OPEN

Safety and Tolerance of a Novel Anti-Regurgitation Formula: A Double-Blind, Randomized, Controlled Trial

*Marc Bellaiche, †Thomas Ludwig, ‡Małgorzata Arciszewska, †Anke Bongers, §Cédrico Gomes, ||¶Agnieszka Świat, #Faouzi Dakhlia, **Anne Piollet, † Raish Oozeer, and ††Yvan Vandenplas

ABSTRACT

Objectives: A novel anti-regurgitation (AR) formula has been designed to support gut health and improve gastrointestinal (GI) symptoms beyond regurgitation. This study assessed the tolerance and safety of this new AR formula.

Methods: This was a 4-week double-blind, randomized, controlled trial with a 4-week extension in formula-fed infants with regurgitation. The new AR (Test) formula contained 0.4 g/100 mL locust bean gum (LBG) as thickener, partly fermented formula with postbiotics, and short-chain galacto-oligosaccharides (scGOS) and long-chain fructo-oligosaccharides (lcFOS) (0.4 g/100 mL, ratio 9:1). The Control AR formula contained LBG (0.4 g/100 mL) with postbiotics and has a history of safe use. The primary outcome was the Infant Gastrointestinal Symptom Questionnaire (IGSQ) sum score including stooling, spitting-up/vomiting, crying, fussiness and flatulence.

Results: All 182 infants screened were enrolled in the study. The primary analysis showed the equivalence of the IGSQ sum scores at Week 4 between groups. IGSQ sum scores improved significantly within 1 week (Mixed Model Repeated Measurement [MMRM], $P < 0.001$). Post-hoc analyses showed a bigger improvement of the IGSQ score in the Test (n = 38) versus Control (n = 44) group (MMRM, $P = 0.008$) in infants with more severe gastrointestinal (GI) symptoms (IGSQ score $\geq$35). Stool characteristics were comparable between groups. Growth related z scores were in line with the WHO child growth standards and both groups showed improvement of regurgitation. Adverse events did not show any safety concerns.

Conclusions: The novel AR formula combining LBG, scGOS/lcFOS and postbiotics is well-tolerated, safe and supports adequate growth during the intervention. Post-hoc analyses suggest that the formula results in more improvement of GI symptom burden in infants with more severe symptoms.

Key Words: functional gastrointestinal disorder, gastroesophageal reflux, locust bean gum, regurgitation, thickened formula

(*JPGN* 2021;73: 579–585)

What Is Known

- Regurgitation is a gastrointestinal (GI) disorder that is commonly associated with other GI symptoms, such as infant colic and gassiness.
- Regurgitation should be managed by parental reassurance and nutritional advice. In non-exclusively breastfed children, this may include the use of thickened anti-regurgitation (AR) formulae.

What Is New

- The new AR formula combining locust bean gum with pre- and postbiotics is well-tolerated and safe.
- Both AR formulae improved regurgitation and most GI symptoms within one week of intervention.
- Post-hoc analyses show greater improvement of GI burden in infants with more severe symptoms who received the new formula.

Expert reviews suggest that the management of infant regurgitation should build on parental reassurance and nutritional advice (1,2). Even in infants suspected of gastro-esophageal reflux disease, thickened formula should be considered according to the latest guidelines as a first-line therapy option for non-exclusively breastfed infants (2). Thickeners include different starches or plant-derived fibers such as locust/carob bean gum (LBG) (3). LBG is a fiber that has been demonstrated to be safe and effective in reducing the frequency and volume of regurgitation (4–9). LBG has a lower caloric value and has been reported to have a higher viscosity in vitro as compared to starches (3,10).

Regurgitation has been reported to be associated with other gastrointestinal (GI) symptoms, such as infant colic, gassiness and constipation (11,12). Previously, studies have reported beneficial GI effects of a specific fermented infant formula (13–15). Fermentation processes generate bioactive compounds which are also

Received February 10, 2021; accepted May 29, 2021.

From the *Hospital Robert-Debré, GI department, Paris, France, the †Danone Nutricia Research, Utrecht, The Netherlands, the ‡Poliklinika Ginekologiczno-Poloznicza Sp. Z.O.O. Sp.K, Bialystok, Poland, the §Senlis Hospital, Pediatric Service, Taverny, France, the ||Centrum Medyczne Promed, the ¶Centrum Medyczne Plejady, Krakow, Poland, the #Centre Condorcet, Hôpital Privé d'Antony, Antony, France, the **Chamalières, France, and the ††KidZ Health Castle, UZ Brussel, Vrije Universiteit Brussel, Brussels, Belgium.

Address correspondence and reprint requests to Yvan Vandenplas, KidZ Health Castle, UZ Brussel, Laarbeeklaan 101 - 1090 Brussel, Brussels, Belgium (e-mail: yvan.vandenplas@uzbrussel.be).

Supplemental digital content is available for this article. Direct URL citations appear in the printed text, and links to the digital files are provided in the HTML text of this article on the journal's Web site (*www.jpgn.org*).

Source of Funding: The study was funded by Danone Nutricia Research, Utrecht, The Netherlands.

Conflicts of Interest: T.L., A.B. and R.O. are employees of Danone Nutricia Research (Utrecht, The Netherlands). None of the other authors have a conflict of interest.

Trial registration: ClinicalTrials.gov, Identifier: NCT03371615.

known as postbiotics (16), provisionally defined as compounds produced by microorganisms and released from food components or microbial constituents, including non-viable cells that, when administered in adequate amounts, promote health and well-being (17). The combination of a specific prebiotic mixture of short-chain galacto-oligosaccharides (scGOS) and long-chain fructo-oligosaccharides (lcFOS) and postbiotics in formulae for healthy term infants was demonstrated to be safe, well tolerated, and to improve gut health, including stool consistency and microbiota composition (18–20).

In a newly developed AR formula, LBG is combined with scGOS/lcFOS and postbiotics, which is expected to address GI symptoms beyond regurgitation. The purpose of this study was to investigate the tolerance and safety of this new AR formula. As a primary aim, the equivalence of GI tolerance was examined between the new AR formula and an AR formula that has a history of safe and efficient usage for more than 10 years.

METHODS

Design and Participants

This was a multi-center, randomized, controlled, double-blind, two parallel-group study, consisting of a 4-week study main phase, followed by an optional 4-week extension. The study was performed in accordance with the principles of the Declaration of Helsinki and approval was obtained from the independent Ethics Committees. Participants were recruited from 18 centers in Poland, France and Germany. Only parents who already decided to fully formula feed were informed about the study. After parents provided written informed consent, screening took place. Eligible infants were singleton, term-born (>37 and <42 weeks gestational age), ages between 21 and 91 days, diagnosed with uncomplicated regurgitation based on adapted Rome IV criteria (21,22) (ie, >2 episodes of regurgitation per day for a period of at least 1 week). Further, eligible infants had normal birth weight (10th to 90th percentile according to applicable growth charts) and were fully formula fed. Infants were excluded in case of history of retching, hematemesis, aspiration, apnea, failure to thrive, feeding or swallowing difficulties or abnormal posturing, GI infection, congenital conditions, presence of any other gastrointestinal (GI) symptoms that were not functional in nature, and known allergy to any of the products' ingredients. Other exclusion criteria were the use of systemic antibiotics, prokinetics, proton pump inhibitors and/or complementary feeding and participation in any other clinical intervention study.

Intervention

Subjects were randomly assigned in a 1:1 ratio to the novel or control AR formula, using a computer randomization program with the permuted block randomization stratified per center. The allocation sequence was generated by a study independent statistician. Both products were cow milk-based, nutritionally complete infant formulae in accordance with European regulations (Table, Supplemental Digital Content 1, *http://links.lww.com/MPG/C496*). The novel AR (Test) formula contained 0.4 g/100 mL LBG, 0.4 g/100 mL scGOS/lcFOS (ratio 9:1) and 26% fermented formula with postbiotics derived from the Lactofidus™ fermentation process, including 3′-galactosyllactose. The control AR product was commercially available and contained 0.4 g/100 mL LBG and 11% fermented formula with postbiotics but did not contain scGOS/lcFOS. Parents and all study staff were blinded to the study products. Products were delivered as a powder to be dissolved in water, had a similar taste, smell and appearance and were manufactured by Danone Nutricia.

Outcome Measures

The primary outcome parameter was the sum score resulting from the Infant Gastrointestinal Symptom Questionnaire (IGSQ), a validated 13-item questionnaire that allows parents to describe the frequency and intensity of their infant's GI symptoms in the preceding 7-days (23). Blinded research staff administered the IGSQ during study visits at Baseline (Visit 1), Week 2 (Visit 2) and Week 4 (Visit 3). At Weeks 1 and 3, parents completed the questionnaire at home. The IGSQ sum score ranged from 13 (very low GI symptom burden) to 65 (very high GI symptom burden). Secondary outcome parameters included the single IGSQ item scores, stool frequency, stool consistency (24) and the Adapted Vandenplas scale on regurgitation severity (7,25), which were completed by parents in daily diaries. Based on the stool data the occurrence of diarrhea was derived, applying the WHO criteria for diarrhea as the passage of >3 watery stools per day (26). Investigators measured infant anthropometrics, reported the occurrence of adverse events (AEs) and the use of concomitant medication at each study visit, until and including the visit at the end of the extension (Week 8, Visit 4).

Statistical Analyses

Assuming no difference between study groups, 70 evaluable infants per group would be required to achieve 80% power to show equivalence between groups of the IGSQ sum score at Week 4 ($\alpha = 0.05$; equivalence margins based on 0.5 standard deviation [SD] that was assumed to be 4.1). Anticipating a non-evaluability rate of ~20% resulted in a total number of 180 infants to be randomized. A pre-specified interim analysis including a semi-blinded comparison of the study groups was performed to evaluate GI tolerance and safety data of the first 73 subjects. Results were reviewed by the independent Data Monitoring Committee who recommended the continuation of the trial without modification.

The primary analysis was based on the Per-Protocol (PP) population, as defined before database lock. Equivalence between groups was examined by analyzing whether the two-sided 90% confidence interval (CI) of the difference in the mean IGSQ sum score at Week 4 laid within the predefined equivalence margins ($\alpha = 0.05$). Data were analyzed using a linear Mixed Model Repeated Measurement (MMRM) approach on the post-baseline IGSQ scores

Author Contributions: M.B. and Y.V. have contributed to the methodology and supervision of the study. M.A., C.G., A.S., F.D., A.P. and R.O. have performed work on the investigation and acquisition of the data. T.L. and Y.V. designed the study. A.B. performed work on writing of the statistical analysis plan, interpretation of the data and writing of the first draft of the manuscript. All authors were involved in the interpretation of the data, contributed to the writing and critical revision of the manuscript for important intellectual content, approved the final version and agreed to be accountable for all aspects of the work.

DOI: 10.1097/MPG.0000000000003289

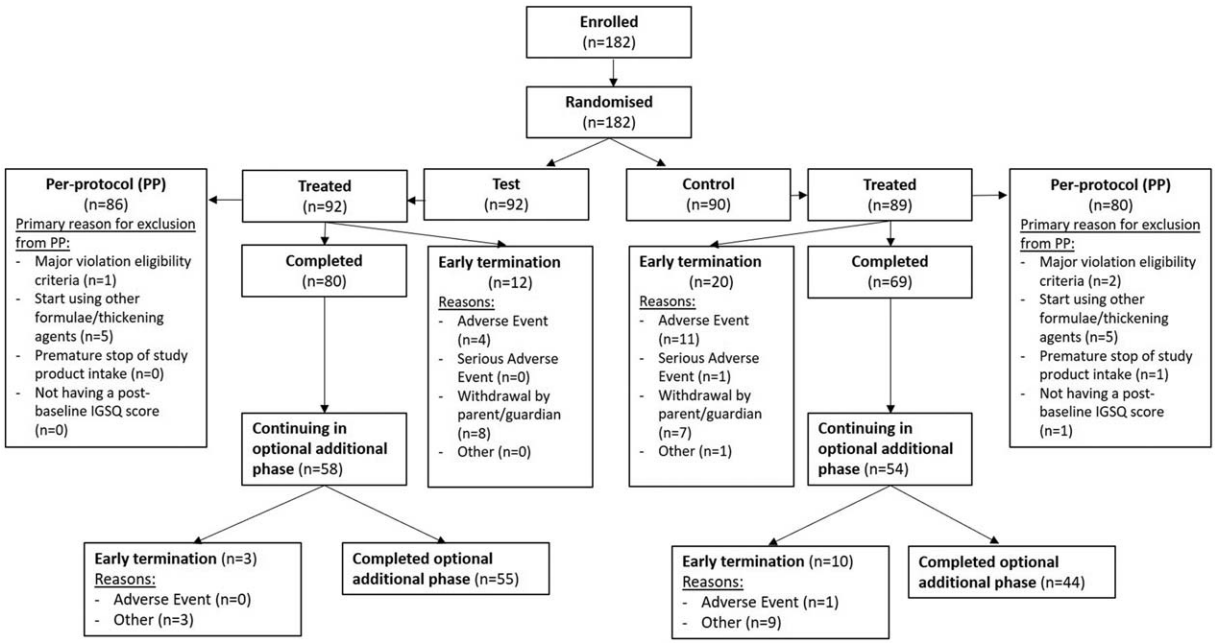

FIGURE 1. Flow diagram of participants through study, from enrolment to study completion.

with treatment, time (categorical) and interaction between treatment and time as fixed effects, baseline IGSQ score as adjustment covariate and center as a random effect. Sensitivity analyses included the analysis of the all-subjects-randomized population, assessing the missing at random assumption and adjustment for potential covariates. A prespecified interaction test was performed to analyze the impact of the baseline IGSQ score on the intervention effect (predefined cut-off P-value < 0.1 used to keep interaction in the model), followed by post-hoc subgroup analyses using a similar MMRM model as described above. Stool frequency and the Adapted Vandenplas Scale scores were compared between groups using a Mann-Whitney test. A Cochran–Mantel–Haenszel test was used to compare groups for the distribution over the different stool consistency categories. All statistical analyses were performed using SAS Life Science Analytics Framework version 4.7.3 (SAS Institute Inc, Cary, NC, USA).

RESULTS

Subjects

Between November 2017 and July 2019, all 182 screened infants were randomized to the Test or Control group (Fig. 1). Demographic data were not apparently different between study groups. The mean age of the population at enrolment was 44 days and the mean duration of regurgitation before randomization was 29 days (Table, Supplemental Digital Content 2, *http://links.lww.com/MPG/C497*). One infant allocated to the Control group did not start intervention and was therefore excluded from the all-subjects-treated (AST) population (Test, n = 92; Control, n = 89). Twelve subjects (13.0%) in the Test group, and 20 subjects (22.5%) in the Control group dropped out during the 4-week study main phase. Reasons for drop-out in the Test group were withdrawal by parent (n = 8) and AEs (n = 4; crying, dyschezia, n = 2 cow's milk allergy). In the Control group, reasons for early termination were AEs (n = 11; abdominal pain, flatulence, hard feces, hematochezia, worsening of regurgitation, crying and GI infection, n = 4 diarrhea), withdrawal by parent (n = 7), a serious AE (n = 1; gastroesophageal reflux disease), and other reasons (n = 1). The early termination rate was not statistically significantly different between groups (Fisher exact test, $P = 0.120$), but the early termination rate due to the occurrence of an (S)AE was significantly higher in the Control versus Test group (n = 12 [13.5%] vs n = 4 [4.3%]; Fisher exact test, $P = 0.037$).

Primary Results

Equivalence within 0.5 SD equivalence margins (+3.1 and −3.1) of the IGSQ sum score at Week 4 was demonstrated for the Test

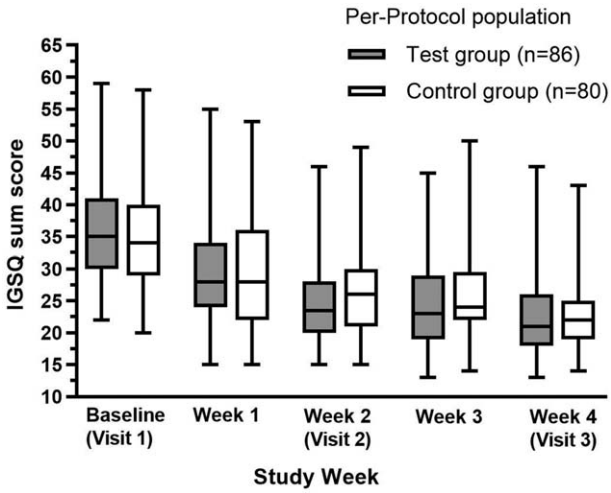

FIGURE 2. Distribution of Infant Gastrointestinal Symptom Questionnaire (IGSQ) sum scores from baseline to Week 4 in the per-protocol population. The bottom and top edges of the box are located at the sample 25th and 75th percentiles. The median is represented by the horizontal line. The whiskers of the box plots show the minimum and maximum values.

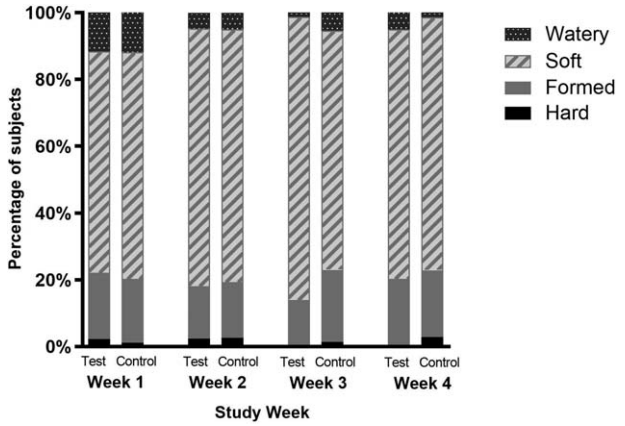

FIGURE 3. Percentage of subjects with specific stool consistency category based on parent reported diary in the all-subjects-treated population.

versus Control group in both the PP population (difference in estimated means of −1.41, 90% CI [−3.04; 0.22]) and the all-subjects-randomized population. IGSQ sum scores improved from on average 36 at Baseline to 23 at Week 4 (Fig. 2), which was statistically significant after 1 week of intervention (MMRM, $P < 0.001$). There was no significant difference between groups at any time point.

Secondary Results

All single IGSQ items showed significantly improved scores at Week 4 compared to baseline, of which the majority of items (9 out of 13) significantly improved within 1 week (Table, Supplemental Digital Content 3, *http://links.lww.com/MPG/C498*).

There were no significant differences between groups in stool frequency, stool consistency and diarrhea (>3 watery stools per day). The majority of infants in both study groups had a stool consistency categorized as 'soft' (varying from 67% in Week 1 to 75% in Week 4) whereas a relatively low proportion of subjects were reported to have watery (varying from 12% in Week 1 up to 3% in Week 4) or hard stools (varying from 2% in Week 1 to 1% in Week 4) (Fig. 3).

Mean weight-for-age, length-for-age, weight-for-length and head circumference-for-age WHO *z* score values were not statistically significant different between study groups and were close to zero, indicating adequate infant growth during the intervention.

Infants presenting with >2 episodes of regurgitation per day were enrolled in the study. The IGSQ showed that 67% of the infants had >4 episodes of regurgitation per day at baseline. Scores significantly improved within 1 week (paired Wilcoxon test, $P < 0.001$). In addition, the Adapted Vandenplas Scale showed that a majority (75%) of infants were reported to have 0–2 regurgitation episodes per day within the first study week (Table, Supplemental Digital Content 3, *http://links.lww.com/MPG/C498*).

Post-Hoc Analysis of Primary Results

The response on the primary outcome parameter was explored by studying the impact of the baseline level of GI symptom burden. This effect modifier analysis showed that the baseline IGSQ score impacted the intervention effect on the 4-week IGSQ sum score (baseline by intervention interaction $P = 0.052$; prespecified cut-off of $P < 0.1$ used to keep interaction in the model). The subsequent post-hoc analysis showed a statistically significant improvement of the IGSQ scores in the Test (n = 38) versus Control group (n = 44) (MMRM, $P = 0.008$), in infants with a baseline IGSQ sum score ≥35 (the overall median). This difference was not significant in the subgroup of infants with a baseline IGSQ sum score <35 (MMRM, post hoc, $P = 0.320$) (Fig. 4).

Adverse Events and Concomitant Medication

The most commonly reported AEs during the 8-week study were GI disorders, infections and skin disorders. The number of subjects with any AE was relatively higher in the Control versus Test group (n = 33 [37.1%] vs n = 29 [31.5%]; Fishers exact test, $P = 0.439$). In addition, there were more subjects with AEs (in most cases GI disorders) that were assessed as (potentially) product related by the investigators in the Control versus Test group (n = 14 [15.7%] vs n=10 [10.9%]).

In the Test group, three serious AEs (pneumonia due to Respiratory Syncytial Virus, upper respiratory tract infection, and exanthema) were reported in three subjects (3.3%). In the Control group, eight serious AEs (pylorus hypertrophy, difficulty in feeding,

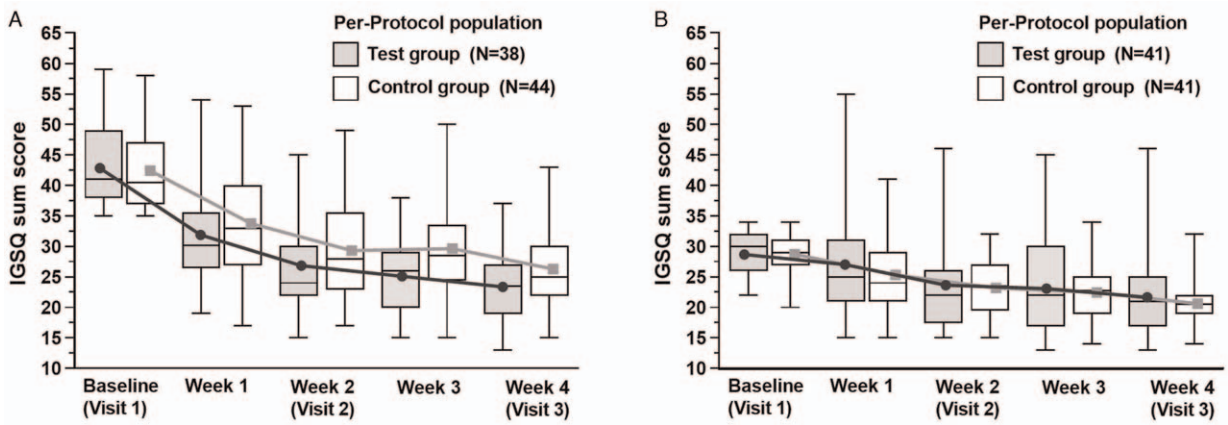

FIGURE 4. Distribution of Infant Gastrointestinal Symptom Questionnaire (IGSQ) sum scores for subpopulations with (A) baseline IGSQ sum scores ≥35 and with (B) baseline IGSQ sum scores <35 in the per-protocol population. The bottom and top edges of the box are located at the sample 25th and 75th percentiles. The median is represented by the horizontal line and the mean values in each group are connected with lines. The whiskers of the box plots show the minimum and maximum values.

gastroesophageal reflux disease without esophagitis, bronchitis, bronchiolitis, pneumonia (two cases), and skull fracture) were reported in seven subjects (7.9%). All these events required hospitalization and were recovered at the end of the study. These serious AEs were not assessed as product related by the investigators. One serious AE in the Control group that was not unexpected (gastroesophageal reflux disease) was assessed as possibly product related by the investigator.

There were more skin disorders in the Test versus Control group (n = 9 [9.8%] vs n = 1 [1.1%]; Fisher exact test, $P = 0.018$), which were distributed across different types of events (dermatitis, eczema, miliaria, rash, skin irritation). The investigators assessed these as not being related to the study products. In the Control group, there were more GI disorders in comparison to the Test group (n = 21 [23.6%] vs n = 11 [12.0%]; Fisher exact test, $P = 0.051$) (Table, Supplemental Digital Content 4, *http://links.lww.com/MPG/C499*). Apart from a difference between groups in the occurrence of flatulence (Control n = 5 [5.6%] vs Test n = 0 [0%], Fisher exact test, $P = 0.027$), there were no significant differences between study groups in any other type of GI events.

There were no apparent differences between groups in the use of medication. Throughout the study, a few subjects needed medication for acid-related disorders, that is, n = 3 [3.4%] of the subjects in the Control group, versus none in the Test group. Also, slightly more subjects used medication for functional GI disorders in the Control versus Test group (n = 12 [13.5%] vs n = 10 [10.9%], respectively). The use of dermatological medication was slightly higher in the Test versus Control group (n = 3 [3.3%] vs n = 0 [0%]).

DISCUSSION

The newly developed AR formula combining LBG with scGOS/lcFOS and partly fermented formula with postbiotics was well tolerated by infants with regurgitation in this double-blind, randomized, controlled multi-center study. The primary analysis showed that the 4-week IGSQ sum score for the evaluation of GI symptom burden was equivalent for the novel AR formula compared to the control AR formula, which had a history of safe use.

International recommendations emphasize parental reassurance and dietary advice as the preferred management of regurgitation, including the optional usage of thickened AR formulae in non-exclusively breastfed children (1,2). Results from the present study showed that both LBG containing formulae resulted in a significant reduction of regurgitation and improvement of specific GI symptoms in infants with regurgitation.

The IGSQ sum score has a range from 13–65 and sum scores >30–35 are considered to indicate relevant GI symptom burden (23,27). The average baseline IGSQ sum score of 36 in this study confirms findings from previous studies that regurgitation is frequently associated with other GI symptoms (11,12). IGSQ sum scores improved significantly within 1 week and after 4 weeks, scores were comparable to values reported in healthy term born infants without specific GI issues (23,27–31).

Adding fibers such as prebiotics to infant formula has been reported to increase stool frequency in healthy infants and to result in softer stools, to a pattern closer to that of breastfed infants (32,33). The higher total level of fiber and postbiotics in the novel AR formula compared to the control AR formula did not lead to a significantly higher stool frequency or to any significant differences in stool consistency. Stool data were in the range of what has been reported in healthy term born infants who received formulae with pre- and/or postbiotics (19,20). In addition, the incidence of GI disorders reported as AEs, including the occurrence of diarrhea and flatulence, was not higher compared to what is known from a healthy infant population (11,34–36).

Both the early termination rate due to AEs that were mainly GI related and the occurrence of GI type of AEs were lower in the group receiving the new AR formula compared to the control group. These differences may suggest beneficial GI effects of the new AR formula. Post-hoc analyses indeed suggested a significantly bigger improvement of GI burden after intervention with the new AR formula in the subpopulation of infants with more severe GI symptoms. Infants with more severe GI distress could be considered at higher risk to be prescribed medication, especially proton pump inhibitors (37–39), which have been reported to have side effects (40,41). Therefore, expert reviews have discouraged the use of medication in the management of regurgitation (1,2).

The greater improvement of the IGSQ score in the subpopulation with a more severe GI burden could be mediated by the effect of the combination of fermented formula and scGOS/lcFOS on the microbiome. Functional GI symptoms have been associated with the composition and function of gut microbiota as reviewed recently (42). The gut microbiome has been described to play a role in mucin production and gut barrier (43) and to be involved in lactate metabolism in the gut, therefore directly influencing the production and accumulation of gas (44). In addition, alteration of the gut microbiota can be associated with low-grade systemic inflammation as recently shown in infants with colics (45). Moreover, the combination of partly fermented formula with postbiotics and scGOS/lcFOS has been reported to modulate the gut microbiome toward a pattern closer to that of breast-fed infants (18,46,47). Considering these findings and the association of microbiome dysbiosis with functional GI disorders (42), the combination of fermented formula and scGOS/lcFOS is postulated to have a beneficial GI effect by impacting gut microbiota.

Regurgitation was included as a secondary parameter in the study to assess efficacy. Based on the Adapted Vandenplas Scale and the IGSQ items, a reduced frequency and severity of regurgitation were observed within 1 week of intervention in both study groups, confirming the efficacy within one week that was shown in earlier investigations with LBG thickened formulae (6,7).

This study has some limitations. In the study, infants were included based on the adapted Rome IV criteria for the diagnosis of regurgitation, including the presence of regurgitation >2 times per day for at least 1 week before randomization, whereas Rome IV criteria refer to at least 3 weeks for diagnosing regurgitation (21); however, as the average duration of regurgitation before study inclusion was 29 days, study results should be considered to be representative for infants fulfilling the Rome IV criteria. In addition, the improvement of symptoms related to functional GI disorders with increasing age should be considered. A recent study in healthy term infants showed that IGSQ scores decreased over time with increasing age, in particular after 4 months of age, indicating that symptoms improve with maturation (27). As the present study shows a larger decline in IGSQ scores in infants with GI issues under the age of 4 months, these findings suggest that both AR study formulae contributed to the improvement of GI burden.

Though this study has been designed as a tolerance and safety study, the post-hoc analysis provides interesting leads for future efficacy studies. This analysis first of all indicates that there are no tolerance concerns in infants with a higher burden of GI symptoms upon inclusion in the study. In addition, the post-hoc analysis suggests a bigger improvement of the IGSQ sum score in infants with a higher GI burden. This might provide a higher potential to show an improvement, and may represent a more specific study population.

This intervention among infants with regurgitation who were otherwise healthy demonstrated that both AR formulae containing either LBG and the partly fermented formula with postbiotics, with or without the addition of scGOS/lcFOS were well tolerated, safe,

and supported adequate infant growth during the intervention. This study also confirmed the consistent association of regurgitation with other gastrointestinal symptoms and indicated a beneficial effect of the triple combination of LBG with the specific prebiotic mixture scGOS/lcFOS (9:1) and postbiotics derived from the Lactofidus™ process.

Acknowledgments: We are grateful to the families who participated in this study, as well as to the participating investigators and site staff members in the LeopARd Study Group who conducted the research. We would also like to acknowledge the contribution of the LeopARd study team. Thank you to Prof. Dr med. S. Koletzko, Kinderpoliklinik Munich, Germany; Dr M. van Wijk, Academic Medical Centre Amsterdam, The Netherlands and Prof. T. Stijnen, Leiden University Medical Center, The Netherlands for their role in the Data Monitoring Committee of this study.

REFERENCES

1. Rosen R, Vandenplas Y, Singendonk M, et al. Pediatric gastroesophageal reflux clinical practice guidelines: joint recommendations of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition. *J Pediatr Gastroenterol Nutr* 2018;66:516–54.
2. Salvatore S, Abkari A, Cai W, et al. Review shows that parental reassurance and nutritional advice help to optimise the management of functional gastrointestinal disorders in infants. *Acta Paediatr* 2018;107:1512–20.
3. Salvatore S, Savino F, Singendonk M, et al. Thickened infant formula: what to know. *Nutrition* 2018;49:51–6.
4. Borrelli O, Salvia G, Campanozzi A, et al. Use of a new thickened formula for treatment of symptomatic gastrooesophageal reflux in infants. *Ital J Gastroenterol Hepatol* 1997;29:237–42.
5. Grosse K, Boctor L, Hilber U, et al. Spezialnahrung für vermehrt spuckende Säuglinge. *Kinderärtzliche Praxis* 1998;69:204–10.
6. Hegar B, Rantos R, Firmansyah A, et al. Natural evolution of infantile regurgitation versus the efficacy of thickened formula. *J Pediatr Gastroenterol Nutr* 2008;47:26–30.
7. Vandenplas Y, Hachimi-Idrissi S, Casteels A, et al. A clinical trial with an ''anti-regurgitation'' formula. *Eur J Pediatr* 1994;153:419–23.
8. Wenzl TG, Schneider S, Scheele F, et al. Effects of thickened feeding on gastroesophageal reflux in infants: a placebo-controlled crossover study using intraluminal impedance. *Pediatrics* 2003;111 (Pt 1):e355–9.
9. Tounian P, Meunier L, Speijers G, et al. Effectiveness and tolerance of a locust bean gum thickened formula: a real-life study. *Pediatr Gastroenterol Hepatol Nutr* 2020;23:511–20.
10. Gonzalez-Bermudez CA, Frontela-Saseta C, Lopez-Nicolas R, et al. Effect of adding different thickening agents on the viscosity properties and in vitro mineral availability of infant formula. *Food Chem* 2014;159:5–11.
11. Bellaiche M, Oozeer R, Gerardi-Temporel G, et al. Multiple functional gastrointestinal disorders are frequent in formula-fed infants and decrease their quality of life. *Acta Paediatr* 2018;107:1276–82.
12. Bellaiche M, Ategbo S, Krumholz F, et al. A large-scale study to describe the prevalence, characteristics and management of functional gastrointestinal disorders in African infants. *Acta Paediatr* 2020;109:2366–73.
13. Thibault H, Aubert-Jacquin C, Goulet O. Effects of long-term consumption of a fermented infant formula (with *Bifidobacterium breve* c50 and *Streptococcus thermophilus* 065) on acute diarrhea in healthy infants. *J Pediatr Gastroenterol Nutr* 2004;39:147–52.
14. Mullie C, Yazourh A, Thibault H, et al. Increased poliovirus-specific intestinal antibody response coincides with promotion of *Bifidobacterium longum*-infantis and *Bifidobacterium breve* in infants: a randomized, double-blind, placebo-controlled trial. *Pediatr Res* 2004;56:791–5.
15. Wegh CAM, Geerlings SY, Knol J, et al. Postbiotics and their potential applications in early life nutrition and beyond. *Int J Mol Sci* 2019;20:4673.
16. Salminen S, Stahl B, Vinderola G, et al. Infant formula supplemented with biotics: current knowledge and future perspectives. *Nutrients* 2020;12:1952.
17. Collado MC, Vinderola G, Salminen S. Postbiotics: facts and open questions. A position paper on the need for a consensus definition. *Benef Microbes* 2019;10:711–9.
18. Huet F, Abrahamse-Berkeveld M, Tims S, et al. Partly fermented infant formulae with specific oligosaccharides support adequate infant growth and are well-tolerated. *J Pediatr Gastroenterol Nutr* 2016;63:e43–53.
19. Vandenplas Y, Ludwig T, Bouritius H, et al. Randomised controlled trial demonstrates that fermented infant formula with short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides reduces the incidence of infantile colic. *Acta Paediatr* 2017;106:1150–8.
20. Rodriguez-Herrera A, Mulder K, Bouritius H, et al. Gastrointestinal tolerance, growth and safety of a partly fermented formula with specific prebiotics in healthy infants: a double-blind, randomized. *Controlled Trial Nutr* 2019;11:1530.
21. Benninga MA, Nurko S, Faure C, et al. Childhood functional gastrointestinal disorders: neonate/toddler. *Gastroenterology* 2016;150:1443–55.
22. Zeevenhooven J, Koppen IJ, Benninga MA, et al. Rome IV criteria for functional gastrointestinal disorders in infants and toddlers. *Pediatr Gastroenterol Hepatol Nutr* 2017;20:1–13.
23. Riley AW, Trabulsi J, Yao M, et al. Validation of a parent report questionnaire: the infant gastrointestinal symptom questionnaire. *Clin Pediatr (Phila)* 2015;54:1167–74.
24. Bekkali N, Hamers SL, Reitsma JB, et al. Infant stool form scale: development and results. *J Pediatr* 2009;154:521.e1–6.e1.
25. Dupont C, Vandenplas Y. Efficacy and tolerance of a new anti-regurgitation formula. *Pediatr Gastroenterol Hepatol Nutr* 2016;19:104–9.
26. World Health Organisation. Diarrhoea. https://www.who.int/health-topics/diarrhoea#tab=tab_2. [Accessed 17 August 2021].
27. Pados BF, Basler A. Gastrointestinal symptoms in healthy, full-term infants under 7 months of age. *J Pediatr Nurs* 2020;53:1–5.
28. Storm HM, Shepard J, Czerkies LM, et al. 2′-Fucosyllactose is well tolerated in a 100% whey, partially hydrolyzed infant formula with *Bifidobacterium lactis*: a randomized controlled trial. *Glob Pediatr Health* 2019;6:1–10.
29. Yao M, Lien EL, Capeding MRZ, et al. Effects of term infant formulas containing high sn-2 palmitate with and without oligofructose on stool composition, stool characteristics, and bifidogenicity. *JPGN* 2014;59:440–8.
30. Mao M, Zhang L, Ge J, et al. Infant feeding regimens and gastrointestinal tolerance: a multicenter, prospective, observational cohort study in China. *Glob Pediatr Health* 2018;5:1–12.
31. Nowacki J, Lee HC, Lien R, et al. Stool fatty acid soaps, stool consistency and gastrointestinal tolerance in term infants fed infant formulas containing high sn-2 palmitate with or without oligofructose: a double-blind, randomized clinical trial. *Nutr J* 2014;13:105.
32. Vandenplas Y, De Greef E, Veereman G. Prebiotics in infant formula. *Gut Microbes* 2014;5:681–7.
33. Scholtens PA, Goossens DA, Staiano A. Stool characteristics of infants receiving short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides: a review. *World J Gastroenterol* 2014;20:13446–52.
34. Iacono G, Merolla R, D'Amico D, et al. Gastrointestinal symptoms in infancy: a population-based prospective study. *Dig Liver Dis* 2005;37:432–8.
35. van Tilburg MA, Hyman PE, Walker L, et al. Prevalence of functional gastrointestinal disorders in infants and toddlers. *J Pediatr* 2015;166:684–9.
36. Vandenplas Y, Abkari A, Bellaiche M, et al. Prevalence and health outcomes of functional gastrointestinal symptoms in infants from birth to 12 months of age. *J Pediatr Gastroenterol Nutr* 2015;61:531–7.
37. Hinds R, Loveridge N, Lemberg DA, et al. Functional gastrointestinal disorders in infants: practice, knowledge and needs of Australian pharmacists. *J Paediatr Child Health* 2019;56:1769–73.
38. Mahon J, Lifschitz C, Ludwig T, et al. The costs of functional gastrointestinal disorders and related signs and symptoms in infants: a systematic literature review and cost calculation for England. *BMJ Open* 2017;7:e015594.
39. Salvatore S, Barberi S, Borrelli O, et al. Pharmacological interventions on early functional gastrointestinal disorders. *Ital J Pediatr* 2016;42:68.
40. Corsonello A, Lattanzio F, Bustacchini S, et al. Adverse events of proton pump inhibitors: potential mechanisms. *Curr Drug Metab* 2018;19:142–54.

41. Levy EI, Hoang DM, Vandenplas Y. The effects of proton pump inhibitors on the microbiome in young children. *Acta Paediatr* 2020;109:1531–8.
42. Zeevenhooven J, Browne PD, L'Hoir MP, et al. Infant colic: mechanisms and management. *Nat Rev Gastroenterol Hepatol* 2018;15:479–96.
43. Willemsen LE, Koetsier MA, van Deventer SJ, et al. Short chain fatty acids stimulate epithelial mucin 2 expression through differential effects on prostaglandin E(1) and E(2) production by intestinal myofibroblasts. *Gut* 2003;52:1442–7.
44. Pham VT, Lacroix C, Braegger CP, et al. Early colonization of functional groups of microbes in the infant gut. *Environ Microbiol* 2016;18:2246–58.
45. Partty A, Kalliomaki M, Salminen S, et al. Infantile colic is associated with low-grade systemic inflammation. *J Pediatr Gastroenterol Nutr* 2017;64:691–5.
46. Beghin L, Tims S, Roelofs M, et al. Fermented infant formula (with *Bifidobacterium breve* C50 and *Streptococcus thermophilus* O65) with prebiotic oligosaccharides is safe and modulates the gut microbiota towards a microbiota closer to that of breastfed infants. *Clin Nutr* 2020;40:778–87.
47. Tims S, Rodriguez-Herrera A, Polman J, et al. A partly fermented infant formula with prebiotics scGOS/lcFOS modulates the gut microbiota functioning towards a more breastfed-like microbiota. *J Pediatr Gastroenterol Nutr* 2018;66:1177. (abstract).