Next generation of infant formula - prebiotics, postbiotics and HMOs to support the immune system through the gut

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The early stages of life are a period of rapid growth and development. From the first day of pregnancy until 2 years of age, the so-called "first 1000 days", all the major organs in the body grow and mature in function. Therefore, this period offers a critical window of opportunity to shape both short- and long-term health.

Nutrition in early life has a key impact on optimal physical growth and body composition, cognitive development, immune maturation, development of the gastrointestinal system and development of healthy appropriate eating habits. An imbalanced intake of nutrients in terms of quality and quantity (under- and overnutrition) can have profound effects on the development of the child, including on his or her risk of non-communicable diseases in later life. Increasing scientific evidence shows that meeting the specific nutritional needs during the first 1000 days and beyond, will positively influence health outcomes.

Breast milk is the best source of nutrition for all infants and breastfeeding has numerous short- and long-term benefits for both infants and mothers. We acknowledge the importance of WHO code and support their recommendation for exclusive breastfeeding for at least six months and introduction of safe and appropriate complementary foods thereafter.

Nutricia supports breastfeeding by investigating the unique complexities of breast milk. We want to better understand its composition and related benefits so we can use it as inspiration to develop innovative nutritional solutions for infants who are not fully breastfed. Based on deep knowledge on the complex and diverse composition and function of breast milk, we add probiotics, Human Milk Oligosaccharides (HMOs), synbiotics and/or postbiotics to infant formula depending on the need of the infants.

At Nutricia, we aim to deliver specialised nutritional solutions and services for each stage of development to ensure an optimal start in life.
CRITICAL MILESTONES IN INFANT FORMULA – HOW CLOSE WE CAN GET TO BREAST MILK

If breastfeeding did not already exist, someone who invented it today would deserve a dual Nobel Prize in medicine and economics.’ (Keith Hanson, World Bank Group). Breast is best, but it has never been the only option. There was, and will always be, a need for human milk substitutes, no matter how successful the promotion of breastfeeding. Currently, two approaches to the development of infant formulas include matching human milk composition or matching breastfeeding performance. Both approaches are challenging. For example, the composition of human milk is variable, not only between mothers (and populations), but within an individual over the course of lactation. In contrast, the composition of infant formula remains rather constant. To address the latter, more advanced staging of infant formula has been proposed by some experts. Moreover, not all human milk components may be added to formulas (e.g., live cells) or are essential, as they may be synthesized (e.g., long-chain polyunsaturated fatty acids in term infants). The presence of a component in human milk by itself is not considered a sufficient reason for adding it to infant formula (e.g., taurine, nucleotides). Finally, high costs of the innovative, sophisticated infant formulas may make them less affordable to some populations. Thus, multiple factors complicate the matching of human milk composition. Similarly, matching breastfeeding performance is challenging.

It is clear, for example, that not all of the advantages of breastfeeding are necessarily attributable to the nutritional content of human milk. Currently available infant formulas do not raise safety concerns with regard to growth and adverse effects. However, differences between breastfed and formula-fed infants with respect to short- and long-term outcomes exist. Thus, opportunities for further improvement of infant formulas exist. Despite the challenges, research on further improving infant formulas continues. Among others, efforts focus on metabolic programming and the potential impact on infant gut microbiota of adding probiotics, prebiotics, including human milk oligosaccharides, or postbiotics. The development of modifications with documented short- and long-term effects assessed according to current standards continues. Ideally, all potential stakeholders, including those in academia and industry, should be working together. Recommendations for the conduct of public-private research collaborations have been developed. Such collaborations are likely to optimise infant formula. Still, infant formula will always remain a compromise; breast milk will remain the best.

Prof. Hania Szajewska (Chair)
The Medical University of Warsaw, Department of Paediatrics, Poland

Hania Szajewska, MD is Professor and Chair of the Department of Paediatrics at the Medical University of Warsaw. Since 2014, she has been serving as the Editor-in-Chief of the Journal of Pediatric Gastroenterology and Nutrition. Prof. Szajewska has broad interests in paediatric nutrition but her research focuses on the effects of early dietary interventions on later outcomes; the gut microbiota modifications such as with probiotics and/or prebiotics; acute and chronic diarrheal diseases, and coeliac disease. She is or has been actively involved in several European Union-funded projects. She is an enthusiastic advocate for the practice of evidence-based medicine. Previously, Prof. Szajewska served as a member of the Council and then as the General Secretary of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN).

She also served as the Secretary of the ESPGHAN Committee on Nutrition. Currently, Hania Szajewska serves as the co-chairperson of the ESPGHAN Working Group on Probiotics and Prebiotics. Most recently (January 2019), she joined the Board of Directors of the International Scientific Association for Probiotics and Prebiotics (ISAPP). She has co-authored more than 300 publications and 25 book chapters. Citations over 14,000; Hirsh index 63 (Scopus, January 2019).
Dr. Bernd Stahl
R&I director Human milk Research at Danone Nutricia Research, the Netherlands

Bernd Stahl received his education and his PhD as biologist at the Westfälische Wilhelms-Universität Muenster, Germany. His research was focused on characterisation of biologically relevant molecules obtained from human milk and other natural sources by mass spectrometry in connection with other analytical methods. He has a strong background for more than 20 years in human milk research with a significant contribution to research on and implementation of the specific prebiotic principle for early life nutrition. Bernd was conferred the Mattauch-Herzog Award of the German Society for Mass Spectrometry. Since 2008 he is chair of ILSI Europe Task Force Prebiotics.

He is author and co-author of more than 100 scientific publications, reviews and book chapters. He is (co)inventor of more than 30 patents. Currently his work is focused on scientific understanding of factors influencing and being influenced by breastfeeding and human milk beyond just nutrition. To understand the variation of human milk composition (influenced by the maternal diet and health, the physical and social environment, and genetics) and the consequences for the breastfed infant is relevant for global public health. The research will help to deliver further inspiring innovations for optimising infant and maternal nutrition.

Bernd Stahl is member of the American Society for Nutrition (ASN), American Society for Mass Spectrometry (ASMS), American Chemical Society (ACS), German Biochemical Society (GBM), German Society for Mass Spectrometry (DGMS), European Milk Bank Association (EMBA), International Milk Genomics Consortium (MGC) and the International Society for Research in Human Milk and Lactation (ISRHL).

Intense research on human milk and lactation over the last decades resulted into major innovations in infant formula. The drivers of adding ingredients especially beyond the mandatory composition to infant formula has been and still is human milk. Its various, structurally diverse bioactive compounds altogether ensuring unique functionality, human milk remaining the "golden standard". One hallmark insight in human milk composition is its diversity and complexity of its components. Besides its complex protein and lipid composition still after a millennium of human milk research many things are still to be discovered.

Nutricia, active in specialised nutrition for more than 120 years, has been studying human milk for more than 40 years, decoding the science to create the next best infant formula generation. One striking discovery is that human milk contains bioactive compounds of which a large part is HMOs. They are the third largest fraction of human milk comprising of more than 200 different short- and long-chain oligosaccharide structures. HMOs are complex carbohydrates that provide many benefits to the infant.

To mimic the structure and function of this complex HMOs pool, Nutricia has already introduced a unique oligosaccharide blend consisting of scGOS/lcFOS (9:1) to infant formula in 2000, comprising of more than 100 structures. The effects and benefits of scGOS/ lcFOS (9:1) are intensively studied in more than 30 studies described in more than 55 scientific publications. scGOS/lcFOS (9:1) remains unparalleled in its clinically proven benefits: stimulating a healthy, diverse gut colonisation dominated by the growth of Bifidobacteria as seen in healthy breastfed infants, which is associated with the reduction of gastro-intestinal and airway infections plus reduced antibiotics use. Moreover, it is still the only oligosaccharides mixture which is endorsed by scientific medical societies such as the World Allergy Organisation (WAO), the International Science Association of Probiotics & Prebiotics (ISAPP) and the European Food Safety Agency (EFSA).

To summarise, Nutricia’s new nutritional concept with the exclusive combination of the well-known mixture scGOS/lcFOS (9:1), 3’-FL and 3’-GL mimics the essence of human milk content, including the quality, quantity, diversity and functionality of the complex oligosaccharide structures in human milk.

Therefore, Nutricia has now even further enriched its unique oligosaccharides blend by combining scGOS/lcFOS (9:1) with 2’-FL* and 3’-GL*, both identical HMO structures as present in human milk. This pool of oligosaccharides in our new nutritional concept will further mimic the quantity, complexity and functionality of the total pool of HMOs. 2’-FL is known to inhibit pathogen binding and new data has shown that 2’-FL is more efficient in presence of scGOS/ lcFOS (9:1). 3’-GL is reported to protect the gut barrier and is produced during Nutricia’s unique, patented Lactofidus™ fermentation process, also introducing specific postbiotics in its formulations.

Postbiotics are defined as bioactive compounds produced by food-grade micro-organisms in a fermentation process. The introduction of postbiotics is a revolutionary breakthrough paving the way to the future of infant formula and has been inspired by the presence of bioactive compounds in human milk like bacteria and their metabolites, which are known to have immune benefits.

Nutricia’s new nutritional concept with the exclusive combination of the well-known mixture scGOS/lcFOS (9:1), 2’-FL and 3’-GL comprises more than 100 oligosaccharide structures, supporting immune system through gut via various modes of action. Together with specific postbiotics, this new concept most closely reflects complexity, diversity and functionality of human milk. Nutricia’s new nutritional concept with the exclusive combination of the well-known mixture scGOS/lcFOS (9:1), 2’-FL and 3’-GL comprises more than 100 oligosaccharide structures, supporting immune system through gut via various modes of action. Together with specific postbiotics, this new concept most closely reflects complexity, diversity and functionality of human milk.
Flavia Indrio received her Medical degree at Bari University, Italy. The residence program in Pediatrics was done in Bari Italy, Karolinska Institute Stockholm, Sweden and Gasthuisberg Hospital Leuven, Belgium.

Her research field has been focused on neonatal gastrointestinal disorders, GI motility, prebiotics and probiotics. Her current position is Associate Professor of Pediatrics, University of Bari, Italy where she is responsible of the Paediatric Gastroenterology and Motility Unit. Next to that, Flavia Indrio is teacher of Paediatric Gastroenterology and Nutrition for the Resident in Paediatric University of Bari School of Medicine and tutor for Medical Student at University of Bari School of Medicine.

Flavia Indrio currently published more than 100 peer reviewed papers. She is member of Italian Society of Paediatrics, Italian Society of Paediatric Gastroenterology, Italian Society of Neonatology and European Society of Paediatric Gastroenterology. She is honorary Member of South African Society of Paediatrics. Finally Flavia Indrio is Member of Committee of Nutrition ESPGHAN and eLearning Program for ESPGHAN and Chair of 4th International Congress on Prebiotics and Probiotics in Paediatrics.

The exciting potential of prebiotics, postbiotics and HMOS to support immune system through gut

The gut and gut microbiota play a critical role in the establishment and maintenance of a healthy immune system. Human milk contains many bioactive compounds known to support the development of a healthy gut microbiota and immune system. A new nutritional concept combining specific oligosaccharides and postbiotics in infant formula has been developed:

Postbiotics
In this concept, postbiotics are being produced using the Lactofidus™ fermentation process, with the specific strains Bifidobacterium breve C50 and Streptococcus thermophilus 065. Postbiotics derived from these strains have a history of safe use in infant formula and benefits on immune and gut, have been described in various clinical studies. Postbiotics are an emerging field with great potential. The effects of infant formula containing specific postbiotics are well-documented including enhancing the response to vaccines and strengthening the mucosal and sub-mucosal immune system over more than two decades.

There is accumulating evidence showing that infant formula with postbiotics derived from Bifidobacterium breve C50 and Streptococcus thermophilus 065 provide gut and immune benefits, like favourable effects on acute diarrhea, increased poliovirus-specific intestinal antibody response, production of IgA and the number of Bifidobacteria.

Additionally, thymus size and stool pH are close or almost the same when using formula with postbiotics compared to breast milk. Finally, infants fed with formula containing postbiotics were carrying more Bifidobacteria infants long than infants fed a standard formula. The difference was also related to the carrying of adolescents, showing that this formula may influence not only the pH size of the thymus but also the microbiota. Based on available insights, postbiotics in infant formula are safe and could favor a healthy and resilient gut and immune system in infants.

Unique combination of specific oligosaccharides and postbiotics
In clinical studies, the combination of prebiotics and postbiotics has been shown to be safe and well-tolerated resulting in normal growth according to WHO standards in healthy infants. In addition, microbiota composition and activity were closer to breastfed infants and investigators reported reduced incidence of colics (as AE) all anticipated to positively impact a resilient immune and gut functioning in healthy infants.

To summarise, there is exciting potential on the unique combination of scGOS/lcFOS (9:1), postbiotics and HMOS in infant formula bringing immune through gut benefits, closer to breast milk functionality.
Human milk oligosaccharide 2’-fucosyllactose more efficiently modulates immunogenicity during maturation of human dendritic cells in the presence of scGOS/lcFOS prebiotics
Saskia A. Overbeek, Bernadet Blijenberg, Nienke Kettelarij, Ling Xiao, Bernd Stahl, Johan Garssen, Belinda van’t Land
Type: oral presentation
Abstract No.: A-1071-0019-00242
Abstract Presenter: Saskia Overbeek
Session: Parallel Session: Nutrition 1
Presentation Number: N-O-008
Presentation Time: 17:15 - 17:25
Length of Presentation: 7 min. + 3 min. discussion
17:15 - 18:15

Combination of specific pre-and postbiotics in infant formula induces gut barrier maturation closer to mother’s milk and supports gut functionality in mice
Mona Mischke, Audrey Vincent, Belinda Duchêne, Jan Krol, Isabelle van Sauningen, Ingrid Renes
Type: oral presentation
Abstract No.: A-1071-0022-00849
Abstract Presenter: Mona Mischke
Session: Parallel Session: Nutrition 1
Presentation Number: N-O-011
Presentation Time: 17:45 - 17:55
Length of Presentation: 7 min. + 3 min. discussion
17:15 - 18:15

Automated stool consistency scoring for non-toilet trained children by machine learning algorithms
Thomas Ludwig, Xi Wang, Ines Oukid, Koen Huysentruyt, Puspita Roy, Agathe C. Foussat, Yvan Vandenberg
Type: oral presentation
Abstract No.: A-1071-0010-00190
Abstract Presenter: Thomas Ludwig
Session: Parallel Session: Gastroenterology 2
Presentation Number: G-O-018
Presentation Time: 17:15 - 17:25
Length of Presentation: 7 min. + 3 min. discussion
17:15 - 18:15

Human milk oligosaccharide 3’-galactosyllactose can protect the intestinal barrier to challenges
S. Varasteh, Vani Land B, I. Giziakis, M. Mark, B. Stahl, S. Wiertsama, G. Folkerts, J. Garssen and S. Braber
Type: poster selected for Poster Walk
Abstract No.: A-1071-0019-00293
Abstract Presenter: Soheil Varasteh
Session: Parallel Session: Gastroenterology 2
Presentation Number: G-O-018
Presentation Time: 17:15 - 17:25
Length of Presentation: 7 min. + 3 min. discussion
17:15 - 18:15

The gasel study: family impact, management, and overlap of functional gastrointestinal disorders in African infants
Marc Bellachie, Simon Ategbo, Faïlia Benhassine, Fanny Krumholz, Thomas Ludwig, Abdihelak Askale
Type: poster
Abstract No.: A-1071-0010-00189
Abstract Presenter: Marc Bellachie
14:00 - 14:45

Quantification of infant crying and fussing by parental electronic diaries versus automated machine based learning: an in-home observational study
Thomas Ludwig, Steven Ting, Jeffrey A. Richards, Kimberly K. Coulter, Agathe C. Foussat, Stephen M. Hannon, Hania Szajewska
Type: poster
Abstract No.: A-1071-0010-00219
Abstract Presenter: Thomas Ludwig
14:00 - 14:45

Observational study shows high degree of inter-observer agreement for the Brussels Infant and toddler stool scale for non-toilet trained children
Thomas Ludwig, Koen Huysentruyt, Jill Wang, Steven Ting, Agathe C. Foussat, Puspita Roy, Yvan Vandenberg
Type: poster
Abstract No.: A-1071-0010-00262
Abstract Presenter: Thomas Ludwig
14:00 - 14:45

Relationship between liver size and liver function in mice
O.A.H.O. Ronda, R. Havinga, B. J. M. van de Heijning, F. Kuipers, H.J. Verkade
Type: poster
Abstract No.: A-1071-0016-01207
Abstract Presenter: Onne Ronda
14:00 - 14:45

OVERVIEW OF ORAL SESSIONS, POSTERS AND E-POSTERS
Thursday 6 June 2019
Friday 7 June 2019
Combination of prebiotic oligosaccharides and fermented infant formula (with Bifidobacterium breve C50 and Streptococcus thermophilus O65) is safe and modulates the gut microbiota towards a microbiota closer to that of breastfed infants. Laurent Béghin, Sebastian Tims, Mieke Rodofs, Carole Rougé, Raish Oozeer, Thameur Rakza, Gaetano Chirico, Christoph Grüber, Guus Roestants, Jan Knol, Jean Christophe Rozé, Dominique Turck

Type: oral presentation
Abstract No.: A-1071-0020-01258
Abstract Presenter: Laurent Béghin
Session: Parallel Symposium: Probiotics for infants
Presentation Number: P-O-039
Presentation Time: 09:50 - 10:00
Length of Presentation: 7 min. + 3 min. discussion

Combination of short chain galacto-oligosaccharides and long chain fructo-oligosaccharides (scGOS/lcFOS 9:1) with 2’-fucosyllactose (2’-FL) positively impact the infant gut microbiota composition and metabolic activity in a simulator of the human intestinal microbial ecosystem (SHIME®). Chee Yong Goh, Kees van Limpt, Roger Bongers, June Su Yin Low, Nana Banike, Jan Knol and Kaouther Ben Amor

Type: ePoster presentation
Abstract No.: A-1071-0022-00852
Abstract Presenter: Kaouther Ben Amor

Fatty acid profile during lactation of Chinese women: a pooled data analysis. Linde Flors, Maneke Abrahamse-Berkeveld, Bernd Stahl, Inga C. Teller

Type: ePoster presentation
Abstract No.: A-1071-0022-00836
Abstract Presenter: Bernd Stahl
10:40 - 11:30

Label free targeted LC-ESI-MS2 analysis of 3’- and 6’-galactosyllactose in human milk with enhanced structural selectivity. Marko Mank, Bernadet Blijenberg, Bernd Stahl

Type: ePoster presentation
Abstract No.: A-1071-0022-00363
Abstract Presenter: Marko Mank
10:40 - 11:30

Native whey protein improves maturation of the immature intestine of preterm and near term piglets. Marit Navis, Vanesa Muncan, Per Torp Sangild, Line Møller Willumsen, Pim J Koelink, Marion E. Wijdeveld, Evan Abrahamse, Thomas Thyrmann, Ruud M. van Elburg and Ingrid B. Renes

Type: ePoster presentation
Abstract No.: A-1071-0002-00807
Abstract Presenter: Marit Navis
10:40 - 11:30

Mineral bioaccessibility from amino acid based medical nutrition formulas for infants and children under different digestive conditions in vitro. Francina Dijk, Evan Abrahamse, Ingrid Renes, Ardy van Helvoort

Type: ePoster presentation
Abstract No.: N-P-015
Abstract Station: ePoster Session 9
Abstract No.: 957
10:40 – 11:30
**Objectives and Study**

The neonatal immune system is polarised towards Th2-type immunity with dampening of Th1-type immune responses and cytokines. Human milk is uniquely suited to provide optimal nutrition and immune protection to infants, in which human milk oligosaccharides (HMOS) consist of short and long chain oligosaccharides, typically present in a 9:1 ratio. 2'-Fucosyllactose (2'-FL) is one of the most prominent short chain HMOS and has been associated with anti-infective capacity of human milk.

We recently demonstrated that dietary 2'-FL improves humoral and cellular immune responses in an influenza-specific murine vaccination model, suggesting that 2'-FL improves neonatal vaccine efficacy. To further elucidate these observations, the effect of 2'-FL, combined with a specific mixture of dietary short chain galacto- and long chain fructo-oligosaccharides (scGOS/lcFOS, 9:1 ratio) during human DC maturation was studied.

**Methods**

During differentiation towards immature DCs (mDCs) CD14+ monocytes (isolated by negative selection from the PBMC fraction from healthy human buffy coats) were cultured for 6 days with IL4 and GM-CSF. Subsequently maturation for 24 hours with LPS (100 ng/ml) co-incubated with or without 2'-FL (0.25-1%) and scGOS/lcFOS (9:1 ratio; 1% w/v) was studied using IL10 and IL12 production detection. Moreover, the cross-talk between these DCs and naïve CD4+ T-cells was studied in an allogenic mixed lymphocyte reaction (MLR).

**Results**

LPS matured DCs significantly induced IL10 and IL12 (P<0.001), which was concentration-dependently decreased by 2'-FL (IL10 ns, downrend trend; IL12 P<0.05; ≥0.2% 2'-FL) Interestingly, 2'-FL was more efficient in the presence of complex oligosaccharide structures scGOS/lcFOS (IL10 P<0.01; ≥0.2% 2'-FL; IL12 P<0.01; ≥0.1% 2'-FL). The direct interaction of 2'-FL during DC maturation was significantly (P<0.05; 0.5% 2'-FL; P<0.01; 1% 2'-FL) increased the IL10/IL12 ratio, suggesting the development of a tolerogenic DC phenotype. Furthermore, although within the alogenic MLR no differences in IFNγ levels were detected, the combination of 2'-FL and scGOS/lcFOS significantly suppressed IL13 release by the co-cultured T-cells (P<0.001).

**Conclusion**

These results suggest that 2'-FL has a direct immune-modulatory effect on DC maturation, which was more pronounced in the presence of scGOS/lcFOS. Moreover, combined exposure of 2'-FL and scGOS/lcFOS resulted in a dampening of the release of Th2 cytokine IL13 whereas the release of Th1 cytokine IFNγ did not change, suggesting the importance of diversity in probiotic oligosaccharides within early life nutrition.

**Objectives and Study**

The gastrointestinal tract (gut) undergoes significant and well-timed growth and maturation during the perinatal period, leading to the establishment of proper digestive and absorptive functions, intestinal barrier and immune homeostasis, which are crucial factors for health and well-being. Early life nutrition is known to affect this developmental trajectory of the gut. Compared to mother’s milk, formula concepts have been shown to induce precocious maturation of the small intestine in preclinical models. With this study we aimed to elucidate, if infant formula (IF) that contains specific prebiotics or specific pre- and probiotics as compared to IF without pre- or probiotics, stimulates gut maturation more similar to the mother-fed situation, in a mouse model.

**Methods**

From postnatal day (PN)14 to PN175, mouse pups were separated from dams and received either IF with prebiotics (scGOS/lcFOS, 9:1; PRE), with a combination of pre- and probiotics (PRE+POST), or control (CTRL) IF without pre- and probiotics ad libitum. Postbiotics were derived from the Lactobacillus TM fermentation process, using bacterial strains Streptococcus thermophilus D65 and Bifidobacterium breve CS5. On PN175 morphology and functional parameters for gut maturation were assessed. Age-matched, non-separated mother-fed pups (MF) served as reference group.

**Results**

Morphologically, we observed more non-vascularized, mature crypts in ileal epithelium of all mice that received IF while separated from dams compared to MF mice. Crye-villus length in fecum of PRE and CTRL mice was significantly greater than in MF mice, while PRE+POST mice rather resembled the MF situation. Functionally, we found that ileal succrase activity was increased in mice receiving PRE+POST IF compared to MF, while lactase activity was similarly high in all IF groups and comparable to MF levels. Pre- weaning increases in succrase activity might suggest accelerated gut maturation. However, since lactase activity levels in PRE+POST mice were maintained similarly high as in MF, our findings rather suggest increased disaccharidase functionality, which might support an easier weaning transition. Gut permeability was measured by FITC-dextran and was similar in PRE+POST and MF mice, while it was significantly lower in mice receiving CTRL IF compared to both, PRE+POST and MF mice. Precociously decreased permeability as seen in CTRL IF could affect health long lasting by altering immune maturation.

**Conclusion**

Overall, maternal separation in combination with IF feeding induces precocious maturation of morphological gut parameters (i.e. replacement of vacuolated cells), but not of functional gut parameters (i.e. disaccharidase activities) IF containing a combination of specific pre- and postbiotics induces gut barrier maturation closer to the mother-fed situation.
AUTOMATED STOOL CONSISTENCY SCORING FOR NON-TOILET TRAINED CHILDREN BY MACHINE LEARNING ALGORITHMS

Thomas Ludwig1, Jill Wong1, Ivo Oukid2, Koen Huysentruyt3, Puspita C. Foussat1, Yvan Vanderplas1

Objectives and Study
The accurate classification of stool consistency of non-toilet trained children remains challenging. The Brussels Infant and Toddler Stool Scale (BITSS) has recently been established as a reliable measure, though differences in inter-rater reliability between parents and healthcare professionals have been reported. The aim of this study was to develop and evaluate machine learning based algorithms for automated objective, and reliable recognition of the consistency of stools in diapers from photos. This technology can be utilised for example by mobile phone applications for clinical study purposes or home assessment of stool consistency of stools in diapers.

Methods
A total of 1681 photos of diapers with stool from children between 0 and 24 months of age were obtained in a study after ethical approval. The stool consistency of each uploaded diaper photo was assessed independently by the 7 photos of the BITSS by two mothers that were not included in the study. Disagreements in scoring were solved by a healthcare professional to obtain the final scoring. Transfer learning was used as an approach to train a deep convolutional neural network utilising an open source library (Keras) for the training of the neural network will increase the accuracy of the original BITSS validation study.

Conclusion
This proof of concept study shows that it is possible to develop machine learning algorithms for the automated and objective scoring of the consistency of stools in diapers. A higher number of photos for the training of the neural network will increase the accuracy of the predicted scores.

HUMAN MILK OLIGOSACCHARIDE 3’-GALACTOSYLLACTOSE CAN PROTECT THE INTESTINAL BARRIER TO CHALLENGES

S. Varasteh1, Varri Land B1,2, Gisák1, M. Markt1, B. Stahl1, S. Wiersma1, G. Folkerts1, R. Garssen1,3 and S. Braber1

Introduction and Objective
Human milk contains more than 1000 individual oligosaccharide structures. These human milk oligosaccharides (HMOS) play an essential role in the postnatal growth and development of the mucosal immune system. The unique diversity of HMOS include galacto-oligosaccharides. One specific functional structure is based on the 3’-galactosyllactose (3’GL), 4’-galactosyllactose (4’-GL) and 6’-galactosyllactose (6’GL) (Newburg et al., 2016). The aim of the current study was to investigate the ability of structurally different HMOS-like galactosyllactoses, to protect the intestinal epithelial barrier and to unravel the importance of their structure (linkages) in these microbiosa-independent effects.

Methods
Human intestinal epithelial Caco-2 cell monolayers grown in a transwell system, model for intestinal barrier function (Akbari et al., 2017), were pretreated with different galactosyllactoses, including 3’GL, 4’GL and 6’GL for 24h, before being exposed to the fungal toxin deoxynivalenol (DON, a trigger for intestinal integrity breakdown). In addition, the difference between 3’GL with an α1-3 and a α1-3 glycosidic linkage was examined.

Conclusion
The structures of specific galactosyllactoses show different effects in protecting the intestinal epithelial barrier under challenges with 3’GL with a α1-3 glycosidic linkage showing a protective effect on the epithelial barrier. These data further support the notation that it is of key importance to understand the function and diversity of the structures within the total pool of HMOS, including the specific benefits of 3’GL within early life nutrition.

References
THE GASEL STUDY: FAMILY IMPACT, MANAGEMENT, AND OVERLAP OF FUNCTIONAL GASTROINTESTINAL DISORDERS IN AFRICAN INFANTS
Marc Bellache1, Simon Ategbo2, Fadila Benhassine3, Fanny Krumholz4, Thomas Ludwig5, Abdelhak Abkari6

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2. Bologhine Hospital, Department of Paediatrics, Algiers, Algeria.
3. Danone Nutricia, Africa & Overseas, Limonest, France.
4. Paediatrics, Algiers, Algeria.
5. Paediatrics, Algeria.
6. Paediatrics, Casablanca, Morocco.

Objectives and Study
Functional gastrointestinal disorders (FGIDs) and related symptoms are common in infancy. Guidelines emphasize as the first-line management of the most common FGIDs parental reassurance and nutritional advice. Management recommendations also underscore that pharmaceutical intervention is usually not required, if not even harmful. For Africa important gaps in the understanding of the impact of FGIDs on families and management practices have been highlighted in reviews. This cross-sectional, observational study aimed to evaluate the pattern, impact on quality of life of families and the management of FGIDs and related signs and symptoms in infants below one year of age in Africa.

Methods
From June to November 2017, 759 doctors practicing in 10 African countries (Morocco n = 203, Algeria n = 182, Ivory Coast n = 107, Cameroon n = 62, Tunisia n = 50, Congo n = 39, Madagascar n = 38, Gabon n = 36, Mauritius n = 28, and Senegal n = 15) participated in the study. Doctors completed questionnaires for a total of 10 812 families with infants aged between 0 and 12 months that were consulting them for the first time for FGID symptoms as the main complaint. FGIDs were assessed according to adapted Rome IV criteria for infant regurgitation, colic and functional constipation, and in addition gas and bloating. In addition, doctors were asked about management recommendations. The quality of life of infants was evaluated on a scale extracted from the quality of life in infants (QUALIN) questionnaire validated for paediatrics.

Results
In brief, of the families that consulted a doctor for gastrointestinal symptoms 57.6% of the infants were diagnosed with infant colic, 29.3% with regurgitation, 31.4% with functional constipation and 43.2% with gas and/or bloating. Interestingly, the incidence differed between countries. For example, regurgitation varied between 28.5% and 47.7%. Overall, more than 50% of infants presented with more than one FGID. For example, infant colic was combined with gas and/or bloating in 24.4% of all infants, and with regurgitation in 15.0%. 14.2% of infants had a combination of infant colic, gas and/or bloating, and regurgitation. The mean score for quality of life of infants and their families linked to FGIDs was 6.1/10, and there was a significant difference between infants with one or more FGIDs (5.9 versus 6.5 respectively, p = 0.001). Overall, 96.8% of doctors reported to provide parental reassurance, 88.3% reported to give dietary and hygiene advice, and 62.4% reported to prescribe drugs. Most children receive a combination of the different treatments. The most commonly prescribed drugs included antispasmodics, oral laxatives, prokinetics, antacids and combinations of these and other prescription medications. All interventions were significantly more often reported for infants with a combination of FGIDs.

Conclusion
The study findings as described here indicate a frequent concomitant occurrence of FGIDs and a high level of drug prescriptions in the daily practice of doctors in African countries. This is in line with similar observations in other geographies and future studies will have to clarify the incidence of FGIDs in the general population, and the impact of FGIDs on quality of life compared to unaffected infants.

FUNCTIONAL GASTROINTESTINAL DISORDERS IN AFRICAN INFANTS

Objectives and Study
Excessive crying and fussing in infants are common and of relevant parental concern. The Language Environment Analysis (LENA) system’s novel machine based learning algorithms aim to automatically identify, quantify and distinguish periods of crying and fussing. The aim of this study was to compare infant crying and fussing documented by parental electronic diaries with those detected by the LENA system.

Methods
For this observational study, 22 families with healthy term infants younger than 24 weeks of age were enrolled. The child’s primary caretakers were asked to make regular entries into an electronic diary regarding periods of crying and fussing and to use LENA to collect information on infant crying and fussing parameters for 14 days within a 16-day period. Data from families that supplied a concurrent electronic diary and LENA data for at least 20 hours within one day during the study period were considered for further analysis.

Results
Eleven families provided a total of 142 days of concurrent electronic diary reporting and LENA recording. Of these families, 8 families provided 14 days of concurrent recording, and one family each provided 13,15, and 5 days of concurrent recording. In brief, differences in absolute crying and fussing durations, time resolution, and distribution were observed between parental diary reports and the LENA system. Parents reported a longer daily mean crying duration (± SD) as compared to that detected with the LENA (34.0 ± 5.79 minutes versus 145 ± 202 minutes, respectively, p = 0.0001), however, there was no significant difference between parental reported and LENA detected fussing duration (200 ± 250 minutes and 247 ± 254 minutes, respectively, p=0.09). Per event, crying and fussing durations reported by the parents were significantly longer than those detected by LENA (both p<0.05). Accordingly, 9/11 families reported a higher total crying and fussing duration as compared to the LENA system, whereas 8/11 families also reported a lower total number of events of crying and fussing compared to the LENA system. In addition to mean differences between parental diaries and the LENA system, systematic differences in individual reporting between families became apparent. The degrees of correlation between parental reported and LENA detected crying and fussing time varied between families (Spearman Correlations between -0.5 and 0.8). For example, in one family the LENA system detected a lower mean total duration of crying and fussing compared to what parents reported (374 minutes versus 65.0 minutes, respectively). In another family the LENA system detected a higher mean total duration of crying and fussing compared to what the parents reported (695 minutes versus 284.4 minutes, respectively).

Conclusion
This study provides first insights into parental reporting of crying and fussing in healthy infants compared to that obtained with a digital device that objectively measured crying and fussing. Subsequent investigations will further unravel distinct effects and how the perception of crying and fussing varies among individual families.

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OBJECTIVE STUDY SHOWS HIGH DEGREE OF INTER-OBSERVER AGREEMENT FOR THE BRUSSELS INFANT AND TODDLER STOOL SCALE FOR NON-TOILET TRAINED CHILDREN

Thomas Ludwig1, Koen Huysentruyt2, Jill Wong1, Steven Ting1, Agathe C. Foussat1, Puspita Roy1, Yvan Vanderplas3

Objectives and Study
The Brussels Infant and Toddler Stool Scale (BITSS) was recently presented as a classification tool to describe stool consistency for children that are not toilet trained. It was the primary objective of this observational study to create an electronic database of at least 200 pictures of stool containing diapers for each of the seven stool consistency score photos of the BITSS. In addition, differences in stool consistency scoring between mothers who were enrolled in the study and scored photos of the diapers directly, versus mothers who were not enrolled and scored the uploaded photos, were explored.

Methods
106 mothers with children between 0 and 24 months of age who were using commercial disposable diapers were screened to be included in the study. Along with each uploaded stool photo, the study mother rated the stool consistency (type 1 to 7) according to its resemblance to the seven photos of the BITSS. Photos and scores were automatically uploaded into a database. Two mothers who were not enrolled in the study also assessed the stool consistency based on the same photo independently and blinded from each other as well as from the enrolled mother. The percentage of agreement between both non-study mothers was 79.0% with a mean level of disagreement of -0.13.

Results
Out of the 106 screened mothers, 95 mothers were included in the study. In total, 2579 valid photos of diapers with stool from the ongoing study were considered for the analysis presented here. The percentages of agreement between the study mother, and each of the non-study mothers were 48.8% and 58.2%, with mean levels of disagreement of -0.40 and -0.38, respectively. The percentage of agreement between both non-study mothers was 78.5% with a mean level of disagreement of -0.13.

Conclusion
This is the first study that utilises the new BITSS for stool consistency. Overall, there is fair agreement of scoring of stool consistency between study and non-study mothers, with an aggregate level of disagreement of less than half a stool scale. The latter may indicate a trend towards scoring stools harder by the non-study mothers as compared to the mothers included in the study. However, there was a trend towards scoring stools softer by the non-study mothers as well as from the enrolled mother. The percentage of agreement between both non-study mothers was calculated as the average difference between study mother and each non-study mother, and between the two non-study mothers.
Prebiotics, probiotics, and fermentation metabolites, also referred to as postbiotics, are major levers to positively modulate early life microbiota development, but their specific effects on the microbiota and their safety need to be demonstrated. We performed a randomised, controlled, multi-center study (NTR2726) assessing the effects of infant formulae (IF) containing oligosaccharides and/or bacterial fermentation metabolites on the composition and metabolic activity of the intestinal microbiota and on the immune status in healthy infants. Growth and safety were also investigated. The same outcomes were assessed in exclusively breastfed healthy term infants.

Methods
Infants were randomly assigned at 4-7 days of age to one of the 4 IF groups (n=70 by group) or entered the breastfed (BF) reference group (n=70). The 4 IF tested were: (1) a non-fermented cow’s milk based IF (FERM); (2) a fermented cow’s milk based IF with prebiotics (90%scGOS/10%lcFOS); (3) a fermented cow’s milk based IF with prebiotics (FERM/scGOS/lcFOS); (4) a fermented cow’s milk based IF (FERM/scGOS/lcFOS/scGOS/lcFOS/2'-FL). All infants in the study were exclusively breastfed and did not receive any additional feeding or medication. Faecal samples were collected throughout a 2-week simulation period, and microbiota fermentation metabolites on the composition and metabolic activity of the intestinal microbiota and on the immune status in healthy infants. Growth and safety were also investigated. The same outcome were assessed in exclusively breastfed healthy term infants.

Results
The median siG A concentration at 4 months of age in the FERM/scGOS/lcFOS group was statistically significantly higher compared to the siG A concentration in the Control group (p<0.05), and was similar to that of the BF IF group. In all study groups the SCFA content, mainly due to acetate, increased over time (between baseline and month 4) at month 4, SCFA, lactate, and calprotectin levels were not different between the study groups. Median (Q1-Q3) pH was significantly lower (p<0.05) in the scGOS/lcFOS (5.9; 5.5-6.8) and FERM/scGOS/IF (5.4; 5.2-6.1) groups compared to the FERM (6.7; 6.1-6.9) and control groups (6.8; 6.3-6.4). No significant differences in the levels of Bifidobacteria showed an increase over time. At month 4 the bacterial groups Bacteroides distasonis, Blautia coccoides, Clostidium histolyticum, and Clostidium luteum showed reduced levels in the FERM/scGOS/lcFOS/IF or FERM/scGOS/scGOS/lcFOS/IF arms as compared to the control IF. Of note, the levels of B. coccoides in the IF with prebiotics (FERM/scGOS/lcFOS/IF) was similar to levels found in BF infants. No differences were observed with respect to growth and in the frequency of adverse events, except for GI adverse events, which were more frequently reported in the scGOS/lcFOS/IF and scGOS/lcFOS/IF arms. Overall, there was no safety concern.

Conclusion
Infants consuming FERM/scGOS/lcFOS/IF formula have a gut microbiota closer to that of BF infants in terms of composition and metabolic activity, as compared to the control IF group.

Combinations of Prebiotics, Probiotics, and Fermentation Metabolites on the Composition and Metabolic Activity of the Human Intestinal Microbial Ecosystem (Shime)

The SCFA profiles of all the interventions showed that acetate was the most abundant in the proximal and distal colon compartments following supplementation. The concentrations of acetate and propionate were significantly higher in the presence of scGOS/lcFOS/IF and scGOS/lcFOS/2’-FL than in the control and 2’-FL groups. Interestingly, butyrate was generated earlier in the distal colon and at a significantly higher concentration in the presence of 2’-FL and scGOS/lcFOS/2’-FL relative to the control and the scGOS/lcFOS/IF group. Lactate levels were low in all groups. The 16S RNA sequencing data revealed that scGOS/lcFOS and scGOS/lcFOS/2’-FL stimulated the growth of Bifidobacteria to a comparable level for both interventions. No bifidogenic effect was observed when 2’-FL was the only source of carbohydrate. Supplementation with scGOS/lcFOS/2’-FL resulted in a higher level of Velotrobia, a lactate-utiliser bacterium that forms postbiotics, as compared to all other intervention groups including scGOS/lcFOS. Furthermore, supplementation with scGOS/lcFOS/2’-FL induced a decrease in the level of potential pathogens which was not observed in the presence of only 2’-FL.

Conclusion
Our data show that 2’-FL is only fermented in the presence of scGOS/lcFOS, resulting in a bacterial composition that is suggested to confer health benefits for infants by increasing the level of Bifidobacterium, Velotrobia and reducing potential pathogens comparable to what is reported for breastfed infants. Furthermore, this combination enhanced the production of butyrate suggesting that the combination of 2’-FL with scGOS/lcFOS, but not 2’-FL alone, might confer a positive effect on the gut maturation and development in early life.
**Objective**

Human milk (HM) contains an arrangement of more than 200 fatty acids (FA), mostly in the form of triglycerides, some embedded into complex molecules like phospho- or glycosphingolipids. Their concentrations are influenced by many factors including genetic and diet. In general, the Chinese diet is quite different from a Western diet, especially during confinement phase, and reported variation between studies is high. This prompted us to perform a pooled data analysis from existing literature to provide representative means of the predominant FA of Chinese HM over the course of lactation.

**Methods**

FA composition in HM samples from healthy Chinese mothers with term infants were searched for in Medline, English only, between JAN 1980 to AUG 2018. Reports on less than 12 FA, outdated methods, concentrations are influenced by many factors including genetic and diet. In general, the Chinese diet is quite different from a Western diet, especially during confinement phase, and reported variation between studies is high. This prompted us to perform a pooled data analysis from existing literature to provide representative means of the predominant FA of Chinese HM over the course of lactation. We observed emerging patterns throughout lactation for some of the 35 FA WLS means in our analysis. Oic: (C18:1 n9), Inositol, and docosahexaenoic acid (DHA, C22:6 n3) WLS means seemed to remain relatively stable, whereas ALA (10.8% ± 0.2 > 15.2% ± 0.24), capric (C10:0; 0.48% ± 0.01 > 1.39% ± 0.11), lauric acid (2.76% ± 0.16 > 4.81% ± 0.32) WLS means increased with lactation maturation. In contrast, the less abundant eicosadienoic acid (C20:2 n6; 1% ± 0.14 > 0.45% ± 0.05) and DGLA (0.7% ± 0.01 > 0.45% ± 0.04) decreased with maturation. Overall, MCFAs contribute on average 3.4% and 6.4% in colostrum and mature milk, respectively, of which at least 75% was lauric acid (C12:0). Probably due to methodological challenges and the volatile nature of SCFA, only one study reported butyric acid (C4:0). With our novel approach, we were able to detect 3'-GL and 6'-GL by liquid chromatography electrospray ionisation tandem mass spectrometry (LC-ESI-MS2), employing diagnostic CID-fragments in a multiple reaction monitoring (MRM) LC-ESI-MS2 approach allowing for targeted analysis and distinction of native GL-isomers from HM. For example, 3'-galactosyllactose (3'-GL) and 6'-Galactosyllactose (6'-GL) which only differ in the structural selectivity are needed to overcome these drawbacks. Once available, they may help to reveal new early-life health benefits linked to known or yet unknown GI-GLs present in human milk or infant milk formula.

**Results**

Twelve studies comprising 3192 samples were included. Most data were for mature milk and for 12 FA comprising 90% of total HMFA from medium-chain (C6-12) and long-chain (C12-FA) species.

Concentrations of polyunsaturated FA such as linoleic (C18:2 n6), gamma-linolenic (C18:3 n6), arachidonic (C20:4 n6) and 5-linolenic (ALA, C18:3 n3) acids were highly variable among individual studies as characterised by larger 95% CIs, whereas variation for other FA such as lignoceric (C24:0), dithomo-gamma linolenic (DGLA, C20:3 n3), ecosatrienoic (C20:3 n3), and docosapentaenoic (C22:5 n3) acids was very low. We observed emerging patterns throughout lactation for some of the 35 FA WLS means in our analysis. Oic: (C18:1 n9), Inositol, and docosahexaenoic acid (DHA, C22:6 n3) WLS means seemed to remain relatively stable, whereas ALA (10.8% ± 0.2 > 15.2% ± 0.24), capric (C10:0; 0.48% ± 0.01 > 1.39% ± 0.11), lauric acid (2.76% ± 0.16 > 4.81% ± 0.32) WLS means increased with lactation maturation. In contrast, the less abundant eicosadienoic acid (C20:2 n6; 1% ± 0.14 > 0.45% ± 0.05) and DGLA (0.7% ± 0.01 > 0.45% ± 0.04) decreased with maturation. Overall, MCFAs contribute on average 3.4% and 6.4% in colostrum and mature milk, respectively, of which at least 75% was lauric acid (C12:0). Probably due to methodological challenges and the volatile nature of SCFA, only one study reported butyric acid (C4:0). With our novel approach, we were able to detect 3'-GL and 6'-GL by liquid chromatography electrospray ionisation tandem mass spectrometry (LC-ESI-MS2). LC-separation of GLs and other human milk compounds was facilitated by 2.1 x 30mm x 2.1 x 10mm porous graphitised carbon HPLC columns connected in line with a linear on trap mass spectrometer. Gradient elution of GLs started with 0.3% NH4OH in H2O at 0min and ended with 0.3% NH4OH in 95% methanol at 27 min. Constant flow rate was at 0.4 ml/min. All peaks were identified by collision induced dissociation (CID) fragmentation.

**Objectives and Study**

The healthy development of neonates is supported by human milk (HM). Among other bioactive compounds, HM may comprise more than 1000 individual oligosaccharide structures which also include Galactosyllactosyls (GLs) GLs may appear in form of several structural distinct isomers like for example 3'-galactosyllactose (3'-GL) and 6'-Galactosyllactose (6'-GL) which only differ in the glycosidic linkage of the terminal galactose. 3'-GL and 6'-GL have recently been reported to modulate major immunologic pathways of immature human intestinal cells and to attenuate inflammation. Anti-inflammatory effects of e.g. 3'-GL may also be mediated via inhibition of toll like receptor 3 (TLR3) signalling. However, the impact of currently known human milk GLs on the healthy development of infants is far from being completely understood. This is partly due to sensitivity aspects and difficulties in the analytical distinction of low abundant GI-GLs in HM. Therefore, analytical methodologies with enhanced structural selectivity are needed to overcome these drawbacks. Our analysis provides for the first time an overview of representative FA concentration means across lactational stages from a variety of HMFA. Insights in the HMFA profile is important to advance our understanding in the function and interplay of the different FA in the growth and development of infants, an understanding that is currently lacking for many FA.

**Results**

Twelve studies comprising 3192 samples were included. Most data were for mature milk and for 12 FA comprising 90% of total HMFA from medium-chain (C6-12) and long-chain (C12-FA) species.

Concentrations of polyunsaturated FA such as linoleic (C18:2 n6), gamma-linolenic (C18:3 n6), arachidonic (C20:4 n6) and 5-linolenic (ALA, C18:3 n3) acids were highly variable among individual studies as characterised by larger 95% CIs, whereas variation for other FA such as lignoceric (C24:0), dithomo-gamma linolenic (DGLA, C20:3 n3), ecosatrienoic (C20:3 n3), and docosapentaenoic (C22:5 n3) acids was very low. We observed emerging patterns throughout lactation for some of the 35 FA WLS means in our analysis. Oic: (C18:1 n9), Inositol, and docosahexaenoic acid (DHA, C22:6 n3) WLS means seemed to remain relatively stable, whereas ALA (10.8% ± 0.2 > 15.2% ± 0.24), capric (C10:0; 0.48% ± 0.01 > 1.39% ± 0.11), lauric acid (2.76% ± 0.16 > 4.81% ± 0.32) WLS means increased with lactation maturation. In contrast, the less abundant eicosadienoic acid (C20:2 n6; 1% ± 0.14 > 0.45% ± 0.05) and DGLA (0.7% ± 0.01 > 0.45% ± 0.04) decreased with maturation. Overall, MCFAs contribute on average 3.4% and 6.4% in colostrum and mature milk, respectively, of which at least 75% was lauric acid (C12:0). Probably due to methodological challenges and the volatile nature of SCFA, only one study reported butyric acid (C4:0).

**Conclusion**

Our analysis provides for the first time an overview of representative FA concentration means across lactational stages from a variety of geoographies within China. We observed temporal patterns in the course of lactation for several FA means and confirm the complexity, high variation, and diversity of different HMFA. Insights in the HMFA profile is important to advance our understanding in the function and interplay of the different FA in the growth and development of infants, an understanding that is currently lacking for many FA.

**Methods**

Fresh human milk volunteer samples were spiked with internal standards including e.g. α-Arabinopentose. Then, anonymised spiked milk specimens were diluted 1:10 with H2O and subjected to ultrafiltration (UF) with 30k cut off UF permeates were analysed by liquid chromatography electrospray ionisation tandem mass spectrometry (LC-ESI-MS2).
NATIVE WHEY PROTEIN IMPROVES MATURATION OF THE IMMATURE INTESTINE OF PRETERM AND NEAR TERM PIGLETS

Marc Navis1, Vanesa Muncan1, Per Torp, Sangil2, Line Moller Willumsen1, Prim J. Kochler3, Manon E. Widenberg, Evan Abrahamse, Thomas Thymann2, Ruurd M. van Elburg3,5 and Ingrid B. Renes1,5

Objectives
Intestinal immaturity predisposes preterm infants to nutritional challenges, which might lead to clinical complications such as feeding intolerance and necrotising enteroenteritis (NEC). Feeding preterm infants with infant milk formulas (IMFs) is associated with an increased risk to develop NEC compared to human milk. Heat treatments are part of the IMF production process and heating is linked to whey protein denaturation and bioactivity loss, which might impact gut maturation. Aim of the current study was to determine if native whey protein concentrate (N-WPC) has beneficial effects over denatured WPC (D-WPC) on intestinal maturation in preterm born piglets as model for preterm infants.

Methods
In total 34 preterm piglets (90% gestational age, two litters) and 18 near term piglets (96% gestational age, one litter) were delivered by cesarean section. Piglets in each litter were block-randomised based on birth weight in two experimental groups: 1) N-WPC group received formula based on pasteurised WPC (i.e. WPC heated 73°C, 30 sec, which maintains proteins in their native form) and 2) D-WPC group received formula with WPC that was pasteurised and additional heat treated (i.e. heated 73°C, 30 sec + 80°C, 6 min, which results in extensive protein denaturation). The WPC used in this study was prepared from raw cow’s milk by a process that did not involve heating. Piglets received minimal enteral nutrition for 5 days and parenteral nutrition support. Clinical symptoms were monitored daily. On day 5, a lactulose/mannitol test was performed to evaluate gut permeability. Histological scoring based on the integrity of the epithelium, presence of edema and erythrocyte and immune infiltration, showed less damage in the colon of piglets fed N-WPC in comparison with piglets fed D-WPC. The lower damage resulted in less epithelial hyperproliferation, as measured by Ki67+ cells and crypt depth. In addition, colonic expression levels of IL1β, IL8, TNFα and TLR4 were reduced in D-WPC fed piglets compared to N-WPC fed piglets.

Conclusion
Compared to denatured WPC, native WPC decreased NEC incidence and resulted in less histological damage and acute inflammation in piglets with an immature intestine. N-WPC, but not D-WPC decreased intestinal permeability suggesting that N-WPC stimulates gut barrier maturation. The increased ALP activity in N-WPC fed piglets might dampen the colonic inflammatory response and thereby improve intestinal barrier function. Overall, these data show that a formula with N-WPC has beneficial effects on gut maturation in preterm and near term piglets and might therefore also support intestinal maturation in preterm infants and (near) term infants.

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Conflict of interest
EA, RvE and IR are employees of Danone Nutricia Research.

Table 1: Native whey protein improves intestinal maturation in preterm and near term piglets

<table>
<thead>
<tr>
<th></th>
<th>Native WPC</th>
<th>Denatured WPC</th>
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<tbody>
<tr>
<td>NEC incidence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preterm piglets</td>
<td>59%</td>
<td>82%</td>
</tr>
<tr>
<td>Near term piglets</td>
<td>0%</td>
<td>66%</td>
</tr>
<tr>
<td>Lac/man ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preterm piglets</td>
<td>0.10</td>
<td>0.11</td>
</tr>
<tr>
<td>Near term piglets</td>
<td>0.09</td>
<td>0.13</td>
</tr>
<tr>
<td>Total histology score (0-12)</td>
<td>4.8</td>
<td>7.5</td>
</tr>
<tr>
<td>IL8 levels</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preterm piglets</td>
<td>67.1 pg/mg</td>
<td>131.9 pg/mg</td>
</tr>
<tr>
<td>Near term piglets</td>
<td>18.7 pg/mg</td>
<td>54.4 pg/mg</td>
</tr>
<tr>
<td>iALP activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preterm piglets</td>
<td>88.2 U/mg</td>
<td>54.5 U/mg</td>
</tr>
<tr>
<td>Near term piglets</td>
<td>53.6 U/mg</td>
<td>37.8 U/mg</td>
</tr>
</tbody>
</table>

Intestinal alkaline phosphatase (iALP), expressed by colonic enterocytes and marker for intestinal maturation and epithelial defense, showed increased activity in the colon of piglets fed N-WPC.
MINERAL BIOACCESSIBILITY FROM AMINO ACID BASED MEDICAL NUTRITION FORMULAS FOR INFANTS AND CHILDREN UNDER DIFFERENT DIGESTIVE CONDITIONS IN VITRO

Francina Dijk, Evan Abrahamse1, Ingrid Renes1, Ardy van Helvoort1

Objectives and Study

Mineral bioavailability from food is influenced by many factors including, but not limited to, the food matrix and viscosity, food processing, mineral composition (chemical source of salt and concentration), co-digestion of other food items, route of administration (e.g. oral intake vs. post pyloric tube feeding), as well as physiological factors like mineral status, functional state of the gastrointestinal tract, age and use of medication. Generally, amino acid based medical nutrition formulas (AAF’s) are provided orally and undergo digestion and acidification in the stomach before arrival in the small intestine. However, in some specific conditions infants and children are exclusively fed with AAFs directly into the small intestine (i.e. post pyloric feeding). This special type of feeding, where digestion and acidification of the stomach is bypassed, is one of the most challenging conditions experienced in clinical nutrition. Furthermore, there are clinical situations where gastric digestion is compromised due to specific medical conditions or use of medication that interferes with gastric acidification (e.g. commonly used proton pump inhibitors (PPIs)).

In-vivo bioavailability is difficult to study in infants and children; therefore, we developed in vitro methods to evaluate mineral bioaccessibility (fraction of mineral that is potentially available for absorption) from AAFs under different digestive conditions. In this research we have investigated 5 infant and three junior AAF’s from 3 manufactures.

Methods

To assess bioaccessibility of calcium and phosphorus from AAFs, products were analysed for dialyzability of minerals from the formula during and after digestion at three different digestive conditions mimicking:

1. Normal digestion: saliva, stomach pH 3.5 followed by intestinal digestion at neutral pH
2. Post pyloric feeding: intestinal digestion at neutral pH only
3. PPI digestion: saliva, stomach pH 7.5 followed by intestinal digestion at neutral pH

Results

After normal digestion, bioaccessibility ranged between 33% and 65% for calcium, and 57% and 85% for phosphorus (see figure). Under conditions where gastric acidification was absent or bypassed bioaccessibility decreased considerably for both minerals. Furthermore, under these conditions differences in bioaccessibility were more profound and related to the amount and type of mineral salt in the product. Whereas the salts used in product A and B, and C and D were different, bioaccessibility was quite similar for both minerals. Under these conditions differences in bioaccessibility were more profound and related to the amount and type of mineral salt in the product. Whereas the salts used in product A and B, and C and D were different, bioaccessibility was quite similar for both minerals.

Conclusion

Mineral bioaccessibility from AAFs can be influenced by the route of administration, specific digestive conditions, product pH, and the amount and type of mineral salt in the formulation. The developed in vitro methodology enables optimisation of mineral composition in the design of medical nutrition products, which may be helpful for patients with medical conditions requiring the use of prolonged high dose PPIs and/or administration into the small intestine.
THE BIOTICS FAMILY IN EARLY LIFE

This new book explains the impact of the biotics family. It discusses Prebiotics, Probiotics, Synbiotics and the new member of the family, Postbiotics, and their effect on the infant gut and immune system.

Breast milk is the gold standard for infant nutrition. Besides nutritional compounds, it contains many bioactive compounds, such as Human Milk Oligosaccharides (HMOS), bacteria and their metabolites, known to support the development of a healthy gut microbiota and immune system.

This book discusses concepts and how active modulation of the gut microbiota through the use of the Biotics Family may help optimise health outcomes in non-exclusively breastfed infants.

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