mean 18.4 g/kg/day) was non-inferior to that of controls (mean 18.5 g/kg/day), with a difference in estimated means of –0.175 g/kg/day (90% CI –1.34 to +0.99 g/kg/day; per-protocol population). No significant differences between groups were observed for gain in length, head circumference or anthropometric Z-scores. Rates of digestive intolerance, stool frequency and consistency were comparable. No significant differences were reported in common neonatal morbidities including necrotising enterocolitis (test: 2.9%, control: 6.9%, mean difference –4.0% (95% CI –11.1% to 2.2%); all subjects treated population).

Conclusions Use of the novel HMF containing lipids, higher protein and lower carbohydrate levels supports adequate postnatal growth and appears safe and well tolerated in very preterm infants.

Trial registration number NCT03315221

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Fortification of human milk with a multinutrient human milk fortifier (HMF) is recommended to promote optimal growth in preterm infants with a weight <1800 g.

⇒ The optimal composition of multinutrient HMFs is still a topic of debate.

WHAT THIS STUDY ADDS

⇒ Very low birth weight (VLBW) infants receiving a novel HMF with lipids, a lower carbohydrate and higher protein content had a non-inferior weight growth velocity compared with VLBW infants receiving a conventional HMF.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Use of the novel lower osmolality HMF was safe and well tolerated in this population of VLBW infants and supported adequate postnatal growth.

INTRODUCTION

Human milk (HM) is the preferred nutrition for preterm infants.[1 2] However, levels of macronutrients and micronutrients in HM are considered inadequate to support their optimal growth, body composition and neurodevelopment, especially in very preterm infants.[1–5] Therefore, multinutrient fortification of HM is recommended to enhance nutrient content and promote growth in preterm infants with a birthweight <1800 g.[2 6 7] Human milk fortifiers (HMFs) are especially important to reduce cumulative nutrient deficits and postnatal growth restriction because poor growth is associated with impaired neurodevelopmental outcomes.[8 9]

Although HM fortification is standard practice in very low birth weight (VLBW; <1500 g) infants, the optimal composition of multinutrient HMFs is a topic of debate. Optimal protein content of HMFs is unclear, as that of mothers' own milk varies between and within individuals, is influenced by many factors including time after delivery,[10 11] and is often lower than needed to support postnatal growth.[10 12]

As preterm infants are at risk of long-chain polyunsaturated fatty acid (LCPUFA) deficiency, dietary supplementation with docosahexaenoic acid (DHA) and arachidonic acid (ARA) is recommended.[2 11–14] Addition of lipids to HMFs, at the expense of carbohydrates, allows for a reduction in osmolality and an enhanced LCPUFA supply.

A novel multicomponent lower osmolality HMF was developed to improve fatty acid supply when compared with conventional HMF. It contains

DHA and ARA in equal amounts, easily absorbed fats (ie, medium chain triglycerides and anhydrous milk fat), higher protein and lower carbohydrate levels. This study's objectives were to: (1) demonstrate non-inferiority of weight growth velocity during the first 21 days of intervention for the novel HMF compared with control HMF and (2) confirm that the novel HMF is safe and well-tolerated in VLBW infants.

METHODS

Participants

Infants born <32 weeks gestational age (GA) with birthweight <1500 g who were fed HM, enterally and needing HMF for minimum 21 days, were eligible. Nine neonatal intensive care units (NICUs) in the United Kingdom, France, Netherlands and Germany participated.

Exclusion criteria were chromosomal anomaly, metabolic disorder, genetic syndrome, congenital central nervous system malformation, gastrointestinal malformation/compromise, no realistic prospect of survival or participation in another investigational study. All parents/guardians provided written informed consent before enrolment.

Study design and intervention

A double-blind parallel-group, non-inferiority RCT was conducted (online supplemental figure 1). Infants were randomly allocated (block size of four), on a 1:1 basis, stratified by site to receive the test or control HMF daily. For multiples, other eligible siblings were allocated the same HMF as the first sibling randomised. Site staff responsible for feeding administration and study assessments were blinded to the actual HMF allocation (online supplemental methods). The intervention lasted ≥21 days, from the first use of HMF until the infant no longer required HMF, or was discharged home or to a non-participating NICU, whichever came first. Infants were followed up until 24 months corrected age (CA). The study was conducted in compliance with the principles of the Declaration of Helsinki, according to ICH-GCP, and approved by Ethics Review Boards of participating centres/countries.

Feeding regimen

During the study period, infants were fed mother's own milk or pasteurised donor milk when the former was insufficient or not available. Preterm infant formula was provided if fortified HM was insufficient. Fortification of HM was started when a sufficient enteral feeding volume was reached according to each unit's protocol. Both study products were powdered multinutrient HMFs (200 g tins). The novel HMF (test) provided 17 kcal, 0.7 g lipids, 1.3 g protein and 1.5 g carbohydrates per 4.0 g (dose per 100 mL HM; table 1). The added lipids included (phospholipid- and triglyceride-bound) DHA and ARA, medium-chain triglycerides and anhydrous milk fat as source of beta-palmitate. The control HMF, a product in widespread clinical use at the time of study conduct, provided 15 kcal, 0.0 g lipids, 1.1 g protein and 2.7 g carbohydrates per 4.4 g (dose per 100 mL HM). Both HMFs contained extensively hydrolysed protein (casein:whey ratio 50:50) and maltodextrin, as this glucose polymer is preferred to glucose due to its lower osmolality.[2] The test HMF had a higher protein content than the control HMF, as recent studies have shown that a HMF protein level of 1.0–1.1 g/100 mL may result in lower than recommended protein intakes.[2] Products were manufactured by Danone Nutricia according to Good Manufacturing Practices (FSSC 22000 standard). Osmolalities of HM fortified with HMF were: test 410 mOsm/kg; control 450 mOsm/kg.

Primary and secondary outcomes

The primary outcome was weight growth velocity (g/kg/day) between the start of intervention (baseline) and intervention day 21. Secondary outcomes were gains in length, head circumference (HC) and anthropometric z-scores, digestive tolerance parameters and adverse events (AEs). Weight-for-age, length-for-age, HC-for-age z-scores and percentiles were calculated using Fenton growth charts for preterm infants[15] (online supplemental methods).

Data collection

Data were collected from randomisation until 24 months CA, captured into an electronic database (online supplemental methods).

Safety monitoring

Information on AEs, including onset, duration, relationship with study product, severity, seriousness, actions taken, outcomes, concomitant medication use and medical interventions, were recorded (online supplemental methods).

Statistical analysis

A sample size of 91 per group was calculated with a one-sided test (α=0.05, power=0.80, dropout rate=20%, potential loss of df due to number of sites (>5)). With the predefined non-inferiority margin of −1.6 g/kg/day,[16] difference between groups of 0.2 g/kg/day[17] and standard deviation (SD) of 4.3 g/kg/day.[18] Analyses for weight growth velocity were performed using a parametric growth curve mixed-effects model with a quadratic effect of time adjusted for potentially prognostic fixed effect covariates and a family-specific random effect to account for the correlation of the growth outcomes in twins/multiples (online supplemental methods).[19 20] The all subjects treated (AST) population, which included infants with at least one feeding with study HMF, was used for the analyses of safety and digestive tolerance parameters. The per protocol (PP) population was the primary dataset for the efficacy analyses. In non-inferiority trials, there is a general concern that by including participants who did not receive the planned interventions, the two comparison groups become more similar, resulting in incorrect conclusions of non-inferiority.[21–23] Supplementary analyses were performed in the all subjects randomised (ASR) population to evaluate the robustness of the results obtained in the PP population.

RESULTS

Infant characteristics

Between March 2018 and July 2020, 205 infants (102 test, 103 control) were enrolled, of whom 183 were randomised and 22 siblings of multiples were allocated (figure 1). One infant did not consume any study HMF and was excluded from the AST population. The PP population included 155 infants. Dropout rates and reasons for early termination were similar in both groups.

Baseline characteristics were similar between groups in both PP (table 2) and ASR populations (online supplemental table 1). Infants were born at a mean GA of 27.8 (SD: 2.1) weeks with a mean birth weight of 986 g (SD: 264 g). Thirteen per cent of infants were small for GA (birthweight <10th percentile).

Table 1 Nutrient composition of intervention human milk fortifiers (HMFs)

		Test HMF per serving of 4.0 g (4 scoops)†	Per 100 mL fortified human milk‡	Control HMF per serving of 4.4 g (4 scoops)†	Per 100 mL fortified human milk‡
Macronutrients					
Energy	kcal (kJ)	17 (72)	82 (352)	15 (65)	80 (345)
Protein	g (%Energy)	1.3 (30)	2.8	1.1 (29)	2.6
Carbohydrate	g (%Energy)	1.5 (33)	8.4	2.7 (71)	9.6
Fat	g (%Energy)	0.7 (37)	4.2	–	3.5
Arachidonic acid (ARA)*	mg	5.0	25	–	20
Docosahexaenoic acid (DHA)*	mg	5.0	20	–	15
Medium chain fatty acids	g	0.3	0.59	–	0.29
Milk fat	g	0.4	0.4	–	–
Vitamins					
Vitamin A	µg RE	232	298	232	298
Vitamin D3	µg	5.54	5.74	5.0	5.2
Vitamin E	mg α-TE	2.6	2.8	2.6	2.8
Vitamin K	µg	16	16.3	6.4	6.7
Vitamin C	mg	12	16.5	12	16.5
Thiamin	mg	0.13	0.14	0.13	0.14
Riboflavin	mg	0.17	0.20	0.17	0.20
Niacin	mg	2.3	2.8	2.3	2.8
Pantothenic acid	mg	0.75	0.95	0.76	0.96
Vitamin B6	mg	0.11	0.12	0.11	0.12
Biotin	µg	2.5	3.0	2.5	3.0
Folic acid	µg	30	38	30	38
Vitamin B12	µg	0.20	0.22	0.20	0.22
Minerals					
Sodium	mg	33	62	35	64
Potassium	mg	23	73	23	73
Chloride	mg	25	79	25	79
Calcium	mg	70	95	66	91
Phosphorus	mg	38	52	38	52
Magnesium	mg	5.0	8.1	5.0	8.1
Iron	mg	≤0.02	0.035	≤0,02	0.035
Zinc	mg	0.60	0.97	0.61	0.97
Copper	µg	41	81	35	75
Manganese	µg	7	7.4	8.1	8.5
Selenium	µg	1.8	3.4	1.7	3.3
Iodine	µg	11	24.6	11	24.6
Osmolality					
Osmolality	mOsm/kg		410		450

*ARA source is egg lipid and single-cell oil derived from *Mortierella alpina*, DHA source is fish oil and egg lipid.
†Amount to be added to 100 mL human milk.
‡The composition of (preterm) human milk varies greatly. Therefore, the nutrient concentrations in human milk are estimated from literature[43–46] and internal data.

Growth

The primary outcome measure, that is, weight growth velocity during the first 21 days of intervention, of the test group (mean 18.4 g/kg/day), was non-inferior to that of controls (mean 18.5 g/kg/day). The difference in estimated means between groups was –0.175 g/kg/day (90% CI, –1.34 to +0.99) for the PP population, with the lower CI above the pre-defined non-inferiority margin (table 3). No significant differences between groups were observed in length gain, HC gain or changes in anthropometric z-scores (table 3). Results were similar for ASR analyses (data not shown).

At the start of the intervention, 33 (38.8%) test and 21 (30.4%) control infants had a weight <10th percentile (PP population). At day 21 and day 28 after the start of intervention, 35 (41.2%) and 31 (41.3%) of the test and 28 (40.6%) and 17 (29.8%) of the control infants had a weight <10th percentile respectively.

Model estimates for weight, length, HC and anthropometric z-scores during the first 21 days of intervention are presented in online supplemental figures 2 and 3. The weight-for-age z-scores were stable in both groups.

HMF intake and digestive tolerance

Infants reached enteral feeding volumes of ≥150 mL/kg/day at an average age of 13.7 (test) and 13.4 days (control; AST population). Mean (SD) enteral feeding intake during the first 21 days of intervention was 144 (19.4) mL/kg/day in test and 143 (18.8) mL/kg/day in control groups. In both groups, the

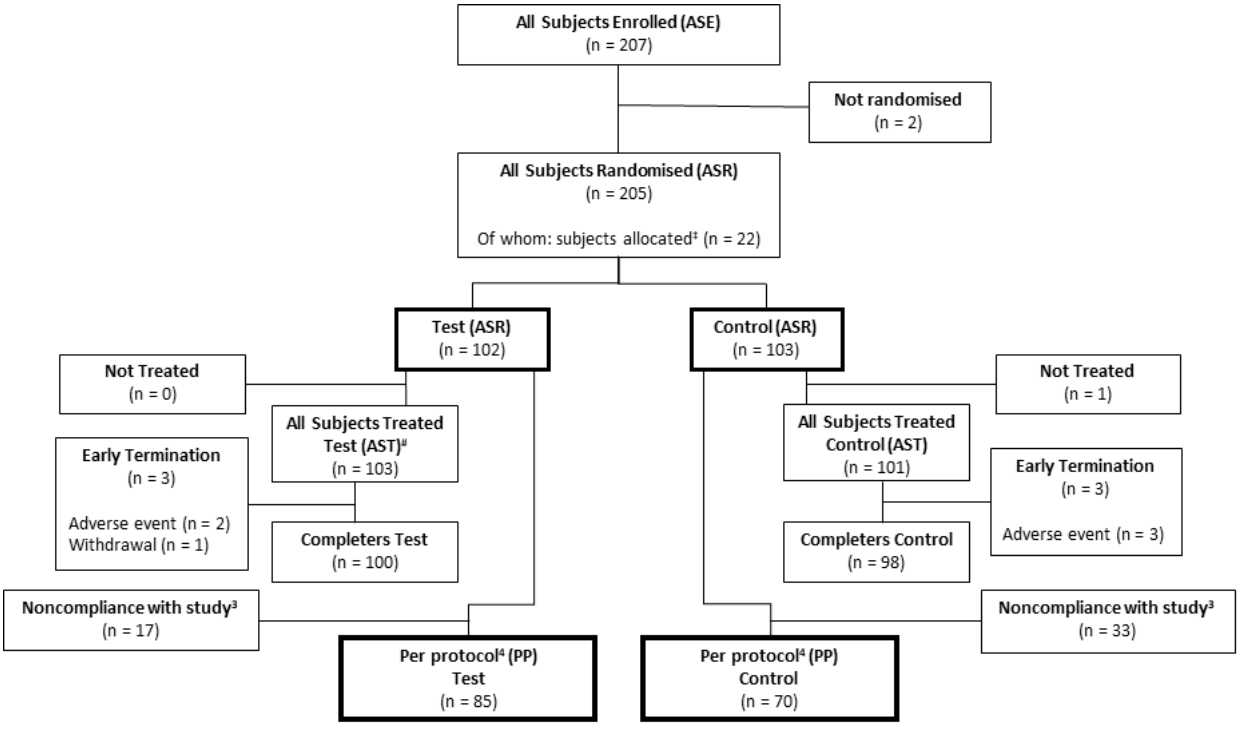

Figure 1 Subject flow. HMF, human milk fortifier. ‡Allocated subjects were siblings assigned to the same group. #One subject randomised to the Control group received Test HMF. [3]Noncompliance with study: subjects with major protocol deviations: A. Major violation of the inclusion or exclusion criteria: n=0. B. Non-compliance with study product intake, as indicated by exposure to study product for <21 days: n=50. C. Not having any post-baseline measurements of weight – no additional exclusions. [4]PP subjects were all subjects without major protocol deviations.

Table 2 Demographics and infant characteristics of the per protocol population

		Test (N=85)	Control (N=70)	Total (N=155)
Sex	Female (%)	48.2	40.0	44.5
	Male (%)	51.8	60.0	55.5
Country	Germany (%)	16.5	18.6	17.4
	France (%)	15.3	20.0	17.4
	The Netherlands (%)	29.4	18.6	24.5
	United Kingdom (%)	38.8	42.9	40.6
Gestational age at birth (weeks)	Mean (SD)	27.8 (2.18)	27.9 (2.04)	27.8 (2.11)
	Min–max	23.7–31.7	23.3–31.7	23.3–31.7
Birth weight (g)	Mean (SD)	966.7 (270.8)	1009.1 (254.6)	985.8 (263.6)
	Min–max	415–1490	450–1457	415–1490
Length at birth (cm)	Mean (SD)	36.0 (3.3)	36.2 (3.1)	36.1 (3.2)
Head circumference at birth (cm)	Mean (SD)	25.2 (2.3)	25.2 (2.3)	25.2 (2.3)
Mode of delivery	Vaginal (%)	35.3	42.9	38.7
	Caesarean section (%)	64.7	57.1	61.3
Small for gestational age*	Yes (%)	15.3	10.0	12.9
	No (%)	85.3	89.3	87.3
Birth plurality	Singleton (%)	70.6	71.4	71.0
	Twin (%)	27.1	27.1	27.1
	Triplet or quadruplet (%)	2.4	1.4	1.9
Apgar score at 5 min	Median (Q1, Q3)	8 (7, 9)	8 (7, 9)	8 (7, 9)
Need for resuscitation in delivery room	Yes (%)	11.8	14.3	12.9
Number of days before baseline on (invasive) mechanical ventilation (days)	Median (Q1, Q3)	6 (2, 10)	6 (2, 9)	6 (2, 9)
Postnatal age at randomisation (days)	Mean (SD)	10.2 (5.2)	9.9 (5.5)	10.0 (5.4)

*Defined as birth weight<10th percentile for gestational age, based on Fenton growth charts for preterm infants.
n, number; Q, quartile; SD, standard deviation.

Table 3 Weight growth velocity, length gain, head circumference gain, anthropometric z-scores and changes in anthropometric z-scores during the first 21 days of the intervention (per protocol population)

Anthropometric parameter	Timepoint	Test (N=85)		Control (N=70)		Test vs control
		n (nmiss)	Mean (SE)	n (nmiss)	Mean (SE)	Mean diff (90% CI)
Weight growth velocity* (g/kg/day)			18.36 (0.47)		18.53 (0.52)	−0.175 (−1.34, 0.99)
						Mean diff (95% CI)
Length gain*(cm/week)			1.03 (0.10)		0.95 (0.08)	0.080 (−0.100, 0.261)
Head circumference gain* (cm/week)			0.94 (0.03)		0.90 (0.04)	0.045 (−0.054, 0.145)
			Mean (SD)		Mean (SD)	
Weight-for-age z-score (unit)	Baseline (day 0)	85 (0)	−1.02 (0.70)	69 (1)	−1.02 (0.57)	
	Intervention day 21	85 (0)	−1.07 (0.72)	69 (1)	−1.02 (0.70)	
	Intervention day 28	74 (11)	−1.05 (0.74)	57 (13)	−0.87 (0.76)	
	36 weeks PMA†	54 (31)	−1.13 (0.94)	30 (40)	−1.09 (0.98)	
			Mean (SE)		Mean (SE)	Mean diff (95% CI)
Weight-for-age z-score change‡ (unit/week)			−0.01 (0.01)		−0.01 (0.01)	−0.006 (−0.037, 0.025)
			Mean (SD)		Mean (SD)	
Length-for-age z-score (unit)	Baseline (day 0)	78 (7)	−0.93 (1.07)	62 (8)	−0.90 (0.89)	
	Intervention day 21	76 (9)	−1.32 (1.03)	67 (3)	−1.31 (0.76)	
	Intervention day 28	64 (21)	−1.36 (0.91)	53 (17)	−1.31 (0.82)	
	36 weeks PMA†	55 (30)	−1.39 (0.98)	37 (33)	−1.44 (0.97)	
			Mean (SE)		Mean (SE)	Mean diff (95% CI)
Length-for-age z-score change‡ (unit/week)			−0.13		−0.15	0.012 (−0.061, 0.085)
			Mean (SD)		Mean (SD)	
Head circumference-for-age z-score (unit)	Baseline (day 0)	75 (10)	−1.11 (0.77)	65 (5)	−1.07 (1.01)	
	Intervention day 21	80 (5)	−1.00 (0.82)	70 (0)	−1.08 (1.02)	
	Intervention day 28	69 (16)	−0.93 (0.80)	56 (14)	−1.03 (1.13)	
	36 weeks PMA†	57 (28)	−0.66 (0.82)	38 (32)	−0.62 (1.10)	
			Mean (SE)		Mean (SE)	Mean diff (95% CI)
Head circumference-for-age z-score change‡ (unit/week)			0.03		0.00	0.033 (−0.037, 0.103)

*Parametric growth curve model with: (1) fixed terms for study group, t (time), t^2, sex, gestational age, postnatal age at baseline, birth weight and interactions study group by t, study group by t^2 and (2) random effects for intercept, t, and t^2 with unstructured covariance matrix, a random intercept for site-ID (for a subject), and a random intercept for family ID (for a subject).
†36 weeks ±4 days; for length and HC 36 weeks ±7 days.
‡Parametric growth curve model with: (1) fixed terms for study group, t (time), t^2, sex, postnatal age at baseline, birth weight and interactions study group by t, study group by t^2 and (2) random effects for intercept, and t with unstructured covariance matrix, a random intercept for site-ID (for a subject), and a random intercept for family ID (for a subject).
§§*For weight growth velocity, the 90% CI of the difference is presented.*
CI, confidence interval; diff, difference; HC, head circumference; N, number; nmiss, number of missing data items; PMA, postmenstrual age; SD, standard deviation; SE, standard error.

dominant feeding was own mother's milk (81%–87%), with donor milk and preterm formula comprising 10%–15% and 1%–6% of the feeding volumes, respectively. During the first 21 days of intervention, 99 (96.1%) infants in the test group received own mother's milk, 28 (27.2%) received donor milk and 20 (19.4%) infants received preterm infant formula next to the supplemented HM. In the control group, this was 95 (94.1%), 32 (31.7%) and 29 (28.7%), respectively (AST; online supplemental table 2). Overall, infants started with HMF at a mean (SD) postnatal age of 10.6 (5.6) days. With a mean (SD) number of 40 (20.8) days on study HMF in test and 32 (19.8) days in Control groups.

Average stool frequency was 2.9 stools/day in the first three intervention weeks, without significant differences between groups. In both groups, most infants (>75%) had soft stools.

Percentages of infants with occurrences of vomiting, regurgitation or clinically significant gastric residuals during the first 21 days of intervention were similar between groups (online supplemental table 3). There was a small difference in the number of vomiting occurrences per day (medians: 0.3 vs 0.1, $p<0.001$) in infants with vomiting (test: n=35; control: n=33). A higher percentage of infants with any vomiting in test versus control groups was seen in the first week only, with the difference statistically significant in one site (online supplemental table 4).

AEs and clinical parameters

Out of 204 infants in the AST population, at least one AE was reported for 77.7% of test subjects and 75.2% of controls. Similar incidence of AEs was observed between groups.

At least one serious AE (SAE) was reported in 41.7% of test subjects and 36.6% of controls. No significant differences between groups were observed for any SAE category, except for a lower incidence of 'gastrointestinal disorders' in the test group (7.8% in test and 16.8% in control; Δ −9.1% (95% CI −18.56% to −0.04%); online supplemental table 5).

No significant differences were observed between groups in rates of common neonatal morbidities: necrotising enterocolitis, retinopathy of prematurity, bronchopulmonary dysplasia, periventricular leukomalacia and intraventricular haemorrhage (table 4).

Table 4 Neonatal morbidities; percentages of occurrence* and point estimate of difference between study groups, with 95% CIs for the all subjects treated population

	Test (N=103) n (%)	Control (N=101) n (%)	Test vs control† % difference (95% CI)
Necrotising enterocolitis (Bell's stage≥2)	3 (2.9%)	7 (6.9%)	−4.02% (−11.12%, 2.24%)
Retinopathy of prematurity	7 (6.8%)	10 (9.9%)	−3.10% (−11.41%, 4.82%)
Bronchopulmonary dysplasia	26 (25.2%)	17 (16.8%)	8.41% (−2.86%, 19.61%)
► Mild	7 (6.8%)	10 (9.9%)	
► Moderate	15 (14.6%)	5 (5.0%)	
► Severe	4 (3.9%)	2 (2.0%)	
Intraventricular haemorrhage (≥ grade 3)/periventricular leukomalacia	2 (1.9%)	1 (1.0%)	0.95% (-3.65%, 5.95%)

*Occurrence is presented as the percentage of infants with at least one adverse event starting during study product use.
†Miettinen-Nurminen confidence limits (including bias correction factor). Definitions: Necrotising enterocolitis with Bell's stage ≥2; Retinopathy of prematurity: based on International Committee for the Classification of Retinopathy of Prematurity; Bronchopulmonary dysplasia: categorised into mild/moderate/severe based on Sahni *et al*[22], Intraventricular haemorrhage ≥grade 3 based on Papile *et al*[23]/periventricular leukomalacia assessed on cranial ultrasound.
CI, class interval; n, number.

The number of infants with an AE leading to product withdrawal was similar between groups: test, n=7 (6.8%); control, n=9 (8.9%), as was the number of infants who terminated the study early: test, n=3 (2.9%); control, n=4 (3.9%), reasons for termination were also similar.

Blood levels of urea, blood urea nitrogen, pH, bicarbonate, sodium, potassium and albumin were available for a subgroup when collected as part of standard care. In this subgroup (n=46), mean (SD) urea levels for test versus control were comparable; 5.9 (3.7) versus 5.4 (2.7) mmol/L in the third week of intervention, respectively.

There was no difference between groups in postnatal age at discharge from NICU, postnatal age at discharge home or mortality during hospital stay (online supplemental table 6).

DISCUSSION

In this double-blind randomised, controlled trial, we demonstrated non-inferiority in weight growth velocity during the first 21 days of intervention in VLBW infants receiving a novel lower osmolality HMF containing lipids (including DHA and ARA), higher protein and lower carbohydrate levels compared with those receiving a control HMF without lipids.

The primary outcome measure of average weight growth velocity during the first 21 days of intervention was similar in both groups, just above 18 g/kg/day, in line with recommendations,[24] and similar to growth rates observed in a study with other lipid-containing HMFs.[25] Average weight-for-age z-scores and percentages of infants with a weight <10th percentile were stable during the first 28 days of intervention in both groups, suggesting that accumulation of postnatal growth deficit could be prevented in most infants with routine use of HMF.[26] This contrasts with the findings of Martin *et al*, who observed that the proportion of ELBW infants with a weight <10th percentile increased from 18% at birth to 75% at 28 days of life, despite growth velocity rates above 15 g/kg/day and HMF.[27]

The comparable growth rates of test and control groups might come from the relatively small difference in protein content (0.2 g/100 mL fortified HM) between study HMFs.[1 28 29] Other studies in preterm infants did not show improved growth comparing HMFs with different protein levels (1.8 g vs 1.0 g per 100 mL HM,[30 31] pointing towards a ceiling effect for enteral protein supply). Increasing protein intake enhances protein accretion and clinical outcome such as weight gain in a linear dose–response manner. It is hypothesised that beyond a so-called 'beneficial upper limit', higher protein intakes do not yield further improvements in clinical outcome[32]

More studies have compared HMFs with varying protein and lipid levels in preterm infants.[20 33–36] Rigo *et al* compared a new fortifier adding 0.7 g fat (including 6.3 mg DHA) and 1.4 g partially hydrolysed protein to 100 mL of HM, to an isocaloric fortifier with 0.0 g fat and 1.0 g extensively hydrolysed protein. Average weight growth velocity in the new fortifier group was 18.3 g/kg/day compared with 16.8 g/kg/day in controls.[25] Notably, the growth rate observed in their new fortifier group was similar to those of both groups in the current study. Another study compared a liquid HMF with a higher protein content (and ARA and DHA) to a traditional powder HMF in VLBW infants. Those who completed a 2-week intervention period showed no differences in weight, length, HC or knee–heel length gains.[37] This is in contrast to a study investigating the same liquid versus powder HMF, which showed significantly higher length growth in the liquid group.[18]

The novel HMF tested in this study contains components that may have benefits for preterm infants. First, it contains lipids at the expense of carbohydrates, reducing osmolality compared with the conventional HMF. The osmolality of the novel HMF fortified to HM of 410 mOsm/kg is well below the maximum tolerable level of 450–500 mOsm/kg indicated in recent reviews.[33 38] Second, the novel HMF includes DHA and ARA in equal amounts. A balanced DHA and ARA supplementation is needed for optimal infant growth and cognitive development.[2 13 14] Moreover, the DHA and ARA are partially bound to phospholipids, which are more efficiently absorbed by preterm infants than triglyceride-bound DHA,[34] and possibly more efficiently incorporated in body tissues.[35 36] Furthermore, the novel HMF contains medium-chain triglycerides, an easily absorbable source of energy,[39] and anhydrous milk fat with 38% of palmitic acid at the beta-position. Benefits of beta-palmitate are improved fat and calcium absorption and softer stools.[40 41] Finally, it contains extensively hydrolysed proteins, in line with clinicians' preferences.[1] Recently, Doshi *et al* showed decreased levels of faecal calprotectin in preterm infants after using a HMF with hydrolysed protein, suggesting that intestinal inflammation may be lower compared with fortifiers containing intact protein.[42]

Overall, AEs reported in this study were typical for preterm infants and no clinically relevant differences in AEs were observed between groups. Hence, there are no safety concerns for the use of this novel HMF in VLBW infants. The low incidence of

necrotising enterocolitis, a condition often linked with preterm enteral nutrition, in this study (test: 2.9% and control: 6.9%) is reassuring. Furthermore, it is encouraging that the incidence of gastrointestinal SAEs was lower in test compared with control infants. We hypothesise that this is related to the lower osmolality of the novel HMF.

In the first week of the intervention, a higher percentage of infants with vomiting was reported for test versus control. Vomiting was not defined *a priori* and was assessed according to local investigators' judgement. This led to high inter-hospital variability in its recording with incidence ranging from 8% to 91% between sites. In the centre with a significant diffrence between groups, it was due to the joint recording of vomiting and aspirates. Their reports of vomiting should be best interpreted as a combination of 'spitting up/posseting' and true vomiting. Overall, infants with vomiting had on average <1 vomiting episode per day. No subject terminated the study prematurely due to vomiting, while all infants grew adequately. We therefore consider these findings as not clinically relevant.

Strengths of our study include the randomised controlled trial design, strict blinding and a relatively large study group. Nine hospitals in four European countries participated, with variations in fortification approaches and standard clinical care. This enhances external validity of the results, as it mimics reality. The study included infants who were fed with their own mother's milk, donor human milk and/or preterm formula, reflecting reality as a mother's own milk is not always available in sufficient amounts.

An important limitation is that the intervention period had a minimum duration of 21 days, which is relatively short. Data were available for a longer period, but only for a subset of the population. This hindered evaluation of growth during a longer time frame. Also, no data on individual HM composition were available. It is known that there is large variation in the composition of HM between mothers,[10–12] and that donor milk on average has a different composition to own mother's milk, including lower protein and LCPUFA levels.[28 43] No blood sampling was done to analyse fatty acid profiles, which would have provided valuable insights into the LCPUFA status. Finally, the PP approach instead of the intention to treat approach could be questioned. The PP population was, by design, the primary dataset for the efficacy analyses, as it is the preferred approach in non-inferiority trials believed to lead to more conservative conclusions.[21] However, analysis in the PP population can lead to biased estimates of the treatment effect, as non-compliant subjects are removed from the comparison. In a sensitivity analysis, non-inferiority was confirmed in the ASR population indicating robustness of our conclusions. In addition, comparison of the baseline covariates between the PP and ASR population showed similar characteristics in both population arms, which confirms the balance of randomisation was not severely influenced by excluding non-adhering participants in the PP population, although this cannot be fully ruled out.

In summary, in a population of VLBW infants, we observed that a novel multicomponent HMF containing LCPUFA and well-absorbable fats appears to support adequate postnatal growth and to be safe and well-tolerated.

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REFERENCES

1 Arslanoglu S, Boquien C-Y, King C, *et al*. Fortification of Human Milk for Preterm Infants: Update and Recommendations of the European Milk Bank Association (EMBA) Working Group on Human Milk Fortification. *Front Pediatr* 2019;7:76.
2 Embleton ND, Jennifer Moltu S, Lapillonne A, *et al*. Enteral Nutrition in Preterm Infants (2022): A Position Paper From the ESPGHAN Committee on Nutrition and Invited Experts. *J Pediatr Gastroenterol Nutr* 2023;76:248–68.
3 Brown JV, Lin L, Embleton ND, *et al*. Multi-nutrient fortification of human milk for preterm infants. *Cochrane Database Syst Rev* 2020;6:CD000343.
4 Embleton ND. Optimal protein and energy intakes in preterm infants. *Early Hum Dev* 2007;83:831–7.
5 Picaud JC, Vincent M, Buffin R. Human milk fortification for preterm infants: a review. In: Koletzko B, Cheah F-C, Domellöf M, et al, eds. *Nutritional care of preterm infants scientific basis and practical guidelines*. Basel, Switzerland: Karger, 2021: 122. 225–47.
6 Ziegler EE. Human milk and human milk fortifiers. In: Koletzko B, Poindexter B, Uauy R, eds. *Nutritional care of preterm infants scientific basis and practical guidelines*. Basel, Switzerland: Karger, 2014: 215–27.
7 American Academy of Pediatrics Committee on Nutrition. Nutritional needs of the preterm infant. In: Kleinman RE, Greer FR, eds. *Pediatric nutrition*. 7th edn. Elk Grove Village, IL, USA, 2014: 83–110.
8 Ehrenkranz RA, Dusick AM, Vohr BR, *et al*. Growth in the neonatal intensive care unit influences neurodevelopmental and growth outcomes of extremely low birth weight infants. *Pediatrics* 2006;117:1253–61.
9 Latal-Hajnal B, von Siebenthal K, Kovari H, *et al*. Postnatal growth in VLBW infants: significant association with neurodevelopmental outcome. *J Pediatr* 2003;143:163–70.
10 Maly J, Burianova I, Vitkova V, *et al*. Preterm human milk macronutrient concentration is independent of gestational age at birth. *Arch Dis Child Fetal Neonatal Ed* 2019;104:F50–6.
11 Tudehope D, Vento M, Bhutta Z, *et al*. Nutritional requirements and feeding recommendations for small for gestational age infants. *J Pediatr* 2013;162:S81–9.
12 Rochow N, Landau-Crangle E, Fusch C. Challenges in breast milk fortification for preterm infants. *Curr Opin Clin Nutr Metab Care* 2015;18:276–84.
13 Hadley KB, Ryan AS, Forsyth S, *et al*. The Essentiality of Arachidonic Acid in Infant Development. *Nutrients* 2016;8:216.
14 Harris WS, Baack ML. Beyond building better brains: bridging the docosahexaenoic acid (DHA) gap of prematurity. *J Perinatol* 2015;35:1–7.
15 Fenton TR, Kim JH. A systematic review and meta-analysis to revise the Fenton growth chart for preterm infants. *BMC Pediatr* 2013;13:59.
16 Lucas A, Fewtrell MS, Morley R, *et al*. Randomized outcome trial of human milk fortification and developmental outcome in preterm infants. *Am J Clin Nutr* 1996;64:142–51.
17 Kim JH, Chan G, Schanler R, *et al*. Growth and Tolerance of Preterm Infants Fed a New Extensively Hydrolyzed Liquid Human Milk Fortifier. *J Pediatr Gastroenterol Nutr* 2015;61:665–71.
18 Moya F, Sisk PM, Walsh KR, *et al*. A new liquid human milk fortifier and linear growth in preterm infants. *Pediatrics* 2012;130:e928–35.
19 Patel AL, Engstrom JL, Meier PP, *et al*. Accuracy of methods for calculating postnatal growth velocity for extremely low birth weight infants. *Pediatrics* 2005;116:1466–73.
20 Cormack BE, Embleton ND, van Goudoever JB, *et al*. Comparing apples with apples: it is time for standardized reporting of neonatal nutrition and growth studies. *Pediatr Res* 2016;79:810–20.
21 ICH. E9 statistical principles for clinical trials. European Medicines Agency; 1998.
22 Sahni R, Ammari A, Suri MS, *et al*. Is the new definition of bronchopulmonary dysplasia more useful? *J Perinatol* 2005;25:41–6.
23 Papile LA, Burstein J, Burstein R, *et al*. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm. *J Pediatr* 1978;92:529–34.
24 Fenton TR, Chan HT, Madhu A, *et al*. Preterm Infant Growth Velocity Calculations: A Systematic Review. *Pediatrics* 2017;139:e20162045.
25 Rigo J, Hascoët J-M, Billeaud C, *et al*. Growth and Nutritional Biomarkers of Preterm Infants Fed a New Powdered Human Milk Fortifier: A Randomized Trial. *J Pediatr Gastroenterol Nutr* 2017;65:e83–93.
26 Senterre T, Rigo J. Reduction in postnatal cumulative nutritional deficit and improvement of growth in extremely preterm infants. *Acta Paediatr* 2012;101:e64–70.
27 Martin CR, Brown YF, Ehrenkranz RA, *et al*. Nutritional practices and growth velocity in the first month of life in extremely premature infants. *Pediatrics* 2009;124:649–57.
28 Amissah EA, Brown J, Harding JE. Protein supplementation of human milk for promoting growth in preterm infants. *Cochrane Database Syst Rev* 2020;9:CD000433.
29 Gao C, Miller J, Collins CT, *et al*. Comparison of different protein concentrations of human milk fortifier for promoting growth and neurological development in preterm infants. *Cochrane Database Syst Rev* 2020;11:CD007090.
30 Maas C, Mathes M, Bleeker C, *et al*. Effect of Increased Enteral Protein Intake on Growth in Human Milk-Fed Preterm Infants: A Randomized Clinical Trial. *JAMA Pediatr* 2017;171:16–22.
31 Reid J, Makrides M, McPhee AJ, *et al*. The Effect of Increasing the Protein Content of Human Milk Fortifier to 1.8 g/100 mL on Growth in Preterm Infants: A Randomised Controlled Trial. *Nutrients* 2018;10:634.
32 Embleton ND, van den Akker CHP. Protein intakes to optimize outcomes for preterm infants. *Semin Perinatol* 2019;43:151154.
33 Pearson F, Johnson MJ, Leaf AA. Milk osmolality: does it matter? *Arch Dis Child Fetal Neonatal Ed* 2013;98:F166–9.
34 Carnielli VP, Verlato G, Pederzini F, *et al*. Intestinal absorption of long-chain polyunsaturated fatty acids in preterm infants fed breast milk or formula. *Am J Clin Nutr* 1998;67:97–103.
35 Liu L, Bartke N, Van Daele H, *et al*. Higher efficacy of dietary DHA provided as a phospholipid than as a triglyceride for brain DHA accretion in neonatal piglets. *J Lipid Res* 2014;55:531–9.
36 Wijendran V, Huang M-C, Diau G-Y, *et al*. Efficacy of dietary arachidonic acid provided as triglyceride or phospholipid as substrates for brain arachidonic acid accretion in baboon neonates. *Pediatr Res* 2002;51:265–72.
37 Masoli D, Mena P, Dominguez A, *et al*. Growth of Very Low Birth Weight Infants Who Received a Liquid Human Milk Fortifier: A Randomized, Controlled Multicenter Trial. *J Pediatr Gastroenterol Nutr* 2021;74:424–30.
38 Ellis Z-M, Tan HSG, Embleton ND, *et al*. Milk feed osmolality and adverse events in newborn infants and animals: a systematic review. *Arch Dis Child Fetal Neonatal Ed* 2019;104:F333–40.
39 Lapillonne A. Enteral and parenteral lipid requirements of preterm infants. In: Koletzko B, Pointdexter B, Uauy R, eds. *Nutritional care of preterm infants: scientific basis and practical guidelines*. Basel, Switzerland: Karger, 2014: 110. 82–98.
40 Havlicekova Z, Jesenak M, Banovcin P, *et al*. Beta-palmitate - a natural component of human milk in supplemental milk formulas. *Nutr J* 2016;15:28.
41 Miles EA, Calder PC. The influence of the position of palmitate in infant formula triacylglycerols on health outcomes. *Nutr Res* 2017;44:1–8.
42 Doshi H, Pandya S, Codipilly CN, *et al*. Does Human Milk Fortifier Affect Intestinal Inflammation in Preterm Infants? *Breastfeed Med* 2020;15:776–8.
43 Floris LM, Stahl B, Abrahamse-Berkeveld M, *et al*. Human milk fatty acid profile across lactational stages after term and preterm delivery: A pooled data analysis. *Prostaglandins Leukot Essent Fatty Acids* 2020;156:102023.
44 Koletzko B, Innis S. Lipids. In: Tsang R, Uauy R, Koletzko B, et al, eds. *Nutrition of the preterm infant: scientific basis and practical guidelines*. 2nd edn. Cincinnati, Ohio, USA: Digital Educational Publishing Inc, 2005: 97–139.
45 Picciano MF. Representative values for constituents of human milk. *Pediatr Clin North Am* 2001;48:263–4.
46 Klein CJ. Nutrient requirements for preterm infant formulas. *J Nutr* 2002;132:1395S–577S.