Early Nutrition Influence — Preventive and Therapeutic Aspects

6th September, 2018
NRC | Vers-chez-les Blanc | Lausanne | Switzerland
## List of Abbreviations

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<tr>
<td>AA</td>
<td>Amino Acids</td>
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<tr>
<td>BF</td>
<td>Breast-Fed / Breast-Feeding</td>
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<td>CD</td>
<td>Coeliac Disease</td>
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<td>CMF</td>
<td>Cow’s Milk Formula</td>
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<td>DHM</td>
<td>Donor human milk</td>
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<tr>
<td>eHF</td>
<td>Extensive Hydrolysate Formula</td>
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<td>FAP</td>
<td>Functional abdominal pain</td>
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<td>FGID</td>
<td>Functional gastrointestinal disorders</td>
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<td>GA</td>
<td>Gestational age</td>
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<td>HC</td>
<td>Head Circumference</td>
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<td>HMO</td>
<td>Human Milk Oligosaccharides</td>
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<td>IBS</td>
<td>Irritable bowel syndrome</td>
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<td>ID</td>
<td>Iron Deficiency</td>
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<td>IGF1</td>
<td>Insulin-like Growth Factor 1</td>
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<td>LBW</td>
<td>Low Birth Weight</td>
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<td>MFGM</td>
<td>Milk fat globule membrane</td>
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<td>MOM</td>
<td>Mother’s own milk</td>
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<td>NCD</td>
<td>Non-Communicable Diseases</td>
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<td>FGID</td>
<td>Functional gastrointestinal disorders</td>
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<td>NICU</td>
<td>Neonatal Intensive Care Unit</td>
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<td>pHF</td>
<td>Partial Hydrolysate Formula</td>
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<td>PBMC</td>
<td>Human peripheral blood mononuclear cells</td>
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<td>PEG</td>
<td>Polyethylene glycol</td>
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<td>PN</td>
<td>Parenteral nutrition</td>
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<td>PPI</td>
<td>Proton pump inhibitors</td>
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<td>QoL</td>
<td>Quality of life</td>
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<tr>
<td>RCT</td>
<td>Randomized Controlled Trial</td>
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<td>SGA</td>
<td>Small for Gestational Age</td>
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<tr>
<td>TLR</td>
<td>Toll-like receptors</td>
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<td>VOC</td>
<td>Volatile Organic Compounds</td>
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<td>day / days</td>
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At this NNI European Meeting we have three main chapters we would like to discuss: Functional gastrointestinal disorders, the influence of microbiota and last but not least nutrition and cognition.

Functional gastrointestinal disorders (FGID) are not a strictly medical issue. But when we look at the frequency of the families asking practitioners, doctors in their daily work, it is a really huge problem that generates a large number of visits. And it always creates trouble – and everybody who has kids can confirm this, even if we are specialists. It has a large impact on the quality of life for the whole family. So it is right that we also discuss this from the scientific point of view: What we can do, what we cannot do and what are the underlying facts.

Of course over there we know that the microbiome plays an important role overall and we are now all aware that we do not have only “bad” bacteria inside us. It is just a question of the right balance. And we all know that the microbiome can have a positive influence on FGID.

When it comes to the microbiome, we had last year at the 4th European Meeting a big session on human milk oligosaccharides (HMO). More and more companies – not only the classical infant formula producers – now produce HMOs. Big pharmaceutical companies already think about supplementation and treatment products. It is really a new way and it is a pleasure that we can discuss the future here a little bit more.

Brain development is also a very important part and still especially now with all these alternative diets. One of the more surprising discoveries related to the gut was that of the so-called “gut brain axis” – the close connection between the brain and the “gut brain.” We will be looking at another link between the gut and the brain – namely, the impact of early nutrition on brain outcomes.

Mike Poßner
Medical Director Europe, Nestlé Nutrition Institute
“I know of no symptom which can be more obscure in its causation than colicky abdominal pain in childhood.”

George Frederic Still, 1909
Early Nutrition Influence – Preventive and Therapeutic Aspects

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FGIDs are very frequent. Indeed, for many of us, it’s one of the most common diagnoses of the patients that we see is that they suffer from FGIDs. I will focus on regurgitation, infant colic and constipation because they are definitely the most frequent.

According to data from France, up to 80% of the clinical visits in early life are due to gastrointestinal discomfort. Most of the time, patients present a combination of symptoms. And again, in these data from France gathered from questionnaires completed by 2,700 parents of infants, we observed that more than half of them, or more than three-quarters (78%) of the infants, were diagnosed with combined FGID- 63% presented 2 symptoms and that as much as 15% presented 3 or more symptoms (Bellaiche M 2018;107:1276). This is something that we should consider if we make guidelines (Chart 1).

A couple of years ago a review of the literature tried to calculate how frequently FGID occurred and showed, according to 13 studies, that the median value that we found in the literature: 25% of all the infants presented troubles involving regurgitation.

### Regurgitation

We have no data at all that intervention decreases regurgitation at the age of 2–3 mo and will change the long term evolution. What we do know is that thickened formula is a very effective treatment for obvious regurgitation. The number of episodes of regurgitation decreased significantly. A study compared intact protein thickened with locust bean to partial hydrolysate thickened with low lactose. Statistically it was slightly better on this score, but whether or not this is clinically meaningful is a totally different question.

The only medication that we have to treat GI reflux disease (not regurgitation) is, of course, antacid-blocking medication.

### Infantile colic

Infantile colic is a challenge:
- it is very common with an incidence of 20–25%
- it has an impact on wellbeing and quality of life and it increases parental insecurity and anxiousness and also adversely affects parents’ social and emotional behavior
- it has a long term effect on functional gastrointestinal disorders because there is a higher prevalence of FGID later in life.

Infantile colic is still a mysterious disorder of the microbial-gut-brain axis. There are a lot of factors which will influence colic, and more and more interest is focusing on the intestinal barrier function and the microbiome:
- pathogenic bacteria such as enteropathogenic E. coli
- high-fat diet
- lipopolysaccharides (LPS)
- drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) and PPIs
- various food allergens and the gluten component gliadin

And infantile colic is associated with gut microbiota and immunity. It is associated with dysbiosis, with increased gut sensitivity and causes low-grade systemic inflammation.
Compared with control group, colicky infants have slower colonization, lower diversity and stability. They express less butyrate-producing species, more proteobacteria (including species producing gas and inflammation) and also less lactobacilli and bifidobacteria (including species with anti-inflammatory effects). Escherichia coli are more abundant in the feces of infants with colic than in the feces of healthy infants.

In all children, during the first months of life, immaturity of the intestinal mucosa implies incomplete gut integrity, thus allowing the passage of large molecules into the bloodstream. Breastfed and formula-fed infants with infantile colic have an increased transmission of the macromolecule human α-lactalbumin across the gut compared with healthy age-matched infants. Fecal calprotectin levels were approximately 2-fold higher in infants with colic than in control infants.

Every functional gastrointestinal disorder is at least two times more frequent in infants with colic. When the pediatricians or the family doctors see an infant with colic, often that child also presents other FGID function prevalence (at least 2 or 3 times higher).

Pain-relieving agents for infants with colic have been shown to be not effective. The literature shows 2 interventions which may be effective: probiotic intervention, and you can try to have an active intervention with heat-killed probiotics together with a substance which decreases the permeability of the intestine (Chart 2).

Like a lot of our colleagues, we like to prescribe proton pump inhibitors (PPI) for crying babies. But there is no effect of PPI on crying and irritability in infants. However, 25% of the infants develop small bacterial overgrowth as a side-effect.

Functional constipation

The estimated prevalence of functional GI symptoms in infants < 12 months is 8–10% (median value). A lot of treatment recommendations have been published. Partial hydrolysate, reduced lactose, prebiotics and palm-oil-free might be useful (Bongers ME. Nutr J. 2007 Apr 11; 6:8). High magnesium content has also been shown to be effective in infants with constipation (ChaoHC, Vandenplas Y. Nutrition 2007;23:469).

For probiotics for functional constipation, the literature is divided in “yes” and “no,” but most of the time it is considered to be not particularly effective. Palm-oil-free formula might be effective in the management of infant constipation. But again focusing on reassurance and diet management.

Lactose

A few words about lactose because lactose is important as lactose in infant formula stimulates a healthy microbiota and stimulates the growth of lactobacilli; and with lactose-containing formula, you’ll get better absorption of calcium than with lactose-free formula. That is also illustrated in this slide, where you have better calcium absorption according to a higher calcium ingestion. And lactose plays an important role in the absorption of calcium. Yet on the other side, if you have reduced lactose in your formula, you have a significant decrease in many functional gastrointestinal symptoms. So the right balance is very important (Chart 3).

**Conclusion**

Functional GI disorders
- **Time is the cure**
- Reassurance!
- **Dietary treatment**
  - Effective
  - Safe
- Postpone medical treatment
- Breastfeeding is an unequalled way of providing ideal food for the healthy growth and development of infants. (WHO, 2013)
FGIDs are defined by the Rome criteria, which were established by working groups of the Rome Foundation. These criteria are symptom based and based on literature and consensus meetings. The name “Rome” comes from the place where the first meetings took place.

The first Rome criteria were established between 1990-1994, but they were only for adults. In 1999 the first pediatric Rome criteria were published and these were basically a copy of the adult criteria. Then in 2006 much was changed: now the pediatric Rome criteria were based on pediatric research and a subdivision was made between neonates/toddlers and children/adolescents because they show different kinds of FGIDs. The most recent publication was in 2016, where still the same subdivision was made (Chart 1).

Reflux and Regurgitation

There are a lot of difficulties with the semantics of reflux and regurgitation because both clinicians and parents use the terms interchangeably.

- Reflux (GER): passage of gastric contents into the esophagus
- Regurgitation: reflux, which can be seen
- Reflux disease (GERD): reflux causing troublesome symptoms and/or complications

To define troublesome symptoms is a very difficult thing to do in young infants. The definition for infant regurgitation by Rome IV is that at least 2 events of regurgitation should occur per day for at least 3 wk in infants 3 wk – 12 mo of age and no retching, hematemesis, aspiration, apnea, FTT, feeding difficulties, abnormal posturing should occur and no other signs should be present.

The prevalence varies greatly from study to study and they say that between 8 – 26% of infants < 1 year of age have infant regurgitation and the peak is usually around 4 mo of age. Treatment consists of education reassurance, thickening of feedings and sometimes a positioning advice, although that is debatable.

GERD

There are rarer disorders like infant rumination syndrome, which is included in the Rome IV criteria. It is the habitual regurgitation of stomach contents into the mouth for the purpose of self-stimulation. In Rome IV it is defined as:

- Repetitive contractions of the abdominal muscles diaphragm, and tongue
- Effortless regurgitation of gastric contents, which are either expelled from the mouth or rechewed and reswallowed
- Three or more of the following:
  - Onset between 3 and 8 mo.
  - Does not respond to management for gastroesophageal reflux disease and regurgitation
  - Unaccompanied by signs of distress
  - Does not occur during sleep and when the infant is interacting with individuals in the environment (must include all for ≥ 2 mo.)

According to prevalence studies, this would occur in 1.7–7.2% in infants < 1 year of age and in 1.9–2.9% in 1- to 3-year-old children, based on questionnaire and interview studies. However, this could be an overestimation.

Rumination is considered to be caused by social deprivation and parental behavior that does not meet the child’s social needs, a child develops a behavior to self-stimulate. There is no diagnostic test required, but ask the parents to videotape. This is easy as now everybody has a mobile phone. Treatment consists of empathetic and responsive nurturing and parental training to deal with their child’s social needs in an appropriate matter.

Cyclic vomiting syndrome

For Cyclic vomiting syndrome Rome IV criteria stated:

1. Two or more periods of unremitting paroxysmal vomiting with or without retching, lasting hours to days within a 6-mo period
2. Episodes are stereotypical in each patient
3. Episodes are separated by weeks to months with return to baseline health between episodes of vomiting

Prevalence: 0.0–3.8% in infants < 1 year of age, 3.4–6.1% in children 1–3 years. The etiology is not completely understood, but it is associated with a maternal history of migraine headaches.

Infant colic

Infant colic consists of prolonged episodes of crying, often in the afternoon or evening, with peaks 4–6 wk of age, usually resolved by 3–4 mo of age. It can be very distressing for parents and is often a reason...
for consultation. But it has also been said to be part of normal physiological and behavioral behavior and that it's even part of normal physiology for children as well (Chart 2).

Rome III criteria were based on Wessel's "rule of threes" ($\geq 3$ hours/day, $\geq 3$ days/wk, $\geq 3$ wk). During the meeting for Rome IV, they asked: What is the evidence basis to use 3 hours as a limit? And is a child that cries for 2½ hours a less severe case? So they defined criteria for clinical purposes and for research purposes.

**For the clinical diagnostic criteria, Rome IV states:**

1. $< 5$ mo of age when symptoms start and stop.
2. Caregivers report recurrent and prolonged periods of infant crying, fussing or irritability that occur without obvious cause and cannot be prevented or resolved by caregivers.
3. No evidence of failure to thrive, fever or illness.

**Additional for clinical research purposes:**

4. In a telephone or face-to-face screening interview with a researcher or clinician, the caregiver reports that the infant has cried or fussed for $\geq 3$ hours per day during $\geq 3$ days in 7 days
5. Total 24-hour crying plus fussing is confirmed to be $\geq 3$ hours measured by $\geq 1$ prospectively kept 24-hour behavior diary

The prevalence for Infant colic is very high: 17–25% in infants $\leq 6$ wk of age. The treatment consists of addressing parental coping in reassuring patients.

**Functional diarrhea**

A different kind of problem is functional diarrhea, which is considered as "frequent unformed stools in otherwise healthy toddlers" ("toddler's diarrhea"). There are a lot of dietary factors to be considered, especially overfeeding, excessive fruit juice intake, other carbohydrates (fructose) ingestion with low fat intake, sorbitol.

**Rome IV criteria:**

1. Daily painless, recurrent passage of $\geq 4$ large, unformed stools
2. Symptoms last $\geq 4$ wk
3. Onset between 6 and 60 mo of age
4. No failure to thrive if caloric intake is adequate

The prevalence in infants is not very common; it is more commonly seen in 1- to 3-year-old children.

**Infant dyschezia**

This is a very different problem, which is considered to be caused by failure to coordinate intra-abdominal pressure with relaxation of the pelvic floor.

**Rome IV criteria:**

1. $\geq 10$ minutes of straining and crying before successful or unsuccessful passage of soft stools.
2. No other health problems.

(This is in infants $< 9$ mo of age instead of $< 6$ mo in the Rome III criteria)

Prevalence is 0–3.2% in infants $< 1$ year of age. Treatment consists of education, reassurance and avoidance of rectal stimulation. There is no need for laxatives.

**Functional constipation**

Infrequent, difficult to pass, hard stools are suggestive of functional constipation. In toilet-trained children, fecal impaction can result in fecal incontinence. Its prevalence is very high, both in infants and toddlers (4.7–16.1% in infants $< 1$ year of age / 9.4–26.8% in 1- to 3-year-old children).

**For Rome IV criteria at least 2 of the incidents must include 1 mo in children up to 4 years of age:**

- 2 or fewer defecations per week
- History of excessive stool retention
- History of painful or hard bowel movements
- History of large-diameter stools
- Presence of a large fecal mass in the rectum
- Neonates In toilet-trained children, the following additional criteria may be used:
  - At least 1 episode/wk of incontinence after the acquisition of toileting skills
  - History of large-diameter stools that may obstruct the toilet

Constipation in early infancy is often a reason to think of potential organic pathology (e.g. Hirschsprung, malformation). Later in life feeding changes are often related to the development of constipation and at a later age – when they’re getting toilet trained – withholding behavior is a very big issue and big peak incidence of functional constipation is at 2–3 years.

First line of treatment for all children of all ages is polyethylene glycol. In children who have constipation and haven’t defecated for a while, disimpaction should be considered.

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**Conclusion**

- FGIDs in infants/toddlers is diagnosed according to the Rome IV criteria
- Infant regurgitation and functional constipation are the most common FGIDs in infancy
- Functional constipation is the most common FGID in toddlers
- Not every FGID requires treatment (other than education and reassurance)
Before the widespread use of probiotics, we basically did not have any therapeutically effective treatment (Chart 1).

I will focus on Lactobacillus reuteri. This strain has shown to have an effect on the gut-brain-axis, on visceral hypersensitivity and on motility. There are a lot of studies of colic which show the effect of this strain in the different organs involved in the pathophysiology of colic.

A summary of in vitro and in-animal studies shows that these strains of L. reuteri modulate the immune response (Rhoads et al., 2012) and intestinal inflammation is one of the most important factors in the pathophysiology of colic. Another study shows that L. reuteri DSM 17938 or its conditioned medium reduces the nerve-firing capsaicin in jejunal segments of mice (Perez-Burgos et al., 2015).

**Clinical scientific evidence**

Looking at RCTs, we have more studies on L. reuteri than any other strain. A new meta-analysis confirmed that among all therapeutic approaches, L. reuteri is the best in colic (Sung et al., 2018). A paper collected and evaluated the data of the randomized control trials in order to create this kind of individual patient data meta-analysis. They examined patient by patient and put together all the data. The result was that crying or fussing was improved by these strains of probiotic. And they also divided breastfed infants and formula-fed infants. In this meta-analysis, the number of breastfed infants was the majority.

This is the first Individual Patients Data Meta-Analysis (IPDMA) and the most definitive method to assess the effectiveness of L. reuteri DSM17938 in managing infants with colic. L. reuteri DSM17938 is effective in breastfed infants and should be recommended in this subgroup of infants with colic.

Our group of research in Bari University try to go further in the field of prevention.

We retrospectively analyzed that a baby affected by colic during regurgitation und constipation in infancy has a high incidence of FGID and abdominal pain. We started a multicenter randomized double-blind clinical trial on a prophylactic use of this strain of probiotic to prevent and just to avoid those two or three weeks of inflammation. Around 500 children were included in the study, and we randomized to receive this strain of L. reuteri or placebo. After three months of treatment, there was a lower incidence of colic regurgitation and a higher number of evacuations. We also measured the economical impact on the pediatric visits, on the loss of parents’ work, emergency department access and the use of cimetropium bromide and other herbal products, and what we find saved 88 euros for the family and 104 euros for the community.

So the conclusion of this study on colic is: probiotics are needed for the treatment of infant colic, with parental reassurance. These two are the only therapeutic procedure that work. But more studies are needed for more evidence regarding the preventive use of probiotic. The treatment for colic should be continued until intestinal maturation is complete around 4 to 5 months of age.
Conclusion

With parental reassurance, the use of probiotics is the only therapeutic procedure that works. Lactobacillus reuteri DSM19738 is the most studied. And the final word seems to have been written about its efficacy on breastfed babies. For the prevention of infantile colic, more studies are needed. The treatment needs to be continued until intestinal maturation is complete.

Regurgitation happens frequently in infants and is a common cause of concern for parents. 43% of mothers regard regurgitation as a health problem. Decreasing the regurgitation is often seen as the most welcomed intervention that physicians can provide.

In treatment, there is much confusion between physicians and parents who ask for PPI.

- 88% of infants with regurgitation symptoms were classified as GERD
- The use of PPIs in infants has been debated:
  - 39% were prescribed for unexplained crying
  - 36% were prescribed for uncomplicated regurgitation
- Only 1.8% of PPI use is completely adherent to GERD practice guidelines
- Use of PPI dramatically alters the intestinal microbiota
- Evidence is sparse and of low quality

It is important to distinguish regurgitation from GERD.

If a baby who weighs five kilograms eats 180 ml, it is comparable to an adult who consumes 3 litres of liquid in 10 min. So, of course, we can have the possibility of regurgitation.

Possible therapeutic options are:
- No seated position for the infant
- Do not overfeed
- Thickened feedings
- No smoking by the caregiver

What about L. reuteri? In 2008, there was the first study in my group; our aim was to show there was a different maturation in preterm babies and if we can improve maturation. So we evaluated the motility of premature babies in the gastric emptying rate and we matched not only with a placebo but also with breastfed babies. L. reuteri Protectis improved gastric motility in preterm infants (Indrio 2008). Oral supplementation of Protectis for 30 days improved the gastric emptying rate in formula-fed preterms and brought it very much closer to the rate among the breastfed babies.

After this, we evaluated the supplementation of this strain L.reuteri DSM 17598 in formula-fed infants affected by regurgitation. We diagnosed it with functional regurgitation and in this study we compared the strain to the placebo and we found an improved gastric emptying rate and we found a fasting antral area. That is an area of the stomach where the babies are fasting. If the diameter is big, it can act as a trigger to lower regurgitation.

And there was a new study in 2017 where we used a partially hydrolysed whey formula, supplemented with starch and L. Reuteri DSM 17598 and the other with placebo. We analyzed 72 randomized infants (37 test/35 placebo formula). All babies were diagnosed with functional regurgitation on the basis of Rome III and Rome IV criteria. We found lower fasting antral area. And the daily number of regurgitations decreased during the second week of using that formula (Chart 2).

Conclusion

- Probiotics could represent another therapeutic option together with parental reassurance and thickened formula (using both procedures together also be an option).
- For the prevention of functional regurgitation with probiotic, more studies are needed (only 1 study so far).
- Lactobacillus reuteri DSM 17938 is the only strain studied so far.
- The treatment needs to be continued until intestinal maturation is complete or until complementary feeding is added.
- L. Reuterii DSM 17938 is the more thoroughly studied strain and may be considered for the management of infantile colic
- L. Reuterii DSM 17938 could be considered as a therapeutic option for functional regurgitation.

2: Effects of a Partially Hydrolysed Whey Infant Formula
Supplemented with Starch and Lactobacillus reuteri DSM 17936 on Regurgitation and Gastric Motility

Assessed for eligibility: n=97

Excluded: associated symptoms (dysphagia, arching): n=10
  - beastfed: n=3
  - allergic colitis: n=2

Declined participation: n=7

Randomized: n=80

Lost to follow-up: n=3

Test formula: n=40

Control formula: n=40

Included in the analysis: n=77

Indrio et al. Nutrients 2017
FGID and the role of probiotics

For the functional abdominal pain disorders or pain-related FGID, we have four categories in the Rome IV criteria: functional dyspepsia, irritable bowel syndrome (IBS), abdominal migraine and functional abdominal pain, now characterized as “not otherwise specified.”

Irritable bowel syndrome (IBS) and functional abdominal pain (FAP) are the most frequent functional abdominal pain disorders and furthermore we have much more evidence of the role of probiotics with those two FGID. The etiology seems to be similar in infants and older kids. We know that those patients do have genetic predisposition, they have higher incidence of early life events or psychological factors. We know that the FAP or IBS can start unusually often after an infection and it has been shown that those patients who have a mild inflammation have altered gut microbiome. Importantly, it has been shown that patients with FAP have altered visceral sensitivity or hypersensitivity which is a part of the brain-gut axis.

**Diagnosis and treatment**

The problem that every physician is facing is how to diagnose the FGID the lack of proper biomarker led to the development of different criteria, from which widely accepted are Rome criteria. Newest version of the Rome criteria, are Rome IV criteria which emphasize the importance of the positive rather than the negative diagnosis. We should aim that the parents and the child will leave the physician’s office knowing that they have some sort of FGID. We have shown in our study that patients who were diagnosed with FAP on the first visit had a tendency to decrease their symptoms on the second visit compared to children who were not aware of the nature of their disease at the beginning. Sometimes, physicians tend to underestimate importance of symptoms in children with functional problems, however, it has been shown that the emotional burden associated with chronic abdominal pain is enormous, regardless whether the pain is functional or caused by organic disease. Furthermore, quality of life is significantly decreased.

Therefore, we are still trying to find proper treatment strategy. As pharmacological treatments, we use different antispasmodics, antidepressants, antihistaminic and laxatives. However, in general we lack high-quality, placebo-controlled, randomized studies in children which will indicate which of the pharmacological treatments are effective.

For non-pharmacological treatment, we have diet modifications. We know that lactose-free diet is not efficacious in the FAP. For FODMAP diet for IBS, we have one randomized control trial (RCT) which included 33 children and they showed improvement of symptoms in adolescents with IBS. There is more evidence for hypnotherapy and cognitive behavioral therapy, even the meta-analysis shows that they are effective, however, they are not widely available.

**Probiotics**

There are 10 RCT which investigated the effectiveness of probiotics in pain-related GI disorders, where 745 children were included. They evaluated altogether 4 strains or combinations of strains. Three trials investigated LGG, 5 Lactobacillus reuteri DSM 17938 and 2 multi-strain probiotic products. LGG studies mainly focused on IBS, while the L reuteri DSM 17938 focused more on FPA “not otherwise specified” (Chart 1).

With LGG, the design differs between the trials. What is the meta-analysis saying about the effectiveness of LGG in the treatment of a FGID? In general, the responders’ rate was significantly better in the arm when the LGG was used and that was most pronounced in the IBS, at the FAP there was no difference. Frequency of the pain significantly decreased in the LGG group compared to placebo. That was also more pronounced in IBS compared to FGID, where there was no difference. Severity of the pain decreased also most pronouncedly in the IBS, while in the FGID there was no difference.

**1: LGG Responders rate**

<table>
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<th>Strain</th>
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<th>Control</th>
<th>Weight</th>
<th>Rate ratio</th>
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<td>32</td>
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<td>2.4</td>
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<td>Francavilla 2010</td>
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<tr>
<td>Ackerman 2005</td>
<td>12</td>
<td>14</td>
<td>1.8</td>
<td>0.01</td>
<td>0.00 (0.00, 0.05)</td>
</tr>
<tr>
<td>Lognormal/IBS (12)</td>
<td>85</td>
<td>82</td>
<td>100.0%</td>
<td>1.00 (0.99, 1.01)</td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: p<0.00, df=12, (P=0.23), I²=33%

Test for overall effect: Z=3.56, P=0.0004

**2: Pain intensity (Jadresin)**

Horvath et al. APT 2011

One thing that I want to stress is the importance of placebo as well. It was shown in our study, and it was also found by others, that there is significant decrease in the severity of symptoms in the place-
3: Pain intensity (Weizman)

<table>
<thead>
<tr>
<th>Week</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Week 4</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Week 8</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
</tr>
</tbody>
</table>

4: Pain intensity (Romano)

<table>
<thead>
<tr>
<th>Week</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Week 4</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Week 8</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
</tr>
</tbody>
</table>

5: Probiotics modus operandi

<table>
<thead>
<tr>
<th>Study</th>
<th>Baseline</th>
<th>Week 4</th>
<th>Week 8</th>
<th>Week 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ma et al. Infect Immun 2004</td>
<td>1.0</td>
<td>0.8</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>Lin et al. Inflamm Bowel Dis. 2008</td>
<td>0.8</td>
<td>0.6</td>
<td>0.4</td>
<td>0.2</td>
</tr>
</tbody>
</table>

bo arms. Almost 40–50% improved only by taking the placebo.

Probiotics can improve or change the microbiome at least during the time that we are giving them. We know that some probiotics strains have the ability to decrease the amount in vitro studies of pro inflammatory cytokine and L. reuteri was able to decrease in vitro but also in vivo the sensory of pain which was triggered by capsaicin- and distension-evoked firing of spinal nerve action potentials (Chart 5).

Can we introduce the probiotics earlier, especially taking into account the new RCT, but also taking into account effectiveness of placebo? So in some proportion of patients we can consider the probiotics as a first step together with reassurance and education.

**Constipation and probiotics**

Constipation has a high prevalence of around 9% worldwide and more than 90% have no underlying organic cause. The etiology is multifactorial according to age: withholding behavior is very important especially in toddlers, psychological factors and social conditions but also alterations in intestinal microbiota. Based on the ESPGHAN and the NASPghan guidelines for the treatment of functional constipation, it has been proposed that polyethylene glycol (PEG) should be the first-line treatment and these guidelines do not recommend the usage of the probiotics for the treatment of childhood constipation.

As shown in the chart, ¼ of children who are having constipation during childhood will still have the problem in adolescence and also during adulthood.

Probiotics seem to have some promising effect, at least in the in vitro studies, where it has been shown that they reduce the transit time in the colon.

That was furthermore pronounced in adult studies, where the meta-analysis shows that probiotics in general decrease the gut transit time by 12 hours. They also increase the stool frequency by a 1.3 bowel movements/wk. There is also a strain- and species-specific effect, where all these meta-analyses advocate for specific probiotic strains.

**Probiotics and constipation in children**

However, in children, we have 9 RCT, with 677 included patients. But most of those studies use different probiotics and different strains; they had different protocols, different designs and they used the probiotics at different ages. The meta-analysis from last year shows that there is no effect on the usage of probiotics as an add-on treatment to the laxative in children with functional constipation.

This meta-analysis was published before these last 2 RCT, which were quite big in toddlers and older children. One is the Polish study, where they used the L. reuteri DSM 17938 in children who were > 3 years of age together with PEG and they found no effect. Similar as in our study, where we used the L. reuteri together with lactulose and we found no extra effect of adding probiotics to the lactulose. These two studies are quite different to the Italian study published in 2010 which was performed in infants and no laxative treatment was given. The difference could be at least partially explained by the fact that last two studies included toddlers, so the pathomechanism of the constipation is quite different compared to infants. There is also the difference in the laxative because these two studies use laxatives and we know that PEG and lactulose can influence the intestinal microbiome.

**Conclusion**

- Probiotics are not the same
- Only strains proven for specific clinical condition/indication should be recommended
- Advising the usage of probiotics, we should consider:
  - FAP/IBS – give positive diagnosis
  - To consider:
    - LGG for IBS
    - L. reuteri DSM 17938 for FAP
  - For constipation:
    - Current evidence limited
Functional gastrointestinal disorders (FGID) are very common during infancy, leading to frequent medical consultations. Parents are often worried about the health of their child and they want appropriate medical management. For the most of the times, it is not so easy and the family’s quality of life could be impaired.

The aim of this large observational prospective study was to assess the quality of life (QoL) and clinical management of infants with FGID. There are some studies on QoL in children. In the study by Youssef N. et al. (2006), the quality of life score was much lower in children with functional abdominal pain compared to healthy children and even compared to children with IBD or GERD. However, we have few data on quality of life in infants. That is why we wanted to conduct this study and we had different aims:

- To evaluate the quality of life of infants with FGID,
- Outcome of the FGID symptomatology and QoL
- Medical management

This is a prospective observational and longitudinal study conducted in France between May 2014 and April 2015*. Children where followed during one month to medical consultation, and QoL was assessed at inclusion and at day fifteen using the QUALIN questionnaire. Infants < 5 mo where included: they should be at least partially fed with an infant formula and they should consult for the first time for FGID. Infants with allergy or exclusively breastfed infants were excluded.

The investigators were pediatricians in private practice and they were located in different parts of France. So we used a 34-item QUALIN questionnaire, which is available in French and in many other languages such as English, Spanish, Italian and even Russian. It has been specially developed to evaluate the quality of life of infants. It includes 4 subcategories: behavior and communication, the ability to remain alone, the family environment, and the psychological and somatic wellbeing of the child.

Primary endpoint was a comparison of the Qualin score at day 0 and day 15; and we basically used the paired-student test. We also compared the evolution of the symptomatology between inclusion and day 30.

Overall, 1122 infants were recruited; 307 were excluded from the analysis for different reasons: missing questionnaires; infants who didn’t meet the inclusion criteria; time between the visits not respected. Finally, we had data for 815 infants with functional GI disorder. These data were analyzed.

At inclusion, we had 52 percent male infants (426/815) with a mean age at diagnosis of 2.1±1.2 mo. 30% were partially breastfed, whereas 4% had an early weaning. The main symptoms were regurgitation in ¾ of the included infants, followed by constipation in 31%. Some had diarrhea (10%) and constant crying (3%). The question was about the duration of the crying (>3 hours per day) which could explain this low percent of constant crying. About 20% had a mix of different symptoms: regurgitation plus diarrhea or regurgitation plus constipation. The principal outcome was the evaluation of the Qualin score between day 15 and day 0; the score increased from 27 to 38 and it was significant (Chart 1).

**1: Functional Gastrointestinal Disorders**

<table>
<thead>
<tr>
<th>Condition</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td>3%</td>
<td>3%</td>
<td>2%</td>
<td>2%</td>
<td>16%</td>
<td>16%</td>
<td>54%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constipation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regurgitation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>13%</td>
<td>13%</td>
<td>12%</td>
<td>12%</td>
<td>44%</td>
<td>44%</td>
<td>88%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**2. Course of FGID**

<table>
<thead>
<tr>
<th>Condition</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constipation</td>
<td>7%</td>
<td>7%</td>
<td>7%</td>
<td>7%</td>
<td>20%</td>
<td>20%</td>
<td>30%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regurgitation</td>
<td>3%</td>
<td>3%</td>
<td>3%</td>
<td>3%</td>
<td>10%</td>
<td>10%</td>
<td>20%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Number of stools/w in case of constipation (N=250)**

**Number of bottles feeds followed by regurgitation**

Jung C et al. 2017
QoL was better after medical management. And this was also the case for all the subcategories of the score, notably for the subcategories “behavior and communication” and “psychological and somatic wellbeing.”

Concerning the course of FGID, the symptomatology improved in most of the infants. This was the case for constipation, with an increase of the numbers of stool/weight and also for regurgitation with a diminution of the frequency, intensity and volume of regurgitation for most of the infants. Symptomatology of diarrhea improved in 55% of the infants, associated with the diminution of the number of stools/wk; and the number of infants with constant crying dramatically diminished to 12% at day 30 (Charts 2/3).

The proposed medical management was, first, a change of infant formula for an infant formula targeting the FGID symptoms. Some pediatricians gave lifestyle and dietary advice, but only in 34% of the cases. Some prescribed medication to about ¼ of the cohort, some prescribed probiotics or plant extracts as first prescriptions.

The infant formulas prescribed were anti-reflux formula (77% in case of regurgitation), acidified formula with low lactose level and probiotics for constipation (79% in case of constipation), low lactose level and probiotics for diarrhea (30%) / infant colic (56%). In most cases, the tolerance was considered very good (95% of cases).

The rate of medication was surprising high notably in case of regurgitation. About half of these infants received alginate agent or simeticone, 27% received anti-spasmodic agent (trimebutine) and 15% of infants with regurgitation received PPI, which was surprising for us because the investigators were pediatricians (Chart 4).

Concerning the dietary and lifestyle advice, they were given in 34% of the cases and among them some pediatricians advise the anti-Trelenbourg sleeping position (44%) – that was surprising because it is not recommended – abdominal massages (16%), changing of water or early weaning (11%).

We performed a multivariate analysis to identify factors that were independently associated with an increase of the QoL score and it appeared that at a younger age (< 3 mo), partial breast feeding and dietary advice were associated with an increase of quality in the Qualin score.

Conclusions

- QoL rapidly increased after medical management, whatever the FGID at inclusion was.
- There was no control group to compare the quality of life at inclusion.
- This underscores the importance of dietary advice and reassurance for the parents. And the recommendation to continue with partial breastfeeding was also confirmed in this study.
- High rate of medication prescriptions … even if they are not recommended.

Because QoL is the principal target of clinical care, we think this could be a useful tool to help parents and also practitioners manage these disorders and it could be used regularly in consultation, first visits and then the next appointment to follow the evaluation.
Age-appropriate microbiome maturation and the role of HMO

Specific features of infant microbiota

Infant microbiota dynamically develops during the first weeks and months of life. Bacterial diversity generally increases with age, accompanied by changes in microbiota composition. Microbiota consists of numerous taxa; this complexity can be described as “microbiota types” where samples with similar microbiota composition are clustered using complex algorithms. The concept of microbiota types was first developed for adult gut microbiota as “enterotypes”. At the very beginning of life, a large proportion of infants show microbiota type which consist mainly of Enterobacteriaceae. Within few weeks a microbiota types rich in bifidobacteria become dominant. Starting at approximately six months, and triggered by the introduction of complementary foods, a progression towards adult-like much more diverse microbiota types is observed.

What is normal infant microbiota?

A recent meta-analysis including 34 studies delineated healthy development of infant gut microbiota and identified the impact of common disruptors of its development. In Cesarean-section delivered infants the colonization with bifidobacteria shows the clearest contribution of maternal strains to infant microbiota. In particular, Bacteroides and bifidobacteria were the taxa with the highest similarity with the maternal microbiota of Cesarean-section delivered infants, and Cesarean section implies antibiotic usage, at least in the mother, so it is inherently difficult to separate the effects of transfer disruption by the delivery procedure from disruption induced by antibiotics.

Microbiota and later health

Is the disruption of age-specific microbiota composition linked to later immune and metabolic health disorders? The evidence continues to accumulate and most compelling examples are related to broadly understood immune disorders. For example, microbiota diversity and the abundance of butyrate producers is associated with decreased eczema severity. For example, in a cohort of Singaporean infants. An independent study including infants from Finland and the Netherlands reported that the abundance of Streptococcus species at three months positively correlated with the adiposity increase between birth and 18 months in a cohort of Singaporean infants. An independent study including infants from Finland and the Netherlands reported that the abundance of two Streptococcus species at three months positively correlated with higher than expected BMI at the age of 5 to 6 years (Chart 1).

1: Age-specific microbiota composition is linked to later immune and metabolic health disorders

Low abundance of butyrate producers and low microbiota diversity at 6-18 m associated with eczema severity

High Streptococcus abundance associated with higher adiposity and BMI

The speaker: Olga Sakwinska, Switzerland
What are the drivers of microbiota development?

Human milk is the optimal nutrition for infants. What follows, the best way to learn how to improve infant nutrition is from human milk. In particular, human milk oligosaccharides (HMO) are the third largest component of human milk by dry weight. Human milk is unique in terms of HMO quantity (typically >10g/L) and diversity (over 200 structures), which is suggestive of their importance for infant nutrition. Despite high diversity, some compounds are predominant, for example 2'-fucosyllactose (2'FL) constitutes a large proportion of HMO in human milk. Surprisingly, HMO cannot be directly used by human body, rising questions about their biological functions. On the other hand, HMO are accessible to bacteria. How HMO shape microbiota composition and function is the topic of active research and it is one of the leading hypotheses regarding the influence of HMO on infant health outcomes.

The quantities of HMO in human milk vary among individuals and depend largely on the milk group and the time of lactation. Milk groups are genetically determined. The best understood is the variability in fucosyltransferase 2 gene (FUT2). Approximately 20% of the individuals do not have the functional variant of FUT2 leading to the lack of fucosylated compounds, such as 2'FL, in milk (so called “Non-Secretors”) (Chart 2). Time of lactation is also an important modifier of HMO composition. For example, the amount of 2'FL is high at birth and decreases with the time of lactation, suggesting that such changes could be the driver of the development of infant microbiota.

The fact that 2'FL and other fucosylated compounds are entirely absent from milk of FUT2 negative women allows to link HMO composition in milk with the health outcomes of the infant. In a prospective cohort study conducted in Bangladesh, Dhaka, where most infants were exclusively breastfed until six months, we have observed that infants of FUT negative (“Non-Secretor”) mothers had increased risk of respiratory infections in the first six months of life. This suggested that 2'FL and fucosylated compounds had protective effect from respiratory infections.

Further, a randomized double-blinded clinical trial compared feeding formula with two HMO, 2’FL and LNnT, with standard formula. Reference group of breastfed infants was also included. With the safety as the primary outcome, it was observed that formula with the two HMO was well-tolerated and supported appropriate growth. In addition, as secondary outcome, a decreased risk of respiratory infections, in particular more severe infections and reduced antibiotics and antipyretics use was reported in the group receiving formula with HMO (Chart 3). HMO are hypothesized to have a role in shaping infant gut microbiota. Accordingly, the analysis of gut microbiota at three months of age revealed that HMO increased the proportion of specific microbiota type present in breastfed infants and absent in the group fed standard formula. Interestingly, this shift was also associated with decreased antibiotic usage during the first year of life, as the infants with breastfed-specific microbiota type required less frequent antibiotics treatment (50% reduction).

### Conclusion

- Gut microbiome develops dynamically in the first months and years of life.
- Transfer of bifidobacteria and Bacteroides from maternal gut is essential for the seeding of normal infant gut microbiota and it is severely disrupted by cesarean section.
- Age-appropriate microbiota maturation is linked to optimal immune development.
- Clinical intervention trial and observational data suggest that 2’Fucosyll-HMO provides protection against respiratory infections and decreases the need for antibiotic use.
- The protection appears to be mediated through support of age-appropriate microbiome development by HMO.

---

**Table 2: Learning from Human Milk**

<table>
<thead>
<tr>
<th>Oligosaccharides</th>
<th>Human Breast Milk</th>
<th>Human Milk Oligosaccharides HMO</th>
<th>Cow Milk</th>
<th>Infant Formula Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>α1-2, β1-3, β1-4</td>
<td>Protein, lactose, galactose</td>
<td>Protein</td>
<td>Protein, lactose</td>
</tr>
<tr>
<td>Fat</td>
<td>α1-2, β1-3, β1-4</td>
<td>Fucose, sialic acid</td>
<td>Fat</td>
<td>Fucose, sialic acid</td>
</tr>
<tr>
<td>Glucose</td>
<td>β1-2, β1-3, β1-4</td>
<td>Glucose, sialic acid</td>
<td>Glucose</td>
<td>Glucose, sialic acid</td>
</tr>
<tr>
<td>Galactose</td>
<td>β1-2, β1-3, β1-4</td>
<td>Glucose, sialic acid</td>
<td>Galactose</td>
<td>Glucose, sialic acid</td>
</tr>
<tr>
<td>N-acetyl-glucosamine</td>
<td>β1-2, β1-3, β1-4</td>
<td>Glucose, sialic acid</td>
<td>N-acetyl-glucosamine</td>
<td>Glucose, sialic acid</td>
</tr>
</tbody>
</table>

**Table 3: Infants fed HMO formula had less respiratory infections and related antibiotic use in the first year of life**

<table>
<thead>
<tr>
<th>Reported event</th>
<th>Control (n = 87)</th>
<th>Test (n = 88)</th>
<th>P-value (95% CI)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bronchiolitis</td>
<td>15 (17.3)</td>
<td>6 (6.8)</td>
<td>0.26 (0.08, 0.78)</td>
<td>0.64 (0.19, 2.06)</td>
</tr>
<tr>
<td>0-12 months</td>
<td>16 (18.6)</td>
<td>10 (11.3)</td>
<td>0.30 (0.11, 0.81)</td>
<td>0.66 (0.21, 2.11)</td>
</tr>
<tr>
<td>LRT (Ad. cluster)</td>
<td>12 (13.9)</td>
<td>7 (8.0)</td>
<td>0.25 (0.10, 0.62)</td>
<td>0.41 (0.15, 1.02)</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>0-6 months 34 (39.1)</td>
<td>25 (28.4)</td>
<td>0.54 (0.21, 1.35)</td>
<td>0.32 (0.13, 0.79)</td>
</tr>
<tr>
<td>0-12 months</td>
<td>17 (19.3)</td>
<td>13 (14.8)</td>
<td>0.52 (0.22, 1.21)</td>
<td>0.52 (0.22, 1.21)</td>
</tr>
<tr>
<td>0-6 months</td>
<td>42 (48)</td>
<td>30 (34.1)</td>
<td>0.30 (0.13, 0.69)</td>
<td>0.41 (0.24, 0.69)</td>
</tr>
<tr>
<td>0-12 months</td>
<td>59 (68.4)</td>
<td>31 (35.3)</td>
<td>0.26 (0.10, 0.68)</td>
<td>0.42 (0.24, 0.74)</td>
</tr>
</tbody>
</table>

**Note:** Designs and number with event + (%) OR (95% Cl)
Necrotising enterocolitis (NEC) is an inflammation and ischemia of intestinal mucosa. 10% of neonates < 1500 gram develop NEC and the mortality is still very high: 15–30%. There are many complications, e.g. short bowel syndrome, stenosis and developmental delay. The cause is considered multifactorial: immature gastrointestinal tract is a risk factor, as is enteral feeding, especially formula feeding and colonization with pathogens. Intestinal hypoxia-ischemia-reperfusion is a risk factor too. The staging system of NEC according to Walsh and Kliegman extends from stage I, “suspected”, to stage IIIb, the most severe phenotype. Usually the diagnosis of this disease is established in an advanced stage. So there is a great need for novel diagnostic preclinical biomarkers. Combining different biomarkers gives you high accuracy. NEC biomarkers can be found in different fluids, e.g. in urine, blood and even stool, and it is a crosstalk between these biomarkers. E.g. NEC is associated with a change of microbiota.

**Microbiota in NEC**

Gut microbiota is a key factor in NEC pathogenesis:

- Without bacteria, no NEC
- Antibiotics are still the cornerstone in therapy
- Probiotics and breastmilk are considered protective
- NEC outbreaks usually occur in clusters
- (Early) microbial differences are described between NEC and controls.

This makes microbiota an interesting potential biomarker.

**Studies on microbiota and NEC**

The largest study of microbiota on NEC thus far (Warner et al, Lancet 2016) was done in 3 US hospitals and included 46 children with NEC > 1lb. It showed that increased Proteobacteria were involved or associated with onset of NEC a few days before NEC developed. However, this microbiome risk profile was only present in children GA < 27 wk and, more importantly, in children who developed NEC after 30 days postnatal. This is a very small selective group; the majority develops NEC 2 or 3 wk after birth.

Another study (Heida et al. CID 2016) describes the differences in the microbiome composition already present in the meconium. They followed these children and also saw some differences in the microbiome composition several days before NEC developed. The microbiome signatures in the meconium and in the stool samples before NEC were different. No uniform microbial NEC signature has been detected thus far, limiting its potential for use as a diagnostic biomarker.

**The Dutch experience**

In the search for novel biomarkers for NEC and sepsis, an ongoing study performed 9 NICUs in the Netherlands and Belgium since 2014. We collect fecal samples from children GA < 30 wk daily up to day 28. Thus far, we have included 1,500 infants and > 20,000 samples. We look at the microbiota before NEC or sepsis develops and we also look at the fecal VOC profile. Volatile Organic Compounds (VOC) are molecules responsible for the smell. VOCs are responsible for the odor of stools and > 90% are produced by the intestinal microbiota.

VOC can be detected by various techniques. The most important are chemical-analytical-based techniques such as gas chromatography mass spectrometer, which gives you plenty of information, but is not useful in clinical practice. On the other hand, we have pattern-based techniques such as electronic-nose devices. These are sensors which work similarly to the human nose. We use an electronic-nose device consisting of multiple sensors and based on pattern recognition. It can be used at bedside, so you get direct information within seconds and it is quite cheap.

We collect feces samples from NEC, from sepsis patients, and also from controls. In a first pilot study, we compared profiles of 13 NEC patients with 26 controls and we defined different time intervals. When the child diagnosed NEC at day 0, we looked several days back at the VOC and compared it with the sample of children who did not develop NEC. 4–5 days before the diagnosis was made, there was no difference in the profiles. But 2–3 days prior to the onset of NECESSARY, we found increased NEC profiles compared to controls (Chart 1).

The focus of the next study was to compare fecal VOCs from infants who developed late-onset sepsis (LOS). We collected samples from 127 LOS children from these 9 NICUs and we matched them with fecal VOCs from controls. The majority of these infants have CoNS, Staphylococcus aureus was also important.

First of all, we put all these data together and we look to see whether we could discriminate fecal VOCs profiles of children with late-onset sepsis from controls. Clinically, this was not very relevant. We decided to focus on different specific pathogens, so we compared VOCs from gram-negative bacteria and gram-positive bacteria and compared these to the controls. We found that they increased in the days before sepsis.

3 days before Staph aureus sepsis develops, you can see that the VOC patterns of these children are already different from controls and this also accounts for E. coli and Staph. epidermis.

**1: Fecal VOC profiles before NEC**

<table>
<thead>
<tr>
<th>Time interval</th>
<th>μAUC ± 95%CI</th>
<th>p-value</th>
<th>Sens</th>
<th>Spec</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-1</td>
<td>0.94±0.04</td>
<td>&lt;0.001</td>
<td>88.9</td>
<td>88.9</td>
</tr>
<tr>
<td>T-2/-3</td>
<td>0.77±0.21</td>
<td>0.024</td>
<td>83.3</td>
<td>75.0</td>
</tr>
<tr>
<td>T-4/-5</td>
<td>0.65±0.25</td>
<td>0.257</td>
<td>60.0</td>
<td>60.0</td>
</tr>
</tbody>
</table>

De Meij et al, J Pediatr 2015
A study by Bos et al. (Plos Pathog. 2013) looked into different VOCs deriving from different pathogens and found that each different pathogen produces only unique VOCs. If you combine all these profiles, then you get rubbish. So that’s why you have to focus on different pathogens, also in the clinical practice.

In the same group, we looked at the microbiota in the NEC and collected fecal samples from 53 NEC cases and compared them to controls. We performed microbiota analysis up to 3 days prior to the onset of the clinical diagnosis of NEC by means of IS-pro. This technique combines bacterial species differentiation by the length of the 16S-23S rDNA interspace region with instant taxonomic classification by phylum-specific fluorescent labeling of PCR primers (Budding et al FASEB 2010). One of the advantages of IS-pro is that you can get a profile within 5 hours.

**Comparing the NEC profiles in 1, 2, 3 days before NEC to controls was a little bit disappointing because we didn’t see any significant differences at the phylum levels. Then we looked at the diversity of the samples. We found that diversity is also differentiated: first, it is associated with NEC. In the diversity too, we did not see any significant alterations between NEC and controls at 3-1 day prior to NEC at phylum level. Then we looked at the species level, at the cumulative profile of the controls and at NEC: we found no major differences. However, looking into details, you see that the NEC case has more C. perfringens and more S. pyogenes, while controls have a greater abundance of Suterella spp. We took a closer look at the most NEC III phenotype. This outcome is interesting because in this specific group with the most NEC III phenotype, we were able to find differences between NEC and controls which are not present if you look at the overall group. We have a decreased absolute abundance of firmicutes and the difference in the first years as well (Chart 2).

Interestingly, many children among the controls had bacteroides. So it might also play a protective role against the development of NEC. If you look at survivors of NEC for five years, we were able to find microbial differences between NEC and controls.

**How could this information be used in the future NICU?**

If used to complete NICU population, it might be possible to use one single test with a very high sensitivity and relatively low specificity to select those children who have a higher risk of developing NEC. And if you know which group might develop NEC, you can add another biomarker with a fairly high specificity, e.g. with microbiota: this is more expensive and more complex, but you already need a selection of children. It might be useful for progressively more definite classification. This is just a little bit of dreaming about the future of the NICU, but this is how it might be.
The immune system is a system of cells and tissues that protects us against invading pathogens. It must learn how to provide tolerance to “non-threats” such as food components, commensal microbiota and to the organism itself. So it must actually learn how to distinguish “harmless” from “dangerous” – and this is an active process.

**Immune System Development**

Immune cells and organs proliferate rapidly in the first trimester and the development of secondary lymphoid organs is nearly complete at birth. However, quite recent work indicates that these organs, particularly the GALT, remain highly responsive to environmental stimuli (antigens and allergens) throughout life.

Declining biodiversity and aberrant intestinal colonization is a main theme in the “allergy epidemic.” Complex microbial communities in the gastrointestinal tract have a symbiotic relationship with the host, and microbial exposures in the perinatal period seem to be critical for the ontogeny of both innate and adaptive immunity. Colonization of the mucosal surfaces appears to be vital for the establishment of tolerance. Delay or disruption in the colonization of the gastrointestinal tract may result in dysregulated immune responses. This has been clearly shown in animal models and there is also indirect evidence in humans. For example, delivery by cesarean section is associated with disturbed intestinal colonization patterns and increased risk of both allergic and autoimmune disease. But we do not yet know exactly how the mechanism of tolerance establishes itself in humans.

Gut microbiota have also been demonstrated to enhance the integrity and functions of the gut barrier. Gut microbiota will increase secretory IgA production locally and also induce the goblet cells to produce mucin, thereby enhancing the gut barrier. The gut microbiota can also be involved in crosstalk with distant organs as bacterial ligands, bacterial metabolites and immune cells may enter the systemic circulation and seed distant organs e.g. the lung.

Toll-like receptors (TLR) are believed to be ancient gatekeepers in innate immunity; they are expressed on a number of epithelial and endothelial cells, leukocyte subsets in blood. TLR activation can either increase or decrease the suppressor activity of Tregulatory cells, thus providing an important link between innate and adaptive immunity.

In a study within a high-risk cohort in Perth, where blood was first sampled at birth and afterwards during the first five years of life, infants that later developed allergic disease showed increased inflammatory responses following TLR activation in early life compared with healthy children. However, these responses failed to develop into mature Th1 type responses in allergic children (Tulic MK et al., JACI 2011).

The emerging in utero colonization hypothesis posits that the placenta harbors its own microbiota and that the colonization of the gut begins already in utero. More research is needed to confirm the latter hypothesis.

Building on the results from the high-risk cohort in Perth, where babies who went on to develop allergic disease had exaggerated innate immune responses (Tulic MK et al., JACI 2011), we hypothesized that this immune dysfunction might be dependent on microbial stimuli very early in life. So in another high-risk cohort, in which atopic mothers were included, we sampled feces from the mothers in the 3rd trimester and then afterwards from their infants at 1 week, 1 mo and 12 mo of age. Blood was drawn at 6 mo of age and PBMC were activated with microbial ligands. We studied microbial development in babies that had eczema, plus evidence of sensitization or had food allergy. We paired the microbial development to healthy controls, which despite their heredity, were non-sensitized and had no eczema (Chart 1).

There were both maternal effects and also postnatal effects. In pregnancy, there was reduced diversity

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**Chart 1: Programming of the microbiota and immune system**

- **Atopic mothers**
  - 3rd trimester
  - 1 week
  - 1 month
  - 12 months
  - 2.5 years

- **SPT+ milk, egg and/or peanut**
  - Eczema

- **Not IgE-sensitized**
  - No eczema

of the main phylum Bacteroidetes in mothers whose infants developed IgE-associated eczema. Although this was not seen in their infants, it could be consistent with results from a Swedish study that found reduced Bacteroides in infants who developed IgE-associated eczema (Abrahamsson TR et al., JACI 2012). In our study, we found reduced Ruminococcaceae in infants that developed IgE-associated eczema. Notably, the underrepresentation of Ruminococcus in these infants was associated with exaggerated innate inflammatory immune responses (West CE et al., Allergy in press). Interestingly, underrepresentation of Ruminococcus was also a feature in food sensitized 1-year-olds in a Canadian cohort (Azad MB et al., Clin Exp Allergy 2015).

Very recently, we studied the longitudinal gut microbiota development in allergic disease in more detail as we had collected stool samples in a probiotic allergy prevention trial in infancy. We did a follow up at 8 years of age including a very thorough clinical assessment and collection of biological specimens (West CE et al., Allergy 2013). We clinically phenotyped the children at 8 years of age and found 21 children which had IgE-associated allergic disease. Seventy-two children served as healthy controls as they were neither IgE sensitized nor did they show any other allergic manifestations.

Because we could study these children longitudinally from infancy to school age, we could identify both temporal and consistent underrepresentation of specific taxa in allergic diseases (Sjödin KS et al., Allergy in press). Ruminococcus was transiently underrepresented whereas Prevotella, Coprococcus and Bacteroides were consistently underrepresented. Another novel finding is that Faecalibacterium, which is suggested as a bacterial biomarker for gut health, correlated with the T cell regulatory markers IL10 and FOXP3 in allergic children at 8 years of age. In addition, we found transient and minor effects of probiotic intake so, during the intervention, there was a higher abundance of Lactobacillus at 6 mo of age; when we looked at 8 years of age there were no effects on the global microbiota nor were there any long-lasting effects on the Lactobacillus population.

Importance of Bacteroidetes

One of the functions of Bacteroidetes is that they stimulate epithelial mucin production and, together with Prevotella, Coprococcus and Bacteroides, they have the capacity to ferment carbohydrates to produce butyrate, with both nutritive and anti-inflammatory effects. Very recently, Bacteroides-derived fecal sphingolipids were negatively associated with food allergy (Lee-Sarwar K et al., JACI 2018).

In summary, this is the first study to have actually looked at the longitudinal development of gut microbiota in relation to IgE associated diseases (Sjödin KS et al., Allergy in press). Although there was a temporal underrepresentation of Ruminococcus, one important finding is that it was actually consistent underrepresentation of Bacteroides, Prevotella and Coprococcus.

The dysbiosis seen in a number of inflammatory non-communicable diseases has aroused huge interest and prompted researchers to use various microbiota modulation techniques to try to favorably modulate gut microbiota to improve immunological and metabolic outcomes and also influence the gut-brain axis. We do have to keep in mind that nutrition and gut microbiota are interrelated, so there are a number of dietary exposures that can have effects both on immunological programming and the gut microbiota. These include pre- and probiotics, dietary fibers, folic acid and antioxidants, when and how we introduce allergenic foods and PUFAs. So the aim of optimized intervention at this stage would be to try to induce the tolerogenic environment in the gut and also to promote tolerance acquisition.

Fiber is one option and I am involved in one study where dietary fiber is given in pregnancy to improve maternal gut microbiota with possible effects on a later colonization and immune outcomes in their children. Pre-, pro- and probiotics are still of interest and it will be highly interesting to disentangle the effects of HMOS in tolerance acquisition in the human setting. In addition, we are becoming increasingly aware that environmental microbes also have a huge impact on our microbiota thereby giving rise to other interventions than diet.

I think that during the last two years, we have come a little bit closer toward understanding this process. We have to take into account the prenatal and postnatal period in order to promote establishment of oral tolerance (Chart 2).

2: Microbial transmission and oral intake

<table>
<thead>
<tr>
<th>Prenatal period</th>
<th>Postnatal period</th>
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<tr>
<td>Oral intake of pro- and prebiotics</td>
<td>Microbial transmission through vaginal birth</td>
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<tr>
<td>The newborn acquires the maternal vaginal and gut microbiome (Lactobacillus, Bifidobacterium, Bacteroides etc.), favoring further commensal colonization.</td>
<td>The newborn acquires the maternal vaginal and gut microbiome (Lactobacillus, Bifidobacterium, Bacteroides etc.), favoring further commensal colonization.</td>
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<tr>
<td>Intestinal colonization by symbiotic bacteria</td>
<td>Intestinal colonization by symbiotic bacteria</td>
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<tr>
<td>Oral tolerance</td>
<td>Oral intake of pre- and prebiotics</td>
</tr>
<tr>
<td>Breastfeeding:</td>
<td>May support and benefit a homeostatic microbial colonization of the infant’s gut.</td>
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<tr>
<td>- Antigen transmission</td>
<td>- Antigen transmission</td>
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<tr>
<td>- Tolerogenic immune mediators</td>
<td>- Tolerogenic immune mediators</td>
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<tr>
<td>- TGF-β, IL-10, IgA, IgG</td>
<td>- TGF-β, IL-10, IgA, IgG</td>
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<tr>
<td>- Human milk microbiota</td>
<td>- Human milk microbiota</td>
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<td>- Microbiota modulating factor</td>
<td>- Microbiota modulating factor</td>
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<tr>
<td>Transfer of tolerogenic immune mediators to the baby</td>
<td>Enhanced epithelial barrier integrity</td>
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<tr>
<td>IgA</td>
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Summary

- Our commensal gut microbiota is important for the development of immune tolerance
- Dysbiosis is implicated in allergic diseases; more studies investigating the functional aspects of the gut microbiota are needed
- The gut microbiota is tunable
- Gut microbiota modulation strategies should aim to optimize both maternal and infant colonization patterns
What is the evidence that nutrition affects brain outcomes in preterm infants?

The last 30 years have seen a dramatic increase in survival of infants born preterm. The reasons for these improvements are complex, but there is strong reason to believe that improved attention to nutritional management has had a key impact on improving short- and long-term outcomes.

In turn, improvements in survival have meant that we are caring for increasingly fragile infants, some of whom have complex nutritional requirements. Despite advances in understanding and practice, adverse cognitive outcome remains the most serious consequence in preterm infants who survive.

The energy requirements of the brain are very high. To illustrate this, take this example from Geraint Thomas, who won the Tour de France in 2018. This athlete cycles up and down mountains for 3 wk and during this time he consumes 7,000 kcal/day, which is equivalent to 100 kcal/kg/day. In contrast, our smallest babies in the NICU require at least 120–130 kcal/kg/day. Most of this energy expenditure happens in the brain. So it is very easy to understand how even a short-term energy deficit is associated with a high risk of poor brain development.

Brain development is particularly rapid in the 3rd trimester and infancy: between 24 wk gestation and 2 years of age, human infants acquire around 90% of their brain size. During this period, a series of neuro-anatomical developmental processes must take place in a time-coordinated fashion if complex behavioural, cognitive and motor circuits are to develop normally. Normal brain development may be prevented due to damage associated with hypoxic or ischaemic events, brain haemorrhage, or ‘cytokine’ damage associated with systemic sepsis syndromes. Nutritional management – feeding rates, breast milk, etc. – may impact on the incidence of sepsis and necrotising enterocolitis (NEC) and thus, in turn, affect brain outcomes (Chart 1).

When you put together the complex neurological process, the complexity of the NICU environment and the fact that we can see a sub-optimal nutritional status, it is perhaps not surprising that we see very large numbers of preterm babies with abnormal neurocognitive outcome, neurocognitive phenotype.

At least five different interrelated mechanisms associate nutrition with brain growth. We need:

- Nutrients for tissue substrate: macro- and micronutrients
- Energy to drive the system: carbohydrates, lipids, proteins
- Signaling and growth factors: energy intake, branched-chain amino acids, IGF-1, etc.
- Impacts on gene expression: folate, B12, iron, choline, etc.
- Prevention of disease: breast milk reduces NEC and sepsis and everything that reduces NEC and sepsis improves brain growth.

Why is malnutrition so common? I think it is the complex situation in the NICU. The focus is what we can see and measure – and we measure a lot. But malnutrition is invisible and there is no machine or monitor that sounds an alarm when nutrient intakes are inadequate. The impact of nutrient deficiency – or excess – on preterm brain depends on the amount and type of nutrient, but also on the timing and duration of the exposure.

In the first few days and weeks, parenteral nutrition (PN) is the only reliable means of delivering adequate macronutrients to very preterm infants. Failure to provide adequate macronutrients in just the first week is associated with worse developmental outcome in infancy; but despite the strong observational evidence, there are no controlled trials of PN that clearly show improvements in brain outcomes.

A study shows 1 wk protein and energy intakes associated with 18 mo outcomes (n = 124; ≤ 1,000 g; mean 25 wk gestation). For 10 kcal/kg/day energy intake, you have 4.6 advantage in the MDI Bayley at 18 mo and an advantage of 8.2 for 1 g/kg/day protein intake (Stephens at al. Peds 2009) (Chart 2).

2 RCTs in the UK show different results: in SCAMP (Morgan et al. 2015), there was better head growth;
What is the evidence that nutrition affects brain outcomes in preterm infants?

2: First week protein & energy intakes associated with 18 m outcomes

3: Mother’s own milk (MOM): brain outcome better despite slower growth

Observational data point to the importance of breast milk even in the post-discharge period, despite the slower weight gain that is often observed. Studies have also begun to explore the role of increased levels of specific nutrients such as choline, with one recent trial suggesting that this may improve developmental outcomes in preterm and other infants at high risk of brain injury (Caudill et al. FASEB journal 2018).

Conclusions

- Focus on mother’s own milk (MOM)
- Deficient macronutrient intakes remain common
  - Optimal intakes remain uncertain
  - Macronutrient enrichment post-discharge in high-risk
- Ensure micronutrients are not deficient
  - Iron, zinc, iodine, essential lipids, etc.
- ‘Nutritional’ prevention of NEC or sepsis
- Urgent need for well-designed trials
- Multiple areas of future promise
Iron deficiency

Iron deficiency (ID) is often stated as the most common nutrient deficiency in the world. Young children are a high-risk group. About 25% of preschool children have ID anemia and up to 50% in poor areas as South Asia and Africa.

We know from animal studies and observational studies that iron is one of the nutrients which are essential for brain development. There have been numerous intervention studies and a meta-analysis showing that there seems to be an overall effect size of about 2 IQ points (Sachdev, Publ Health Nutr 2005). The most recent Cochrane meta-analysis shows that there is actually no convincing evidence that iron supplement improves motor or mental development in young children with iron deficiency anemia. This implies that prevention iron deficiency is really the most important factor.

We studied this in a high-risk group, which consists of marginally low-birth-weight infants (2,000 – 2,500 grams). This is actually a large proportion: about 3–5% of all newborns or even as much as 15% of neonates in some countries. These infants are usually perceived as healthy. It’s known from previous studies that these babies are at increased risk of behavioral problems and scholastic problems; theoretically, they could have a risk of iron deficiency. We performed the RCT of iron supplements in 285 otherwise healthy infants with gestational age 32 – 37 weeks. (Chart 1)

In the placebo group, 36% had iron deficiency and 10% had iron deficiency anemia. This was substantially reduced by iron supplementation and there was no IDA at all in the 2 mg group. But the aim was not only to observe the effects on iron status, but also the effects on the actual health of these children. It was very interesting to observe them at 3 years of age using the Achenbach child-behavior checklist. Among those babies who were not supplemented; 13% had behavioral problems, compared to only 3% in the two iron-supplemented groups. This was similar to a group of normal birth weight babies who were not at a high risk of iron deficiency (Chart 1).

So this really suggests that prevention of early iron deficiency in this risk group reduces the risk of later behavioral problems. We followed these children up to 7 years of age. These findings were published this year and the same pattern remains.

Delayed Cord Clamping

A totally different way of preventing iron deficiency is to delay the clamping of the umbilical cord at birth. In another RCT, we showed that delayed umbilical cord clamping at delivery of term infants improved early iron status and resulted in improved fine-motor function: 400 low-risk pregnancies were included and the intervention was early cord clamping (< 10 sec - ECC) or delayed clamping (> 3 min - DCC). The babies in the delayed clamping group had almost 100 grams heavier birth weight and also significantly higher hemoglobin. We followed those babies and found that at 4 mo there was no longer any difference in hemoglobin, but there were still significant differences in iron status, as well as a difference in the proportion with iron deficiency. Up to 4 years of age, we observed significant effect on fine-motor function in 3 different tests. The differences mostly involved boys (Chart 2).

Iodine

Severe iodine deficiency during pregnancy results in impaired brain development (cretinism) in the offspring. Mild/moderate iodine deficiency in pregnant women has been associated with lower IQ in the offspring. Mild/moderate iodine deficiency in pregnant women has been associated with lower IQ in the offspring. Consumption of iodized salt has decreased; mild iodine deficiency is now common in several European countries, including Sweden.

We are currently conducting a randomized, controlled trial of iodine supplementation of pregnant Swedish women (n=1,275) to determine whether this will improve cognitive development of their children up to school age. Among the first 200 participants, there is a significant effect on iodine status. This seems to have a good effect on preventing iron deficiency in the mothers. So it remains to be seen if this favorably affects neurodevelopment in the offspring.

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<th>1: Iron supplementation of LBW infants reduces behavioural problems at 3 years</th>
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<td>Placebo</td>
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The speaker: Magnus Domellöf, Sweden

The brain is the fastest-growing organ in young children. Furthermore, the brain develops very rapidly during the latter part of gestation and the first 2–3 years of life, and adequate nutrition is essential for the processes of neural proliferation, neural migration, axonal and dendrite growth and arborization, synapse formation and myelination.

**Iron deficiency**

Iron is essential for brain development. There have been numerous intervention studies and a meta-analysis showing that there seems to be an overall effect size of about 2 IQ points (Sachdev, Publ Health Nutr 2005). The most recent Cochrane meta-analysis shows that there is actually no convincing evidence that iron supplement improves motor or mental development in young children with iron deficiency anemia. This implies that prevention iron deficiency is really the most important factor.

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Breastfeeding and MFGM

Many observational studies have shown that breastfeeding is associated with higher IQ (about 4 points higher IQ at school age), but the mechanism behind this correlation is unknown. Several components in the breast milk are suspected to have an impact on development. We focused on the milk fat globule membrane (MFGM), which surrounds the lipid droplets that are excreted by the mammary epithelial cells. MFGM is also present in cow’s milk, but it is not present in standard infant formulas, in which the dairy fat is usually discarded and replaced by vegetable fats.

We did RCT of MFGM added to infant formula; 160 babies (0–2 months) were randomized to MFGM formula or standard formula. The intervention lasted until 6 mo and the babies were followed for the first 12 mo. In the Bayley score at 12 mo, there was no significant difference between MFGM and standard formula group in the verbal or motor scores, but there was a significant difference in the cognitive domain of the Bayley III test, where the MFGM-supplemented group had a 4 points higher Bayley score. They ended up at the same level as the breastfed control. We are currently following these children to 6 years of age to see if this effect persists (Chart 3).

Which of the MFGM components could cause this mechanism? One component is really interesting: the gangliosides. They comprise 10% of total brain lipids and play a role in the formation of synapses, neural growth and neural function. Brain gangliosides are highest in the prenatal and early postnatal periods. Breastmilk contains gangliosides at significantly higher levels than cow’s-milk-based infant formulas; 32% higher ganglioside-bound sialic acid concentrations were observed in the brains of infants with SIDS compared to breast-fed infants.

There was a small RCT (n= 59) randomized formula with or without supplementation of complex milk lipids to increase ganglioside content to 11–12 µg/mL. Significantly increased ganglioside serum levels were observed; at 6 mo, scores increased (to the level of BF group) for Griffith Scales general score, hand and eye coordination and performance. Of course, it is difficult to measure neurodevelopment at 6 mo, but it goes in the same direction as our study. Looking at the Lipodomics, we have not yet measured gangliosides, but we have measured a lot of lipids and we find a significant difference between the standard formula and the MFGM formula. This requires more studies to elucidate which factor in the MFGM would be responsible for this effect on neurodevelopment.

Conclusions

Early nutritional interventions can influence brain development in term infants

Examples:
- Iodine supplementation of pregnant women (study ongoing)
- Delayed cord clamping at delivery
- Iron supplementation of low-birthweight infants
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<thead>
<tr>
<th>Speakers</th>
<th>Institution</th>
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<tr>
<td>Magnus Domellöf</td>
<td>Departement of Clinical Science</td>
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Our **Mission**

The Nestlé Nutrition Institute shares leading science-based information and education with health professionals, scientists and nutrition communities and stakeholders, in an interactive way.

**What we do**

As well as being the world’s largest publisher of nutritional papers and journals, the NNI organizes and hosts internationally acclaimed workshops and symposia, making their findings available worldwide — often making the most of new technology to do so. It provides accredited continuing education programs for a wide range of healthcare professionals including doctors, nurses and dietitians. The NNI also offers grants to young clinicians and scientists globally, with a particular focus on the developing world.

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By sharing science-based information and education, the Institute fosters “Science for Better Nutrition” to contribute to the enhancement of the quality of people’s lives all over the world:

- Support your clinical practice with validated tools
- Access to the latest publications, videos and news
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