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Comparative study of preterm infants fed new and existing human milk fortifiers showed favourable markers of gastrointestinal status

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Abstract

Aim: This study examined the influence of different human milk fortifiers on biomarkers of gastrointestinal immaturity and inflammation in preterm infants.

Methods: We report secondary outcomes from a controlled, double-blind, randomised, parallel group study conducted from 2011 to 2014 in neonatal intensive care units at 11 metropolitan hospitals in France, Belgium, Germany, Switzerland and Italy. Preterm infants born at up to 32 weeks or weighing up to 1500 g were randomised to a new powdered human milk fortifier (n = 77) or a control fortifier (n = 76) for a minimum of 21 days. We analysed faecal markers of gut inflammation, namely alpha-1 antitrypsin and calprotectin, and maturity, namely elastase-1.

Abbreviations: ANCOVA, analysis of covariance; FS, fortification strength; NEC, necrotising enterocolitis.

Results: Faecal alpha-1 antitrypsin was slightly lower in the new than control forti-fier group after 21 days of full enteral feeding, with a geometric mean and standard deviation of 1.52 ± 1.32 vs 1.82 ± 1.44 mg/g stools (*P* = .01). There was no significant difference in faecal calprotectin (median [Q1-Q3] of 296 [136-565] µg/g stools in both groups combined at study day 21). Faecal elastase-1 was lower in the new fortifier than control fortifier group (202.5 ± 1.6 vs 257.7 ± 1.5 µg/g stools, *P* = .016).

Conclusion: Mean values for each parameter were within the ranges in healthy term infants, indicating favourable markers of gastrointestinal status in both groups. In ad-dition, for faecal calprotectin, the relatively high concentration observed in preterm infants fed fortified human milk suggests that the threshold level for detecting necro-tising enterocolitis should be revised.

KEYWORDS

alpha-1 antitrypsin, calprotectin, elastase-1, low birthweight infant, prematurity

1 | INTRODUCTION

Preterm infants have immature development at birth[1] and are more likely to need specialised care in the immediate postpartum period than term infants. Immaturity of the gastrointestinal system is a par-ticular concern, as it has been associated with severe morbidities, in-cluding necrotising enterocolitis (NEC).[2] It also influences the ability of the infant to digest, absorb and use the nutrients needed to support further growth and development.[3] Gut immaturity, and the associated increased risk in morbidity and feeding intolerance, makes the choice of appropriate enteral nutrition extremely important. Although giving a preterm infant human milk provides many them with benefits,[4,5] the nutritional profile of unfortified human milk is insufficient to meet the increased demands for growth in this population.[6] As a result, human milk fortification is recommended for all infants born weighing less than 1800 g.[7] While feeding fortified human milk has been shown to have no adverse effect on feeding tolerance in preterm infants,[8] the influence of different human milk fortifiers on markers of gut maturity and gastrointestinal inflammation has not been well studied.

Maturity of gastrointestinal function and inflammation can be assessed using faecal biomarkers, which provide a non-invasive and early method for detecting any increased risk for these conditions.[9] Alpha-1 antitrypsin is a serum trypsin inhibitor that is highly resis-tant to intestinal proteolysis. The extravasation of alpha-1 antitryp-sin into the gut can be measured in the stools and is a classic marker for protein-losing enteropathies or conditions that result in loss of blood proteins into the intestinal tract.[10] This process happens in intestinal inflammation, as a result of mucosal ulceration or aug-mented permeability. The total protein in stools is another marker of protein-losing enteropathy and gut inflammation. Faecal calpro-tectin reflects various pathological processes that occur in the mu-cosa of paediatric patients, with elevated concentrations indicating pathogenesis.[11] Faecal elastase-1 is a highly specific and sensitive test for determining gut functional maturity, specifically pancreatic

Key notes

- This study examined the influence of different human milk fortifiers on biomarkers of gastrointestinal immatu-rity and inflammation in preterm infants.
- We report secondary outcomes from a controlled, dou-ble-blind, randomised, parallel group study in neona-tal intensive care units at 11 metropolitan hospitals in France, Belgium, Germany, Switzerland and Italy.
- Mean values for each parameter were within the ranges in healthy term infants, indicating favourable markers of gastrointestinal status in both groups.

exocrine function, and differentiating severe from milder pancreatic insufficiency.[12] In preterm infants, these parameters can be used as non-invasive markers for pancreatic function, intestinal permeability and intestinal complications such as NEC.[12-14]

The purpose of this study was to assess the effect of two human milk fortifiers with different composition on faecal biomarkers of gastrointestinal inflammation and maturity of gut function in clini-cally stable preterm infants. Faecal biomarkers were the secondary endpoints of a trial that evaluated weight gain velocity, other growth parameters and nutritional biomarkers, including lipid profiles, in clin-ically stable preterm infants The new fortifier was PreNAN Human Milk Fortifier (Nestlé, Vevey, Switzerland), and the control fortifier was FM85 Human Milk Fortifier (Nestlé, Vevey, Switzerland). The primary results of the trial have already been reported.[15,16] Briefly, they showed that the unadjusted weight gain rate was 18.3 g/kg/d for the new fortifier and 16.8 g/kg/d for the control fortifier and that both fortifiers were well tolerated, with a similar incidence of adverse gastrointestinal events. The adjusted mean weight gain was 2.3 g/d greater for the new fortifier and the lower limit of the 95%

confidence interval of 0.4 g/d exceeded both the noninferiority and superiority margins.

2 | METHODS

This report presents secondary outcomes from a controlled, double-blind, randomised, parallel group study that was conducted in neonatal intensive care units at 11 metropolitan hospitals in France, Belgium, Germany, Switzerland and Italy. Each provided between 25 and 45 beds. We included clinically stable preterm infants born from April 2011 to March 2014 at up to 32 weeks of gestation or with a birthweight of up to 1500 g. The mothers agreed to provide expressed or donor breast milk for the 21-day study duration. The inclusion and exclusion criteria have previously been reported.[15,16]

The fortifiers were both based on cows' milk and they provided similar energy, namely 17 kcal per 100 mL of human milk. For every 100 mL of HM, the new human milk formula provided 1.4 g partially hydrolysed whey protein, 0.7 g lipids, 1.3 g carbohydrate and a blend of micronutrients. The control human milk fortifier provided 1.0 g extensively hydrolysed whey protein, no lipids, 3.3 g carbohydrate and a blend of micronutrients. The new fortifier was developed with a higher protein to energy ratio, with protein provided as partially hydrolysed whey and non-protein energy from lipids, as medium-chain triglycerides and docosahexaenoic acid. It also contained higher electrolyte and vitamin levels, while achieving a lower osmolality. The aim was to comply with the recommendation from the European Society for Paediatric Gastroenterology Hepatology and Nutrition[17] and the discussions by an expert group reported by Koletzko et al.[18]

Infants tolerating ≥100 mL/kg/d of human milk for >24 hours were randomised to receive either the new or control fortifier for 21 days after they had achieved full enteral feeding. The fortifiers that were given to the infants started at half-strength, and this was called fortification strength (increase) day one (FS day one). It was then increased in line with hospital practice, with the full-strength fortification being reached when infants could maintain intakes of 150-180 mL/kg/d, namely full enteral feeds and full fortification. This was called study day one and occurred approximately 3 days after fortification began. The measurements then continued for another 21 days. FS day one occurred at a median (Q1-Q3) of 13 (11-18) days in the new group and 14 (10-20) days in the control group. The mean days of life for study day one were 16 (13-20) vs 17 (13-23) days, respectively, and 36 (33-40) vs 37 (33-43) for study day 21.

Stool samples were collected on FS day one or the day after and at 21 ± 1 days (day 21). They were analysed for the following parameters: gastrointestinal inflammation, namely faecal alpha-1 antitrypsin, calprotectin and total protein, and maturity of gut function, namely faecal elastase-1. Approximately 5-8 g was collected from each infant within 2 hours of a bowel movement. Samples were stored frozen at −20°C and shipped for analysis on dry ice. If the stool sample quantity was below 5.5 g, which was judged to

be insufficient, a second collection was made from a later bowel movement on the same or following day. Concentrations of faecal calprotectin and elastase-1 were assessed by enzyme-linked immunosorbent assays, following sample treatment with the Smart Prep faecal extraction device (Roche Diagnostics). These were as follows: EK-Cal (Bühlmann Laboratories); ScheBo Pancreatic Elastase 1 (ScheBo Biotech AG) and Euroimmun Analyzer A1 (Euroimmun). Faecal alpha-1 antitrypsin concentration was assessed by immunonephelometry, using the IMMAGE 800 (Beckman Coulter) and faecal total protein by near-infrared spectroscopy with the Fenir 8820 (Perten Instruments). All the analyses were completed in a central laboratory (Rothen Medizinische Laboratorien AG).

FS day one values were log-transformed and groups were compared using t tests computed with the Satterthwaite method. Day 21 values were log-transformed and analysed using analysis of covariance (ANCOVA), adjusted for the FS day one value of the relevant parameter, sex and centre (random effect). Changes from FS day one to day 21 were analysed using ANCOVA, adjusted for postmenstrual age and weight at study day one, when full enteral feeding and full fortification had been achieved, the FS day one value of the relevant parameter, sex and centre (random effect). As many data were missing for total faecal protein, only summary statistics were calculated for this parameter.

The study was reviewed and approved by the institutional review board or independent ethics committee at each hospital and the parent or legal representative of each participant provided written, informed consent prior to enrolment. The Clinical Trial Registration for the study was NCT01771588.

3 | RESULTS

The study flow diagram, baseline subject characteristics and parental demographics have previously been reported.[15] A total of 153 infants were enrolled and randomised to either the new fortifier (n = 77) or the control fortifier (n = 76). One infant in the new group was excluded due to a history of systemic disease, and two in the control group were small for gestational age, leaving 76 and 74, respectively. The infant characteristics were similar between the new and control groups: 50% and 53% of girls, 32% and 27% of vaginal births and 24% and 22% of twins, respectively. The mean birthweights were 1147 ± 258 g and 1156 ± 289 g, and the mean gestational ages at birth were 28.8 ± 2.1 weeks and 28.7 ± 1.18 weeks. As shown in Figure 1, only 56%-94% of the population provided data for the stool analyses (depending on time point and parameter), due to the difficulty in obtaining sufficient stool quantities for all subjects.

There were no significant differences in any faecal biomarkers at FS day one (Figure 2). The geometric mean concentrations of faecal alpha-1 antitrypsin (Figure 2A) and faecal elastase-1 (Figure 2C) were significantly lower in the new fortifier group compared to the control fortifier group at study day 21 (P = .010 and .016, respectively). In addition, the increase from FS day one to study day 21 for both of these parameters was significantly less in the new
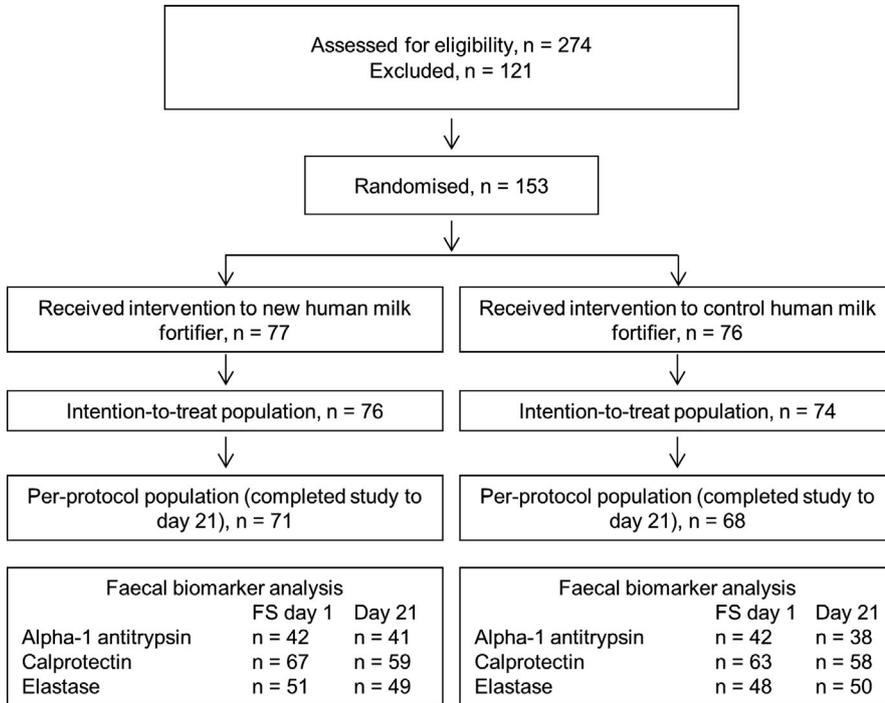
FIGURE 1 Flow of study participants and sample sizes for stool analyses. FS day 1 = fortification strength day one. Day 21 = study day 21

fortifier group compared to the control fortifier group (P = .007 and .004, respectively). Faecal calprotectin concentrations (Figure 2B) tended to increase in both groups during the study, according to the Satterthwaite test. The P-values associated with the test of mean changes from FS day one to study day 21 in the new fortifier and control fortifier groups were P = .051 and P = .112, respectively, with no significant difference between groups demonstrated at study day 21 or in the adjusted change over time. The evaluation of the total protein concentration in stools was limited due to inadequate stool material in 89% of cases at FS day one and 85% of cases on study day 21. Nevertheless, no significant difference was observed between the groups on FS day one when we looked at the medians and inter-quartile ranges: these were 1.70 (1.45-1.80) for the new fortifier vs 1.40 (1.30-1.53) for the control fortifier (P = .100). The values were also similar in both groups at study day 21, namely 1.60 (1.50-1.63) for the new fortifier vs 1.60 (1.50-1.70) for the control fortifier. No inferential statistics were performed at study day 21 because 85% of the values were missing.

4 | DISCUSSION

This report presents faecal biomarkers of gastrointestinal inflammation and gut maturity in clinically stable, preterm infants fed human milk fortified with either a new or control multinutrient, powdered fortifier. Our results indicate that the different human milk fortifiers led to limited differences in faecal biomarkers of gastrointestinal status in this population.

The faecal alpha-1 antitrypsin results observed here may have been due to several factors. First, alpha-1 antitrypsin is present in human milk[19,20] and appears to increase with lactation.[19] Human milk alpha-1 antitrypsin is also resistant to pasteurisation[21] and proteolysis in young infants,[22] meaning that faecal concentrations may be high, or change over time, even in the absence of enteropathy. It is likely that this accounted for the higher levels of this protein in the two study groups when they were compared to infants who were predominantly fed preterm formula.[19,20,23] For example, Sivan et al[23] reported concentrations of faecal alpha-1 antitrypsin in a sample of preterm infants who were predominantly fed formula. These ranged from 0.34 to 1.05 mg/g stools, which was lower than the geometric mean values observed in our study (Figure 2). However, it is unlikely that the alpha-1 antitrypsin content of human milk contributed to the small, but significant, difference between the groups on study day 21, since each group consumed a very similar volume of fortified milk during the study period. This was 153 mL/kg/d in both groups, with 49% of the volume from donor milk in the new fortifier group and 51% in the control fortifier group.[15] Alternatively, it can be speculated that these results indicate a slight, but significant, increase in gastrointestinal protein loss and, or, inflammation among infants in the control than new fortifier groups. However, the geometric mean concentrations in both groups were substantially lower than the cut-off value previously associated with various gastrointestinal diseases in children (2.6 mg/g stools)[24] and the difference between the groups was quite small (0.30 mg/g stools).

In a 2016 systematic review, Pergialiotis et al[25] concluded that faecal calprotectin was elevated in newborns with NEC. However, faecal calprotectin has also been shown to be higher in healthy, exclusively breastfed infants than in infants fed formula or a mixture of breast milk and formula,[26,27] suggesting that higher levels
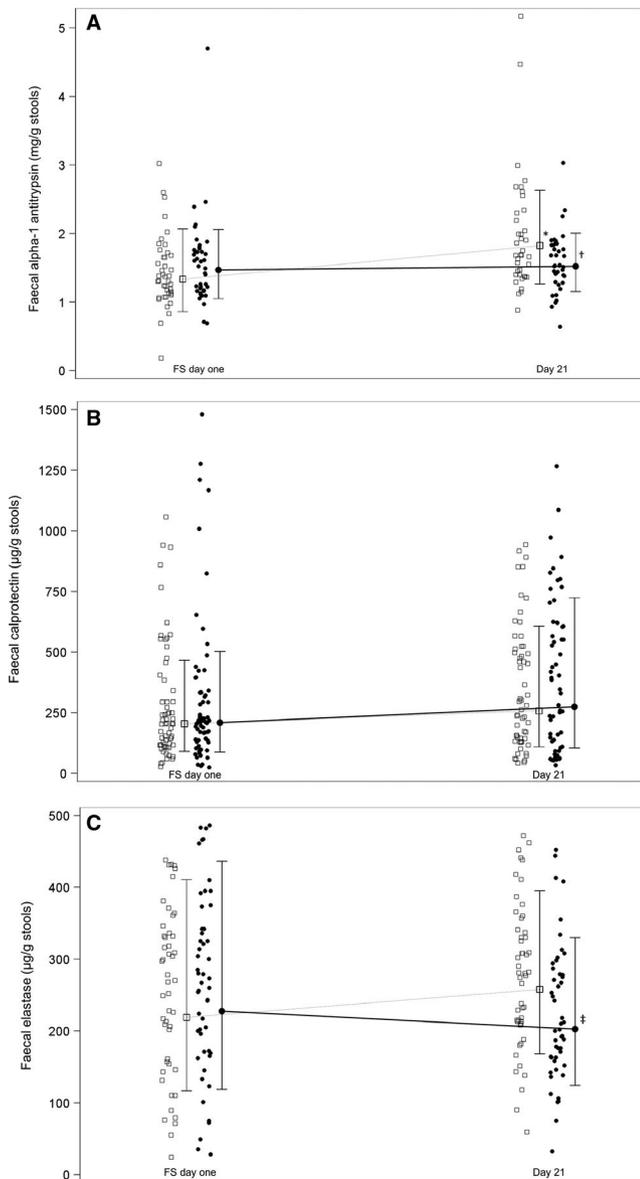
FIGURE 2 Geometric mean */÷ geometric SD for faecal alpha-1 antitrypsin (panel A), faecal calprotectin (panel B) and faecal elastase (panel C). Black circles/solid line represent new fortifier and open squares/dashed line represent control fortifier. FS day one = fortification strength day one and day 21 = study day 21. *P = .017 vs FS day one value (by t test computed using Satterthwaite method); †P = .010 vs control fortifier (by analysis of covariance, adjusting for value at FS day one, sex and centre); ‡P = .016 vs control fortifier (by analysis of covariance, adjusting for value at FS day one, sex and centre)

may not always indicate pathology. Consistent with this hypothesis, Groer et al[28] showed that infants who received their mother's own milk had increasing faecal calprotectin levels over time, while infants who received mixed feeding or pasteurised donor milk did not show this increase over time. Together, these previous findings suggest that faecal calprotectin may not always represent pathological inflammation in preterm infants, but rather feeding pattern and, or, normal maturation of the gut immune axis.[28]

Furthermore, no infants in our study experienced NEC, despite the fact that many had calprotectin levels (ie median [Q1-Q3] of 296 [136-565] µg/g stools in both groups combined at study day 21) that greatly exceeded those reported to be indicative of this condition.[25,29] In our study, the slight increase in faecal calprotectin over time in both the fortifier groups was likely to have reflected both feeding with human milk fortifier plus mixed sources of human milk and normal gastrointestinal maturation and development. The lack of a significant difference between the groups was consistent with a previous study that showed no differences in this biomarker prior to commencing fortification or 14 days after fortification with either a human milk or bovine milk-based fortifier.[30] Our results also provide further evidence that more work is needed to identify an appropriate diagnostic cut-off value for NEC. This finding was consistent with the conclusions reached by Pergialiotis et al.[25]

Reduced elastase-1 secretion in stools is considered to be a marker of pancreatic insufficiency, with values of less than 200 µg/g stools indicating impaired exocrine pancreatic function.[31] Values have been shown to normalise for most infants, including preterm infants, within the first few weeks after birth.[12,32] Preterm infants have been shown to exhibit pancreatic immaturity and high sensitivity to environmental stress, including inappropriate nutrient uptake related to defects in the gut barrier function.[3] Given that nutrients within the duodenal lumen are the most important stimulators of the pancreatic exocrine response,[33] it can be hypothesised that such inappropriate uptake might lead to excessive elastase production and secretion into the gut lumen. Excessive elastase activity in the gut lumen may contribute to maintenance or exacerbation of inflammatory status,[34] leading to metabolic stress and an increased risk of feeding intolerance and NEC.[35] In the present study, preterm infants fed with the new fortifier presented with slightly lower levels of faecal elastase at study day 21 than the group who received the control fortifier. This finding suggests that the new fortifier may have had a modest impact on gut homeostasis.

To our knowledge, this was the first study to assess multiple faecal markers of gastrointestinal inflammation and maturity of gut function in response to different forms of human milk fortification in preterm infants. A limitation was that the number of samples of sufficient quantity for all analyses ranged from 10% to 85% of the full sample size.

5 | CONCLUSION

These results indicate that different human milk fortifiers can lead to modest differences in markers of gastrointestinal status in clinically stable, preterm infants. The mean values of each marker in both fortifier groups were within the ranges observed in healthy term infants. In addition, these results suggest that the faecal calprotectin threshold value for early detection of NEC should be revised in light of the relatively high concentrations observed in our subjects. When they are combined with previously reported

anthropometry and clinical biochemistry data, these results further illustrate that the new fortifier was safe and well tolerated by our cohort.

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